XVIII ISAH Congress 2017

March 19 – 23, 2017, Mazatlán, Sinaloa, Mexico

PROCEEDINGS

of the XVIII International Congress of the International Society for Animal Hygiene

"International Co-operation and Solidarity in Animal Hygiene towards One Health"

International Society for Animal Hygiene
and
Autonomous University of Sinaloa, Mexico

Preface and Welcome

On behalf of the organizing committee, the scientific committee and the Autonomous University of Sinaloa, it is our great pleasure to welcome you in Mazatlán, Mexico to participate in the XVIII International Congress of the International Society for Animal Hygiene (ISAH–2017) from 19th to 23rd March, 2017.

More than 47 years after the foundation of ISAH in Hungary 1970 and 14 years after the XIth ISAH Congress 2003 in Mexico City ISAH again reside in Mexico, this time in the state of Sinaloa with an important farm animal production.

The motto of the XVIIIth ISAH 2017 congress is **"**International Co-operation and Solidarity in Animal Hygiene towards One Health". It puts the focus of the congress right into the centre of the three principle domains of the science of hygiene, namely preserving animal health, human health and the health of the environment by preventing diseases according to the principle of "One Health".

The Congress therefore gives emphasis to all recent, novel and innovative research

on animal hygiene, animal health and welfare and sustainable livestock production.

A special focus of XVIIIth ISAH 2017 lies on the cooperation between veterinary and human health and will give incentives to improve the interaction of animal hygiene, veterinary and human public health.

Particular attention will be paid to animal welfare, health and behavior and to prevention strategies to avoid harm, pain and suffering. An important topic is the spread of diseases and pathogens in animals including those that pose a risk to human health (zoonoses). A wide range of further topics covers research and applied science in the fields of herd health, food quality and food safety, environmental pollution by animal production, emerging diseases, feed and additives, sustainable animal production, biosecurity, use of antibiotics and resistance, animal production in developing countries and husbandry of farmed animals, fishing, apiculture and aquaculture, precision livestock farming and disease prevention by new anti-infective approaches.

We do hope the congress will provide to you all a unique opportunity to present recent research results, to meet and get together with international experts and professionals to discuss interesting results and ponder new problems in a stimulating intellectual atmosphere and last not least to enjoy the charms of the relaxing beaches of Mazatlán and its beautiful surroundings.

We wish you a very prosperous congress and a nice stay in Mazatlán.

Dr. Juan Eulogio Liera Guerra
Rector Autonomous University of Sinaloa
2nd Vice-President of ISAH

Dr. Ruben Barajas Cruz
President of Organizing Committee
XVIII ISAH Congress

XVIII ISAH Congress 2017 Organizing Committee

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List of former ISAH Congresses:

Hungary 1973,
Yugoslavy, 1976
Austria, 1980
Czechoslovakia, 1982
Germany, 1985
Sweden, 1988
Germany, 1991
USA, 1994
Finland, 1997
Netherlands, 2000
Mexico, 2003
Poland, 2005
Estonia, 2007
Germany, 2009
Austria, 2011
China, 2013
Slovakia, 2015

Welcome address of ISAH President

Dear colleagues, friends and participants,

It is my great pleasure to welcome you all on behalf of the Executive Board of the International Society for Animal Hygiene in Mazatlán hosting our XVIIIth International Congress 2017 and invite you to share the congress with us. It is the second time that we are guests in Mexico, this time not in the metropole of Mexico City but in the beautiful north of the country with a variety of animal farming.

Animal hygiene, represented by ISAH, is a unique scientific interdisciplinary field of research and applied sciences where health and welfare of both animals and humans are closely intertwined and hygienic measures are in service of animal welfare, public health, diseases prevention, sustainable production of food from farm animals, bio-security, behavioural needs of animals and environmental protection in livestock production making optimal use of resources.

The promotion of interdisciplinary networks of scientists working in the field of animal hygiene and related areas and the transfer of "cutting-edge" knowledge on animal hygiene to veterinarians, animal scientists, animal producers, physicians and public health professionals as well as to decision makers in agribusiness and politics is one of the aims of the ISAH congresses.

The core device is "Prevention is better than Cure" which is in accordance with the EU future principle to prevent diseases instead of costly treatments. Veterinarians and related professions like agricultural engineers, biologists, epidemiologists and experts from human medicine are working closely together in order to prevent or reduce the risk of outbreaks of zoonotic diseases in the “One World – One Health” concept.

ISAH plays this role, in close cooperation with organizations like OIE, with increasing success over the last nearly 50 years. It is a non-profit science organization driven by an honorary Executive Board and an Extended Executive Board composed of the ISAH country representatives in a network of 55 countries throughout the world. (see also ISAH-soc.org). You are all invited to join us as members for a small contribution fee of 20 Euro per year.

Prof. Dr. Dr. h. c. Jörg Hartung
President of ISAH
On behalf of the Executive Board

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Program

18th International Congress on Animal Hygiene
ISAH-2017 Mazatlán, Mexico

"International Co-operation and Solidarity in Animal Hygiene towards One Health"

19 – 23 March, 2017

Sunday, March 19 of 2017

Place:

Centro Cultural Universitario

Address: Cruz 2, Paseo Olas Altas CP 82 000

Mazatlán, Sinaloa

Time	Activity	Place
12:00 to 21:00	Registration	Centro Cultural Universitario
19:00 to 21:00	Welcome cocktail	Centro Cultural Universitario

Monday, March 20 of 2017

Plenary

Scientific Program
Place: Mazatlán International Center
Av. del Delfín 6303, Fracc. Marina Mazatlán, 82103 Mazatlán, Sin.

Morning Session
Chairs:
Dr. Jörg Hartung, University of Veterinary Medicine Hannover and Dr. Andres Aland, Estonian University of Life Sciences

Time	Activity	Speaker	Title	Hall
9:00-9:30	**Plenary Lecture 1 (Conference)**	**Joaquin Braulio Delgadillo, Ph.D SAGARPA, Mexico**	**Animal Health in Mexico and International trade in livestock products**	"Mazatlán III"
9:30-10:00	**Plenary Lecture 2 (Conference)**	**Deyanira Barrero, Ph.D FAO**	**Importance of the initiative One Health** **One Health: within the framework of the Sustainable Development Goals (SDG) and its contribution to Food and Nutrition Security (FNS)**	"Mazatlán III"
10:00-10:30	**Plenary Lecture 3 (Conference)**	**Ignacio García Bocanegra, Ph.D. University of Cordoba**	**The Wildlife Population Health**	"Mazatlán III"
10:30-11:00		**Coffee Break**		
11:00-11:40	**Plenary Lecture 4 (Conference)**	**Donald M. Broom, Ph.D. University of Cambridge**	**Animal Welfare and Sustainability**	
11:40-12:00		**Coffee Break**		
12:00-13:00		**Inauguration Ceremony**		"Mazatlán III"
13:00-13:40	**Plenary Lecture 5 (Conference)**	**José Narro Robles, Ph.D. Secretary of Health Government of Mexico**	**Added later**	"Mazatlán III"
13:40-15:20		**Lunch Brake**		

Afternoon Session
Chairs:
Dr. Andres Aland, Estonian University of Life Sciences and Dr. Jörg Hartung, University of Veterinary Medicine Hannover

Time	Activity	Speaker	Title	Hall
15:20-16:00	**Plenary Lecture 6 (Conference)**	**Anthony Wilson, Ph.D. Pirbright Institute**	**One health and vector-borne diseases: what can we learn from comparing human and veterinary emergence events**	"Mazatlán III"
16:00-16:40	**Plenary Lecture 7 (Conference)**	**Frank van Erdenburg, Ph.D. University of Ultrech**	**In pursuit of happiness, the key to evolution**	"Mazatlán III"
16:40-17:10		**Coffee break**		
17:10-17:50	**Plenary Lecture 8 (Conference)**	**Francisco Suarez Guemes, Ph.D** **Alex Morrow, PhD. STAR-IDAZ**	**Add latrr**	"Mazatlán III"
17:50-18:35	Meeting	**Meeting of the Extended Executive Board of ISAH Country Representatives**		"Mazatlán III"

Monday, March 20 of 2017

Concert

Place: Theatre "Angela Peralta"

Andador Plaza Machado, Col. Centro 82000 Mazatlán, Sinaloa

19:30-21:30	Concert	**Symphonic Orchestra of the Autonomous University of Sinaloa**	Theatre "Angela Peralta"

Tuesday, March 21 of 2017

Scientific Program					
	Place: Mazatlán International Center Av. del Delfín 6303, Fracc. Marina Mazatlán, 82103 Mazatlán, Sin.				
Morning Session Chair: Dr. Leonel Avendaño Reyes Autonomous University of Baja California					
Time	Activity	Speaker	Title		Hall
8:15	**Hanging of posters in designed stand**				Isla de Pajaros
9:00-9:45	**Plenary Lecture 9 (Conference)**	**Richard A. Zinn, Ph.D. University of California, Davis**	**Heat Stress in Beef Cattle**		**University Theatre**
9:45-10:30	Coffee Break and Poster Viewing				Lobby
	Oral Presentations: **Animal health, welfare and behaviour I**				University Theatre
10:30-10:45	**Aspirin upregulates αb-crystallin to protect the myocardium against heat stress in chickens** S. Tang[1#], B. Yin[1#], H. Chen[1], Y. Cheng[1], X. Zhang[1], E. Bao[1*], J. Hartung[2]				
10:45-11:00	**Reasons and risk factors for on-farm mortality in Estonian dairy herds** K. Reimus[1], T. Orro[1], U. Emanuelson[2], A. Viltrop[1], K. Mõtus[1].				
11:00-11:15	**How does changing the feeding bin affect cows' behaviour?** M. Soonberg, D. Arney, T. Kaart, A. Aland				
11:15-11:30	**Air filtration systems to prevent airborne infections in pig facilities under field conditions** C. Wenke[1], J. Pospiech[1], D. Rüster[1], T. Reutter[2], U. Truyen[1], S. Speck[1]				
11:30-11:45	**Animal welfare indicators and body weight of beef cattle in silvopastoral systems of Uruguay** P. Bobadilla[1], H.J. Bueno[2], S.M. Huertas[3]				
11:45:12:00	**Automated assessment of animal welfare indicators in pigs at slaughter** L. Blömke, N. Kemper				
12:00-13:30	Lunch Break				

Tuesday, March 21 of 2017

Afternoon Session

Chair:

Dr. Gustavo Ruiz Lang
Autonomous metropolitan University

13:30-14:15	**Plenary Lecture 10 (Conference)**	**Adelita San Vicente, M.Sc. Seeds of Life Foundation**	**The importance of maize for Mexico and for the World**	Isla de Pajaros
	Oral presentations: **Animal health, welfare and behaviour II**			Isla de Pajaros
14:15-14:30	**Associations between driving actions and animal stress in mobile slaughter of cattle** Jan Hultgren, Charlotte Berg, Bo Algers			
14:30-14:45	**A linkage between non-compliance with animal welfare legislation and environmental emissions** Peta L. Hitchens[1], Jan Hultgren[2], Jenny Frössling[2,3], Ulf Emanuelson[4], Linda J. Keeling[1]			
14:45-15:00	**Ammonia reducing microbial-mineral litter additive for poultry manure treatment** K. Kalus[1], S. Opalinski[1], M. Korczynski[1], K. Matusiak[2], Z. Dobrzanski[1], B. Gutarowska[2], R. Kolacz[1]			
15:00:15:15	**The effect of ambient temperature on joints in the distal forelimbs of healthy racehorses** M. Soroko[1], K. Howell[2], K. Dudek[3], P. Cwynar[4]			
15:15-15:45	**Coffee Break**			
15:45-16:00	**Occurrence of claw lesions in beef suckler cows in Germany** K. Gillandt[1], N. Kemper[1]			
16:00-16:15	**Dairy cow daily time budget as an indicator of welfare, health and biosecurity** [1]P.Novak, [1]G.Mala, [2]S.Smutna, [2]L.Smutny			
16:15-16:30	**Welfare of native pig breeds in different housing conditions** J. Walczak[1], W. Krawczyk, E. Herbut			
16:30-16:45	**Validation of carcass lesions as indicators of pig welfare on farm** N. van Staaveren[1,2], B. Doyle[1], E.G. Manzanilla[1], J. A. Calderón Díaz[1,3], A. Hanlon[2], L.A. Boyle[1,3]			
	Oral presentations: **Husbandry of farmed animals, fishing, apiculture and aquaculture**			Isla de Pajaros
16:45-17:00	**The effect of two technological systems for calf housing to the future performance of dairy cow** G. Mala, P. Novak, M. Stipkova, P. Jiroutova, J. Knizek, D. Prochazka, M. Slavikova			
17:00-17:15	**New tool for average daily gains monitoring on pig farms** Thomas M. Banhazi[1], Mark Dunn[2], Annamarie Banhazi[1]			

Tuesday, March 21 of 2017

Scientific Program In parallel Session

Place: Mazatlán International Center

Av. del Delfín 6303, Fracc. Marina Mazatlán, 82103 Mazatlán, Sin.

Morning Session

Chair:

Dr. Fernando Iván Flores Pérez

Autonomous University of the State of Morelos

Time	Activity	Speaker	Title	Hall
	Oral Presentations: **Animal hygiene and herd health I**			Isla de Venados
10:30-10:45	**A longitudinal study to assess the hygienic quality of disinfection measures on pig farms** P. Münster[1], K. Müller[2] [1]*H. Bröring GmbH & Co. KG, Dinklage, Germany*.			
10:45-11:00	**Factors associated with the age-time to prrsv seroconversion in swine infected herds** C. Fablet[1], C. Marois-Créhan[2], V. Dorenlor1, F. Eono[1], E. Eveno[1], V. Tocqueville[2], S. Gorin[3], S. Quéguiner[3], L. Bigault[4], B. Grasland[4], G. Simon[3], N. Rose[1]			
11:00-11:15	**Rustic model of milk production and quality and the prevalence of pathogenic bacteria in bovine mastitis in Jalisco, Mexico** H. Castañeda Vázquez[1], M. Alicia Castañeda Vázquez[1], E. P. Salas Castañeda[1], J. C. Serratos Arevalo[2], J. R. Estrada González[2], and C. Bedolla Cedeño [3]			
11:15-11:30	**When All-In/All-Out is not 'AIAO': a technical note on its consequences for pig health** J.A. Calderón Díaz[1, 2], L.A. Boyle[1], A. Diana[1, 3], M. McElroy[4], S. McGettrick[4], J. Moriarty[4] E.G. Manzanilla[1]			
11:30-11:45	**Efficacy of sodium hypochlorite against multi-resistant gramnegative bacteria** A. Köhler[1], M. Labahn[1], M. Reinhardt[1], A. Rodloff[2], U. Truyen[1], S. Speck[1]			
11:45:12:00	**DO ESBL- /AMPC-producing enterobacteriaceae survive disinfection measures in broiler farms?** A. Blasse, C. Robé, A. Friese and U. Roesler			
12:00-13:30	**Lunch Break**			

Tuesday, March 21 of 2017

Afternoon Session
Chair:
Dr. Jorge Saltijeral Oaxaca
Autonomous Metropolitan University

	Oral Presentations: **Animal hygiene and herd health II**	Isla de Venados
14:15-14:30	**Transmission of ESBL-/AMPC-producing enterobacteriacae in the broiler production** K. Daehre[1], M. Projahn[1], P. v. Tippelskirch[2], S. Orquera[2], T. Alter[2], A. Friese[1], U. Roesler[1]	
14:30-14:45	**Effect of wintering system on the behaviour of yearling dairy heifers** L.A. Boyle[1,3], R. H. van Reenen[2], K. O'Driscoll[1], F. van Eerdenburg[2], F. Buckley[1]	
	Oral Presentations: **Animal production in developing countries**	Isla de Venados
14:45-15:00	**Involvement of environmental conditions in dairy performances of Tunisian herds** Y. Ressaissi[1], M. Ben Hamouda [2]	
15:00-15:15	**Effect of technology transfer on sustainable livestock production of developing countries** A. Cervantes Nuñez[1], E. Guerra Liera[2], J. Moreno Quiroz[2], J.O. Duarte Atondo[2] F, Inzunza Castro[2], and L. A. López Juarez[2]	
15:15-15:45	**Coffee Break**	
	Oral Presentations: **Nutrition, feed and additives**	Isla de Venados
15:45-16:00	**Influence of *bifidobacterium* species on functional status of rumen** Luboš Zábranský[1], Miloslav Šoch[1], Veronika Hadačová[1], Anna Poborská[1]	
16:00-16:15	**Potential use of olive cake by products in Ossimi sheep in Egypt** K.M., Marzouk[1] ; M. Y.,Mohamed[2] , E.M.M.,Ibarhim[2] and A.I., El Zanouny[1]	
	Oral Presentations: **Sustainable animal production and agro-biodiversity conservation: efficient and alternative farming**	Isla de Venados
16:15-16:30	**The use of photovoltaic cells in a dairy cattle farm** W. Krawczyk[1], J. Walczak, E. Herbut	
16:30-16:45	**The use of tannins as growth promoters in poultry chickens** L. M. Redondo, E. A. Redondo, P. A. Chacana, M. E. Fernandez-Miyakawa	
	Oral Presentations: **Precision livestock farming: techniques, risks and benefits**	Isla de Venados
16:45-17:00	**The calf cough monitor: sound analysis for early detection of bovine respiratory disease** Lenn Carpentier[1], Tomas Norton[1], Dries Berckmans[2], Bernadette Earley[3], Ilaria Fontana[4], Emanuela Tullo[4], Marcella Guarino[4], Daniel Berckmans[1]	
17:00-17:30	First meeting with PTF Students present themselves, PTF presents.	Isla De Venados

Wednesday, March 22 of 2017

Scientific Program

Place: Mazatlán International Center

Av. del Delfín 6303, Fracc. Marina Mazatlán, 82103 Mazatlán, Sin.

Morning Session

Chair

Dr. Stefan Gunnarsson

Swedish University of Agricultural Sciences

Time	Activity	Speaker	Title	Hall
9:00-9:45:00	**Plenary Lecture 11 (Conference)**	**Cornelia Silaghi, Ph.D. University of Zurich**	**Vector Borne Diseases**	Isla de Pajaros
9:45-10:30	**Coffee Break and Poster Viewing**			Lobby
	Oral Presentations: **Animal health, welfare and behaviour III**			Isla de Pajaros
10:30-10:45	**Animal welfare and sostenible human program** E. De Varona Rodríguez[1], L. M. Medina Celis[1], G. Medina Celis[2]			
10:45-11:00	**Homology among *Rhipicephalus microplus* tick populations** C. L. Barraza Tizoc[1], I. Enríquez Verdugo[1], N. Castro del Campo[1], J. D. Solís Carrasco[1], R. Barajas Cruz[1], Y. E. Villalba Robles[1], and S. M. Gaxiola Camacho[1].			
11:00-11:15	**Prevalence of *Cryptosporidium* spp in lambs of the municipality of Culiacan, Mexico.** C.B. De Dios Quiñonez, N. Castro del Campo[1], I. Enríquez Verdugo[1], C.L. Barraza Tizoc, J.D. Solis Carrasco [1], N. Castro del Campo[2], S.M. Gaxiola Camacho[1]			
11:15-11:30	**Evaluation of animal welfare in pigs during discharge, pens staying and stunning effectiveness** G. Dominguez Jimenez[1] R. L. Nogales Acuña[2], F. H. Chamorro Ramirez[1]			
	Oral Presentations: **Animal hygiene, food quality and food safety I**			Isla de Pajaros
11:30-11:45	**Effect inoculant mixed culture on theobromine** **Cocoa pod silage** M. A. Zakariah[1] and M. Zakariah[1]			
11:45:12:00	***Cryptosporidium* in piglets and calves of river basins in Kathmandu: an issue of animal hygiene and health** S. Paudyal[1], S. P. Shrestha[2]			
12:00-13:30	**Lunch Break**			

Wednesday, March 22 of 2017

Afternoon Session

Chair:

Dr. Jan Venglovsky

University of Veterinary Medicine and Pharmacy in Košice

13:30-14:15	**Plenary Lecture 12 (Conference)**	**Jana Mojžišová, Ph.D University of Veterinary Medicine and Pharmacy in Kosice**	**Education and research activities at the University of veterinary medicine and pharmacy in Košice, Slovak Republic**	Isla de Pajaros
	Oral Presentations: **Animal hygiene, food quality and food safety II**			Isla de Pajaros
14:15-14:30	**The importance of microbiological analysis of drinking water, intended for animal consumption** S. Antoniu			
14:30-14:45	**The microbiological monitoring of laboratory environment** S. Antoniu			
14:45-15:15	**Coffee Break**			
15:15-15:30	**Melamine negatively affactes testosterone synthesus in mice** J. Sun[1], Y. Cao[1], X. Zhang, Q. Zhao, E. Bao, Y. Lv *			
15:30-15:45	**Lassa fever risk perception and "one-health" considerations associated with rodent control practices in a Nigerian university** Amienwanlen E. Odigie[1], Babasola O. Olugasa[2]			
15:45-16:00	**Water hygiene: an important parameter on the way towards improved animal health** N. Kemper[1]			
16:00-16:15	**Effects of glyphosate on farm animal-associated bacteria** O. Makarova[1], J. Poeppe[1], K. Bote[1], U. Roesler[1]			
16:15-17-45	**ISAH GENERAL ASSEMBLY**			Isla de Pajaros
17:45- 18:30	**Concluding meeting with PTF Students**			Isla de Pajaros
19:30-22:30	**ISAH CONFERENCE BANQUET (Place TBD)**			

Wednesday, March 22 of 2017

Scientific Program In parallel Session

Place: Mazatlán International Center
Av. del Delfín 6303, Fracc. Marina Mazatlán, 82103 Mazatlán, Sin.

Morning Session
Chair
Dr. Uwe Rösler
Freie Universität Berlin

Time	Activity	Speaker	Title	Hall
9:45-10:30	**Coffee Break and Poster Viewing**			Lobby
	Oral Presentations: **Zoonoses and emerging diseases**			Isla de Venados
10:30-10:45	**Prevalence and risk factors associated with serovars of *Leptospira* in dogs, related human seropositive**	CV. Hernandez,[1-3], SM. Gaxiola.[1], I. Manriquez [1], I. Osuna I[2], JR. Rivas[3].		
10:45-11:00	**Actualization of strategies for one-health in the context of Animal hygiene education in west Africa**	B. O. Olugasa[1], A. E. Odigie[2]		
	Oral Presentations: **Antibiotics use in animal production: resistance and consequences**			Isla de Venados
11:00-11:15	**ESBL-plasmids interfere with biofilm formation, competitive adhesion and serum resistance**	K. Schaufler [1], A. Ranjan [1], T. Semmler [1,2], L. H. Wieler [1,2], C. Ewers [1,3], D. J. Pickard [4], S. Guenther [1, 5]		
11:15-11:30	**Differences of phenotypic resistances between *E. coli* isolates from "old" animal house dust samples**	J. Schulz[1], I. Ruddat[2], J. Hartung[1], N. Kemper[1]		
	Oral Presentations: **Biosecurity**			Isla de Venados
11:30-11:45	**The importance of microbiological analysis of drinking water intended for animal consumption**	S. Antoniu		
11:45:12:00	**Are the "top" 25% irish pig farms doing something different in terms of biosecurity practices related to staff and visitors?**	J.A. Calderón Díaz[1, 2], M. Rodrigues da Costa[1, 3], Pilar Guzmán Medina[1], L.A. Boyle[1], E.G. Manzanilla[1]		
12:00-13:30	**Lunch Break**			

Afternoon Session
Chair
MSc. Marco Antonio Espino García
PROAN, Mexico

	Oral Presentations: **Environmental pollution by animal production**			Isla de Venados
14:15-14:30	**Reduction of gas emissions from poultry production using biofilter with water curtain**	W. Krawczyk[1], J. Walczak, E. Herbut		
14:30-14:45	**Whole genome sequence analysis of ESBL- E. coli of ST 131 AND ST648 from various habitats**	Katharina Schaufler[1], Torsten Semmler[2], Alan McNally[3], Lothar Wieler[2], Derek Pickard[4] Uwe Rösler[4], Christa Ewers[5], Sebastian Guenther[1,4]		
14:45-15:15	**Coffee Break**			
15:15-15:30	**Animal welfare in an environmental perspective; a case study of life cycle analysis of pig production**	Stefan Gunnarsson[1] and Ulf Sonesson		
15:30-15:45	**Reduction of bioaerosols in exhaust air of a biofilter by regulation of the moisture content**	L. Nier[1], N. Volkmann[1] J. Schulz[1], N. Kemper[1]		
15:45-16:00	**Natural fertilizers as a substitute for maize silage in agricultural biogas production**	J. Walczak[1], W. Krawczyk		

Poster Session

Tuesday, March 21 of 2017
9:45 to 10:30 hours

Scientific Program

Place: Lobby of Mazatlán International Center
Av. del Delfín 6303, Fracc. Marina Mazatlán, 82103 Mazatlán, Sin.

Chair: Dr. Briceida Ortiz Lopez and Dr. Leopoldo Raul Flores Aguirre

Autonomus Univerity of Sinaloa

Posters must be placed at 8:15 am and remain displayed throughout the day
The presenting-author (or collaborators) must be available next to his poster during all coffee brakes
Posters must be removed by their authors at the end of the day

Board Number	Poster Title
	Animal health, welfare and behaviour
T1	**A welfare-friendly method alternative to blood sampling for group-housed sows?** C. Fablet, V. Dorenlor, F. Eono, S. Eudier, E. Eveno, D. Liégard-Vanhecke, N. Rose, F. Pol
T2	**Octenidine hydrochloride: disinfection efficacy against mrsa of different origin** A. Köhler[1], M. Labahn[1], C. Cuny[2], U. Truyen[1], S. Speck[1]
T3	**Efficiency of different air filter types at laboratory scale** C. Wenke[1], J. Pospiech[1], D. Rüster[1], T. Reutter[2], U. Truyen[1], S. Speck[1]
T4	**Pen-shade on feedlot performance of calves during their first days in confinement under hot weather conditions** R. Barajas[1], B. J. Cervantes[2], B. Ortiz[1], N. Castro[1], D. Jiménez[1], L. A. Montejo[1], A. Salazar[1], S. Sepúlveda[1], L. Avendaño-Reyes[3]
T5	**The welfare quality® assessment protocol - how can it be adapted to family farming dual purpose cattle raised under extensive systems in tropical conditions?** Adalinda Hernandez[1], Charlotte Berg[1,5], Sofie Eriksson[1], Linnea Edstam[1], Agustin Orihuela[2], Horacio Leon[3], Carlos Galina[4]
T6	**Effect of *Brucella abortus* antibodies on days open and calving interval in cows** [1]A. Córdova Izquierdo A., [1]A. E. Iglesias Reyes, [1]R. Espinosa Cervantes, [2]J.E. Guerra Liera, [2]J.F. Inzunza Castro, [3]R. Huerta Crispín, [4]M.L. Juárez Mosqueda, [5]G. Cansino Arroyo, [5]A. Gómez Vázquez, [6]V. Velázquez Ordoñez, [6]P. Sánchez Aparicio, [7]J. Olivares Pérez, and [1]C.G. Ruiz Lang
T7	**Presence of subclinical mastitis in holstein cows at the time of milking** [1*]Córdova Izquierdo A., [1]Iglesias Reyes A. E., [1]Espinosa Cervantes R., [2]Guerra Liera J.E., [2]Inzunza Castro J.F., [3]Huerta Crispín R., [4]Juárez Mosqueda M. L., [5]Cansino Arroyo G., [5]Gómez Vázquez A., [6]Velázquez Ordoñez V., [6]Sánchez Aparicio P., [7]Olivares Pérez J., and [1]Ruiz Lang C.G..
T8	**Mobile houses for laying hens – both chance and challenge** M. F. Giersberg, B. Spindler, N. Kemper
T9	**Cognitive bias test as a tool for accessing welfare of fish** K. Wojtas[1], R. Kolacz[1]
T10	**Protocol to assess welfare in dairy sheep and dairy goats** J. Saltijeral[2], E. De Varona[1], and G. Ruiz [2]
T11	**Influence of continuous environmental enrichment on aggressive behaviour of piglets** S.L. Rauterberg, N. Kemper, M. Fels
T12	**Abomasal secretion in the milk-fed calves with diarrhea** *Igor N. Zhirkov,*
T13	**Use of ecological pure substance in treatment the diarrheas of preruminant lambs** Igor N. Zhirkov,
T14	**Study of different laboratory methods for diagnosis of bovine leptospirosis** N. Barrandeguy [1], A. Suanes [2], J. Piaggio [2,1], Huertas S [1].
T15	**Turmeric as an anthelmintic alternative in backyard goats** Ma. E. Cervantes-Valencia[1], I. Cruz-Mendoza[2], N. Saldaña-Hernández[2], Y. Alcalá-Canto[2]
T16	***T. pisiformis* induces hormonal and behavioral changes associated with infective dose** R. Domínguez-Roldan[1], C. Hallal-Calleros[1], E. Sciutto[2], M. Hernández[2], V. Aguirre-Flores[1], S. García-Jiménez[3], A. Báez-Saldaña[2], F. I. Flores-Pérez[1]
T17	**Finding of *Libyostrongylus douglassii* in ostriches, through the identification of l111 in the state of Mexico.** J.R. Sánchez Ayala [1], I. Cruz Mendoza [2]

T18	**Effects of stocking density and genotype on some blood chemistry levels of heat stressed heifers** J.A. Aguilar[1], J.E. Guerra[2], L. Avendaño[3], U. Macías[3], M.A. Gastélum[2], A. Correa[3], A.L. Lara[3], A. Vicente[1], J.L. Corrales[3], R. Barajas[1], R. Vicente[3]
T19	**Effects of shade on growth performance of hairsheep ewes in severe heat stress conditions** L. Avendaño[1], J.L. Corrales[1], G. Corrales[2], J.E. Guerra[2], U. Macías[1] M.A. Gastélum[2] A. Correa[1], A.L. Lara[1], A. Vicente[2], R. Vicente[1], J.A. Aguilar[2], J.L. Ponce[3], R. Barajas[4], and M. Mellado[5]
T20	**The use of an algae-based complementary feed helps limiting pedv damage on suckling piglets** M Gallissot, J Laurain, L Diaz, MA Rodriguez
T21	**Serological detection of *Ehrlichia canis* in canines from Culiacan, Mexico** B.E. López Gallegos[1], S.M. Gaxiola Camacho[1], C.L. Barraza Tizoc[1], N. Castro del Campo[1], J.D. Solis Carrasco[1], M.C. Rubio Robles[1], J. Gaxiola Montoya[1], I. Enríquez Verdugo[1.]
T22	**Identification of *Anaplasma marginale* in calves from culiacan, mexico** J.J. Campos Sánchez[1], C.N. Badilla Medina[1], N. Castro del Campo[1], C.L. Barraza Tizoc[1], J.D. Solís Carrasco[1], S.M. Gaxiola Camacho[1], I. Enríquez Verdugo[1].
	Zoonoses and emerging diseases
T23	**Improved protein cocktails complement bovine purified protein derivative for *in vitro* diagnosis of subclinical bovine TB** A.H. Alvarez[1], A. Gutiérrez[1], V. Gómez[1], G. Pérez[1], J. Naranjo[1], F. Milián[2]
T24	**Marbofloxacin action in amastigotes of *leismania chagasi* in macrophages of balb/c mice** J. F. A. A. Amante[1], J. Venturini[2], B. M. Santos, A. R. Santos[2], G. S. Latosinski[1], H. Langoni[1]
T25	**Immuno-stimulating complex as adjuvant for recombinant veterinary vaccine against rabies virus** *Rocca MP [3], Menozzi BD [3], Langoni H [2], Pereira CA [1], Astray RM [1].*
T26	**Novel *Rhodococcus equi* virulence plasmid (pVAPN) type identified in bovines and human from brazil** M.G. Ribeiro[1], G.H.B. Lara[1], P. da Silva[2], A.L. Mattos-Guaraldi[3], M.M.J. Franco[1], A.C. de Vargas[4], R.I. Sakate[5], T. Kakuda[6], F.J.P. Listoni[1], S. Takai[6]
T27	**Genes associated to virulence and *in vitro* antimicrobial susceptibility of *T. pyogenes* isolated from bovine mastitis** M.G. Ribeiro[1], R.M. Risseti[1], A.P.C. de Vargas[2], C.A.D. Bolaños[1,3], C.L. de Paula[1], A.C. Alves[1], G.H.B. Lara[1], M. Twarużek[4], E. Zastempowska[5]
T28	**Histopathological and molecular diagnosis of *Trypanosoma cruzi* in domestic cats from Brazil** S.B. Lucheis[1, 2, 3], M.F. Alves-Martin[3], M. L. Alves[5], M.A.S. Zuque[3], M.S. Paixão[3], W.J. Santos[3], L.M. Guiraldi[3], G.P. Sánchez[1], D.T. Silva[4], Wilma Aparecida Starke-Buzetti[5].
T29	***Trypanosoma cruzi* infection in wildlife in a high-end gated community in southeastern Brazil** L. Moraes Paiz[1], M. R. Donalisio[1], V. Bodelão Richini-Pereira[2], J. E. Tolezano[3], G. Motoie[3], C. L. Castagna[4], H. Langoni[5]
	Disease prevention and new anti-infective approaches, climate change and livestock production
T30	**Current level of GHG emission reductions in polish agriculture** J. Walczak[1], W. Krawczyk
T31	**Immune modulating activities of sulfated polysaccharides of green algae (*Ulva armoricana*) extract** MA Rodriguez[1], M Berri[2], L Diaz[1], P Nyvall-Collen[1]
T32	**Behavioural and neurohormonal analysis** OF DEHORNING PROCEDURE IN CALVES [1]P. Cwynar, [1]R. Kupczyński, [1]A. Burek, [1]K. Pogoda-Sewerniak, [2]M. Soroko
T33	**African swine fever in Poland** **– epidemiological report** Przemysław Cwynar, Witold Janeczek
T34	**Effect of feeding *Trigonella foenum-graecum* on growth performance of broiler chicks** H. Castañeda Vázquez[1], M. A. Castañeda Vázquez[1], E. P. Salas Castañeda[1],J. C. Serratos Arevalo[2], D. S. Covarrubias[2] , C. Bedolla Cedeño
T35	**Morphometric characterization of the teats in holstein cows in Tejaro Michoacán** C. Bedolla[1], R. Lucio[1], A. R. Cruz[1], E. García[1], J. C. Bedolla[1], H. Castañeda[2], V. Velázquez[3] and J. Saltijeral[4]
T36	**An exchange proposal: Jean Monnet Module: Hygiene and Animal Welfare** J.Saltijeral [1], G.Ruiz [1]
T37	**Comparison between hair coat thermal insulation of alpacas and merinos** M. Soroko[1], A. Wyrostek[2], K. Howell[3], K. Dudek[4], P. Cwynar[5], B. Patkowska – Sokoła[2]
T38	**Antigenic and genotypic characterization of rabies virus isolated from bats (*mammalia: chiroptera*) from municipalities in São Paulo State, southeastern brazil** B. D. Menozzi[1], R. de Oliveira Novaes[2], L. Moraes Paiz[3], V. Bodelão Richini Pereira[4], H. Langoni[1]

Poster Session

Wednesday, March 22 of 2017

Scientific Program

Place: Lobby of Mazatlán International Center

Av. del Delfín 6303, Fracc. Marina Mazatlán, 82103 Mazatlán, Sin.

Chair: Dr. Leopoldo Raul Flores Aguirre and Dr. Briceida Ortiz Lopez

Autonomus Univerity of Sinaloa

Posters must be placed at 8:15 am and remain displayed throughout the day
The presenting-author (or collaborators) must be available next to his poster during all coffee brakes
Posters must be removed by their authors at the end of the day

Board Number	Poster Title
	Husbandry of farmed animals, fishing, apiculture and aquaculture
W1	**Deworming practices in sheep: selective deworming, benefits?** P.M.C. Acevedo-Ramírez[1], M.A. Mendoza Nieto[2], C. Juárez Campos[2], A.L. García Soria, A.A. Trejo[2], H. Quiroz Romero[1], I. Cruz Mendoza[1].
W2	**Effect of anthelmintic treatments in horses** P.M.C. Acevedo-Ramírez[1], B. Landeros-Mellado, H. Quiroz-Romero[1], I. Cruz-Mendoza[1]
W3	**Technical performances influenced by infectious and non-infectious factors: a study in 41 swine herds** C. Fablet[1], N. Rose[1], B. Grasland[1], E. Lewandowski[2], M. Gosselin[3]
W4	**Hygienic status of organic enrichment materials in pig production** K. M. Wagner, J. Schulz, N. Kemper
W5	**Comfortable housing of dairy cows – basis for health, welfare and biosecurity** [1]P.Novak, [1]G.Mala, [2]S.Smutna, [2]L.Smutny
	Animal hygiene, food quality and food safety
W6	***Listeria monocytogenes* participation in the production chain of familiar milk husbandry at Botucatu, São Paulo, Brazil** G. C. Oliveira, N. B. Junqueira, A. Salina, F. F. Guimarães, S. F. Joaquim, F. M. Dalanezi, H. Langoni
W7	**Deworming or not? Indiscriminate use of antiparasitics, especially ivermectin** P.m.c. acevedo-ramírez
W8	**Practices of deworming in cattle** P.M.C. Acevedo-Ramírez[1], J.J. Campos-Sánchez[2], H. Quiroz-Romero[1], I. Cruz Mendoza[1]
W9	**Research on *Yersinia enterocolitica* in expansion tanks at dairy farms in Sao Paulo, Brazil** S. B. Lucheis[1, 2, 3], A. B. Bertolini[1], M. F. Alves-Martin[3], M. S. Paixão[3], W. J. Santos[3], L. M. Guiraldi[3], M. F. Toscano[4], M. I. M. Medeiros[2]
W10	**Forage production of three sudan hybrids in two locations with rainfed conditions in Sinaloa, Mexico** MA Gastélum Delgado[1], JE Guerra Liera[1], D González González[2], LA López Juárez[1], MA Cárdenas Contreras[1], HJ López Inzunza[1], M Mejía Delgadillo[1]
	Animal hygiene and herd health
W11	**Airborne detection of swine influenza a virus and *Mycoplasma hyopneumoniae* in french swine farms** C. Fablet, C. Marois-Créhan, S. Hervé, P. Renson, G. Simon, O. Bourry, N. Rose
W12	**Mastitis caused by *Mycoplasma bovis* in Brazil** N. B. Junqueira, G. C. Oliveira, A. Salina, F. F. Guimarães, S. F. Joaquim, G. S. Latosinski, H. Langoni
W13	**Epidemiology of gastrointestinal parasites in ewes from mexico** P.M.C. Acevedo-Ramírez[1], A.L. García-Soria, H. Quiroz-Romero[2], I. Cruz-Mendoza[2]
W14	**Evaluation of the hygiene management in an equine surgery clinic** I. Frank[1], W. Brehm[2], U. Truyen[1], S. Speck[1]
W15	**Microbial load of dust samples from laying hens flocks in egypt: first results** MFE Ahmed[1,2], H Ramadan[2],J Schulz[1] and N Kemper[1]
W16	**Seasonal variation of subclinical mastitis during the summer-autumn period in a dairy herd of family production** G Mancera Cuadros[1; 2; 3], B Valladares Carranza[2; 3], M Talavera Rojas[2; 3], O Castelán Ortega[2], M González Ronquillo[2], J Saltijeral Oaxaca[4], V Velázquez Ordóñez[2; 3]
	Nutrition, feed and additives
W17	**Productive response of growing pigs to organic zinc supplementation** J. M. Romo Valdez[1], J. A. Romo Rubio[1], A. Montero Pardo[1], M. A. Rodríguez Gaxiola[1], C. Urías Castro[1], H.

Thursday, March 23 of 2017

Scientific Program

Place: Farms around Mazatlán
(less than 50 km from Mazatlán)

Time	Activity	Chair
8:30-14:30	**Technical Tours** **Chair** Dr. Ruben Barajas Cruz Autonomous University of Sinaloa	
8:30-14:30	**Ecological Farm**	Dr. Briceida Ortiz Lopez Autonomous University of Sinaloa
8:30-14:30	**Pig open system in hot climate**	MVZ Victor Hernandez Posada Tecnica Mineral Pecuaria, SA de CV
8:30-14:30	**Feedlot cattle in tropical weather**	Dr. Ruben Barajas Cruz Autonomous University of Sinaloa
8:30-14:30	**Shrimp farm**	Dr. Gustavo A. Rodriguez Montes de Oca Autonomous University of Sinaloa

Lectures

Opening Address

Animal Hygiene – interdisciplinary science and research towards "One Health" for a better life of animal, man and environment

Jörg Hartung
Institute of Animal Hygiene, Animal Welfare and Farm Animal Behaviour,
University of Veterinary Medicine Hannover, Foundation
Germany
President of the International Society for Animal Hygiene

Introduction

Hygiene is one of the oldest scientific disciplines in mankind history. The term goes back to the early roots of European civilisation and the mythology of ancient Greece. The importance of hygiene in human medicine of those days is demonstrated by the fact that "Hygiéia" was in the rank of a goddess. She was the daughter of Asklepios (god of surgeon) and Epione (goddess of health care taker). Asklepios was the son of Apollon and Coronis. His teacher had been the centaur Cheiron who taught him to treat humans and heal diseases. Cheiron was a natural scientist we would say today. He knew nature and animals and the forces of the environment. Epione tried to ease pain and to soothe and to comfort sick patients. Both gods recognised very soon that neither his skills to heal nor her abilities to care for sick patients were able to prevent disease and suffering because they came always too late when the patients had fallen ill already. Therefore they engendered her child Hygiéia which is a synonym for "the art to maintain health" both physically and mentally. Her task was to prevent the onset of a disease and all forms of suffering by creating pleasant, harmonious and as far as possible stress-free living conditions (well-being). Thus Hygiéia represents still today the hygienic principle that preventing a disease is the first choice and better than curing a disease. This principle of prevention is clearly expressed in the EU Animal Health Strategy since 2007 by the term "Prevention is better than cure". This is equally true for humans and animals and for veterinary as well as human medicine.

The term "animal hygiene" appears in veterinary text books at the beginning of the 19th century. However, it was not until the beginning of the last century that animal hygiene became a regular component of veterinary science and education focussing on health care and the investigation of the origin of animal diseases, mainly infectious diseases. Klimmer (1913) was one of the first who introduced "hygiene as preventive medicine" in the curriculum of veterinary universities and in practice. His definition of animal hygiene still meets essential topics of today: "The maintenance of the health of (farm) animals is that part of veterinary science which helps us to recognise the causes of animal diseases and teaches us how to prevent factors that cause disease by improving disease resistance without neglecting the economic purpose of livestock production". Klimmer saw "Animal Hygiene" as "Preventive Medicine" which stands opposite to "Curing Medicine". The overall aim of animal hygiene is to keep animals healthy and protect them from all factors that can impair their health and well-being. This holistic approach of preventing disease and discomfort instead of curing is not limited to typical food delivering animals such as cattle, sheep, pigs and poultry, it applies also to domestic and companion animals like dogs, cats, horses or falcons.

The new age in animal production

With the advent of modern animal production since the end of the 1950ies with increasing animal numbers per farm a new thinking and acting was required not only in animal farming but also in veterinary science and animal hygiene. Since 1961 the pig world market rose from about 50,000 t to 160,000 t in 2005, and poultry meat tripled to 240,000 t. These developments in animal farming can be characterised by three terms:

Intensification,
Specialisation
and Regional Concentration.

Intensification of livestock management generally means indoor stock keeping all year round, high animal densities per m² housing, a high degree of mechanisation and automation, e.g. in feeding, water supply, manure removal and ventilation, a low labour requirement and often a small air volume in relation to the number of animals in the housing unit. In the European Agreement on the Protection of Animals in Animal Farming (Chapter I, Article 1) intensification is addressed as: "Modern intensive animal farming systems are systems in which mainly technical facilities are used that are primarily operated automatically and where the animals entirely depend on the care and supply of the farmer". Most typical examples can be found in broiler and pig production and the high animal densities become particularly clear towards the end of the rearing or fattening periods when the animals are almost fully grown and the live animal mass per m² housing area reaches its boundary values.

Specialisation means that only one animals species, specially bred for this branch of production, is kept in specialised buildings on the farm at high stocking densities. In these specialised farms the consumer often has problems in recognizing his still largely traditional concept of a farm on which several animal species, from dairy cows to hens, are kept. The catchword animal factories than spreads quickly and complaints are voiced that the animals are simply considered as "animal machines" under purely commercial conditions. Thus the technical term "specialised intensive animal husbandry" quickly turns into the phrase "mass animal farming" with its negative connotations.

The third point is regional concentration which means the pronounced agglomeration of such intensive livestock production units in certain regions, as can be observed in many countries of Europe, such as e.g. in North-West Germany, or some parts of the Netherlands. This involves all resulting consequences of dung and slurry application on the only limited amount of land for water, soil and air caused by a surplus of nitrates, phosphates or atmospheric pollution with odours and other air-borne substances such as gasses, dusts and micro-organisms. Not least there is a risk of swift spread of infectious diseases in these regions with high animal densities, as the periodic epidemics of e.g. swine fever or avian flu showed and still show.

These developments to large and efficient production units, which admittedly supply ever-cheaper products such as meat and eggs, but in which ever fewer people are employed (in Germany today less than 2 % of the population works in agriculture) and where work procedures and mechanisms are ever less known and understood by the society, posed new challenges not only to animal farming but also to veterinary medicine and animal hygiene. Soon it became evident that future and success of this modern livestock production would depend to a growing extent on the acceptance of the society and the consumer who increasingly are calling for "sustainable livestock production", a term which packages displeasure, non-comprehension, criticism and fears, as well as hopes for a kind of animal farming that can exist in the future.

Foundation of ISAH

Scientists of veterinary medicine and animal production from 11 countries came together in the beginning of the 1970ies and founded the International Society for Animal Hygiene (ISAH) in order to find scientific and practical answers to these new challenges and they adopted the immortal and universal goals of Hygiéia – following the comprehensive concept of animal hygiene as a science for the whole life of animal and man.

Two examples of the working fields of Animal Hygiene today

In the following examples are briefly reported of recent research which is going on in the field of animal hygiene and which is in the tradition of the holistic approach of Hygiéia for preventing disease by understanding the reasons.

Air pollutants in and from animal houses

The air of modern livestock houses can contain high amounts of a large variety of air pollutants such as odors, gases like ammonia and carbon dioxide, dust, fungi and bacteria including zoonotic agents, endotoxins, allergens and antibiotic resistant bacteria such as MRSA (Dungan and Leytem, 2009; Seedorf and Hartung, 2002; Hartung, 1998) which can negatively affect animal's and farm workers respiratory health as well as of nearby residents (DGUV 2011, Hartung and Schulz 2008). In numerous research projects dust was identified as an important carrier of these substances (e.g. Seedorf and Hartung 2002). Hamscher et al. (2003) showed that dust in piggeries can contain various antibiotics including tetracyclines, sulfonamides, tylosin and chloramphenicol, and in some samples the concentrations reached 12.5 mg/kg animal house dust.

In a recent study it was demonstrated that animal house dust cannot only carry antibiotics in considerable amounts it also has a long memory for antibiotic resistant bacteria (Figure 1). In a retrospective study, 119 sedimentation dust samples stored between five and 35 years from various barns of intensive livestock farming were evaluated for the occurrence of cultivatable *Escherichia coli*. Growth of *E. coli* occurred in 54 samples. Successful cultivation was achieved in samples from as early as 1994. The frequency of detection increased from earlier to later time periods, but the concentrations, which ranged between 3.4×10^2 and 1.1×10^5 colony-forming units per gram, did not correlate with sample age. 110 *E. coli* isolates were tested on antimicrobial resistance. More than 50% were resistant to a minimum of five out of 10 antibiotics tested. Younger isolates and isolates from fattening poultry barns tended to be resistant to significantly more antibiotics than older isolates and those from laying-hen houses. Overall, it was shown that under particular conditions, dust from farm animal houses can be reservoirs for antimicrobial-resistant *E. coli* for at least 20 years. Obviously, survival strategies allow *E. coli* to survive such long periods in environmental samples like dust.

Research in the last couple of years revealed the fate and survival time of emitted bio-aerosols in the surrounding of farms. Investigations in and around broiler houses showed that the travel distance of *Staphylococcaceae* downwind can be at least 500 m from the source. In stable wind

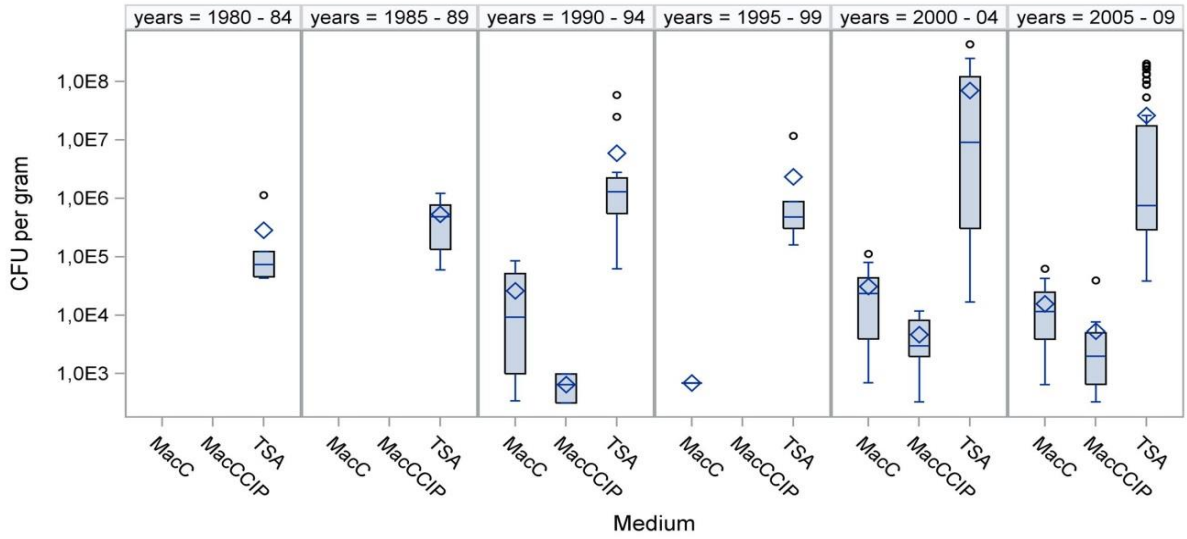

Fig. 1: Bacterial concentrations in dusts collected during various time periods. Total viable bacteria were counted on TSAagar, and *Escherichia coli* were counted on MacConkey agar(MacC) and on MacConkey agar with ciprofloxacin (MacCCIP). ◊ = arithmetic mean, ○ = outlier, − = median. (Schulz et al. 2016).

conditions more than 4000 cfu/m³ were found 477 m downwind the barn (Figure 2). *Staphylococcaceae* are typical bacteria in broiler house air. They can probably serve as indicator bacteria for the bacterial pollution because they do usually not appear in relevant concentrations in normal outside air.

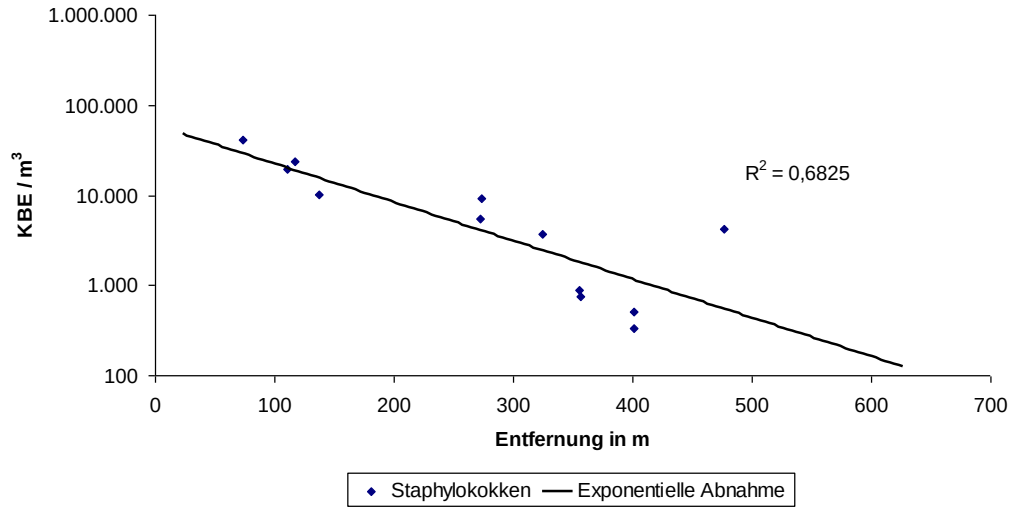

Fig. 2: Decreasing concentrations of *Staphylococcaceae* with increasing distance downwind a forced ventilated broiler barn with 30,000 birds. Sampling 1.5 m above ground. Animals in second half of production cycle. Air temperature about 16 °C, wind speed between 1.7 m/s and 6.3 m/s. n = 12. (Schulz et al. 2004).

Animal welfare - Automatic monitoring of pig aggression

Another important area of research in animal hygiene is the well-being and behaviour of animals. Animal Welfare is a global issue today and societies and people are getting more concerned about the welfare of their animals, farm animal in particular. That holds true for Europe but also for China and Brazil and many other fast developing countries. And the question arises: Do we really know enough about our animals, do we understand their needs, their behaviour?
A little experiment shall help to elucidate the complexity and intelligence of our farmed pigs.

Aggression belongs to the normal behaviour of pigs. In intensive pig farming, however, aggressive fighting occurs repeatedly because the animals are regrouped several times during their life, requiring the reestablishment of group hierarchy, which can lead to considerable stress and serious injuries. Other consequences are reduced growth, low carcass quality and poor maternal abilities (Sonoda et al. 2013a). Therefore, experiments were carried out, firstly, to better understand nature and purpose of aggressive actions among grouped pigs and, secondly, find ways to reduce injurious levels of aggression (Sonoda et al. 2013b). This knowledge could help to improve the welfare of the animals and increase production efficiency. The piglets were monitored by video cameras and aggressive behavioural action sequences were analysed and algorithms developed for behaviours such as activity, speed and orientation towards other piglets. If the algorithm indicates aggressive behaviour a specific actuator was started automatically at a very early stage to distract the fighting piglets from each other.

In practical terms, the piglets were trained on a specific sound followed by a reward in the farrowing pens. The most preferred reward by the piglets was chocolate chips. After mixing when one piglet took an aggressive position (aggressor) and started to fight the sound was released and chocolate smarties fell from a feeder in the trough. The piglets learned rather

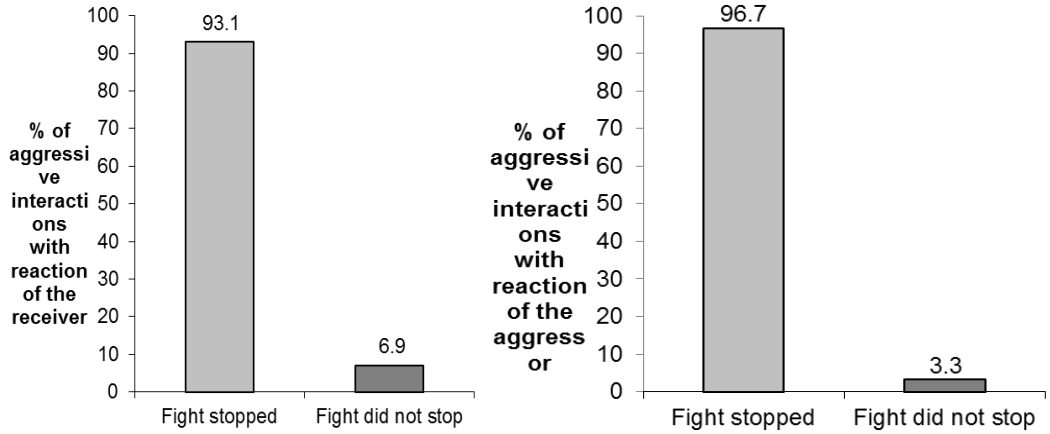

Fig. 3: Percentage of aggressive interactions stopped and not stopped after activation of the feeder during an aggressive interaction of the piglets. Total n=223. LEFT graph only the receiver reacted, n=102, $p<0.05$. RIGHT graph only the aggressor reacted, n=121, $p<0.05$. (Sonoda 2014)

fast (3 to 8 days of training) to associate the sound with the supply of chocolate and very quickly they made a decision between sweets or fighting. In a so called resident-intruder test (Figure 3), more than 90% of aggressive interactions could be interrupted by the sound followed by the activation of the feeder. It was observed that the aggressor was more rarely prepared to give up fighting immediately. The used method relies on the intelligence of the pigs and seems to be a useful environmental

enrichment for piglets with the significant potential to reduce the length and intensity of aggressive interactions among weaned and regrouped piglets (Sonoda 2014). Automatic systems should be further developed.

Concluding Remarks

These are a few examples from the wide field of animal hygiene in research and practice show that animal health is the state of an individual or a herd of complete physical, mental and social well-being and not merely the absence of disease or infirmity. Animal hygiene is that scientific discipline in veterinary medicine which tries to understand the reasons for good physical, mental and social health of an animal. It is a holistic approach taking in account the animal's nature and ambient factors.

Hygiéia, the daughter of Asklepios and Epione, appears sometimes as a difficult child with a complex character. However, taking care of her and working towards her principles of preventing physical and mental diseases while employing different scientific disciplines and developing new analytical tools and methods is a promising way forward to a better future for the benefit of animals, man and environment.

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IN PURSUIT OF HAPPINESS, THE KEY TO EVOLUTION

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SUMMARY. Evolution is the result of the survival of the species that have best adapted to their environment. However, the question is: what determines this adaptation? What is the driving force behind this selection? Here is described that if a species is well adapted, it will receive more rewards. The number of received awards determines the level of (animal) welfare of an individual and it is concluded that (animal) welfare is, therefore, the driving force of evolution.

INTRODUCTION

In his book "On the origin of species", Charles Darwin explained how the evolution process selected and determined the characteristics of each species. 'Survival of the fittest' became a well known term. However, how does one know that he is the fittest? And most important: How does one become the fittest? Is there a way to influence your fitness? In this respect it is interesting to see that public concern about animal welfare has increased during the last thirty years and especially in the last ten years (Broom 2015). But, what is the reason why we want to be 'happy'? What is the biological significance of welfare? Is there an evolutionary benefit of welfare? If so, the perception of well-being must have evolved during the evolution.

The Brambell committee defined in 1965 (animal) welfare: "Welfare is a wide term that embraces both the physical and mental well-being of the animal. Any attempt to evaluate welfare, therefore, must take into account the scientific evidence available concerning the feelings of animals that can be derived from their structure and function and also from their behavior". Broom's (1986) definition of the welfare of an individual, as its state with regard to its attempts to cope with its environment, refers to all coping systems and so includes feelings and health. It is now used, in modified form, by the O.I.E. (World Organization for Animal Health). As explained by Broom (2014) p.28, the O.I.E. text reads like a committee document so has some imprecise parts in it. One of which is: welfare is not "how an animal is coping" but is a state that reflects how well it is coping (Broom 2015). This implies that welfare reflects how well an animal fits in its environment. When used in research on stereotypic behaviour, 'coping' typically refers to a learnt response, which does have benefits (i.e. is at least partially successful) (Würbel et al., 2006). The more flexible an animal is, the broader the differences in environment that it can handle and the more options it has for a good welfare. Spruijt et al. (2001) define welfare as the balance between positive (reward) and negative (stress) experiences or affective states. All these definitions include aspects of feeling and/or mental states, thus implying that welfare is something that requires a brain. However, if the definition of Spruijt et al., (2001) is shortened in: Welfare is the balance between positive (reward) and negative (stress) experiences, it does not require a brain and makes welfare also applicable to species without one. This may seem far-fetched, but could also hold merit as will be explained here.

REWARDS

In general, rewards make one 'happy', so the more rewards, the 'happier' the individual will be. Rewards are related to beneficial resources or events. Cabanac (1971) summarized this in his statement: pleasant = useful. My suggestion is, however, to turn this around into: useful = pleasant. Meaning that those things which are good in the short term, but also in the long run, i.e. have high Darwinian fitness value, are perceived as pleasurable and thus will cause and reinforce the behaviour inducing this. Because there always are several needs to be met, an animal has to decide which is most important. The expected level of the reward, thus the amount of pleasure expected, will determine what an animal will do. In this way an animal can prioritise its behaviour to the current situation (Cabanac, 1992; Spruijt et al., 2001). The development during evolution of taste buds that can detect sugar and fat is thus very rewarding. Animals and people will search for food that has a good taste and in that way minimize the intake of food and thus the time spent for searching and digesting. In general, the behaviour of animals is highly economic. Each behaviour is evaluated for the costs and benefits. The more rewarding (beneficial) a certain type of behaviour will be, the more likely it is that it will be performed (Spruijt et al., 2001).

HOW CAN WELLBEING INFLUENCE EVOLUTION?

If, for example, during a glacier period the habitat of the brown bear is under pressure and becomes smaller, there are not enough resources for everyone (Hassanin 2015). The bears that are looking for food will look for opportunities to obtain other sources than they would normally do in order to satisfy their need for rewards. This implies that they might take a risk and go into the snowy area in search for prey. The ones that have a light coloured coat will be in an advantage over the dark coloured individuals, who were in an advantage in their 'normal' habitat. The lighter ones might thus catch some seals and find their 'taste' very good, because they are rich in fat and thus energy. However, they also need to adapt their digestive system and metabolism to the extreme amount of fat from the seals, which will cause heart problems in other mammals. This had to take place in the same time frame because the evolution of the polar bear took place very rapidly (Liu et al., 2014). The energetic value of seals is very high and thus highly rewarding for a hungry animal. It was thus very attractive to evolve from a brown bear into a polar bear under those conditions. A similar mechanism could have been active for the evolution of the red fox into the arctic fox, who show similar physiological features (Kumar et al., 2015). It is thus the thrive for feeling well (aquire rewards) that pushes for a genetic adaptation. This might be mediated by the activity of mutation-rate modifiers, which result in different mutation rates in various species (Drake et al., 1998)

SENTIENCE

The term sentience generally implies that the individual has the capacity to have feelings (DeGrazia 1996, Kirkwood 2006). This capacity resides in the brain because it involves awareness and cognitive ability. However, recent detailed studies of behaviour and the brain showed that this attitude towards sentience is no longer satisfying and that sentience means more than just having feelings. A definition is: a sentient being is one that has some ability: to evaluate the actions of others in relation to itself and

third parties, to remember some of its own actions and their consequences, to assess risks and benefits, to have some feelings and to have some degree of awareness (Broom 2006, 2014, 2015). How rewards are perceived by the individual depends largely on the way their physiology is developed. For vertebrates this is studied well and is reflected in the brain by the concerted action of opioid and mesolimbic dopaminergic systems (Spruijt et al., 2001). However, for animals with simple nervous systems or without one this is not so clear. Snails do 'know' how and where to find food, as do helminths, who also have a very simple nervous system. So they must have some kind of perception of the benefit of retrieving necessary resources. They spend energy to move to a certain place in order to get their reward in the form of nutrients. They also move away to avoid negative environments. For organisms without even a simple nervous system, however, this is more difficult to understand. The essence of welfare, defined as the balance between positive (reward) and negative (stress) experiences, must thus be incorporated in their DNA. Single cell organisms can move towards nutrients or away from danger (if physically possible). Gilroy and Trewavas (2001) define the classic example of fine sensing of single cells the zygote of the marine alga *Fucus*. This single cell can respond to remarkably slight gradients in temperature, osmotic pressure, light, pH, minerals (K+, Ca2+), solution flow, electrical fields, other chemicals, gases and probably gravity, and direct the orientation of growth accordingly many hours later (Jaffe, 1969). They also mention that a similar remarkable sensitivity to signal gradients is shown by single-celled Euglenoids, which can sense their own cytoplasmic weight and modify swimming activity (Hader and Hemmersbacj, 1997). Furthermore, Freddolino and Tavazoie (2012) stated that beyond the utility for accurate perception of immediate events, microbes make predictions about the trajectory of their sensory environment. They reviewed recently discovered examples of such predictive behavior, their physiological benefits, and the underlying evolutionary forces shaping them. "These observations compel us to go beyond homeostasis and consider a predictive-dynamic framework in which cellular behavior is orchestrated in response to the meaning of an environmental perturbation, not only its direct and immediate fitness consequences" (Freddolino and Tavazoie, 2012). In their review they mention that E. coli growing on poor carbon sources reveal a 'risky' strategy. These bacteria take a voluntary risk of homeostasis for the possibility of future gain. Similar behavior has also been observed in the slime mold Physarum polycephalum [not strictly a microbe, but a syncytial, light fobic, slime mold without internal divisions (Stewart & Stewart 1959)], which exhibits foraging behavior that weighs food value against risk due to light exposure, and in some cases even rejects non-risky, but low-value, food sources in favor of exploration (Latty & Beekman 2010). Although these organisms lack a brain, the data of Latty and Beekman (2015) show that in decision making they use simple heuristics, which can give the impression that they make sophisticated decisions. These authors also described differences in the speed of decision making in Physarum polycephalum, depending on stressors such as light or hunger (Latty and Beekman 2011) and that their decisions are sometimes irrational (Latty and Beekman 2011b). Although this might seem strange at first thought, irrational behavior can be favoured by natural selection. As McNamara et al. (2014) pointed out, this might be caused by the fact that a current food option might disappear in the near future or a better option might (re)appear soon.

More complex lifeforms such as plants grow their roots deeper in the soil during a period of drought and stop at the level where there is water. Plants can also direct their leaves towards the

sunlight and detect minerals at distances in the soil and direct their roots towards patches of these minerals in the soil (Gilroy and Trewavas 2001). Here must be, not yet understood, (reward) feedback mechanisms in function. Although plants do not have a nervous system, they are capable of transmitting a variety of electrical signals through their 'body' and show extensive cross-talk and interactions between signalling systems (Zimmermann et al., 2009; Rhodes et al., 1996; Wildon et al., 1992; Gilroy and Trewavas, 2001). Plants also have a memory and can even forget things, when they are no longer functional (reviewed by Crisp et al., 2016). Just as bacteria can adapt to their environment by losing functions (Hottes et al., 2013).This implies that there are feedback mechanisms in function that are calculating costs and benefits.

Apparently welfare is so important that 'nature' has constructed a very complex system (opioid and mesolimbic dopaminergic systems) for its perception. This system was already present in primitive brains and remained intact through the evolution of the vertebrates. Some of the transmitter molecules used by these systems evolved as much as 1000 million years ago (Walker et al.,1996), and mammalian dopamine, serotonin, and norepinephrine neurotransmitters are also used by invertebrate phyla, such as mollusks and arthropods, that diverged from prevertebrate lines roughly 600 million years ago (Nesse and Berridge, 1997). One could say that the higher a species is evolved during evolution, the better and more differentiated its perception of welfare is.

So I conclude that the perception of a reward or stressor is existing in all species and the number of received rewards determines the level of well-being and how well an individual or species fits in a certain environment. Because of this, at least sentient animals have a drive to increase their level of well-being and thus to find ways to increase the number of rewards. This could be the answer to the question posed by Kirkwood (2006): "What evolutionary advantage does sentience confer?" Adaptation to a certain environment is a way to increase the number of rewards received in that environment and thus the Darwinian fitness. For every environment, with several minimal requirements, a species will evolve that will fit there based on adaptations that maximize the number of rewards. Humans can be considered as highly adaptive because they also adapt to their environment by changing the environment and are so able to survive in very harsh conditions like deserts or the arctic. Because adaptation in the long term is genetically regulated, welfare can thus be seen as the driving force behind evolution.

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The changing epidemiology of vector-borne diseases – driving factors and research approaches

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The occurrence and spread of insect vectors and vector-borne diseases can be affected by many current global events such as changes in climate, demography or travel behaviour. Mosquitoes are among the most important transmitters of human vector-borne diseases worldwide with dramatic effects on human health. To name but a few, amongst them are important viral diseases such as the Dengue and Chikungunya fever, the Zika virus with recent outbreaks in the tropics, as well as important protozoan diseases such as Malaria, still for example a major cause of disease for children in the tropics. Alongside those human disease agents, also zoonotic diseases are transmitted by mosquitoes, such as the West Nile fever virus which circulates endemically between mosquito and bird populations. Biting midges transmit mainly veterinary diseases, and very important ones such as the blue tongue virus and African horse sickness virus. Many mosquito-borne pathogens have long been thought to occur mainly in the tropics due to the tropical habitats of their most common insect vectors, but recent developments have shown that mosquitoes are capable of invading new habitats. In Europe, for example, large invasive and now established populations of *Aedes albopictus*, the Asian tiger mosquito, and of *Aedes japonicus*, the Asian bush mosquito, have been described. Furthermore, *Aedes aegypti*, the yellow fever mosquito has been knocking on the doorsteps of Europe and recently *Aedes koreicus* has been found as a new invasive mosquito species in Italy. These mosquito species all have in common that they are container-breeding species and are able to be transported long distances as desiccated eggs and can therefore potentially establish in new geographic locations. In order to assess the risk of vector-borne disease transmission, certain parameters have to be taken in consideration such as the vector capacity of a certain mosquito species. This value is made up of several factors amongst which are the vector competence, the host preference and biting behaviour and several other bionomic data of the mosquito species, (e.g. the daily biting rate, abundance etc.).

This outline will focus on some of the research goals and possible approaches to tackle the complexity of the vector capacity.

In a large-scale study to assess the risk for introduction of West Nile virus into Switzerland, aside from vector competence and mosquito abundance, the host preferences of Swiss mosquito species were tested with two approaches: Animal baited traps and blood meal analysis by molecular methods. We could demonstrate that the most likely bridge vectors in Switzerland (assessed with abundance, spatio-temperal activity, laboratory vector competence, virus detection in the field and host preference) are *Culex pipiens/torrentium, Aedes vexans and Aedes japonicus.* For the latter species, we could show the first detection of an avian blood meal in nature.

Accurate and high-throughput identification of vector arthropods is of high importance in surveillance and control programmes which are becoming more common due to changes in the geographic range and extent of many arthropod-borne diseases. To this aim, our group is working on the development of molecular identification tools such as Loop mediated isothermal amplification (LAMP) and Protein profiling by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. These techniques fulfil requirements for rapid identification, and in the case of LAMP, are even applicable in the field. For MALDI-TOF mass spectrometry, we have established a large reference database with regard to vector arthropods, including Ceratopogonidae, Culicidae, Ixodidae, Phlebotominae and Simuliidae, and we continually expand it in collaborative work, with the goal of creating a comprehensive, centralized database that comprises the reference spectra of all major arthropod vectors.

The mosquito-borne nematodes *Dirofilaria immitis* and *Dirofilaria repens* are used in our laboratory as a model to study vector-pathogen interactions. The approaches used include the assessment of vector competence in local and invasive mosquito species through experimental infections and observation of developmental stages in the mosquito. It was shown for the first time that the invasive container-breeding species *Ae. japonicus* as well as the indigenous *Aedes geniculatus* are competent vectors for both filarial species. To assess the vector competence of mosquitoes under realistic conditions, we now study the development of the filarial nematodes under realistic fluctuating temperature conditions that mimic Central European summer time. Furthermore, to gain fundamental knowledge on the complex host-pathogen-interactions, we study the genomics and transcriptomics of arthropods and nematodes. We are currently working on a comparative transcriptomic approach of the developmental stages of *D. r*epens in order to understand host-pathogen interactions, metabolic pathways and pathogenicity as well as to identify potential drug and vaccine targets in this nematode which can serve as a model for other future studies.

With continuing global changes, the challenges posed by insect vectors and by the diseases they transmit are likely to grow rather than diminish. To assess the risk and develop control tools for these challenges also in the future, extensive studies and developments on the epidemiology and on fundamental host-pathogen-interaction will continue to be of utmost important.

EDUCATION AND RESEARCH ACTIVITIES AT THE UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE, SLOVAKIA

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Rector
University of Veterinary Medicine and Pharmacy in Košice, Slovakia

The University of Veterinary Medicine and Pharmacy in Košice, not only the sole university for veterinary medicine in Slovakia, but given the quality of its educational and research activities, also a unique institution which is an inseparable part of education and research in Europe. The University of Veterinary Medicine and Pharmacy in Košice was established by an Act of the Slovak National Council on 16 December 1949 as the Veterinary College in Košice. Its founding was the culmination of many years of effort aimed at establishing a school of veterinary medicine in Slovakia. During its decades of existence the university has undergone many changes, some of them principle changes. What hasn't changed, however, is the fact that from its founding up to the present the university has not lagged in its efforts to rank through the quality of its work among the best institutes offering a university education. With its highly erudite educators, scientific workers and other qualified personnel, it offers in its departments, institutes and clinics a broad background to domestic and foreign students for study in accredited study programmes at all three levels of university education.

The university has about 2300 students in each of the three degrees of study in the full-time and external forms, more than 200 of whom are foreign students who each year arrive at the university to study veterinary medicine in the English language, particularly from Israel, Sweden, Norway, Ireland, Great Britain, Greece, Cyprus, Iceland, Belgium, Austria and Japan. International recognition ensures the university a sufficient amount of interest from abroad, with students attracted in particular by the high level of practical preparation and permanent contact with animals. This is reflected, along with the excellent, language-efficient experts and long years of experience, since this particular part of study was implemented in 1991, in the high share of foreign students out of the overall university population.

EDUCATION

The study programmes with the longest tradition are the *general veterinary medicine and the food hygiene* programmes, which in the 2006/2007 academic year was expanded with a study programme in *pharmacy*. Veterinary medicine and pharmacy rank among the so-called regulated professions, the study of which must meet the demands of European Union guidelines. The University of Veterinary Medicine and Pharmacy in Košice meets the standards required by the EU and a diploma conferred by the university is valid in all countries of the European Union.

Graduates of the full-time six-year study programme in *general veterinary medicine*, which since 1991 has been provided in the English language for students from all over the world, obtain the academic title of *doctor of veterinary medicine* (abbreviated as MVDr.). A graduate of this programme can perform work as a veterinary doctor in the state or private sphere with a focus mainly on diagnostic, therapy and the prevention of diseases in domestic, agricultural and exotic animals, but also in the fields of food industry, pharmacy, scientific research fields and environmental protection. A successful

graduate of the other full-time six-year study programme in *food hygiene*, which is focused more on professional subjects from the fields of production of food products of animal origin, also obtains the title *doctor of veterinary medicine*. Such a veterinary doctor is capable of endorsing the health safety of foodstuffs from first production up through consumption, performing inspections of food products of animal and plant origin and their handling, supervising the import and export of foodstuffs on the commercial market. Equally, he or she is also qualified to perform the activities of a veterinary doctor for economic, domestic and exotic animals. The academic title of magister (abbreviated as Mgr.) is conferred on graduates of the full-time fiveyear study programme in *pharmacy*. The study programme is orientated on the education of professionals in the field of pharmacy and pharmaceuticals as a component of health care for people and animals and is specialised in research, production and inspection of drugs and medicines. Graduates are employed in pharmaceutical, hospitals and medical centres, in the pharmaceutical and cosmetics industry, in the system of state inspection of drugs and in the health care insurance industry. In addition to these second degree study programmes, the university offers education of the first degree in three study programmes – *cynology* and *the safety of feeds and foodstuffs and animal science*. Graduates obtain the title of bachelor (Bc.) in the full-time and external form of this three-study programme and can subsequently earn the title of magister (Mgr.) in study programme of the second degree – *market and quality of foodstuffs.* The *cynology* study programme has gained popularity in that it enables the connection of love of dogs with wider possibilities of professional application. A graduate acquires expertise for performance of his or her profession in specialised veterinarian facilities, mainly in the Police Force, in the army, in the railway police, in customs administration or in private security services. A graduate of the study programme *safety of feeds and foodstuffs* can work in state and private laboratories with a focus on the evaluation and quality of foodstuffs and animal feeds.

The university offers a higher education of the third degree in 15 accredited study programmes with a standard length of at 4 years in full-time, or 5 years in the external form of study. After successfully completing study of one of the accredited study programmes the graduate obtains the academic title of Doctor (Doctor of Philosophy, abbreviated as PhD.) The relevant study programmes are: *food hygiene, veterinary morphology and physiology, internal disease of animals, veterinary surgery, orthopaedia and radiology, veterinary obstetrics and gynecology, infectious diseases of animals, parasitic diseases of animals, forensic and public veterinary medicine, animal nutrition and dietetics, animal hygiene, microbiology, immunology, neurosciences, toxicology and virology.*

INTERNATIONAL COOPERATION

Significant, and in the field of veterinary education from the statewide and international point of view, irreplaceable is the role of cooperation with other universities and research institutes both at home and abroad. Cooperation with other universities is realised in the framework of the university's active membership in the European Association of Establishments for Veterinary Education (EAEVE), the European Veterinary Network of Students Staff Transfer (VetNEST), the Wild Animals Vigilance Euromediterranean Society (WAVES), the Association of Carpathian Region Universities (ACRU) and the Slovak Academic Association for International Cooperation (SAAIC). A significant result in the field of international cooperation is the creation and signing of an international contract on cooperation

between the University of Veterinary Medicine and Pharmacy in Košice and Bodø University from Norway. The creation of a common bachelor's study programme for obtaining a common title of Bachelor's degree in Animal Science satisfies the standards of the educational committee of EAEVE per regulation 2005/36. This programme is the first such common study programme; instruction will begin in the 2010/2011 academic year.

RESEARCH

The scientific activities are focused primarily on these areas: infectious and parasitic diseases of agricultural and pet animals, non-infectious diseases of agricultural and pet animals, creation and protection of the environment for animals and people, hygiene, production and processing of healthy, safe foodstuffs, pharmaceutical research. Outstanding results are produced in the Center of excellence for animal contagions and zoonosis INFEKTZOON. The mission of the Center is: to be the leading scientific workplace in the battle with zoonosis and infectious contagions of animals and people, protection for agricultural and social animals from dangerous contagions, leading to significant economic gains and improvements in the quality of animal welfare, better quality and a safer food-products supply chain for our citizens, contributes to a significant degree to the public health of the human population, the training of highly qualified doctoral candidates, veterinary doctors and diagnostic workers from practice.

COMPLETE ACCREDITATION AND EVALUATION

A confirmation of the quality and permanently high level of scientific, research and educational activities of the university is its repeated evaluation by recognised authors on the national and international levels. On the national level, the university is evaluated at six-year intervals by the Accreditation Committee of the Slovak Republic, which thoroughly assesses the educational, research and other creative activities of a university as well as the personnel, technical, informational and other conditions in which these activities take place, and then expresses itself in response to the university regarding accreditation of all the study programmes and regarding the accreditation of all habilitation activities and activities for designating professors in which the university wants the corresponding right acknowledged. On the basis of the evaluation report from the Accreditation Committee of the Slovak Republic, the Slovak Ministry of Education issued a decision on the results of complete accreditation, which acknowledged that the UVLF in Košice has the right to confer the relevant academic titles to graduates of all study programmes offered by the university at all three degrees of university study. The Slovak Ministry of Education issued to the university, on the basis of the complete accreditation, a certificate of its worthiness to perform research and development.

Evaluation on the international level is also very important for the university. In other years an evaluation of the similarities of education in the field of veterinary medicine between the Slovak Republic and the European Union was performed by the EU Commission-TAIEX, a re-evaluation by the Brussels-based European Association Establishments for Veterinary Education (EAEVE), of which the UVLF is a member, and also the institutional evaluation of the European University Association (EUA). The conclusions of all of these evaluations were favourable for the university, and they confirmed that the university has many strong sides, primarily in the field of its unique orientation on

the field of veterinary medicine, food hygiene and pharmacy, and further in the high qualification of the level of academic and technical personnel, the obtaining of revenues for research, the good success of its graduates on the labour market not only in Slovakia but also abroad as well as the provision of education in the English language for students from abroad and the success in evaluation by external rankings and ratings agencies.

The evaluation of our university by the European Association of Establishments for Veterinary Education (EAEVE) in 2016 had successful conclusion - status APPROVED & ACCREDITED. As a result, our university has joined only eleven European veterinary establishments (out of 96) which have been accredited.

Animal health, welfare and behaviour

ASPIRIN UPREGULATES αB-CRYSTALLIN TO PROTECT THE MYOCARDIUM AGAINST HEAT STRESS IN CHICKENS

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SUMMARY. We established in vivo and in vitro models to investigate the role of αB-Crystallin (CryAB) and assess the ability of aspirin (ASA) to protect the myocardium during prolonged heat stress. For vivo, thirty-day-old chickens were divided into three groups (*n*=90): heat stress (HS, 40±1°C); ASA(-)HS(+), 1 mg/kg ASA orally 2 h before heat stress; and ASA(+)HS(-), pretreated with aspirin, no heat stress (25˚C). Hearts were excised after 0, 1, 2, 3, 5, 7, 10, 15 and 24 h. For vitro, primary chicken myocardial cells were isolated from the hearts of twelve-day-old specific pathogen free (SPF) embryos and were divided into four groups (HS: 2 h, ASA+HS: 2 h, HS: 24 h, ASA+HS: 24 h). In vivo, rectal temperature, pathological changes and CryAB expression were studied in broiler chickens, while, in vitro, cell viability and CryAB expression were tested in primary chicken myocardial cells. Our results showed that pre-treated with ASA can reduce the sensitivity to heat and can lower pathological lesion. ELISAs indicated ASA induced CryAB in vivo to protect against heat stress-induced myocardial damage, but ASA did not induce CryAB in primary chicken myocardial cells. In conclusion, the study indicates that ASA can induce expression of CryAB and protect the myocardium in vivo, but not in vitro.

Key words: aspirin, αB-crystallin, myocardium

INTRODUCTION

Heat stress is an environmental and occupational hazard, which can harm poultry, especially broiler chickens, because they have no sweat glands and poor thermotolerance (Sandercock, 2001). However, little attention has been paid to investigation of mechanisms associated with heat stress in broiler chickens. CryAB, which belongs to the small heat shock protein (sHsp) family, is expressed ubiquitously throughout the mammals' body and exerts a variety of highly protective functions to maintain homeostasis (Ashby, 2010). CryAB has also been shown to exert anti-apoptotic properties, as it can prevent cell death in response to conditions such as stroke (Tian, 2013) by maintaining the cell cytoskeleton (Wettstein, 2012). In present study, we established in vivo and in vitro models to investigate CryAB functions on the chicken myocardium during prolonged heat stress. Moreover, we also assessed the ability of ASA to protect the myocardium against heat stress in vivo and in vitro, and investigated the relationship between CryAB and ASA during heat stress.

MATERIALS AND METHODS

Establishment of the in vivo heat stress model: 270 SPF chickens were randomly divided into three groups: the ASA(-)HS(+) (heat stress) group, the ASA(+)HS(+) (aspirin administrated before heat stress) group, and the ASA(+)HS(-) (aspirin administration without heat stress) group. None of the chickens were provided with water for the 12 h leading up to the experiment. Chickens in the ASA-HS

and ASA groups were administered aspirin (Sigma, USA) orally at 1 mg/kg body weight 2 h in advance. Chickens in the HS and ASA-HS group were heat stressed in a preheated air chamber at 40 ± 1°C with 60% ~ 70% humidity respectively for 0 h, 1 h, 2 h, 3 h, 5 h, 7 h, 10 h, 15 h, and 24 h. Birds were allowed free access to food and water ad libitum. 10 chickens in each group were sacrificed humanely by decapitation. Rectum temperature was measured before slaughter. The hearts were excised and fixed in 10% formalin for pathological studies or frozen in liquid nitrogen for ELISA.

Isolation and culture of primary chicken myocardial cells: Primary chicken cells were isolated from the hearts of twelve-day-old specific pathogen free (SPF) embryos and were confirmed using the marker alpha actinin (α-actinin, ab11007, abcam, USA) by immunofluorescence analysis. Four groups of primary chicken cells were established (HS: 2 h, ASA+HS: 2 h, HS: 24 h, ASA+HS: 24 h). After treatment with ASA and/or HS, cell count using Trypan and CryAB expression using ELISA kit (MBS2882479, MyBioSource, USA) were employed.

Statistical analysis: Data was compared with the baseline level (0 h in the HS group) by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test using SPSS version 21 for Windows and Graphpad prism 6.0 software. All raw data presented is expressed as the mean ± standard deviation (SD). All experiments were repeated three times.

RESULTS

Clinical manifestation of heat stress in broiler chickens

The broilers behaved normally after being orally administered ASA in water 2 h before heat stress. Rectal temperatures were tested and were shown in Figure 1. After exposure in heat stress, group ASA(-)HS(+) displayed more sensitivity to heat and had significantly higher body temperatures compared to the ASA(+)HS(+) group at all time points during heat stress ($P < 0.05$). Both groups subjected to heat stress had higher rectal temperatures than the control group housed at RT (25˚C).

Effect of heat stress on the survival of primary myocardial cells in vitro

Primary chicken myocardial cells were treated with or without 1 mg/mL ASA ASA for 2 h, cultured at 42˚C for 2 h or 24 h, and cell numbers were determined (Figure 2). There were significantly more cells in the ASA pretreated group than the HS group after 2 h heat stress ($P < 0.01$) and after 24 h heat stress ($P < 0.01$).

Histopathological changes in the myocardium in the in vivo model of heat stress.

The histopathological changes in the myocardium of the heat stressed groups and control group are shown in Figure 3. Chickens pre-treated with ASA 2 h before heat stress, euthanized at 0 h of heat stress were defined as the ASA control group (Figure 3F). As shown in Figure 3, heat stress resulted in severe damage (observed after 5 to 24 h) in the myocardium characterized by karyolysis and necrosis, signifying cell death. In comparison, the myocardium was less damaged in the ASA pretreated group.

Effect of heat stress on CryAB protein expression in vivo

The expression of CryAB was quantified in the myocardium of the chickens using an ELISA (Figure 4). At 0 h heat stress, the concentration of CryAB in the myocardium of the control group was only 200 pg/mL compared to 1200 pg/mL in the groups pretreated with ASA for 2 h, which represents a 6-fold difference ($P < 0.01$). In the HS group, expression of CryAB increased after 1 h heat stress, reached the highest level after 10 h of heat stress (1400 pg/mL), then slightly decreased but still remained high between 15 and 24 h heat stress (1000 pg/mL). In the ASA group pretreated with ASA for 2 h, CryAB peaked at 0 h and 3 h (i.e., 2 and 5 h after administration of ASA), then sharply decreased after 3 h ($p < 0.01$) and recovered to control levels (200 pg/mL) after 24 h heat stress. In the ASA(+)HS(+) group, the expression of CryAB was lower after 1h heat stress compared to the HS group not pretreated with ASA, then slightly increased at 2 h, but remained lower than the levels in the ASA(-)HS(+) group up to the end of heat stress (24 h) with the exception of a peak at 7 hours (1000 pg/mL).

CryAB expression in primary chicken myocardial cells in the in vitro model of heat stress

CryAB expression was also measured in primary chicken myocardial cells in vitro after different treatments（Figure 5）. Before heat stress, CryAB was expressed at significantly higher levels in ASA-pretreated cells than control cells that had not been treated with ASA ($p < 0.01$). However, cells exposed to 1 h heat stress expressed significantly higher levels of CryAB compared to not only before HS ($P < 0.01$), but also compared to the ASA(+)HS(-) and ASA(+)HS(+) groups after 1 h heat stress. The levels of CryAB remained higher in cells subjected to heat stress [ASA(-)HS(+)] until 15 h compared to control cells. After 24 h heat stress, CryAB expression returned to the same level as control cells before heat stress. In the ASA(+)HS(+) group, CryAB expression decreased after 1 h and remained at a low level until 24 h heat stress.

DISCUSSION

Heat stress is a non-specific stressor that can affect the welfare of livestock and even contribute to death. Pathological lesions, mainly due to necrosis, were observed in the rat myocardium after 40 min of heat stress in vivo, accompanied by lower CryAB expression, indicating CryAB may play an important role to protect the mammalian heart against heat stress (Tang, 2014). The aim of this study was to determine whether CryAB could be induced by ASA and protect the myocardium during heat stress in broiler chickens using in-vivo and in-vitro models.

Our in vivo expression profiling of broiler chickens revealed 2 h pretreatment with ASA increased the expression of CryAB before heat stress and during the early stage of heat stress. This reflects the metabolism of ASA; the half-life of ASA in humans is approximately 2.0 to 4.5 h. Pathological analysis of the in vivo model further confirmed that pretreatment with ASA to induce CryAB expression played a critical role to protect the myocardium against heat stress.

The expression of CryAB was not the same in vivo and in vitro in primary chicken myocardial cells. In vitro, ASA treatment induced lower levels of CryAB than HS in vitro. As previously stated, aspirin controls fever via the prostaglandin system by irreversibly inhibiting the cycloxygenases. However, cells in culture do not possess a hormone regulatory system. The main functional region of CryAB is its αB-crystallin chain, which means it cannot be induced by acid or other stressors like the inducible Hsps. However, CryAB could not be induced by pretreatment with ASA in primary chicken myocardial cells in the present study, which could reflect specific differences between the mammalian and chicken prostaglandin systems.

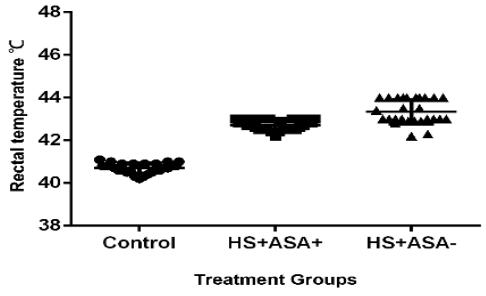

Figure 1. Rectal temperature of the chickens

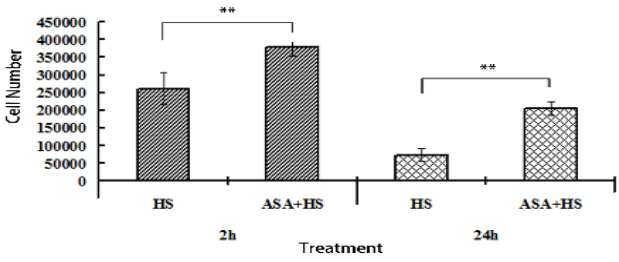

Figure 2: Cell viability of primary chicken myocardial cells during heat stress *in vitro*

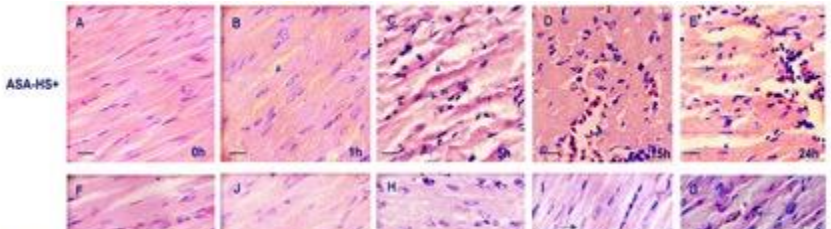

Figure 3. Pathological changes in the chicken myocardium after different durations of heat stress (1bar=10μm)

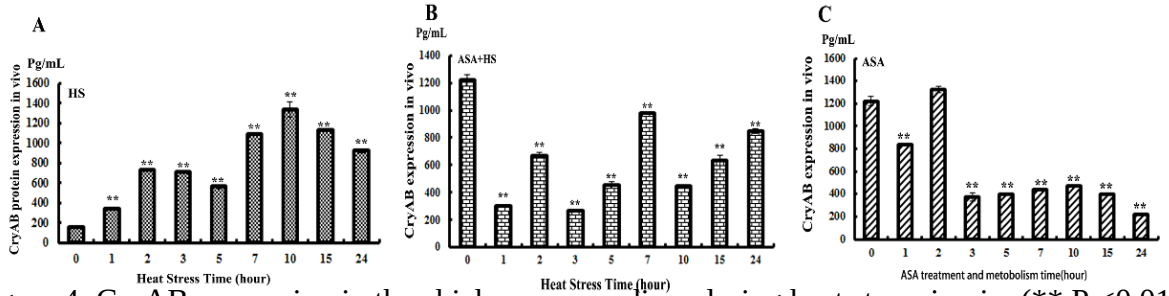

Figure 4: CryAB expression in the chicken myocardium during heat stress in vivo(** P <0.01)

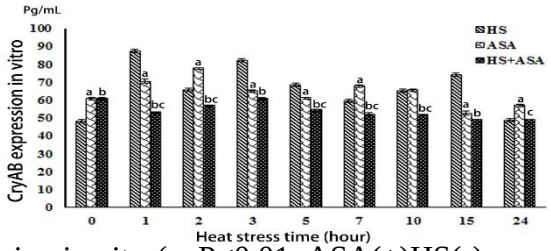

Figure 5. ELISA of CryAB expression in vitro(a: P<0.01: ASA(+)HS(-) compared to HS group; b: P<0.01: ASA(+)HS(+) compared to HS group; c: P<0.01: ASA(+)HS(+) compared to ASA(-)HS(+) group

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REASONS AND RISK FACTORS FOR ON-FARM MORTALITY IN ESTONIAN DAIRY HERDS

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SUMMARY. On-farm mortality of dairy cattle causes financial loss for the farmer and reflects animal welfare status. The aim of this study was to determine reasons, as well as risk factors, for on-farm mortality (unassisted death and euthanasia) in Estonian dairy cattle. Data of years 2013 and 2014 for all cattle from dairy herds in Estonia was collected from the Estonian Agricultural Registers and Information Board. The dataset included records of 363,380 animals from 2,616 herds. Multivariable Weibull proportional hazard models with herd as random effect were composed for detecting significant associations between potential risk factors and on-farm mortality. Data from Estonian Livestock Performance Recording Ltd was used to determine the reasons for on-farm mortality.
The overall mortality rate (MR) was 6.95 per 100 animal-years. Mortality was highest in male (MR = 55.96, 95% CI 54.53; 57.42) and female (MR = 27.70, 95% CI 26.99; 28.43) calves up to three months old. The main farmers´ stated reasons for dairy cow mortality were 'Metabolic and digestive disorders' and 'Feet/claw disorders'. The main reasons for on-farm mortality among young stock were 'Metabolic and digestive disorders', 'Respiratory and infectious diseases' and 'Other/unknown reasons'. The risk factor analysis was carried out in three age categories: 0-3 months, 3-24 months and over 24 months. Estonian Holstein breed was associated with significantly higher risk of mortality compared to Estonian Red breed cattle in every age group. The hazard of on-farm mortality was significantly higher for herds with over 400 animal-years compared to smaller herds in all age groups. Regional differences in mortality hazard were present in the model of young stock over 3 months and adult cattle. Seasonal differences in mortality rate were present and differed by age groups.

Key words: dairy cattle, mortality, survival analysis

INTRODUCTION

Increasing on-farm mortality among dairy cattle has been reported in several countries (Thomsen et al., 2006; McConnel et al., 2008). On-farm mortality is associated with economical loss for the farmer resulting from direct and indirect losses. Also, a higher demand for replacement animals associated with additional cost on buying and raising offspring is an inherent consequence for such herds. On-farm cattle mortality is also an indicator of animal welfare (Thomsen and Houe, 2006). Despite of the availability of registry data, representative overviews over mortality rates in cattle populations of countries are few (Pannwitz, 2015).
Objectives of this study were to assess on-farm mortality (unassisted death and euthanasia) in Estonian dairy cattle and identify risk factors and the frequency of reported reasons of mortality.

MATERIAL AND METHODS

Data was retrieved from the Estonian Agricultural Registers and Information Board (EARIB) and the Estonian Livestock Performance Recording Ltd (ELPR) for the study period from 1st of January 2013 to 31st of December 2014. A dataset was compiled including data of all animals in Estonian dairy herds present in the population in the 1st of January 2013, and animals born and imported between 1st of January 2013 and 31st of December 2014. EARIB data, containing information about the whole dairy cattle population, was used to calculate mortality rates and to perform risk factor analysis. In order to describe reasons for each mortality event ELPR data was used.

The observation period for each individual started from 1st of January 2013 or the date of birth/importation and lasted until the failure (unassisted death or euthanasia) or censoring (slaughter, selling or end of the study period). The study population was categorized into three breed categories: Estonian Holstein, Estonian Red and other breeds. The variable 'herd size' was created based on the number of animal-years in total per calendar year and four categories were made by splitting close to quartile values: herds with 1-399, 400-899, 900-1499 and more than 1500 animal-years. Counties were compiled into four regions: Northeast, Southeast, Southwest and Northwest region.

Survival analysis was applied in order to estimate the mortality rate. Multivariable Weibull proportional hazard models with herd as a gamma distributed frailty effect were composed for detecting significant associations between potential risk factors and on-farm mortality. Due to different baseline risk of different age categories the risk factor analysis was done separately in three age categories: calves aged <3 months, young stock 3-24 months and cattle older than 24 months. Variables of interest in this analysis were age, category, breed, gender, herd size and region, and all variables were included in the multivariable model.

Seasonal distribution of mortality was analysed in four age groups: calves aged up to three months, young stock 3-12 months, young stock 12-24 months and cattle older than 24 months by using survival analysis. In each age category the observation period of each animal was split at the first day of each calendar month. Mortality rate was calculated for each month of the two-year study period.

All statistical analyses were performed using STATA MP version 14 (StataCorp LP, College Station, USA).

RESULTS

The EARIB dataset included in total 363,380 animals from 2,616 herds. During the study period, there were 26,088 deaths, 600 euthanasias, 60,059 animals were slaughtered, 82,652 were sold abroad, 356 disappeared and 3 animals were slaughtered for diagnostic purposes. A total of 193,622 animals survived until the end of the study period and were right censored. On-farm mortality rate (MR) per 100 animal-years was 6.95 (95% confidence interval (CI) 6.87; 7.04).

The main farmers´ stated reasons for dairy cow mortality were 'Metabolic and digestive disorders' (40.1% of mortality cases) and 'Feet/claw disorders' (23.6%). The main reasons for heifer mortality were 'Metabolic and digestive disorders' (43.2%), 'Respiratory and infectious diseases' (26.8%) and 'Other/unknown reasons' (17.6%). Main farmers´ stated reasons for mortality among young bulls were 'Metabolic and digestive disorders' (55.2%), 'Respiratory and infectious diseases' (23.5%) and 'Other/unknown reasons' (13.5%).

Crude mortality rate estimates for female and male cattle are presented in Figure 1. In female cattle the mortality was highest in young calves under 3 months of age (MR = 27.70 per 100 animal-years, 95% CI 26.99; 28.43) and lowest in 12-18 months old heifers (MR = 0.99 per 100 animal-years, 95% CI 0.89; 1.10), after this age category mortality rate increased. In males the MR was highest in the youngest age group (MR = 55.96, 95% CI 54.53; 57.42). Mortality rate declined in the following age

categories whereas it was lowest in the oldest age category (>12 months, MR = 2.59, 95% CI 2.29; 2.93).

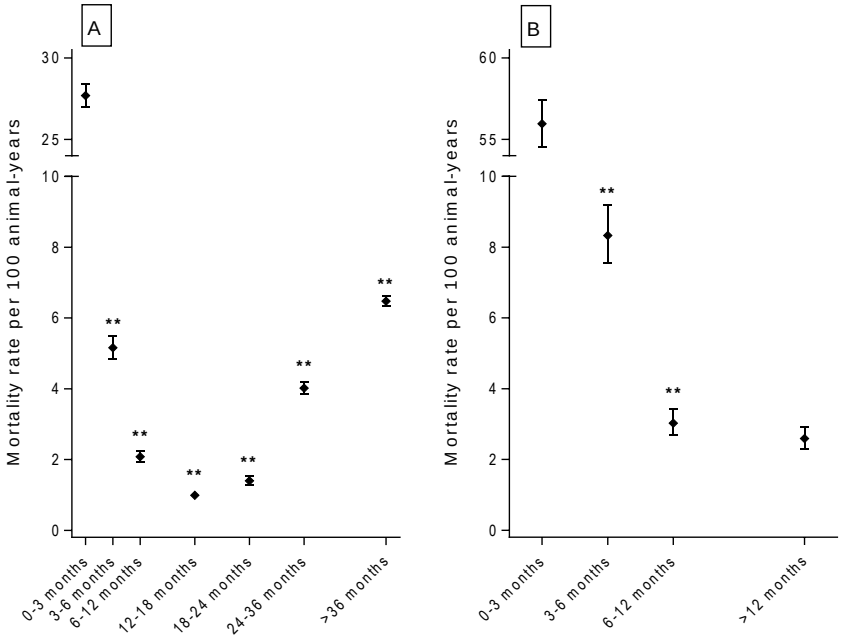

Figure 1. Crude mortality rate estimates in female (A) and male (B) cattle of Estonian dairy herds by age group (** P < 0.001 compared to preceding age category)

According to the multivariable calf (<3 months) model an interaction between sex and breed was present. The highest mortality hazard occurred in Estonian Holstein bull calves (Hazard rate ratio (HR) = 1.97, 95% CI 1.89; 2.05) and Estonian Red bull calves (HR = 1.75, 95% CI 1.59; 1.92) compared to baseline group female Estonian Holstein calves. There were no differences in mortality hazard between breeds in female calves. Compared to male Estonian Holstein calves male Estonian Red calves had significantly lower mortality hazard (HR = 0.89, 95% CI 0.81; 0.97, p = 0.010). Regarding herd size the mortality hazard was significantly higher in herds with over 400 animal-years.

According to the models for 3-24 months and >24 months old cattle Estonian Red breed cattle had lower mortality hazard compared to Estonian Holstein breed cattle. There was significantly higher mortality hazard in animals from herds larger than 400 animal-years compared to smaller herds. The hazard of mortality was lowest in Southwest region compared to baseline category Northeast region.

Calves aged <3 months had higher mortality hazard in January, February and March in both study years. Heifers aged 3-12 months had highest mortality rates in August and September both years. Heifers aged 12-24 months the mortality hazard increased since March 2014 and stayed high during the second half-year of 2014. Among adult cattle (>24 months) the period with increased mortality rate was summer (months July to September) 2013.

DISCUSSION

In order to reduce mortality and improve animal welfare in cows it is necessary to put more effort in prevention of metabolic and digestive tract diseases and feet and claw disorders, which was also highlighted by a Swedish study (Alvåsen et al., 2014). Similarly to previous research (Torsein et al., 2011; Raboisson et al., 2013) it is important to contribute to the prevention of calf diarrhoea and respiratory diseases in order to lower the mortality.

The risk factor analysis identified that Holstein breed was associated with higher mortality hazard which has also been identified in previous studies (Thomsen et al., 2006; Alvåsen et al., 2012). We identified a higher mortality among male cattle which can be related to biological features and lower market value (Pannwitz, 2015).

Herds with >400 animal-years differed from smaller ones by having significantly higher mortality hazard in all age groups. Mortality has been reported to increase with herd size in several studies (Thomsen et al., 2006; McConnel et al., 2008). An explanation may be that in larger herds individual attention of animals is reduced and larger herds have different housing conditions and mechanization compared to smaller farms (Raboisson et al., 2011). Also, herd size is a proxy for many other factors possibly associated with mortality hazard in cattle. Regional differences in mortality hazard were confirmed for cattle over three months of age and 'region' acted as a confounder in all three models. Due to the small size of Estonia the climatic conditions are relatively even but herd management factors may differ by location.

Seasonal distributions of mortality rate differed in the four age categories. Higher mortality in dairy calves during winter period has been identified in previous studies and it is believed that low temperature and more intensive spread of infections may lead to higher mortality (Raboisson et al., 2013). Autumn was a high risk period for young stock but reasons for that are difficult to provide. Higher mortality rate among cows was observed during summer months. With warm and moist weather it is plausible that heat stress may affect the resistance of cows and can be a cause of higher mortality (Bishop-Williams et al., 2015).

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HOW DOES CHANGING THE FEEDING BIN AFFECT COWS' BEHAVIOUR?

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SUMMARY. Understanding the feeding behaviour of dairy cattle in different indoor housing systems is important to optimize production and welfare. Outdoors, grazing cattle walk about 4 km/day, grazes about 4- 14 hours within a 24-hour period and lies down for about 9-12 hours (Broom and Fraser, 2007). Monitoring the locomotion of cows can be used to predict oestrus and lameness. And the same activity monitors can be used to estimate activity and number of feeding visits by cows. In a system in which cows are grouped and given differential access to feeding bins with different rations, and these groups change over time, it is important to find out how a change in the ration with a change in the feeding bin, affects the cow's feeding behaviour, and if so, for how long. Ice tag activity monitors were attached to the right hind leg of ten cows. Walking, standing, lying data and health records were used to record changes before and after a change in the feed ration/feeding bin. Results comparing activity before and after feeding bin change revealed statistically significant increase in motion index, number of steps per minute and number of lying bouts per minute (all $p<0.001$). Comparing the behaviour or cows followed during the dry period showed statistically significant differences in motion index and number of steps per minute ($p<0.001$).

Key words: feeding behaviour, activity, cow

INTRODUCTION

Assessing farm animals' welfare has become a well-researched field of study. Consumers have become more conscious about what is happening on farms and most of them prefer products that come from extensively kept animals.

Welfare assessment is one way for a farmer to understand the bottlenecks to high quality husbandry in his/her farm and how to overcome them. Assessing welfare effectively is not through the consideration of one single measure, it is looking at different aspects that affect animal wellbeing in their environment (Frazer, 1995 reviewed by Fregonesi *et al.* 2001).

Dairy cow behaviour monitoring has become increasingly important and it is relatively common to monitor health and welfare on individual basis (Nielsen, 2013). Available techniques include the Rumiwatch system to monitor feeding, ruminating and activity, Ice Tag Sensors to monitor cows activity, SCR Heatime, Cow Scout and Smartbow for heat detection and health monitoring and Pro Calve that gives pre and post calving distress alerts.

One of the bases for improved quality of animal husbandry is a complete knowledge of behavioural activity (Brzozowska et al., 2014). The level of daily activity of cows has proven to be indicative of physiological and health status and gives indirect feedback about their comfort and welfare (Tolkamp et al., 2010 reviewed by Brzozowska et al., 2014). Cows are social animals and often synchronise their

behaviour. Their behaviour can be affected by fresh feed delivery return from milking, group size, stocking density and housing system. Cows at a lower stocking density or in smaller groups display more behavioural synchrony than cows in a larger group (King *et al.* 2016). When cows change groups their welfare can be compromised (Pavlenko *et al.* 2017). "Changes in behaviours such as activity and resting, can reflect disturbances in a herd, and be related to decreased productivity of the livestock" (Steensels et al., 2012 reviewed by Brzozowska et al., 2014).

MATERIALS AND METHODS

The study was carried out on Märja farm in South- Estonia. The farm houses around 250 cows, including dry cows and young stock, with a zero grazed system. Lactating cows are housed on one side of the farm and young stock and dried cows are in an adjacent building. Cows were loose housed with cubicles covered with rubber mattress bedding. A mixture of peat and sawdust was laid on the mattresses every day to keep them dry. Cows were fed from 30 feeding bins, a total mixed ration *ad libitum* that consisted of a grass and clover silage and a compound feed of barley and rapeseed cake which was fed in portions according to milk yield. Access to water was available all of the time. There were three feeding groups based on milk yield and each group was delivered from 10 bins. The feeding groups were for high (first), medium (second) and low yielding cows (third). The last group consisted of cows who were in preparation for drying off. Feed was delivered additionally at the DeLaval feeding robot two to three times per day.

Ten lactating cows, three primiparous and seven multiparous, were selected based on those that were imminently changing their feeding group either from first to the second group or from the second to the third group. Ice Tag activity monitors were attached to each cows' right hind leg and data were downloaded to a laptop by Ice Manager every week. Health data were collected, but the sample cows did not have any health problems during the time they were monitored.

Cows were monitored for 14 days before and 14 days after group change. Group change meant that the cows had to find new bins to feed from to assess feed. Each bin had a gate which opened when triggered by a transponder which was around each cows' neck. One cow was moved after 14 days of monitoring straight to the dried off area. This cow and four others monitored previously were monitored in dried off area for 5 – 14 days. Due to technical problems, the number of steps for one cow who went straight to the dried off area were not registered. For statistical analyses it was decided to use her data only when dried off area effect was calculated.

From the collected data the average daily values from minute-based observations were calculated. The lying and standing times registered in seconds were converted to proportions per minute. Daily average motion index, number of steps (per minute) and proportion of lying and standing before and after ration change and at dry period were compared with a general linear mixed model. The right skewed distributed number of lying bouts per minute was studied with a generalized linear mixed model with logarithm link function. Both models took into account fixed effects of time period and parity and random effect of cows. The Satterthwaite approximation for the denominator degrees of freedom was applied. The results are expressed as least square means with standard errors and the differences are considered statistically significant at $p \leq 0.05$.

For each variable two separate analyses were made: 1) consideration of nine cows with data before and after ration change, and 2) considering five cows (four cows in number of steps analysis) with data also from the dry period.

For modelling MIXED and GLIMMIX with SAS 9.4 were used. The figures to describe the behaviour of single cow at different time periods were constructed with R 3.2.3.

RESULTS

Comparing the behaviour of nine cows 14 days before and 14 days after ration change revealed statistically significant increase in motion index, number of steps per minute and number of lying bouts per minute (all $p<0.001$). There was no single observed change in lying and standing – some cows tended to lay more and stand less after ration change, while some cows behaved the opposite and some cows did not change their lying and standing behaviour. On average there was no change in standing and lying times ($p=0.650$).

Comparing the behaviour of five cows followed during the dry period revealed statistically significant differences in motion index and number of steps per minute ($p<0.001$). The direction of change after cows went to the dried off area remained the same – the motion indices and number of steps increased. The average values of motion indices and numbers of steps per minute were lower in the dry period compared to before and after feeding bin change. No statistically significant differences in mean number of lying bouts was found. In the dry cow pen, on average, cows stood less and lay more, but those changes varied by cow and the overall time effect was not statistically significant.

DISCUSSION

Grouping of cows is a normal practise in the dairy industry. After feeding bin change, and after moving to the dried off pen it took 3 to 5 days for behaviour to return to normal. It corresponds with work reported by Kondo *et al.* (1984) and Hasegawa *et al.* (1997). They found that it took 5-15 days after social behaviour and locomotion activity return to normal after regrouping or introduction of a new animal into a group. It was interesting to see that although data values normalised it was evident that the motion index, number of steps per minute and lying bouts per minute were higher than previously at the 7^{th}, 8^{th} or even 17^{th} day after feeding bin change. It would have been interesting to video record sample cows and investigate the reasons behind this change.

Von Keyserlingk *et al.* (2008) monitored cows in mid-lactation before and after they were placed into a new social group. They found that after the change cows reduced their time spent feeding, time spent lying down, and time spent allogrooming. We did not video record our sample cows, but lying bouts data from activity monitors showed no statistical differences for both changes. When changing feeding bins, but not group, the behaviour of the sample cows differed within groups. This might possibly have been the result of small sample size and needs to be investigated further.

Cows are diurnal and synchronize their behaviour with their close neighbours (Boyland et al. 2016). After the automatic feeder has delivered feed, or when they return from milking, most cows feed. Cows whose ration has just been changed experience more frustration, because they are not able to get access to their feed from the same bins as before. Those cows who are not so determined to push other cows away or find another bin will return to the lying area, with their motivation to feed unsatisfied. Others, who are more determined to get access to their "old" feeding bin will try to push other cows away and/or by chance find the right bin to which they have access.

In the dried off area there was not enough room for all cows in that pen to feed at the same time. Bewley et al., (2010) and Løvendahl and Munksgaard (2016) have found that during the end of lactation lying times increase. Lying bouts (but not times) decreased in the Løvendahl and Munksgaard

(2016) study and did so too in our study. But an increase in lying time was observed only for two cows out of the five who were removed to the dried off pen.

This study showed that changing feeding place in a group has an effect on the activity of cows and their welfare. Primiparous cows who had never experienced feeding bin change reacted more strongly than multiparous cows, because novelty is a strong stressor, especially if a cow is suddenly confronted with it (Grandin, 1997).

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AUTOMATED ASSESSMENT OF ANIMAL WELFARE INDICATORS IN PIGS AT SLAUGHTER

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SUMMARY
In Germany, farmers are legally obligated to monitor animal welfare indicators. Besides the difficulties in defining valid animal welfare indicators, the comparable assessment is another challenge. Therefore, the aim of this study was to develop a commercial automated camera system for the assessment of animal welfare indicators for pigs in the slaughterhouse. External animal welfare indicators should be recorded directly in a slaughterhouse with 3,500 – 5,000 slaughtered pigs per day with a newly developed camera system (CLK GmbH, Münster, Germany). Following welfare indicators were defined in this project for detailed consideration: Ear necrosis, tail damages, marks and wounds from chasing and tattooing, and swellings of the joints of front and hind limbs. These indicators were in agreement with the German Quality Assurance Initiative (QS, Bonn, Germany). The taken pictures were interpreted by a veterinarian, discussed in a consortium, and used by the manufacturer to develop a computer program for an automated evaluation of the indicators. Moreover, pictures were taken manually in the stables to compare the results of the camera and the original condition before slaughtering. In the next step, the automated results were compared to results assessed by an individual observer. In this project, the camera based, automated rating system of welfare indicators for pigs in the slaughterhouse was developed pre-commercially. The system is a powerful tool to support the objective monitoring of animal welfare, also in large animal numbers, resulting in a better, comparable feedback both to the farmer and the veterinarian.
This study was generously supported by the QS Wissenschaftsfond.

Key words: Pigs, Slaughter, Welfare

INTRODUCTION
During the last years, animal welfare has received special attention in German animal husbandries. Based on the German Protection of Animals Act, § 11 Section 8, the farmer has to assess and evaluate animal welfare indicators directly on the animal within the legally required self-monitoring. Via this self-monitoring, the demands of § 2, German Protection of Animals Act (for instance adequate feed, care, and accommodation appropriate to the species) should be complied. In the official explanatory statement this is commented as follows: "The aim of animal-based self-monitoring is to provide an assessment of the animal's well-being based on appropriate indicators such as foot pad health, mortality rates or organ findings at the abattoir, and to plan and implement improvement measures if necessary." The assessment of animal based welfare indicators at the slaughterhouse represents a particular feasible tool for the retrospective evaluation of the animal's living conditions due to large animal numbers and the realization of comparative observations. However, if assessed by individual observers, the amount of work and the subjectivity of the results represent major problems. In a study at poultry slaughterhouses, significant variations in the evaluation results of broiler feet were found between different experts (Habig, 2013). In order to guarantee consistent, comparable and objective results, food pad condition as welfare indicator is assessed at most larger poultry slaughterhouses in Germany via camera-based monitoring-systems by now. The question arose if such a system can also

be developed and implemented for pigs as well. Therefore, the aim of this study was to test a camera-based rating system for the automated and standardized assessment and documentation of animal welfare indicators in pigs.

MATERIAL AND METHODS

To realize the mentioned aim, a pilot study on the automated assessment of welfare indicators in pigs at slaughter was initiated in summer 2015. Main emphasis was laid on the indicators ear lesions, tail lesions and swellings in joints, based on the recommendations of another pilot study on findings at slaughter, resulting in the guidelines „Leitfaden Befunddaten in der Schweineschlachtung“(QS, 2017). On the participating abattoir, approximately 4,500 pigs are slaughtered daily. The monitoring system (CLK GmbH, Altenberge, Germany) consists of six cameras, taking pictures of the hind legs, the back, and the head and to lateral pictures per pig, respectively, against a blue background. The system was installed after the processes of bleeding, scalding, de-bristling and flame-scarfing. A schematic side-view of the system is represented in Figure 1.

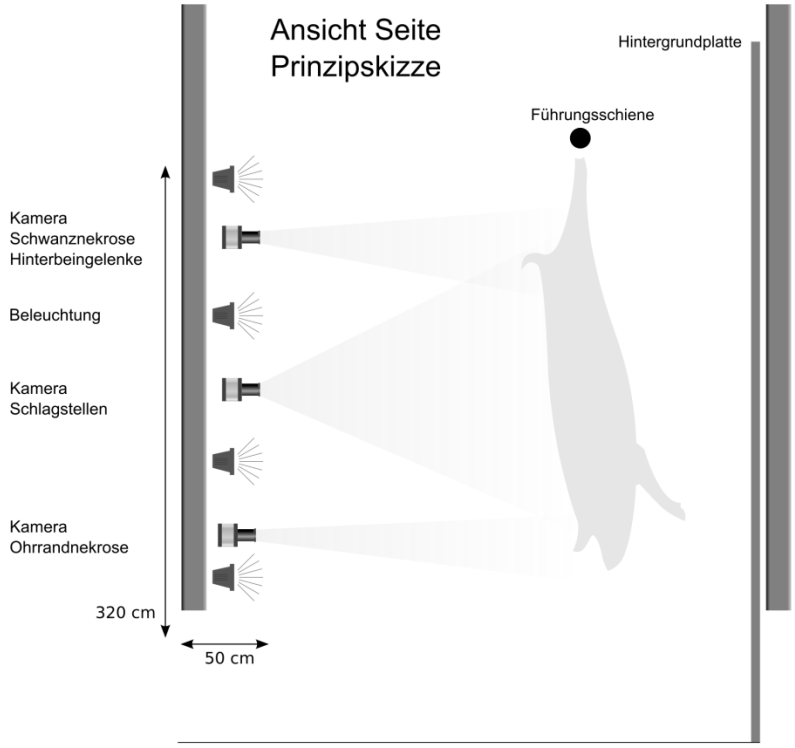

Fig. 1: Side view of the automated rating system (CLK GmbH)

In the developing phase, the system was optimized with regard to the individual assignment of the pictures and findings to each animal. After calibration and parallel assessments by experts, specifications for the defined indicators and the development of respective algorithms, findings were detected automatically. After the developing phase, the system was able to detect the welfare indicators tail lesions and to mark noticeable problems with red circles (Figure 2). Moreover, ear lesions and joint changes, for instance swellings, were detected. With regard to joint changes, discussions with experts regarding the definition of this indicator are planned in the next step

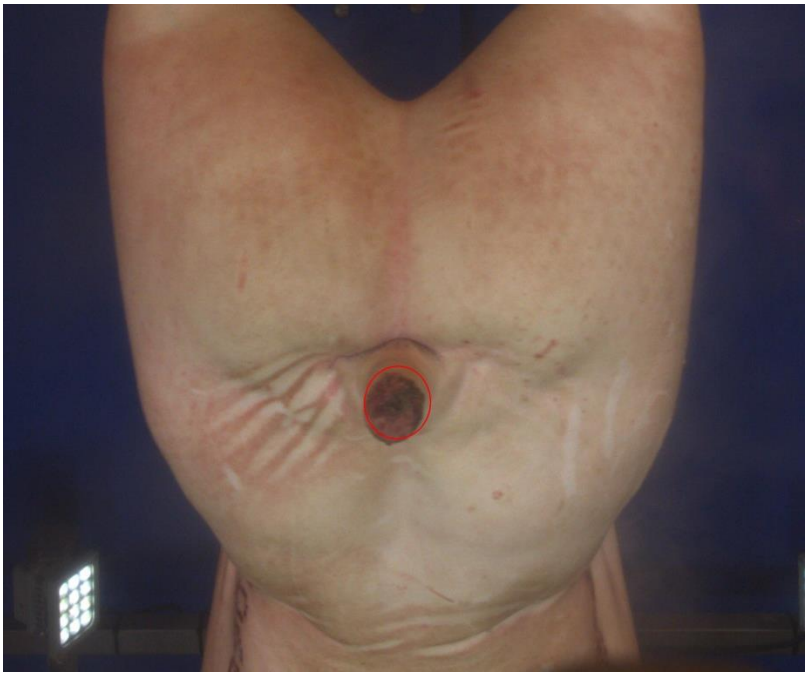

Abb. 2: Marking of the indication „tail lesion“ by the automated rating system (Picture: CLK GmbH)

After the intensive developing phase, sensitivity (true positive rate) and specificity (true negative rate) were calculated based on a comparison of pictures. Moreover, a comparison of the assessed findings by the system and by individual experts took place on several dates. The system runs on every slaughter day and monitors tail and ear lesions. Pictures are saved for further evaluation and documentation. The rate of non-evaluable pictures, for instance based on carcasses hanging askew, is determined as avoid-rate.

RESULTS

Sensitivity and specificity were calculated at three test days as documented in Table 1.

Tab. 1: Sensitivity and specificity of the indicators ear and tail lesions based on pictures from three test days

	sensitivity / specificity
	indicator ear lesions
Tag 1 (n= 3,974)	77.1% / 99.3%
Tag 2 (n= 3,560)	84.0% / 97.5%
	indicator tail lesions
Tag 3 (n= 3,560)	100% / 99.9%

Based on the lesions detected by the system, various evaluations can be performed, for example a comparison of data based on single slaughter dates, as documented in Table 2. Within six days, the prevalence of ear lesions varied between 8.6% and 15.5%, and of tail lesions between 0.6% and 1.3%.

Tab. 2: Prevalence of ear and tail lesions on six consecutive slaughtering days (N= number of evaluated pictures)

prevalence	***indicator ear lesions***	***indicator tail lesions***
day 1 (n= 4,489)	9.1%	1.3%
day 2 (n= 4,206)	10.9%	0.6%
day 3 (n=4,226)	9.5%	0.8%
day 4 (n= 3,697)	15.5%	1.0%
day 5 (n= 4,251)	8.6%	1.3%
day 6 (n= 4,193)	11.4%	1.3%
total (n= 25,062)	10.7%	1.1%

The avoid-rates in the considered period differed daily, too. For the indicator ear lesions they varied between 4.3% and 6.7%, and for tail lesions between 1.9% and 2.9%. This rate should be further reduced.

DISCUSSION

The automated assessment system was implemented successfully for the welfare indicators ear and tail lesions at the test slaughterhouse. The system is very suitable for the daily use. As shown for the joint lesions, besides the technical development, the definitions of indicators and thresholds are of special importance. With regard to the amount of assessed data, a huge variety of different analyses is possible. The system is open for further indicators, e.g. indicators of inappropriate chasing. After the previous operating experience with the automated recording system, the prevalence of ear and tail lesions differs considerably depending on the animal batches, and origins, respectively. For comparison, the system should be installed in several abattoirs, and, moreover, the thresholds, especially for lesions and swellings in the joints, have to be discussed critically and to be standardized, in the best case.

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ASSOCIATIONS BETWEEN DRIVING ACTIONS AND ANIMAL STRESS IN MOBILE SLAUGHTER OF CATTLE

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SUMMARY. Rough handling at slaughter can affect cattle welfare negatively. Aiming to study associations of stress-related animal behaviours and time intervals for driving, stunning and sticking with stockperson driving actions, we made initial observations at a commercial mobile slaughter plant (Hälsingestintan AB, Sweden) on 11 days at nine Swedish cattle farms. The stun box and slaughter unit were housed in a truck trailer. Animals were driven 2.4 to 5.6 m from an inspection pen to the stun box by farm staff (55%), plant staff (13%) or both (32%). Stunning was carried out using a cartridge-driven penetrating captive bolt gun. Data were collected from 183 animals (135 beef and 48 dairy breed) through direct continuous observations. For each animal, the numbers of stress behaviour bouts in driving lane and stun box were recorded separately. Twenty-one driving actions involving physical interaction were observed, counting the total number of bouts per action, as well as the number of stockpersons driving. Associations were assessed using Spearman rank correlation for 18 driving actions that were observed in at least one animal. Most associations were found for stress behaviours during driving and time in driving lane, which were positively correlated with patting using hand or tool, pressing using hand, tail twisting and using electric goad, typically producing rho values of 0.20 to 0.46. Seventeen percent of the animals were not subjected to any driving action and 37% displayed no stress behaviour during driving. Time in stun box and from stunning to sticking were negatively correlated with patting with hand (rho -0.35 and -0.20, respectively). This study indicates moderately strong associations between stress-related behaviours during driving and forceful driving actions by stockpersons at mobile slaughter. Analyses of additional data including comparisons with conventional large-scale slaughter will follow.
Keywords: Cattle, Mobile slaughter, Stress

INTRODUCTION

Slaughter inevitably exposes production animals to risks of poor welfare. Although efforts have been made to reduce suffering, most farm animals still experience considerable stress shortly before slaughter (Warriss 1990; Cockram & Corley 1991), which may compromise their welfare. Pre-slaughter stress may also reduce meat quality due to depletion of glycogen reserves in the muscles (Ferguson & Warner 2008; Warren et al. 2010; Friedrich et al. 2014). In many countries industrialised slaughter undergoes structural changes towards fewer and larger plants, resulting in longer transport distances and increased line speed. This may pose additional threats to animal welfare, increasing the need for well-designed slaughter facilities and proper routines to reduce animal stress (Grandin 1996). Small-scale and farm-based stationary or mobile slaughter may have the potential to reduce animal stress, by shortening or eliminating the transport and the exposure to an unfamiliar environment, and is also associated with lower line speed. A mobile abattoir is a self-contained slaughter and cooling unit which can be moved between farms where animals are reared for slaughter. The effects of mobile slaughter on animal welfare have not been systematically studied before. In 2013 and 2014, a Swedish company (Hälsingestintan AB, Järvsö, Sweden) developed a mobile unit for commercial slaughter of large cattle in Sweden. We aimed to assess the potential for the development of good animal welfare and meat quality in cattle slaughter, by studying associations of stress-related animal behaviours and

time intervals for driving, stunning and sticking with stockperson driving actions in mobile cattle slaughter.

MATERIAL AND METHODS

Data were collected in connection with slaughter at the mobile abattoir of Hälsingestintan at nine farms on a total of 11 days during February to September 2016. The abattoir had a capacity of slaughtering approximately 35 head a day. Stun box and slaughter unit were housed in a truck trailer. Stunning was carried out using a cartridge-driven penetrating captive bolt gun, with cartridges adapted to animal size. The study occasions were spread out in time as evenly as possible but otherwise chosen with regard to practical feasibility. At each occasion as many animals as possible were studied, resulting in a reasonably representative sample of 8-21 animals per study day and 8-38 animals per farm, totally 183 cattle. All animals had been kept on the respective farm for at least 48 h prior to slaughter.

Observations were made during driving from a portable veterinary inspection pen to the stun box (2.4 to 5.6 m, pipe-fence sides), in the stun box and until sticking. All data were collected by two research technicians, one observing the animals up to the stun box and the other one carrying out all remaining observations. For each animal, direct observations were made of number of physical stockperson action bouts at driving, time for driving (from leaving the inspection pen to entering the stun box), time in the stun box (until first shot), time from stunning (last shot) to sticking, number of shots, and number of animal behaviour bouts during driving and in the stun box. Driving actions were categorised as touching, patting or hitting hind part or front part, restraining, pulling or pushing using the hand or a tool, tail twisting, kicking, pricking, hitting with gate, electric prodding, yelling/whistling or creating noise by hitting fittings, as well as number of stockpersons driving. Animal behaviours were recorded during driving and in the stun box separately and categorised as tiptoeing, running, backing, turning, mild slipping, severe slipping, falling, kicking, charging, struggling, freezing, vocalising, eliminating, and exploring. Showing one or more of these behaviours, excluding exploring, was classified as 'stress behaviour'.

Associations between the number of bouts of different driving actions and the total number of stress-related animal behaviour bouts, during driving and in stun box separately, were analysed by Spearman rank correlation. Likewise rank correlations were estimated between the number of driving action bouts and the time for driving, time in the stun box and time to sticking. Due to the large number of tests (90), we applied a 1% significance level.

RESULTS

The studied animals were of beef (74%) or dairy (26%) breed, between 8 and 178 months of age. They were classified as young bulls (54%), steers (17%), heifers (23%) or cows (6%). No calves or adult bulls were included. Driving was carried out by farm staff (55% of animals), abattoir staff (13%) or both (32%). The weather conditions were sunny or partially cloudy when 55% of the animals were observed, and overcast or rainy in the rest of the cases, with temperatures ranging from -9 to +27 ˚C. Forty-eight percent of the animals were driven by one person, 41% by two, 9% by three and 2% by four persons. Totally, 83% were driven using either the hand or a tool, and the number of driving actions per animal varied between 0 and 221 (mean 15.2, median 5 actions). The highest frequencies of driving actions per animal were recorded for patting with hand on hind part (maximum 129 actions per animal), patting with tool on hind part (112) and touching with hand on hind part (79). Violent actions in the form of kicking, pricking and hitting with gate were not observed. Inappropriate actions, including hitting any part of the animal with or without a tool >5 times, electric prodding >5 times, or tail twisting, were observed in 38 animals (21%). The time for driving from the inspection pen to the stun box was between 0:05 and 18:41 min (mean 183, median 48.5 s). The time in the stun box prior to stunning was between 0:08 and 2:08 min (mean 35, median 27 s). The time from stunning (last shot) to

sticking ranged from 0:24 to 3:22 min (mean 98, median 96 s). Young bulls had the longest driving times and cows had the longest times from stunning to sticking, while the times in the stun box did not vary substantially between animal types. Eighteen animals (10%) were reshot, of which one animal four times (3:17 min first to last).

During driving, the total number of stress-related behaviour bouts per animal ranged from 0 to 31 (mean 4.3, median 2 bouts), and 67 animals (37%) displayed no such behaviours at all. In the stun box the total number of bouts per animal ranged from 0 to 14 (mean 2.3, median 2 bouts), whereas 9 animals (5%) displayed no such behaviours. The highest frequencies of stress behaviour bouts per animal were seen for backing (maximum 14 bouts per animal), turning (12) and vocalising (10) during driving, and turning (6), mild slipping (5) and vocalizing (4) in the stun box. For all actions and behaviours observed, the distribution of bouts per animal was heavily positively skewed. There was a significant but weak positive correlation (rho=0.20–0.40) between several types of actions and stress behaviour during driving (no. of persons driving; touching or patting hind or front part with hand; pushing with hand; patting hind or front part with tool; hitting front part with tool; tail twisting; and electric prodding), but not in the stun box. There was also a weak to moderate (rho=0.21-0.46) positive association of some types of actions with driving time (most of the mentioned actions), but not with time in stun box or sticking time; instead negative correlations were found of patting with hand with time in stun box (rho=-0.35) and time to sticking (-0.20), and of pushing and tail twisting with sticking time (-0.21 and -0.20, respectively).

DISCUSSION

In total 83% of the cattle slaughtered at a Swedish mobile abattoir received one or more of the recorded stockperson driving actions, and 95% displayed one or more of the recorded behaviours indicative of negatively affected animal welfare. Inappropriate actions were observed in relation to at least a fifth of the animals. Six percent of the animals received electric prodding, which is within the range usually seen at Swedish slaughterhouses.

Hitting with or without a tool is not an uncommon way to drive cattle at slaughter plants. However, current Swedish legislation prohibits forceful hitting just as it prohibits tail twisting and excessive electric prodding. Crowding can result from a suboptimal driving race design. If many animals move backwards, as observed in this study, or refuse to move forward it might be because they are distracted by e.g. light reflections, shadows, conspecifics or persons entering the flight zone in front of the point of balance (Grandin 2007).

For several driving actions, we found weak correlations between the number of action bouts per animal and the total number of stress-related animal behaviour bouts in the same animal during driving, but not in the stun box. Similarly, Hultgren et al. (2014) found several weak correlations, but no strong ones, between individual driving actions and individual animal behaviours, which they argued might suggest that plant design or events not aimed directly at the animals, such as disturbances close to the driveway, are more important for animal welfare than stockperson-animal interactions. Taken together, our results reveal a moderately strong association between forceful driving and stress-related animal behaviours during driving.

Clearly, short handling times and few negative stockperson-animal interactions are generally beneficial for animal welfare. Improper plant design will probably result in long handling times and high counts. On the other hand, a low line speed means fewer stockperson actions and animal behaviours associated with poor animal welfare per time unit because the animals are less stressed, although the total handling times will be longer. Some animals were patted many times, representing fast sequences of repeated mild actions, which is in accordance with the observations of Hultgren et al. (2014). It is unclear whether or not the stockpersons carried out these actions deliberately, or if they

acted habitually or even unintentionally. Overall, the time from stunning to sticking was unacceptably long, and in single cases exceptionally long, which may be explained by inappropriate stun box design and difficulties to shackle stunned animals rapidly enough.

Ten percent of the animals were reshot, which is consistent with Atkinson et al. (2013). In other countries, studies of cattle shot with penetrating bolt weapons have reported 9 to 32% of the animals being incorrectly stunned (Gregory et al. 2007; Gouveia et al. 2009; von Wenzlawowicz et al. 2012). Inadequate bolt stunning probably has a major negative impact on animal welfare (Grandin 1998; Gregory & Shaw 2000). In this study, however, we did not evaluate stun accuracy or stun quality *per se*, hence the reshoots were made on the initiative of the abattoir staff only.

These results are preliminary and definite conclusions can therefore not be drawn. However, the results indicate that forceful driving to the stun box at mobile slaughter of cattle is associated with increased animal stress and a long driving time, and to some extent with a shorter time in the stun box and until sticking. Further analysis of these and additional project data should include estimation of the variation in driving routines between stockpersons, the influence on driving actions and animal behaviour of extraneous factors like farm conditions and weather, animal factors like breed, sex, age, earlier experience, level of excitation when driving starts, and interaction between animals that are handled together, as well as comparisons with conventional large-scale slaughter.

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We thank Hälsingestintan AB for giving us access to their mobile unit, and their abattoir staff for excellent collaboration. The efforts of research technicians Anne Larsen and Karin Wallin in data collection are highly appreciated. The Marie-Claire Cronstedt Foundation and the Swedish Animal Welfare Association generously provided project funding.

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AMMONIA REDUCING MICROBIAL-MINERAL LITTER ADDITIVE FOR POULTRY MANURE TREATMENT

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SUMMARY. The aim of our research was to invent an effective and innovative microbial-mineral litter additive (MMLA) which can control ammonia (NH_3) emission from poultry production. The MMLA was developed at Lodz University of Technology. Heterotrophic bacteria in a powder form were embedded on a mixture of perlite and bentonite (mineral carrier, MC). The MMLA and MC were mixed with laying hens manure under laboratory conditions and effectiveness in mitigation of NH_3 emission was demonstrated. The NH_3 concentration in the manure headspace air samples was evaluated using spectrophotometric method. The NH_3 reduction rate for MMLA (20 g of MC + 5 g of bacteria powder per 500 g of manure) after 48 h and 96 h was on the level of 71 and 29%, respectively, while the concentration of NH_3 in air samples collected from the control group was about 115 $mg \cdot m^{-3}$ on average.

Key words: Ammonia, Poultry, Manure, Litter, Additive

INTRODUCTION

Poland is the biggest poultry meat producer in the EU. Unfortunately, growing poultry production is strongly linked with livestock odor emission. Main source of odor annoyance is poultry manure, which emits numerous volatile odorous compounds, mainly ammonia (NH_3). Thus, there is a strong need to develop efficient and inexpensive method for mitigation of emission of odorous compounds of livestock origin, including ammonia. There have been many studies on methods for mitigating emissions of livestock odor but most of it focus on the swine production [Maurer et al., 2016; Ubeda et al., 2013]. There have been some evaluations of bacterial additives [Borowski et al., 2010; Matusiak et al., 2015], mineral additives [Czyż et al., 2013; Opaliński et al., 2009; Pillai et al., 2012] or air filtration, [Cai et al.,, 2007; Opaliński et al., 2010] in mitigation of emission from poultry manure, however, those methods are less widely investigated in general. Thus, the aim of the study was to evaluate the effectiveness of the innovative MMLA in mitigating NH_3 from poultry manure, under conditions simulating a typical poultry house environment (air temperature, ventilation rate, stocking density, and amount of manure generated).

MATERIAL AND METHODS

The MMLA was developed at Lodz University of Technology. Six strains of heterotrophic bacteria: Pseudomonas fluorescens (ŁOCK 0961), Bacillus subtilis (ŁOCK 0962), Bacillus megaterium (ŁOCK0963), Leuconostoc mesenteroides (ŁOCK 0964), Enterococcus faecium (ŁOCK 0965) and Streptomyces rutgersensis (ŁOCK0967), were embedded on a mineral carrier (mixture of perlite and bentonite). The procedure of preparation of microbial-mineral litter additive is patent protected (Polish patent no. P393863).

The MMLA was mixed with nearly fresh laying hens manure in order to reduce the emission of NH_3. The trials lasted for 96 h and consisted of six glass treatment containers (220 cm^3 of surface area) filled with 500 g of manure (10 mL of water per 100 g of manure were added to prevent the manure

from drying out and to facilitate manure homogenization). Two of the containers were randomly assigned to the control group (only manure inside), the other two contained the manure and 25 g of MMLA, and the last two contained corresponding amount of MC, to evaluate the influence of the mineral carrier alone. Concentration of bacteria powder in the MMLA for each trial was 20% (5 g of bacteria powder and 20 g of MC per 25 g of the MMLA). All the containers were kept under aerobic conditions with the air flow over the manure at the rate of ~1 $L \cdot min^{-1}$. Air flow was generated by portable aspirators. During sampling, separate aspirators were used and the manure headspace air was pumped through washers, containing 0,1 M H_2SO_4, for 5 minutes with the same flow rate (~1 $L \cdot min^{-1}$). Ammonia concentration in the manure headspace was measured 48 h and 96 h after application of the MMLA. The content of NH_3 was determined according to Polish standards using a UV-3100 PC spectrophotometer (VWR International, Leuven, Belgium).

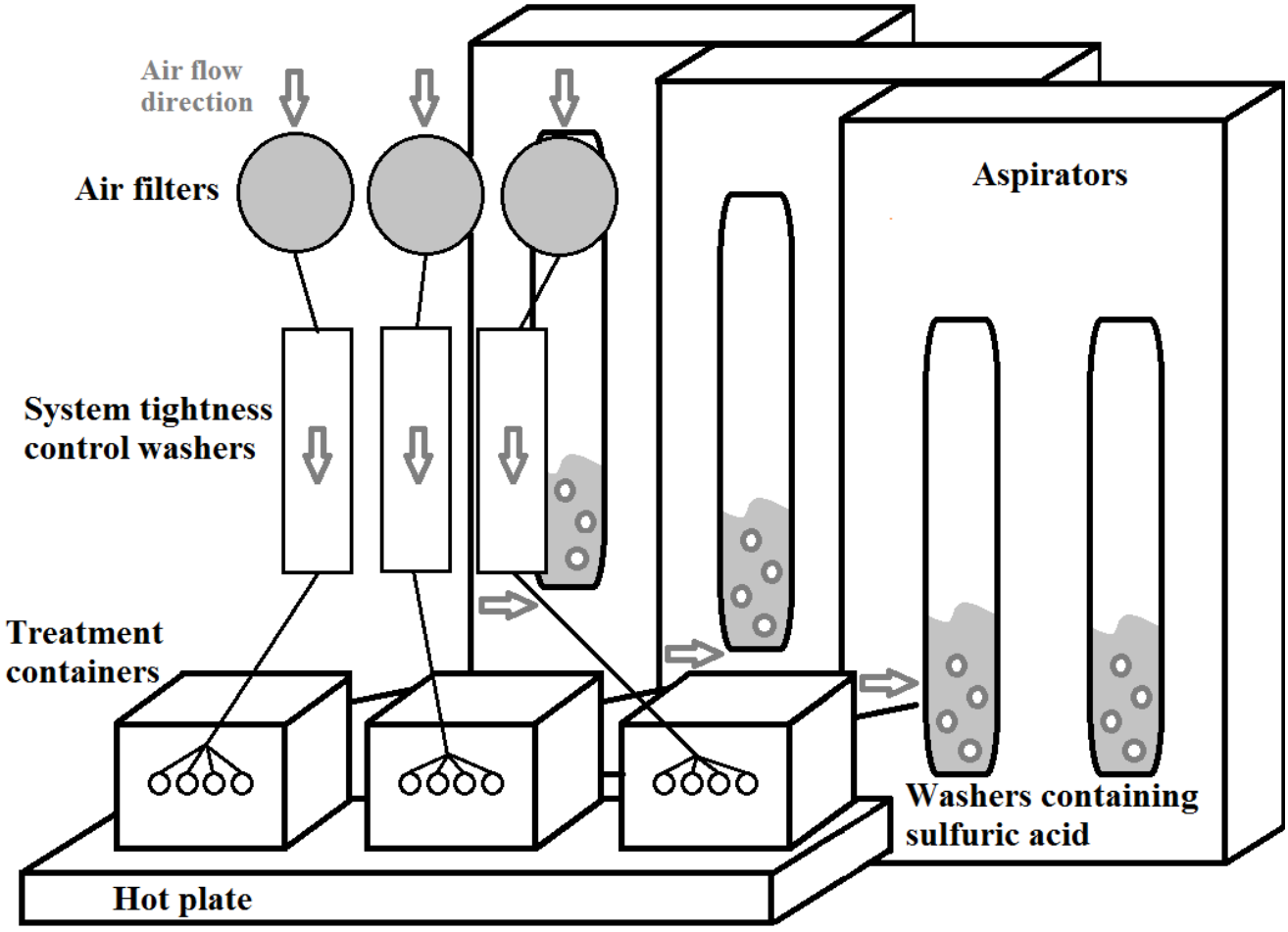

Figure 1. Laboratory-scale system for measuring ammonia emissions from poultry manure. Inlet air was passing through odor filtering cartridges, assuring that clean air is entering the system. Properly working washers at the inlet were ensuring tightness of the system. Manure and the treatment groups (MC, MMLA) were kept in glass containers, with weighted lids, placed on the hot plate (30 ºC). Aspirators were sucking the air from over the manure and pumping it through washers with H_2SO_4 in order to absorb NH_3.

RESULTS

The MMLA treatment reduced NH_3 concentration in the manure headspace air by 71% and 29%, respectively 48 h and 96 h after the application (n=4). The MC treatment increased the NH_3 concentration by 29%, both after 48 h and 96 h (n=4).

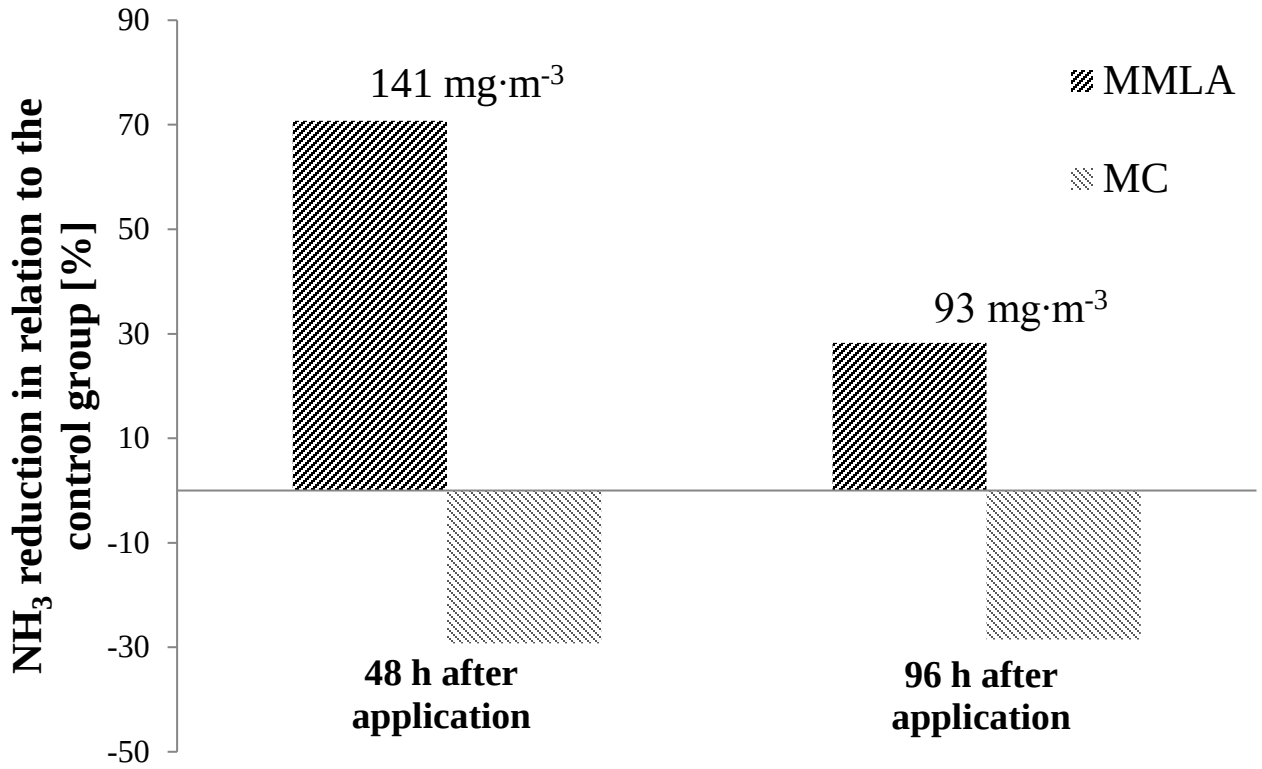

Figure 2. Percentage of NH_3 reduction, 48 h and 96 h after application of used treatments. Values above the bars indicate NH_3 concentration measured inside the containers from the control group.

DISCUSSION

The research showed that investigated dose of the MMLA (25 g per 500 g of the manure) containing 20% of the bacteria powder (5 g of bacteria per 500 g of the manure) is effective in the NH_3 emission mitigation. Evaluated reduction of the NH_3, in comparison to the previous research [Matusiak et al., 2016], is on a higher level, however, the treatment efficacy is relatively short, considering that the level of NH_3 reduction dropped by ~40% between 48 h and 96 h after the application of the treatment. The fact, that use of the MC alone resulted in the increase of NH_3 content requires further investigation. Research on a bigger scale and for a longer period of time during intensive poultry production is necessary, together with economic analyses which will also be needed to estimate full scale application costs.

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OCCURRENCE OF CLAW LESIONS IN BEEF SUCKLER COWS IN GERMANY

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SUMMARY. Poor claw health is one of the most common causes of animal losses in German dairy cows. However, the claw health of beef suckler cows has received little attention so far. In the presented study the health status of the claws of Angus cattle was examined in four farms in Germany to gain further knowledge about claw disorders in extensively kept beef cattle. The farms were located in four representative regions in Germany, from Bavaria up to the Baltic coast region. The cows and their offspring were kept on extensively used pastures during the summer months (April-October) and in stables on straw, fed with hay and silage in the winter months (November to March). The animals of each farm had constant access to mineral feed. The age of the cows varied between first and 10th lactation. Group sizes were between 23 and 225 animals. In total, claws of 312 cattle were scored during the annual claw trimming appointment on each farm, were a professional claw trimmer fixed the cows in a claw trimming chute. The claw health was scored by a single observer using a modified version of the ICAR- Claw Health Atlas. First results revealed that 44 % of the scored cows showed completely intact claws, 1% of the animals were diagnosed with claw ulcer, 2% with double sole, 16% with claw tips broken off, 10% with scissor claws, 9% with horn fissures and 22% with white line disease. Three % of the animals were diagnosed with both, white line disease and horn fissures. This is the first study dealing with claw lesions in Angus suckler cows on a broad data base in Germany. Scoring will be repeated in winter 2016/17 to increase sample size.

Key words: claw health, beef suckler cow, Angus

INTRODUCTION

Poor claw health is one of the most common causes of animal losses in German dairy cows, reducing the productivity due to lower feed and water intake which results elongated laying periods as a result of painful disorders. In addition to the economical impact, poor claw health is also known to be a welfare issue. In other countries such as Canada (Clark, 2004) or Norway (Fjeldaas, 2007) incidence of claw health issues in beef cattle have been reported whereas claw health of beef suckler cows in Germany has received little attention so far. In the presented study, non- infective claw lesions were examined at Angus cattle farms in Germany, between Bavaria and the Baltic coast region, to gain further knowledge about claw disorders in extensively kept beef suckler cows using a modified version of the ICAR Claw Health Atlas (ICAR 2015).

MATERIAL AND METHODS

The claw health of 312 adult beef suckler cows was examined at four German Angus breeding farms (A-D). Scoring was done at the annual claw trimming appointment by a single qualified observer, as

part of the standard claw trimming procedure. The study was conducted during the winter months from November 2015 to March 2016. A second observation is currently in process, though the analysis is not completed yet. All animals were kept on extensively used pastures during the summer months (April to October) and in stables on straw, fed with hay and silage in the winter months (October to April). All animals had constant access to mineral feed. The age of the cows varied between first and 10th lactation, the age of the bulls between one and six years.
The Number of investigated cattle in the scored group per farm varied between 23 and 225 (A_n=27, B_n=36, C_n= 23, D_n= 225) including all animals due for claw trimming on the days of the observation. Using a modified version of the ICAR Claw Health Atlas focus at scoring was put on the following diagnostic findings: Scissor claws (SC), Corkscrew claws (CC), Claw ulcer (CU), White line fissures (WLF), Double Sole (DS), Horn fissures (HF). As an additional parameter, "Claw tips broken off" was observed. This parameter is not part of the ICAR Claw Health Atlas. Cows were considered to be positive for a clinical symptom if it was found on at least one claw of the animal. Symptoms were assessed as 0= no finding, 1= finding on at least one claw. Data were analysed using Excel (Microsoft)

RESULTS

Table 1. Claw disorders of n=312 beef suckler cows at four German farms scored during claw trimming using ICAR nomenclature

Claw disorder	n animals affected	% animals affected
Corkscrew claws (CC)	35	12.5%
Scissor Claws (SC)	31	9.9%
Ulcer (U)	4	1.3%
- Sole Ulcer (SU)	3	1.0%
- Bulb Ulcer (BU)	0	0%
- Toe Ulcer (TU)	1	0.3%
- Toe Necrosis (TN)	0	0%
White line disease (WLD)	70	22.4%
- White line fissures (WLF)	70	22.4%
- White line abscess (WLA)*	1	0.3%
Horn fissure (HF)	27	8.7%
- Axial horn fissure (HFA)	4	1.3%
- Horizontal horn fissure (HFH)	3	1.0%
- Vertical horn fissure (HFV)	21	6.7%
Double sole	7	2.2%
Claw tip broken off	50	16.0%

*One animal affected by WLA as well as WLF

The prevalence for WLD (22.4% of all scored animals) was highest in the observed population, prevalence for CU (1.4% of all scored animals) was lowest. For more detailed information see Table 1.

The levels of parameter occurrence varied between the farms. Results are presented in figure 1-3. While scissor claws were found at Farm B and D, corkscrew claws were found on each farm (Figure 1).

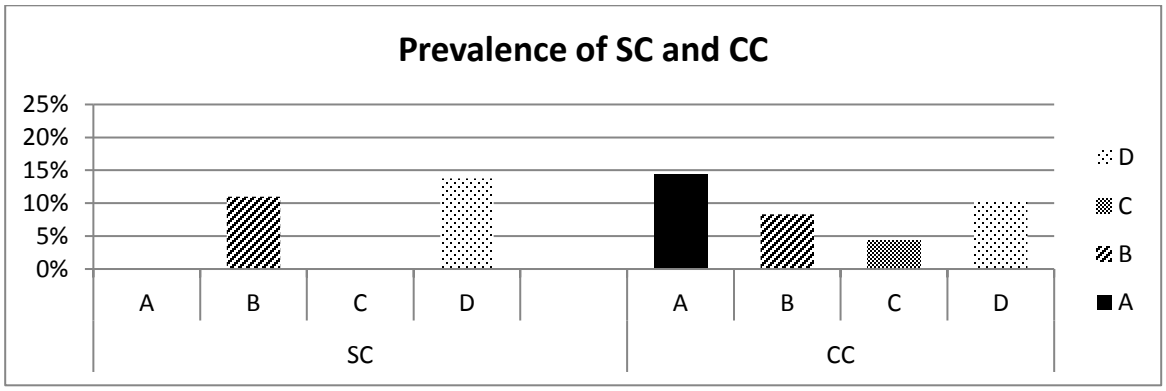

Figure 1. Prevalence of scissor claws (SC) and corkscrew claws (CC) claws

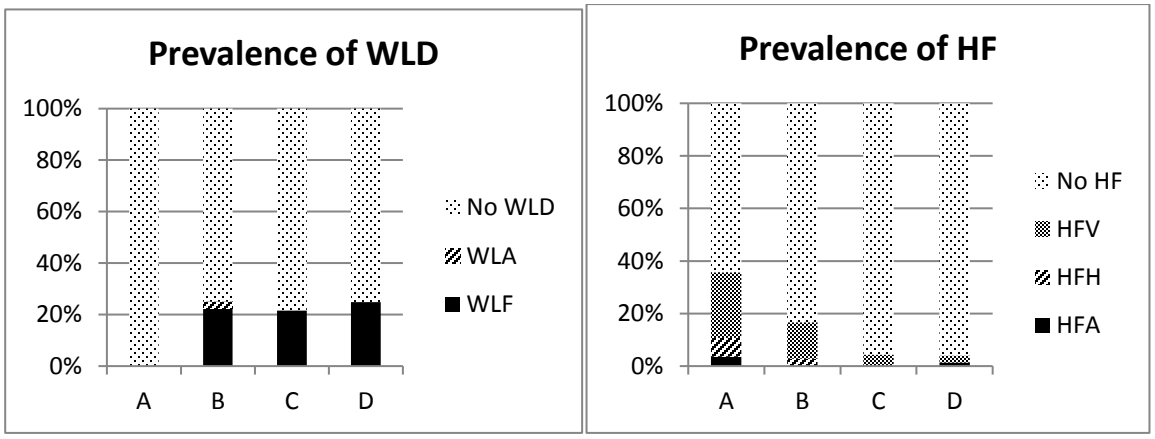

Figure 2 and 3. Prevalence of white line disease (WLD) and horn fissures (HF)

Ulcers were only found on farm B (n=1; 2.78% of the scored animals of farm B) and D (n=3; 1.33% of the scored animals of farm D). Double soles were found on farm C (n=1, 4.35%) and D (n=6, 2.6%).

DISCUSSION

WLF (22.4%), Double Soles (2.4%) and Claw Ulcers (1.3%) are considered laminitis associated claw disorders. The multifactorial pathogenesis of laminitis includes metabolic dysfunction as well as mechanic overload of the claws. Long claws, thin soles or leg trait deficiencies may favour the development of this non infectious inflammation of the claw corium. The findings indicate that chronic laminitis present not only in German dairy herds kept intensively in stables on slatted floor, but also in German beef suckler cowherds kept on straw with permanent access to a pasture in the summer months. This might be one of the reasons for the prevalence being distinctly reduced in beef suckler cow herd compared to dairy herds. WLD was the most frequently observed disease in the presented study, which coincides with the findings of Schöpke et al. (2013) who found WLD to be one of the most frequent claw disorders in German dairy cows. CU, which are considered to be one of the most

severe claw health issues of diary cows, were only found in 1.4% of the observed beef suckler cows. The same was found for Sole haemorragia. With a prevalence of 4.2% this sign of laminitis is clearly distinguishable from findings in German milk cow herds (57.3% Schöpke et al. 2013). CC and SC show a clearly genetic predisposition within the present study, 12.4% of the observed animals showed CC. Other then countries like Norway, the German routine breeding evaluation does not include genetic testing for such diseases. Cases of SC were found in half of the examined herds, 9.9% of the observed animals. As malformed claws can lead to lameness and an associated reduction in productivity a genetic surveillance could be beneficial in the future. Correlations between claw shape and claw lesions were not found to be significant (Fjeldaas et al. 2004). The ICAR Claw Health Atlas was modified by adding the parameter "claw tips broken off". Claw tips can break off as a result of a trauma, poor hoof quality due to malnutrition or chronic laminitis, among other reasons. In order to reveal possible correlations between overgrown claws and broken off claw tips, additional measures of the size of the claws before trimming could be advantageous. Such measures will be included in a second scoring period collected during winter 2016/2017 (data not presented here). In case of a positive correlation, the percentage of broken claw tips might suggest an optimal timeframe between claw trimming appointments. Vertical fissures were recorded in 8.7% of the scored cows, which is less than the average findings of western Canadian beef cattle herds, where about 20% of the animals are affected (Solano et al. 2015). Pathogenesis is not completely known. According to Clark (2004), the age of the animal, dehydration of the horn, claw size and mechanical stress are known risk factors. 44% of the inspected cattle showed completely intact claws in winter 2015/2016. Information recorded at claw trimming is used to improve breeding traits for dairy cows (Gernand et al. 2013). Comparable data in beef suckler cows clearly provides potential to also improve breeding traits. Further studies are needed to investigate the claw health issues of beef suckler cows on a wider scale (for instance by analysing data recorded by professional claw trimmers). Additionally, giving the standardized prevalence for the different diseases might help to reveal management problems and therefore could be used as an important indicator for animal welfare.

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Dairy cow daily time budget as an indicator of welfare, health and biosecurity

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SUMMARY. Dairy cows need to be provided certain environmental requirements to support their natural behaviour, well-being, optimal productivity, fertility and health. The housing environment must provide each cow with unhindered access to feed, water, and a comfortable resting area. The interactions among feeding, resting and rumination are critical to the cow comfort. A free-stall housed dairy cow has six key daily activities. She needs time for eating, lying and resting, social interaction, ruminating, drinking and time spending outside the pens included milking. The aim of the study was to determine dairy cows' daily time budget during the one year monitoring. We analysed 24-hour basic daily activities collected from 96 dairy cows housed in three free stall barns in three farms. The observed parameters were evaluated by Statistica software (non-parametric tests). During our monitoring we found the following structure of daily activities: eating 4.5 hours/day (range 3 – 6) (obviously from 9 to 14 meals/day), lying and resting in the boxes 12.6 hours/day (range 12 to 14), spent 2.4 hours/day (range 2 – 3) in social interaction, ruminate (in standing and lying position) 8.2 hours/day (range 7 – 10), drinking 0.5 hour/day (range 0.2 – 1.5) and 2.6 hours/day (range 2.5 - 3.5) spending outside the pen for travel to and from the parlour, milking. Cows housed in free stall facilities on concrete floors require a minimum of 12 hour/day of rest in a comfortable stall. When their time budget is challenged through increased time out of the pen milking, overstocking, poor stall design, heat stress and prolonged time spent in lock-ups, the primary outcome is increased health problems (lameness, etc.). Competition at feeders leads to increased displacement and reduced time designated for eating. The time budget – the allocation of time to different activities - can vary considerably depending on the environment, management and status of the cow. Only the environment that allows natural resting and feeding behaviour forms the foundation of dairy cow well-being and optimal performance.

Key words: Dairy cows, daily time budget, indicator

INTRODUCTION

Environmental conditions consist of structural, climatic and social factors for cows. Structural environment is constituted to dry, clean, soft and proper sized shelter areas in which animals spend their daily time without stress. Climatic environment consist of climatic conditions of the area in which animals take shelter. Social environment represents groups of animals formed according to social properties of them and group size (Uzal and Ugurlu, 2010).

The cow's management environment influences her ability to practice her natural time budget behaviours (Grant, 2012). Dairy cows have environmental requirements to support natural behaviour, well-being, optimal productivity, fertility and health. Cows will spend between 4.2 and 6.5 hours per day eating, 7.7 to 9.6 hour per day ruminating and have 10–17 rumination periods per day (Albright, 1993). The housing environment must provide each cow with an easy access to feed, water, and a comfortable resting area. A clean, dry and comfortable resting place is associated with greater resting

time, better health and improved productivity. The economic benefits of increased stall comfort include a lower cull rate, increased milk production and a decrease of somatic cell count (Scully, 2016). Changes in the behavioural activity of farm animals are widely used as welfare indicators (Muller and Schrader, 2003) and to investigate animal production parameters (Phillips and Rind, 2001).

The interactions among feeding, resting and rumination are critical to the cow comfort. A free-stall housed dairy cow has six key daily activities. Dairy cows need time for eating, lying and resting, social interaction, ruminating, drinking and time spending outside the pens included milking (Grant, 2012).

The aim of the study was to determine dairy cows' daily time budget during one year monitoring.

MATERIAL AND METHODS

We analysed 24-hour basic daily activities collected from 96 dairy cows (on the 2 – 7 parity) housed in three free stall barns in three farms during the one year period of observing. The housing system was the same in all farms – group housing in pens for 24 – 36 cows with straw bedded cubicles, feeding system - total mix rate two times a day, the average length of the feeding place for one cow was 56 cm (ratio 1.5 : 1), watering system was based on two water bowls (volume 100 – 150 l) which were installed in every pen, dairy cows were being milked in a stationary milking parlour twice a day. Daily cows' activities (eating, lying, rumination, drinking, moving) were recorded by pedometers and vitalimeters installed on cows legs and necks and camera recording the pens space during the 24 hour periods. The milk production data were recovered from the milking parlour software. Health state condition data were obtained from veterinary evidence of records. The observed parameters were evaluated by Statistica software (non-parametric tests).

RESULTS

The median of time and range which spent observed animals performing each of six key behaviour activities is shown in Table 1.

Table 1. Typical daily time budget for lactating dairy cow.

Activity	Mean (hours per day)	Range (hours per day)
Eating	4.5	3.0 – 6.0
Lying in boxes and resting	12.6	12.0 – 14.0
Social interaction	2.4	2.0 – 3.0
Rumination	8.2	7.0 – 10.0
Drinking	0.5	0.2 – 1.5
Outside pen (traveling,milking)	2.6	2.5 – 3.5

During our monitoring we found the following structure of daily activities: eating 4.5 hours/day (range 3 – 6) (obviously from 9 to 14 meals/day), lying and resting in the boxes 12.6 hours/day (range 12 to 14), spent 2.4 hours/day (range 2 – 3), social interaction (amount of time that a cow spends walking around or interacting - grooming, exercising, licking - with other animals around her), ruminate (in standing and lying position) 8.2 hours/day (range 7 – 10), drinking 0.5 hour/day (range 0.2 – 1.5) and 2.6 hours/day (range 2.5 - 3.5) spending outside the pen for travel to and from the parlour and milking.

A major role in cow health and productivity plays the resting and standing time. Cows housed in free stall facilities spent daily the largest time period on lying in boxes and resting in a comfortable stall. The second largest time period was rumination followed by eating. The cattle reduced lying time and

increased standing and feeding time during the observing in winter climatic condition. In spring and autumn climatic periods our observed cows preferred lying instead of walking.
When their time budget is challenged through increased time out of the pen milking, overstocking, poor stall design, heat stress and prolonged time spent in lock-ups, the primary outcome leads to increased health problems (lameness, etc.). Competition at the feeders leads to increased displacement and reduction of times for eating. In order to maintain optimum level of daily water consumption, two water tanks should be located in each pen and cleaned once per day.

DISCUSSION

The time budget – the allocation of time to different activities - can vary considerably depending on the environment, management and status of the cow. Only the environment that allows natural resting and feeding behaviour forms the foundation of dairy cow well-being and optimal performance. According to Grant and Albright (2001) dairy cows spend 3 to 5 hours per day feeding at 100 % stocking density in free-stall housing consuming 9 to 14 meals per day. In addition, they ruminate 7 to 10 hours per day, spend approximately 30 minutes per day drinking, 2 to 3 hours per day outside the pen for milking and other management practices and require approximately 10 to 12 hours per day of resting time.
The effect of season on the time budget activity of dairy cattle is also important indicator. The resting time behaviours of dairy cattle decreased from 50.9% in summer to 40.5% in winter. The measured results of an annual time budget of the dairy cattle are 45.4% lying, 13.7% standing, 25.4% feeding, 1.7% drinking, 9.9% walking, 2.6% milking and 1.3% other behaviours in the research (Uzal and Ugurlu, 2010).
Poor stall designs lead to lower lying times and increased risk for lameness, and cows once they become lame and behave different to non-lame cows in the same stall design, leading to even lower resting times (Cook and Nordlund, 2009).
Espejo and Endres (2007) found that prevalence of lameness in fifty dairy farms was most highly associated with greater time outside the pen. Also greater overstocking had significant impact on milk yield and health indicators such as lameness (Leonard et al., 1996).
Tucker et al. (2009), which summarized the results of studies that measured lying response to the changing amount of bedding, concluded that cows prefer a more compressible (softer) lying surfaces. Some scientists have suggested that there is a linear relationship between the resting time and the milk production; Grant (2012) has proposed that each additional one hour of resting time is associated with 2 to 3.5 more pounds (approx.0.9 – 1.6 kg) of milk/cow/day. Cows produce more milk when they are lying down as blood flow through the external pudic artery increases by around 24-28% when lying compared to standing up (Metcalfe et al., 1992; Rulquin and Caudal, 1992)
In agreement with Friend et al. (1977) and Fregonesi et al. (2007) cows overstocking in the pens decrease the lying time in cubicles.
De Vries et al. (2005) summarized research results of study aimed on frequency of TMR delivery and cow response. Greater feeding frequency may improve ruminal fermentation, rumination time and feeding time, but it also seems to reduce lying time and dry matter intake.
Evaluating the dairy cows comfort using the analysis of the daily time budget of dairy cows and identifying the possible problems is the first step to increase the economical profit for the farmers.

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WELFARE OF NATIVE PIG BREEDS IN DIFFERENT HOUSING CONDITIONS

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SUMMARY

Unlike highly productive breeds of farm animals, phylogenetically older conservation breeds show better adaptability to harsher environmental conditions. In situ conservation of these breeds is often conducted only based on a dedicated breeding programme, under identical conditions as for intensive production, and without considering specific behavioural needs. This approach carries the risk of genetic drift and unintended selection of these breeds, but may also reduce welfare levels. Therefore, the aim of the present study was to determine the effect of some parameters of the living environment on the welfare and productivity of native pig breeds.

The experiment used 84 comparable sows (third and fourth lactation) and 870 fattening pigs (Polish native breeds Złotnicka Spotted and Puławska, and highly productive crossbreds Polish Landrace × Polish Large White). Two research tasks were performed to study the response of the pigs to minimum standards for their protection (Council Directive 2008/120/EC), 30% greater pen area, and management in the outdoor and semi-open housing systems. Analysis was made of the production results, behaviour of the animals, baseline levels of stress hormones (cortisol and ACTH), and blood cell count.

The results indicate that the native breeds managed under minimum standard conditions specified by the European Union regulations showed low welfare levels. Increasing the available area by 30% improved some reproductive parameters of the sows, but increased feed consumption in the fatteners. In both cases, however, positive changes in the stress hormone levels and blood picture were observed. The strongest physiological and behavioural response from the sows and fatteners of the native breeds was induced by the semi-open management. Under this system, the results obtained by the sows were best in the entire study. The strongest reaction to the improved living environment was found in the Złotnicka Spotted pigs.

Key words: pigs, native breeds, welfare standards

INTRODUCTION

Three breeds of pigs are listed as conservation breeds in Poland: Złotnicka White, Złotnicka Spotted, and Puławska. In the National Agri-Environmental Programme, the conservation of animal genetic resources has been a separate package (Conservation of local breeds of farm animals**) since 2005.** Unlike highly productive breeds of farm animals, phylogenetically older conservation breeds show better adaptability to harsher environmental conditions. Research concerning pig welfare levels has shown that many mechanisms may modify the adaptive abilities. Therefore, the aim of the present study was to determine the effect of different values of the major parameters of the living environment on the welfare of native pig breeds.

MATERIALS AND METHODS

The experiment used a total of 84 sows and 870 fattening pigs of the Złotnicka Spotted, Puławska, and Polish Landrace × Polish Large White breeds. Comparable sows (28 animals of each breed) were in their third and fourth lactations during the study. The fatteners (290 animals of each breed) used in the experiment farrowed in spring. Animals were kept in outdoor, semi-open and indoor systems with constant access to water. They were fed complete diets in accordance with national standards regulation. Piglets were weaned at 35 days of age and fattened up to a body weight of 110 kg. In the first task, sows and fatteners of were kept in groups a 30% greater pen area in relation to that recommended by the standards and lactating sows were kept individually indoors without litter. The control group consisted of Polish Landrace × Polish Large White crossbreds, and the experimental groups were comprised of the native breeds. Animals of each group were kept in the same building. In task 2, sows and fatteners of each breed were maintained in groups in a 100% greater pen area in relation to that recommended by the regulation. Lactating sows were kept individually on litter, in a semi-open system with solid-surface runs or outdoors with hutches on pasture.

RESULTS

In task 1, the additional living area of the sows contributed to an increase in the amount of movement and to a decrease in lying time in the daily distribution of behaviour durations for all the breeds (Tab. 1). Significant differences also concerned the decrease in the amount of stereotypic behaviours for each breed. All sows with a greater area were characterized by significantly lower levels of stress hormones and elevated T_4 concentration (Tab. 2). The lower level of stress in these animals also improved the profile of morphotic blood elements (Tab. 2). The outdoor system had a significant effect on increasing the amount of movement in the daily distribution of behaviours of all the breeds (Tab. 5). Differences in the levels of stress and thyroid hormones proved highly significant throughout the study (Tab. 6). The same holds true for the blood count analysis. Thus, the outdoor system turned out to be the most

significant and strongest element that considerably increased the welfare of animals. In terms of behaviour, the crossbreds moved significantly more often in the semi-open system than on pasture (Tab. 7). However, the other breeds responded conversely, as already described for the sows. No stereotypic behaviour was observed in any of the systems. The level of stress hormones in the whole range tested was significantly lower in animals from the outdoor system (Tab. 8). Also the blood count of the fatteners under this system showed a significantly better profile (Tab. 8). For platelet count and lymphocyte percentage, these differences were highly significant.

Table 1. Proportion of the basic types of sow behaviour per day – task 1

Item	**Breed/stocking density**					
	pbzxwbp		Puł		złp	
	Standard	+30%	Standard	+30%	Standard	+30%
Lying	86.6a	81.7b	85.5a	78.8b	78.4a	74.1b
Moving	13.6a	18.3b	16.5a	21.2b	21.2a	25.9b
Feeding	7.2	7.2	6.0	6.5	6.5	6.3
Stereotypies	3.1a	1.1b	4.3a	0.9b	3.1a	0.8b

ab - differences significant at P≥0.05; AB - differences significant at P≥0.01

Table 2. Average level of some blood plasma hormones and morphotic blood elements in sows - task 1

Item	**Breed/stocking density**					
	pbzxwbp		puł		złp	
	Standard	+30%	Standard	+30%	Standard	+30%
Cortisol (nmol/l)	81.2 a	78.3b	90.4 a	81.4b	89.7 a	82.4b
ACTH (pg/ml)	50.0 a	47.8b	53.2 a	48.3b	38.4 a	32.7b
T_4 (µg/dl)	3.61 a	3.79b	3.31 a	3.7b	4.45 a	3.91b
RBC ($Mx10^{12}$/µl)	5.7a	6.1b	5.4a	6.3b	4.9a	5.7b

WBC ($Mx10^3/\mu l$)	21.9	22.1	17.6	19.3	18.9	20.8
PLT($Mx10^9/\mu l$)	156	180	145	167	164	178
% lymphocytes	34	32	34	30	38a	35b

ab - differences significant at P≥0.05; AB - differences significant at P≥0.01

Table 3. Proportion of the basic types of fattener behaviour per day – task 1

Item	**Breed/stocking density**					
	pbzxwbp		Puł		złp	
	Standard	+30%	Standard	+30%	Standard	+30%
Lying	77.6a	64.6b	74.5a	68.6b	69.3a	62.3b
Moving	22.4a	36.4b	25.5a	31.4b	30.3a	37.7b
Incl. feeding	8.6	8.2	8.2	8.5	9.7b	9.3
Stereotypies	2.2a	1.1b	2.1a	1.2b	3.1a	1.8b

ab - differences significant at P≥0.05; AB - differences significant at P≥0.01

Table4. Average level of some blood plasma hormones and morphotic elements in fatteners - task 1

Item	**Breed/stocking density**					
	pbzxwbp		puł		złp	
	Standard	+30%	Standard	+30%	Standard	+30%
Cortisol (nmol/l)	36.79 a	32.45b	36.82 a	32.7b	38.76 a	33.7b
ACTH (pg/ml)	18.05 a	15.4b	21.14 a	17.3b	21.02 a	17.3b
T_4 (μg/dl)	5.9 a	6.4b	5.3 a	5.9 b	5.6 a	6.01b
RBC ($Mx10^{12}/\mu l$)	5.7a	6.1b	5.8a	6.3b	4.9a	5.7b
WBC ($Mx10^3/\mu l$)	21.9	22.1	18.9	19.3	18.9	20.8

PLT (Mx10^9/µl)	156	180	145	167	164	178
% lymphocytes	34	32	32	30	38a	35b

ab - differences significant at P≥0.05; AB - differences significant at P≥0.01

Table 5. Proportion of the basic types of sow behaviour per day – task 2

Breed, line	System	Types of behaviour (% of day)				
		Lying	Moving	Incl. rooting	Incl. feeding	Stereotypies
wbpxpbz	Outdoor	42.3a	57.7a	11.2	5.3a	-
	Indoor	57.8b	42.2b	-	7.7b	3.1
puł	Outdoor	35.4a	64.6a	12.3	5.1a	-
	Indoor	43.8b	56.2b	-	6.3b	2.8
złp	Outdoor	35.3a	64.7a	15.3	5.9a	-
	Indoor	45.4b	54.6b	-	6.2b	4.1

ab - differences significant at P≥0.05; AB - differences significant at P≥0.01

Table 6. Average level of some blood plasma hormones and morphotic elements in sows - task 2

Breed, line	System	Cortisol (nmol/l)	ACTH (pg/ml)	T_4 (µg/dl)	RBC (x/µl) x10^{12}	WBC (x/µl) 10^3	PLT(n/µl) 10^9	% lymphocytes
wbpxpbz	Outdoor	74.6 A	32.3 A	5.35 A	6.1a	21.9a	156A	34 a
	Indoor	92.3 B	51.8 B	3.14 B	5.2b	12.3b	575B	46 b
puł	Outdoor	68.4 A	33.5 A	4.51 A	5.5a	20.1a	189A	38 a
	Indoor	89.2 B	61.3 B	3.12 B	4.7b	16.3b	312B	45 b
złp	Outdoor	62.1 A	29.1 A	4.67 A	6.6A	22.8A	152A	34 a

	Indoor	99.4 B	36.8 B	3.64 B	4.1B	13.9B	434B	58 b

ab - differences significant at P≥0.05; AB - differences significant at P≥0.01

Table 7. Proportion of the basic types of fattener behaviour per day – task 2

Breed, line	System	Types of behaviour (% of the day)				
		Lying	Moving	Incl. rooting	Incl. feeding	Stereotypies
wbp	Outdoor	37.2a	62.8a	8.4	9.8a	-
	Indoor	35.3b	64.7b	-	8.4b	-
puł	Outdoor	35.1a	64.9a	10.2	10.3a	-
	Indoor	41.6b	58.4b	-	8.2b	-
złp	Outdoor	32.1a	67.9a	13.4	12.7a	-
	Indoor	39.8b	60.2b	-	8.3b	-

ab - differences significant at P≥0.05; AB - differences significant at P≥0.01

Table 8. Average level of some blood plasma hormones and morphotic elements in fatteners - task 2

Breed, line	System	Cortisol (nmol/l)	ACTH (pg/ml)	T_4 (µg/dl)	RBC (x/µl) 10^{12}	WBC (x/µl) $x10^9$	PLT (n/µl) $x10^9$	% lymphocytes
wbpxpbz	Outdoor	28.32 A	17.81 a	7.4 a	6.9a	21.8 a	162 A	44 A
	Indoor	45.21 B	22.77 b	5.1 b	5.6b	16.6b	309B	62 B
puł	Outdoor	26.49 a	15.49 a	6.1a	6.2a	21.2a	167A	42 A
	Indoor	36.82 b	21.14 b	5.3 b	4.9b	14.9b	363B	68 B
złp	Outdoor	29.79 A	18.05 a	5.9 a	6.5a	19.4a	117A	43 A
	Indoor	49.03 B	24.35 b	4.9 b	4.8b	11.9b	223B	77 B

ab - differences significant at P≥0.05; AB - differences significant at P≥0.01

DISCUSSION

The results obtained during the study clearly show that the modifications of housing conditions had a favourable effect on the welfare of pigs. This reaction was observed not only in the native breeds, although they (especially the most primitive one) respond the strongest. However, the welfare of pigs cannot be separated from the production results obtained by the animals. Thus viewed, pasture fattening of pigs will not be widely used in production, especially under Polish conditions. In summing up the work, the following conclusions can be made.

1. Native breeds respond unfavourably to the housing conditions defined by minimum area standards. This response applies not only to the behaviour but also to the physiological indicators.
2. Increasing the available area standards has a positive influence on the welfare of pigs by not decreasing the fattening performance and by improving, in some respects, the reproductive parameters of the sows.
3. The highest welfare was observed for the outdoor system, even in relation to housing on solid-surface runs. This system was characterized by higher farrowing rate and weaning rate of piglets.
4. Fattening of pigs in the outdoor system results in significantly poorer production results when compared to the semi-open system.

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A LINKAGE BETWEEN NON-COMPLIANCE WITH ANIMAL WELFARE LEGISLATION AND ENVIRONMENTAL EMISSIONS

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SUMMARY. There is focus on defining goals for sustainable animal production and developing tools to assess sustainability; however, different sectors in society have different goals that may result in conflicts. Drawing on the concept of One Welfare, the objective of this study was to identify findings from animal welfare inspections and environmentally hazardous emission recordings at pig production facilities that are synergetic or conflicting.

We reviewed data routinely collected as part of Swedish official animal welfare control from the Swedish Board of Agriculture and data on environmentally hazardous emissions from the Swedish Environmental Protection Agency, for 2010 to 2014. The official animal welfare control data had been collected using a checklist containing 46 checkpoints related to animal, resource and management based measures of pig welfare. The emissions data included recordings of greenhouse gases and other substances such as nitrous oxide, ammonia (NH_3), ammonium, nitrogen (N), phosphorus (P) and phosphates. Univariable linear regression was used to investigate associations between the emission recordings and animal welfare control checkpoints, adjusting for clustering on pig farm.

In total, 135 animal welfare and emission inspections conducted at 92 premises could be matched. Sufficient data were available and models were generated for three different outcomes: NH_3 air emissions (n=128 recordings), total N and N compound water emissions (n=29); and total P and P compound water emissions (n=29). Factors found to be associated with higher levels of at least one of the environmentally hazardous emissions were insufficient stall hygiene (1.9 times higher NH_3) and poor bedding quality (7.5 times higher total P).

We demonstrated links between official data sources and identified associations between measures of animal welfare status and emissions. Investigation of linkages in other intensive livestock production systems, involving other animal species and other countries, will confirm the value of these findings for sustainable animal production.

Keywords: Legislation, Animal welfare, Environmental emission

INTRODUCTION

Livestock production contributes to climate change and loss of biodiversity (Gerber et al. 2013; Rivera-Ferre et al. 2016). Over the years, legislation and guidelines that aim to reduce negative effects of animal production on the environment, humans, and animals, have been developed. This holistic approach to sustainability is the way of the future, with a common language for sustainability being provided by the Sustainability Assessment of Food and Agriculture (SAFA) system framework (FAO 2014) as well as through the One Welfare concept (Pinillos et al. 2016).

Much is being done to define goals for sustainable animal production and to develop tools to assess sustainability. Increasingly, legislation is written by different competent authorities to promote development towards these goals, and assessments are being made as to whether such goals are being achieved. Very rarely though is there any consideration as to how the differing goals interact. Methods of animal husbandry that reduce the carbon foot print of animal production, but do this at the expense of reduced food safety or animal welfare, will likely provoke strong resistance among consumers and the public. The reverse is also true, as attempts to improve animal welfare in a way that increases any negative environmental impact i.e. increased emission, are unlikely to be sustainable in the long term.

Under SAFA, there has been considerable discussion about conflicts of interest and conflict resolution, particularly relating to governance. Yet it is not identified where these conflicts occur, even though we suspect there are conflicts when trying to achieve several different sustainability goals simultaneously. This can lead to debate in society of which sustainability goal, e.g. improved animal welfare or reducing climate change, is more important. Progress is hampered in both as a consequence. On the other hand, interests can coincide. This would be the ideal state, where improvements in animal welfare, lead to improved health of animals, improved longevity and more efficient production while also having positive effects on the environment. Such win-win areas could be targeted more effectively so that the synergy effect leads to acceleration of progress in both.

In Sweden and elsewhere, there is detailed environmental, animal welfare and food safety legislation. There is also increasingly improved monitoring of the environment, the welfare status of animals and hygiene along the food chain to control the effectiveness of this preventative legislation. Since 2009, as part of Swedish official animal welfare control, data are routinely collected during inspections of farms and other premises that keep animals according to standardised species-specific checklists covering different points in the legislation. The resulting database contains recordings of compliance with animal, resource, and management based measures. Similarly, recordings of emissions are also collected from facilities that engage in activities that result in the release of potentially environmentally hazardous substances.

The goal of this study was to identify areas of conflict and areas of synergy between two routinely assessed sustainability indicators in Sweden today: animal welfare and environmentally hazardous emissions.

MATERIAL AND METHODS

Data from official animal welfare control in all 21 counties of Sweden were provided by the Swedish Board of Agriculture, covering inspections conducted from 1 January 2010 to 31 December 2014. Data pertaining to the keeping and/or production of pigs were retained for this analysis.

The pig welfare checklist contained a total of 46 checkpoints (CPs) covering animal, resource, and management based measures. CP-43 to CP-45 were assessed from 2012 onwards only. The control result for each CP was recorded by the inspector as either compliant, non-compliant, no control carried out (the CP was not assessed), or not applicable. All recordings were conducted by animal welfare inspectors employed by county administrative boards.

The dataset also contained information on the location of the pig production facility, other animal species kept at the facility, number of animals registered, and type of animal-related production or other activity conducted on the site. Inspections were conducted for varying reasons, for example because of a high-risk animal activity, or a notification or complaint about a potential animal welfare issue.

Data on the annual quantities of certain chemical substances that large facilities emit were obtained from the open-source online Swedish Pollutant Release and Transfer Register, administered by the Swedish Environmental Protection Agency (EPA). Emission recordings on approximately 70

substances that are listed in the EPA regulations on environmental reports on hazardous activities requiring permits (NFS 2006:9) are included in the register (EPA 2016). Only those facilities that require environmental permits (SFS 1998:899) have data reported; that is, those that have been deemed to have operations with a heavy environmental impact due to emissions of hazardous substances to air and/or water.

For this study we extracted the data on intensive poultry or pigs (section 7 in the register). The primary substances reported by pig production facilities were for greenhouse and other gases (nitrous oxide, ammonia, ammonium) and inorganic substances (nitrogen, phosphorus, phosphates). Where there were multiple emission recordings in one year at a production facility, we averaged the recorded values for that year, resulting in only one observation per year per production facility. Emission recordings were log-transformed to achieve approximate normality for statistical analysis.

Both databases contained a unique organisation identification number, which was used to merge both datasets by one-to-one matching on this number and the year that the inspection was conducted and the emissions were recorded. However, although all emission recordings had a corresponding organisation identification number, not all official animal welfare controls had one.

Univariable generalised linear regression was used to investigate associations between the emission recordings and CPs. In total, 135 animal welfare and emission inspections conducted at 92 premises could be matched. Models for three of the emission recordings were generated. The outcomes were: Ammonia air emissions (n=128 recordings), total nitrogen and nitrogen compound water emissions (n=29); and total phosphorus and phosphorus compound water emissions (n=29). For zero values, half of the minimum feasible (i.e. non-zero) value was imputed. Geometric mean (GM) ratios were estimated, adjusting for clustering on pig farm. CPs with significant associations based on only one non-compliant inspection were not considered further.

RESULTS

The animal welfare control database contained information about 3865 pig farms registered with the Swedish Board of Agriculture, with 1979 inspections conducted on the pig welfare checklist, and 1353 registered pig farms inspected (35%).

Of the 46 CPs on the pig welfare checklist, nine had a percentage non-compliance at inspection of greater than 10%. These were inadequate outdoor areas, failure to keep veterinary records for at least five years, unclean stalls, inadequate exterior design of facilities (including pasture, exercise areas, ground surfaces, driving routes, fencing), inadequate or poor quality water supply, unclean and/or damp rest areas, poor quality bedding materials, inadequate mechanical and/or emergency ventilation, and other deficiencies not covered by the specific CPs.

The emissions dataset contained information on 1580 emission recordings from 236 intensive livestock production sites including poultry or pig farms of unspecified size (n=14); poultry or pig farms with 40,000 places for poultry (n=859); poultry or pig farms with 2000 places for pigs >30 kg (n=631); and poultry or pig farms with 750 places for sows (n=76). Of these recordings, 1152 were of air emissions, 422 water emissions, and 6 wastewater treatment plant emissions.

Non-compliance with several animal welfare CPs were found to be associated with higher levels of environmentally hazardous emissions in univariable analyses. Ammonia air emission levels were increased at animal welfare inspections non-compliant with stall hygiene (GM ratio 1.9; p=0.009; based on five non-compliant inspections), when compared to compliant inspections. Associations were also apparent for non-compliance with feed and water systems and management of aggressive pigs, but these results were based on only one non-compliant inspection each. Total phosphorus and phosphorus compound water emissions were increased at inspections non-compliant with bedding quality (GM ratio 7.5; p=0.013; based on two non-compliant inspections), while nitrogen and nitrogen compound

water emissions were decreased at inspections non-compliant with interior facility design (GM ratio 0.3; p=0.026; two non-compliant inspections), when compared to compliant inspections.

DISCUSSION

We demonstrated successful linkage between official data sources and identified both synergistic and conflicting associations between measures of animal welfare status and environmental emissions.

A number of limitations should be considered. Some results were based on few observations and should therefore be treated cautiously. Not all facilities that conducted animal-related activities were inspected under official animal welfare control while also having emission recordings. This contributed to significant data loss with less than 3% of registered pig farms with matching animal welfare inspection and environmentally hazardous emissions recordings conducted within the five-year study period. Due to the resultant small sample size, multivariable models could not be fitted. Future models should include facility and inspection factors not considered here. Further, to avoid data loss, we included all types of inspections in analysis. Ideally, only findings from random inspections should be included to avoid bias of results.

Investigation of linkages in other intensive livestock production systems (e.g. poultry and dairy farms) will confirm the value of these findings for sustainable animal production.

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VALIDATION OF CARCASS LESIONS AS INDICATORS OF PIG WELFARE ON FARM

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SUMMARY. Measuring indicators of pig health and welfare (HW) at meat inspection could reduce the need for on-farm assessments. Given their multifactorial aetiology, skin (SL) and tail (TL) lesions are good indicators of pig HW. The aim of this study was to validate the measurement of these lesions on the carcass for the assessment of pig HW on farm. Thirty-one integrated pig farms were assessed using an adapted version of the Welfare Quality® protocol by inspecting 6 randomly selected pens of pigs in the first weaner (S1), second weaner (S2) and finisher stage (S3). The average prevalence of HW outcomes for each stage was calculated. One batch of pigs was observed at slaughter and SL and TL were scored according to severity for each carcass. The average prevalence of carcass lesion outcomes was calculated for each farm. Linear regression models were developed to predict the prevalence of each HW outcome in each stage based on the prevalence of the carcass lesions. The HW outcomes that were best predicted by carcass SL and TL were poor body condition (S1), bursitis (S2), huddling (S1), severe tail lesions (S3) and coughing (S2). To evaluate the potential of carcass lesions as monitoring tools, receiver operating curves (ROC) were created. Problem farms were defined as those above the 75th percentile value for each HW outcome and the overall performance of the models, sensitivity and specificity were calculated at various cut-off values of the predictive carcass lesions. Models for predicting problem farms with poor body condition, bursitis and severe tail lesions were moderately accurate ($0.7<AUC\leq0.9$) with high sensitivity (75-100%) and specificity (70-87%) at the optimal cut-off value of the predictive carcass lesion. Hence, carcass lesions reflect certain pig HW problems on farm and could play a role in reducing the need for on-farm welfare assessments.

Key words: Carcass lesion, Meat inspection, Pig welfare

INTRODUCTION

On-farm welfare assessments are the main method for assessing pig welfare, but these assessments are labor intensive and time consuming (Dalmau et al., 2009). There is an increased interest in using routinely collected data for welfare assessments but, there is limited work on the use of meat inspection data for the purpose of pig health and welfare assessment (Knage-Rasmussen et al., 2015; Nielsen et al., 2015). Tail and skin lesions are among the most frequently cited animal-based indicators of pig welfare and proposed as 'iceberg' indicators giving a picture of the overall health and welfare of the animal (FAWC, 2009; EFSA, 2012). The aim of this study was to validate these carcass lesions as 'iceberg' indicators for on-farm welfare and to evaluate their performance as monitoring tools. It was hypothesized that farms with a higher prevalence of carcass lesions would have a higher prevalence of welfare problems on-farm.

MATERIAL AND METHODS

A list of 45 farrow-to-finish pig farms in the Republic of Ireland was obtained through the Teagasc advisory service with the criteria that they kept records in the Teagasc eProfit Monitor and sent pigs to slaughter to abattoirs that would allow data collection. Thirty-one farmers (69%) agreed to participate in the study representing approx. 12% of Irish pig herds.

Farms were visited during one full day (July to November 2015) where pigs were observed in six randomly selected pens in the first weaning (4 to 8 wks, S1) and second weaning (8 to 13 wks, S2) stages and the finishing stage (13 to 23 wks, S3). In brief, each pen was observed for a 10 min period during which the number of pigs affected by different welfare outcomes (Table1) was recorded using an adapted version of the Welfare Quality protocol (Welfare Quality, 2009).

Table 1. Animal-based welfare measurements collected in 31 farms in the Republic of Ireland adapted from the Welfare Quality protocol

Welfare theme	Welfare criteria	Animal-based measure
Good feeding	Absence of prolonged hunger	Body condition score
Good housing	Comfort around resting	Bursitis, absence of manure on the body[1]
	Thermal comfort	Shivering, panting, huddling[2]
Good health	Absence of injuries	Lameness, skin lesions, tail lesions, ear lesions, flank lesions
	Absence of diseases	Mortality[3], coughing[4], sneezing[4], pumping, twisted snouts, rectal prolapse, scouring[1], skin condition, ruptures and hernia

[1]Recorded on pen level only. [2]Expressed as proportion of resting pigs. [3]Data collected from herd performance data. [4]Frequency during 5 min observation. All other measures are assessed by recording the number of pigs affected in a pen.

Of each farm, one batch of pigs (204.3 ± 25.84 pigs) was observed at the abattoir within 2 weeks of the welfare assessment. Each carcass was scored for tail (TL) and skin (SL) lesions after scalding and dehairing. Tail lesions were scored according to severity on a 5-point (0 = no evidence of tail biting - 4 = evidence of chewing or puncture wounds with severe swelling/infection or open, gaping wound where tail used to be) scale adapted from Kritas and Morrison (2007) and Harley et al. (2012). Skin lesions were scored by assessing the dorsal part of the carcass (area above the loin) according to severity on a 4-point scale (0 = none or a little superficial damage - 3 = much deep damage over a large area) adapted from Aaslyng et al. (2013).

Each farm was considered as the experimental unit. For each production stage, the average percentage of pigs affected by each welfare outcome on farm was calculated. Linear regression models were selected using the adjusted R^2 for each welfare outcome measured on farm (Y). The percentage of pigs with the different tail (1, 2 and ≥3) and skin (1, 2 and 3) lesion scores were included as predictor variables (X). In order to evaluate the potential of carcass lesions to explain different aspects of pig welfare, models which explained the highest variation in the welfare outcome (highest R^2) were selected for the different welfare criteria as defined by the Welfare Quality (2009). Receiver operating curves (ROC) were created for these models to test their ability to identify 'problem' farms. Problem farms were defined as those above the 75th percentile value for each welfare outcome. Sensitivity (Se)

and specificity (Sp) were calculated at various cut-off values of the predictive carcass lesions. Sensitivity and 1-Sp (proportion of false positives) were plotted in ROC (pROC package in R) to determine the optimal cut-off value of the prevalence of carcass lesions used to predict the presence of welfare problems on farm. The accuracy of the model was assessed by calculating the area under the ROC curve (AUC). Values of AUC were interpreted as non-informative (AUC = 0.5), less ($0.5 < AUC \leq 0.7$), moderately ($0.7 < AUC \leq 0.9$), highly ($0.9 < AUC < 1$) accurate, and perfect (AUC = 1) as per Greiner et al. (2000).

RESULTS

The final models with the highest R^2 within each welfare criteria were those for poor body condition (S1, $R^2 = 0.30$), bursitis (S2, $R^2 = 0.28$), huddling (S1, $R^2 = 0.30$), severe tail lesions (S3, $R^2 = 0.31$) and coughing (S2, $R^2 = 0.45$). The results of the ROC analysis for the final models are shown in Table 2.

Table 2. Performance [area under the curve (AUC) and 95% CI], sensitivity (Se) and specificity (Sp) at the optimal cut-off value of carcass lesions used to predict the presence of problem farms where the prevalence of the welfare outcome on farm exceeded the 75th percentile of the study farms (poor body condition in the first weaner stage, bursitis in the second weaner stage, huddling in the first weaner stage, severe tail lesions in the finisher stage and frequency of coughing in the second weaner stage)

Welfare outcome	AUC (95% CI)	Se, %	Sp, %	Optimal cut-off value, %
Poor body condition	0.80 (0.58 – 1.00)	75	87	62.4 Tail score 1
Bursitis	0.82 (0.67 – 0.97)	100	70	14.3 Skin score 2
Huddling	0.61 (0.40 – 0.83)[1]	88	61	49.1 Skin score 1
Severe tail lesions	0.81 (0.62 – 1.00)	88	74	0.98 Tail score ≥3
Coughing/pig	0.71 (0.45 – 0.96)[1]	100	50	44.0 Skin score1

[1]Area under the curve (AUC) was not significantly ($P > 0.05$) higher than 0.5

DISCUSSION

To the authors' knowledge this is the first study aiming to evaluate the use of carcass lesions at meat inspection as indicators for pig health and welfare in the different production stages on farm.

The prevalence of the recorded carcass lesions were capable of explaining up to 45% of the variation observed in the prevalence of the welfare outcome on farm. This suggests that the recording of carcass lesions at meat inspection has value for assessing pig welfare on farm. Knage-Rasmussen et al. (2015) found no relationship between an on-farm welfare index and a welfare index creating from different databases including meat inspection data in sows. However, these authors did not assess the potential of meat inspection records on their own and the formation of the indexes could have masked possible relationships between individual aspects of pig welfare as found in this study.

Prediction of farms identified as having a problem with poor body condition (S1), bursitis (S2) and severe tail lesions (S3) were moderately accurate with high levels of sensitivity and specificity. A combination where both sensitivity and specificity are optimized is especially useful in the context of monitoring and helping inform herd health and welfare management plans (de Vries et al., 2014). The sensitivity in this study ranged between 75 to 100%, showing that the majority of farms with problems with poor body condition (S1), bursitis (S2) and severe tail lesions (S3) were detected. Similarly, specificity ranged from 70 to 87% meaning that few farms will be identified as false positives.

Hence, carcass lesions reflect certain pig health and welfare problems on farm and could play a role in reducing the need for on-farm welfare assessments. However, longitudinal studies are needed to evaluate the recording of carcass lesions over a longer period of time and whether it is capable of picking up changes in welfare status of pigs on farm during production. Additionally, an evaluation of the costs of including carcass lesion scoring at meat inspection, of missing farms with welfare problems and of visiting or penalizing farms without actual problems needs to be determined before this can be applied in a commercial setting.

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ANIMAL WELFARE AND SOSTENIBLE HUMAN PROGRAM

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SUMMARY. Veterinary Sciences are entering a new historical period where the accumulated experience determines a remarkable qualitative leap. The aim is to contribute to the training of specialists with an updated view of animal health, the main approaches and current trends. The teaching methods used are: problem recognition, information search, and hypothesis formulation, choice of resolution strategies and confirmation or rejection of hypothesis. The importance, scope and contribution is that it educates and generates useful knowledge for which it is intended to develop and contribute to solving the problems, assumes responsibility innovation for the improvement of health, productive, economic, social status and environmental, working for environmental sustainability, opening markets and public health. Improving animal welfare and reducing stress require you to learn about animal behavior during handling. Create welfare will help produce leaders in this science, able to recognize both ethical benefits bio-productive and get healthy and safe food that will generate both welfare animal and human.

KEYWORDS: Sustainability, Economic Impact Food Safety, Animal Welfare

INTRODUCTION

Broom's (1991) animal welfare is the situation of an individual in relation to its environment and undoubtedly involves the way of production, and the treatment that the animal receives. The effect of the climate and its changes predicted by simulation models show signs that can affect animal welfare, its productivity, and with it, the direct and indirect demands on the protocols that regulate the treatment, the conditions of production and the Marketing of farm animals. Therefore, the certification of animal welfare in the market, can become a limitation in the commercialization of livestock and its by-products (Galindo et al., 2011).

Actually, evaluations tend to quantify the economic impact of adverse weather conditions on animal welfare, as well as potential losses. Validating with the technologies of mitigation the maintenance of the animals in a state of comfort to maximize their production and to extend the access to markets where the consumers would pay a differentiated price.

The Gross National Product destined to the production and diffusion of the knowledge is meager, ranges from 3 to 5% of GNP is spent on research and development, that is, on the production of new knowledge, which is or should be already the greater Investment in any developed country, as a factor determining its competitiveness. Increasingly, the productivity of knowledge will be decisive in its economic and social success and in its overall economic performance. Detonating differences in the productivity of knowledge; between countries, between industries, between individual organizations (Beltramino, 2011).

The Faculty teaches graduate and postgraduate students, and is organizing for a teaching for life in the way of having "open systems" (Estol, 2010), a new axiom is needed: "The more instruction a person has, more often he or she will need more education."But there is something more important:

maintaining access to open higher education, regardless of age or educational credentials, is a social need in your life to pursue your formal education and to qualify for a work of knowledge (Mota et al., 2012), arriving to conform personal and social learning environments, building a critical citizenship within multidisciplinary groups.

Improving animal welfare and reducing stress require students to learn about animal behavior during their management (Basal, 2013); it is necessary to recognize in Dewey one of the first and most significant contributions to the practical teaching of "learning by doing'' and it's no less influential proposal to form students that combine the capacities of search and investigation with the attitudes of mental opening, responsibility and honesty and when those capacities are put in common, the only thing that remains unalterable is evolution (Taylor, 2014).

The Faculty of Agricultural Sciences since 2008 has participated with very good results in scientific forums such as "Los Días de las Ciencias'', Student Forums and provincial, national and international events, achieving collaborative links and academic exchange at first with the Escuela Agropecuaria Provincial No. 1, Governor Gregores, Santa Cruz, Argentina; the Universidad Autónoma Metropolitana, campus Xochimilco and the Universidad Autónoma de Sinaloa.

The cooperation allows to develop the BA theme with a holistic vision of fundamental importance for the students to complete their vision on Animal Welfare, the main objective of this program has been to contribute to the training of specialists with an updated vision of the world problems Animal health, the main approaches through which their development has gone and current trends, in a classroom, transformed into an exchange space, with multiple ways of seeing, thinking and living life.

MATERIALS AND METHODS

It is intended to contribute to the development of educational actions that get easy the acquisition of abilities research and information skills on animal welfare, allowing the discovery and appropriation of values, principles and methodologies scientific-technological that provide a space for the improvement and exchange of experiences the know as a social construct.

It is recognized that the problem gives us the guideline for the search of information and this, the consequent formulation of hypotheses and choice of resolution strategies for the confirmation or rejection of hypotheses; the Faculty of Agricultural Sciences of our University educate integrally and generates useful knowledge so that it is projected towards the rural development and contributes to the solution of the sensed problems of the agricultural sector, and responsibility is assumed with responsibility for the improvement of the sanitary status, productive, economic, social and environmental, working for agricultural health, environmental sustainability, market opening and public health.

It is recognized for the formation of integral professionals committed to respect for life, the environment and animal welfare, for the generation of applied knowledge and its socialization with impact, for the benefit of those who participate in the agricultural and agro industrial sector, for their capacity to articulate the agricultural and livestock sciences and professions, and finally, by the capacity of its graduates to lead the change in agricultural health, safety and food security, welfare and equity, it is interesting to interact within organizational environments learning.

The individual contribution to the transformation of society is given through constructive, meaningful learning, which in turn demands a reflexive, critical, applied and collaborative pedagogical activity in the educational relationship, motivated by disciplinary modern tendencies, information technology, democracy, justice and equity; with a source of disciplinary knowledge, innovative and creative, the basis for the transfer of know, via academic or social interaction; of its results, publishable and applicable intellectual production is consolidated in order to participate in networks, scientific communities, guilds and producers with transparency and cognitive democracy, permeating the local

business and institutional contexts, with practices and support of a wide range of actions to enhance the positioning university-enterprise with a shared vision.

RESULTS

According to the normative approach, the student is the center of the formative task and is characterized by the fraternal accompaniment by the teacher and the integral development of the dimensions of the human being (Cajiao, 2014) is directed towards the generation of pedagogical processes aimed at practice, reflection and criticism, fostering a flexible environment of constant dynamics of change, student and teacher mobility, with the aim of contributing to the transformation of the society. Activities aimed at generating positive and proactive attitudes towards knowledge, and values such as trust, autonomy and / or self-determination, solidarity and collaborative work, are privileged. With the formation of social values that lead to the improvement of the living and environmental conditions of the community.

The faculty privileges, through the student scientific group of welfare, values such as honesty, responsibility, respect for life, cooperation, and taking into account the ten criteria that are measured by WAP (World Animal Protection) and by the OIE and provides opportunities for research into animal welfare sciences with qualified supervisors and promotes programs of student outreach and participation in community programs and community projects that seek to improve animal and human welfare through partnerships between the university and the company.

Their learning units consider the role of the veterinary professional in animal welfare, bioethics, ethology and motivational behavioral systems, physiological response of stress. Physiological aspects of pain and Evaluation of animal welfare and Physiological Indicators of Animal Welfare, (Galindo, 2014); Cinco Libertades (Gallo, 2010), Animal Welfare and Sustainable Animal Production; The senses of the animal, the principle of the escape zone, design of the facilities, immobilization devices, handling and stress, humanitarian slaughter, and animal welfare during handling and processing (Grandin, 2013), among others.

DISCUSSION

The veterinary is entering a transcendent period, where the accumulated experience will determine a remarkable qualitative leap. It is in this context that we are interested in intervening in this reciprocal action in favor of animal welfare that will result in greater and better quality and quantity of food. Teaching and culture on Animal Welfare contributes to the formation of leaders in this science, capable of recognizing both ethical and bioproductive benefits, as well as:

- It stimulates attitudes, values and vocations.
- It promotes the consolidation of communities of practice, teaching and knowledge, and helps to strengthen ties between them and the community.
- It shows some educational research needs and associated technological development.
- It broadens the vision of the scientific and technological world of those who participate and brings them closer to the national reality.
- It contributes to the establishment of a meaningful human relationship between teachers, students, specialists, family environment and society in general.
- It makes visible the inter and transdisciplinary character of knowledge.

Hence, the indispensable consideration of establishing organizational learning environments so that the individual communicates with the collective and has a shared vision of the complex problems that affect and affect respectively the university and the company, having as part of the binomial student-Teacher who are formed continuously in the day to day with learning to learn taking into consideration a critical citizenship.

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HOMOLOGY AMONG *Rhipicephalus microplus* TICK POPULATIONS

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SUMMARY. Eight hundred and twenty five species of ticks have been described, and are considered the second most important vector in the world; they transmit disease-causing pathogens in domestic and wild animals. The tick *Rhipicephalus microplus* is identified based on morphology and molecular markers such as mitochondrial RNA which reveals the genetic basis between populations. The objective of this work was to identify the morphological and genetic similarity of *Rhipicephalus microplus* among populations of Culiacan, Mexico. The collection of ticks was carried out from March to September of 2015. Fifteen-hundred engorged females were taken from 300 bovines that lived in the Mountain range, the valley, and the coast. After collecting the ticks, they were preserved at -20ºC, and identified morphologically based on pictorial keys. Five ticks from each population were obtained randomly according to geographic region for DNA extraction by the phenol chloroform technique. PCR was performed for amplification of the 460 bp fragment of the miRNA 16S gene, positive samples were sequenced using the Applied Biosystems® 3730XL system, The *In Silico* analysis was performed with the genetic sequences obtained using the Mega6 software and homologous comparison with blast.ncbi.nlm.nih.gov. The morphology of the 1,500 ticks belongs one hundred percent to *R. microplus*. Amplification of the miRNA 16S gene was observed in a band of about 460 bp of *R. microplus*, 14 products of the PCR were sequenced. When performing the alignment, 99 to 100% sequences of ticks are identified between the 3 geographic regions that are being observed. The analysis performed to identify the homology between the sequences and those described in GenBank indicates an identity ranging from 99 to 100%. It is concluded that the populations of *R. microplus* present in Culiacan, Mexico, is the same as the one described in America.

Key words: Homology, *Rhipicephalus microplus,* mitochondrial RNA

INTRODUCTION

The ticks constitute approximately 825 described species (Velayutham et al., 2012), and are considered as the second most important vector in the world, due that transmitted pathogens cause diseases both in domestic and wild animals (Andreotti et al., 2011). Based in molecular studies, six species are allotted in the subgenus *Rhipicephalus* (Boophilus); the most widely distributed are *Rhipicephalus* (Boophilus) *annulatus* y *Rhipicephalus* (Boophilus) *microplus.* The sequences of mitochondrial genome are useful for phylogenetic research, and are the final solution for the establishment of lineages for ticks (Burger et al., 2014). The tick *R. microplus* that inhabits in South Africa and Australia can be considered as a different specie than ticks from Costa Rica, Paraguay, Peru, Uruguay and United States since exhibits genetic homology (Labruna et al., 2009; Low et al., 2015). The mitochondrial genoma, as well as cox1, 12S rRNA, 16S rRNA, and the ITS2 marqueurs are used to determine the phylogenetic relationship of inner subgenus Boophilus (Burger et al., 2014). The obejctive of this research was to identify the morphologycal and genetic similarities across tick *Rhipicephalus microplus* populations in Culiacan, Mexico.

MATERIAL AND METHODS

Three hundred bovines in the municipality of Culiacan were selected using a non probabilistic sampling technique (Thrusfield, 2005). They were coming from three geographical areas: 1) Sierra: 25º47'23.39" N 107º 20'20.16" W, and 630 m o.m.s.l.; 2); Valley: 25º05'34.24" N 107º 27'27.54" W, and 201 m o.m.s.l.; and 3) Coast: 24º20'18.24" N 107º26'56.49" W, and 5 m o.m.s.l. Ticks were collected from selected bovines and frozen (-20ºC) until they were used for lab determinations (Ybanez et al., 2012). Ticks species were determined using both morphological and pictorial keys (DGSA, 2004), observing the ticks with a stereoscopic microscope in a double-blind procedure, and randomly selecting several ticks from each geographical area (Patternina et al., 2016). The DNA was extracted from adult ticks of each geographical area agreeing with the procedure described by Motaghiplisheh et al. (2016). For the amplification of the fragment 460 pb of gen 16S ARN mitochondrial, the following nucleotides were used: 16S+1: 5´-CCG GTC TGA ACT CAG ATC AAG T-3´ and 16S-1: 5´-GCT CAA TGA TTT TTT AAA TTG CTG TG-3´ (Moraes et al., 2011, Mangold et al., 1998). The samples that amplified the gen 16S ARN mitochondrial were sequenced using the system 3730XL Applied Biosystems®, in a commercial laboratory (Macrogen Inc., Seul, Korea). The "*In silico*" analyses of sequences obtained from gen 16S ARN mitochondrial were performed with the software editor of sequences dnasp5.exe and of multiple alignment Mega6 (Low, 2015) (http://www.mbio.ncsu.edu/bioedit/bioedit.html), the 385 pb fraction was obtained, and the homology comparison of its sequences was conducted comparing with existing in the data base (GenBank) of the U.S National Center for Biotechnological Information (NCBI), and the National Library of Medicine of the United States (NIH), through the BLAST program (http://blast.ncbi.nlm.nih.gov).

RESULTS

The morphological structures from the 1,500 ticks that were observed by microscopy and coincided 100% with the described *Rhipicephalus microplus.* The genetic sequences obtained from gen 16s ARNr mitocondrial amplified at 460 pb, indicated that corresponded to *R. microplus*. The ticks population from the three geographical areas had an identity from 99 to 100% across populations; and when they were compared with the description in the GenBank (NCBI), results indicated a homology from 99 to 100% for described ticks in America.

DISCUSSION

The study of phylogenetic relationship of the ticks populations gives a fundamental knowledge about its evolutionary history, and conduct us to a better comprehension of its association with pathogen agents (Beati and Keirans, 2001). Morphological and genetic differences across *R. microplus* strains have been documented (Labruna, 2009). However, results in the three *R. microplus* tick populations in the region of Culiacan show a homology of 100% when they were crossed compared. In the Malaysian peninsula, Low (2015) studied five populations of the tick *R. microplus* coming from five different farms, and when comparison was performed using 16S RNAr mitochondrial a low genetic diversity was found. The high homology (99-100%) observed between *R. microplus* population collected in Culiacan, Mexico, with the one deposited in the GenBank of NCBI, are in close agreement with the results of Sattler et al. (1986), who describes a homogeneity of 98,5 ± 0,013 when compared *R. microplus* tick population coming from North America, North of Mexico, South of Mexico, and Puerto Rico. Results indicate that, in the Culiacan area, they did not suffer isolation enough to conduct to species to separation, and this concept appears be applicable for all North America, agreeing with results of the actual research and relative information in the literature.

CONCLUSIONS

The analysis performed to identify the homology between the sequences and those described in GenBank indicates an identity ranging from 99 to 100%. It is concluded that the populations of *R. microplus* present in Culiacan, Mexico, is the same as that described in America.

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PREVALENCE OF Cryptosporidium spp IN LAMBS OF THE MUNICIPALITY OF CULIACÁN, MEXICO.

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ABSTRACT

Cryptosporidium spp is an intestinal protozoan that infects animals and humans. There are currently 16 recognized species, transmission occurs through the fecal-oral route, following direct contact with oocysts via water and food. The objective of this study was to determine the prevalence of *Cryptosporidium* spp in lambs of the municipality of Culiacán, Mexico. This study was carried out in the FMVZ-UAS parasitology laboratory, located geographically: 24 ° 48'15 "N north latitude and 107 ° 25'52" W west longitude, with an altitude of 54 meters above sea level (MASL). A total of 380 fecal samples were collected from lambs less than 3 months of age. Sampling was carried out in the summer of June and July 2014, samples were collected in latex gloves directly from the anus, then refrigerated in a cooler and transported to the lab. The samples were stained by the modified Ziehl-Neelsen technique and observed under a microscope at 100X. The prevalence of *Cryptosporidium* spp. in lambs' feces was 38% (144/380) and 62% (236/380) negative. According to analysis of data a prevalence of 8.68%, 8.42% and 37.89% was found for one month, two month and three months old lambs. It is concluded that *Cryptosporidium* spp. is present in the lambs of the municipality of Culiacán, Mexico. These results suggest a high risk of infection for both animals and staff working in production units as there is a risk of zoonosis.

KEYWORDS: Cryptosporidium spp, Lambs, Prevalence.

INTRODUCTION

Cryptosporidium is an intestinal protozoan that infects the gastrointestinal tract of humans and animals (Ronald, 2004). At present, 16 species of *Cryptosporidium*, have been isolated from a wide variety of hosts, including all 5 vertebrate groups; (Carey et al., 2004), transmission occurs via the fecal-oral route, or by direct contact with Cryptosporidium oocysts through contaminated water or food (Xiao et al., 2004). *Cryptosporidium* spp. was described in mice in 1907 by Tizzer. The parasite has a monogenic life cycle. The prepatent period varies from 2 to 14 days and the patent period may vary from a few days to several months depending on the species (O'Donoghue, 1995). *Cryptosporidium* spp. infection in lambs with and without diarrhea has been recorded worldwide (Fayer et al., 2000). Several factors play a role in maintaining the infection, high numbers of lambs, overcrowding of animals and hygienic conditions. The incidence of infection is higher at the end of the calving period, as a consequence of progressive contamination of the farm, therefore most diarrheal outbreaks coincide with the calving season in autumn, winter and spring. It should also be considered that asymptomatic parasitized adults excrete small number oocysts, but sufficient to infect newborn lambs (Sánchez et al., 2009). Ozer et al., (1990), carried out the first study in lambs in Elazig province and observed a prevalence of 12%; further studies with *Cryptosporidium* reported a prevalence of 23.3% in the same

animal species in Izmir (Erman et al., 2000). However there are a large number of causes of diarrhea in sheep (Sutherland et al., 2010). A study conducted in Australia by Joshua et al. 2011; Identified negative consequences on carcass weight and carcass yield indicators associated with the identification of *Cryptosporidium*, lambs were sampled from two weeks of age until slaughter which was carried out at 8 months, results indicated an average loss of 1.45 kg of hot carcass weight and 1.79% reduction in carcass yield, which translates to a loss in kilograms of meat. The differences observed in these lambs are supported by previous studies that indicate that these parasites limit the productivity of small ruminants (Alonso et al., 2006). Therefore the objective of this study was to determine the prevalence of *Cryptosporidium* spp. in lambs of the municipality of Culiacán, Mexico.

MATERIALS AND METHODS

The present study was carried out in the municipality of Culiacán, which is located geographically at coordinates 24 ° 47 '21.64 "North and 107 ° 22' 26.74" West, with a height of 76 MASL (INEGI, 2009). For the study, lambs of different breeds and ages from one day to three months were considered. The sample size was determined with the following formula: where n is equal to 1.962 * 0.40 (1-40) / 0.052 = 369

Where:
N= Sample size.
Z = 1.96 for 95% confidence and 2.56 for 99% confidence.
P = Expected frequency of the factor to be studied.
Q = 1-p.
B = Accuracy or error admitted (Jaramillo and Martínez, 2010).

To determine whether there is a statistically significant difference between the ages of the lambs, the results were analyzed by the Chi-square test for homogeneity of proportions (Wayne, 2006), considering a statistically significant value of $P \leq 0.05$. In the study, 380 fecal samples of lambs of varying ages between 1 day and 3 months of age were collected during 2014. Feces were collected in latex gloves directly from the anus, in lambs less than 1 month of age an enema was applied to obtain a sample. After which the samples were refrigerated in a cooler to be transported to the Parasitology Lab at the Facultad de medicina veterinaria y zootecnia of the universidad autónoma de Sinaloa for subsequent staining using the modified Ziehl-Neelsen technique and observed under a 100X double-blind microscope.

RESULTS

The prevalence of Cryptosporidium in lambs in the municipality of Culiacán was 38% ($P \leq 0.03$).

DISCUSSION

The results of this study confirm the presence of *Cryptosporidium* spp. in the municipality of Culiacán with a prevalence of 38%, in a study conducted by Kalman et al. (2012) with 175 sheep in west Romania 24 samples (13.7%) positive to *Cryptosporidium* spp. were found, on the other hand an investigation carried out in Iran by Mohammad and Orkiden (2013) in lambs yielded 10.24% positive samples. The prevalence of *Cryptosporidium* in Belgium was 13.1% in lambs younger than 10 weeks (Guarde et al., 2008). The aforementioned studies present a low percentage compared to the 38% found in the municipality of Culiacán, according to literature this could be explained by epidemiological

conditions, the geographical area studied, clinical history of the herd, exploitation system, Hygiene practices, management and age of sampled animals.

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A WELFARE-FRIENDLY METHOD ALTERNATIVE TO BLOOD SAMPLING FOR GROUP-HOUSED SOWS?

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SUMMARY. Blood is usually used as diagnosis fluid for pigs. Sampling is done by an invasive method which can stress and hurt the animal. Furthermore, it requires at least two trained operators and may be laborious especially in group-housed sows. This study aimed at assessing the feasibility of oral fluid (OF) sampling on a chewing device, as an alternative to blood sampling in group-housed gestating sows. The study was carried out in 30 French herds (1359 sows) selected on the gestation housing type (straw bedding vs. slatted floor). In each herd, paired individual OF and blood samples were taken from at least 30 sows and three pens selected at random. From these pens, pen-based OF samples were collected on a chewing device provided for 45 minutes. Sampling and chewing times per sow and OF quantity were recorded for each sample; individual information was collected for each sow. Every 15 minutes, the lying sows were counted (to measure the pen activity level). Factors associated with individual sampling time and the probability that a sow chew the pen-based sampling device were identified by logistic regression models. Individual OF sample took 2:50min (one operator, 4ml) while blood sample took 1:15min (at least two operators). Sampling time was significantly increased when straw bedding and varied according to the chewing device design and the operator. 45.8% of the sows from 78.8% pens chew the pen-based device. Sows were less attracted by some kinds of collective device, when straw bedding, when multiparous and when the activity of the pen was low. OF sampling is a safe and easy technique performed by a single operator. This is a promising welfare friendly sampling technique for group-housed sows based on the animal cooperation. The type of sampling (individual or pen-based) should be adapted according to animal housing.

Key words: oral fluid, group-housed sows, welfare

INTRODUCTION

Taking blood samples by venipuncture from pigs is the most frequent sampling method used in the field for monitoring or control purposes. It is a labour-intensive, tedious procedure which may sometimes be dangerous for the samplers (e.g. with boars or sows or in group-housed animals). It is also a stressful action for the pigs. Several studies have indicated that oral fluid (OF) sampling may be

a promising method to detect and monitor pig health under field and experimental conditions (Prickett et al., 2008). It is acknowledged as a more welfare-friendly and alternative method to blood sampling. OF specimens may be collected at both the individual or group (pen-based) level. The feasibility of OF has been assessed for growing pigs or boars (Kittawornrat et al., 2012; Gerber et al., 2014; Decorte et al., 2015; Pepin et al., 2015) but rarely for group-housed gestating sows, a mandatory practice in swine European farming system. Hence, this study aimed at assessing the feasibility of OF sampling on a chewing device, as an alternative to blood sampling in group-housed gestating sows.

MATERIAL AND METHODS

The study was carried out in 30 French herds (1359 sows) selected on the gestation housing type (12 herds with straw bedding; 18 with slatted floor/20 herds with less than 15 sows per group and 10 with more than 15 sows per group). In each herd, paired individual OF and blood samples were taken from at least 30 sows and three pens selected at random. The OF was collected by a single operator allowing the animal to chew on dry swabs (Swab cloth, Sodibox®, Nevez, France) until they were thoroughly moistened. The swab was held into the mouth of the pig with a clamp. It was not necessary to restrain the pigs. From these pens, pen-based OF samples were collected on a chewing device provided for 45 minutes. Sampling and chewing times per sow and OF volume were recorded for each sample. Information related to the sows and their living conditions were collected for each animal. Every 15 minutes, the lying sows were counted (to measure the pen activity level). Factors associated with individual sampling time and the probability that a sow chew the pen-based sampling device were identified by logistic regression models ($p<0.05$).

RESULTS

Individual OF sample took on average 2:50min per group-housed gestating sow (one operator, σ=2:38 min, on average 4ml [σ=1.6ml]). The mean blood sampling time per sow was 1:15min (σ=1:04 min). Factors associated with an OF sampling time higher than 2 minutes are given Table 1.

Table 1: Factors associated with an individual oral fluid sampling time> 2 min per sow (30 herds, 1076 sows – Multivariate Generalised Estimation Equations models with a spatial correlation matrix)

Factors		Odds-ratio	Confidence interval 95%	P-value
Type of floor				
	Straw bedding	2.0	1.3;2.9	**<0.01**
	Slatted concrete floor	-		
Swab size				
	270 cm²	2.0	1.4;2.8	**<0.01**
	160 cm²	-		
Operator	-	-		**<0.01**

45.8% of the sows from 78.8% pens chewed the pen-based device. Factors associated with the probability that a sow chewed a pen-based device are presented Table 2.

Table 2: Factors associated with the probability that a sow chews a pen-based OF device (Multivariate Generalised Estimation Equations models with an autoregressive correlation matrix)

Factors	Odds-ratio	Confidence interval 95%	P-value
Type of floor			
Slatted concrete floor	4.2	[2.5;6.9]	<0.01
Straw bedding	-		
Parity			
0 to 1	3.3	[2.3;4.8]	<0.01
2 to 3	1.9	[1.3;2.6]	
≥ 4			
Pen activity			
≤50% lying sows 30 minutes after the beginning of the sampling time	2.6	[1.6;4.3]	<0.01
>50% lying sows 30 minutes after the beginning of the sampling time	-		
Feeding system			
Automatic feeder	1.8	[1.1;2.7]	0.02
Other types	-		
Type of OF device			
Cotton mop	0,8	[0,5 ; 1,2]	0.03
Cotton rope	3,3	[1,7 ; 6,1]	
Synthetic fringing swab	1	-	

DISCUSSION

This study showed that individual OF sampling is an easy method performed by a single operator in group-housed gestating sows. Results indicate that individual OF sampling takes twice more time than

blood sampling in loose sows. However, it also required twice operator less than blood sampling thus rendering both methods comparable in sampling time when human resources are considered. On the other hand, the volume of oral fluid collected per animal is sufficient to perform laboratory tests. Individual OF sampling is a welfare-friendly method based on the animal cooperation. Altogether these results allow validating the feasibility of individual oral fluid collection in group-housed sows under field conditions.

Sows on slatted floors took less time to be sampled. This may be explained by the environment of the pen providing fewer opportunities for rooting and chewing than in straw bedding systems. The sows may be more curious and interested by novelty and may come more easily to chew the swab on slatted floors than in straw bedding systems.

OF sampling time also varied according to the swab size. Perhaps a small swab is easier for the sow to keep in the mouth and to chew. Operators can also influence the time for OF sampling, indicating that some people are more comfortable collecting individual OF than others. If random sampling is not required, a good method should be to choose quiet sows in order to be faster in the sampling procedure. In lock-in lock-out stalls, the sows can be restrained and are quicker to sample.

Similarly to growing pigs, pen-based OF is a good way to rapidly sample a large number of sows. Nevertheless, the degree of success is lower and more variable in group-housed sows than in growing pigs (Seddon et al., 2012). Pen-based OF sampling can especially fail when sows are straw-bedded, multiparous, and fed with an automatic feeder. OF sampling could also be useful in sows in cases of aggressive loose animals when taking blood samples may be dangerous for the operators. Our results are in agreement with previous results showing that faced with a cotton rope, individually housed gestating sows did not demonstrate high motivation via operant responding (Elmore et al., 2012) and group-housed sows showed moderate motivation (Elmore et al., 2011). As parity is correlated with older age, our results are also in accordance with those from Docking et al. (2008) who demonstrated that the interest of animals in the material could be influenced by their age.

As in the previous results from Seddon et al. (2012) in growing pigs, the straw-bedded sows expressed weak chewing behaviour. Straw is a relevant enrichment material for pigs (EFSA, 2014) that elicits exploratory behaviour and for this reason, straw-bedded sows are less interested in a novel object than sows housed in a more barren environment on slatted floor.

The type of device influenced the sow chewing interest for pen-based OF sampling. (Van de Weerd et al., 2003) previously found that the rope was the favorite object for growers to express their functional behaviours among the 70 tested. Attention should therefore be put on the kind of sampling device to increase the sampling success of pen-based OF sampling.

In conclusion, OF sampling a promising welfare friendly sampling technique for group-housed sows based on the animal cooperation. The type of sampling (individual or pen-based) should be adapted according to animal housing. The oral fluid matrix is a promising method that should be validated in regard to the diagnosis test implemented.

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PEN-SHADE ON FEEDLOT PERFORMANCE OF CALVES DURING THEIR FIRST DAYS IN CONFINEMENT UNDER HOT WEATHER CONDITIONS

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SUMMARY. The provision of shade in the feedlot pen helps feedlot cattle to cope with adverse environmental conditions when are under hot weather. However, their impact during the first days of feedlot calves in confinement is poorly explorer. This research was performed with the objective of determine the Influence of pen-shade on feedlot performance of calves during their first days in confinement in hot weather conditions. Sixty-four calves (213.3 ± 5.76 kg) were placed in 16 ground floor pens (6 x 12 m), eight pens were fitted with shade; and the remainders were without shade. Calves were fed with a growing diet (15.1% CP; 1.34 Mcal of NEm/kg DM), and were weighed at days 1 and 21 when ending the experiment. During the experiment climatic conditions were: air temperature 30.8 °C with range of 23.1 to 42.1°C; relative humidity 59.9%, wind speed 2.2 km/h and solar radiation of 925.7 W/m^2. Across 21 days the provision of shade increased (P = 0.06) in 56% the average daily gain (0.574 *vs.* 0.368 kg/day), augmented (P < 0.01) 7% the dry matter intake (4.82 *vs.* 4.50 kg/day), and improved (P = 0.10) in 45% the gain/feed ratio (0.118 *vs.* 0.0.081 kg gain/kg DMI). It is concluded that the provision of shade inside of feedlot pen contributes to promote growth performance of feedlot calves during their first days in confinement under hot weather conditions.

Key words: Pen-shade, Feedlot-performance, bull-calves

INTRODUCTION

Newly arrived light calves in confined feeding system exhibits low dry matter intake (Loerch and Fluharty, 1999; Duff and Galyean, 2007). Additionally, under hot weather conditions the dry matter intake of cattle is depressed too (Gaughan *et al*., 2010; Barajas *et al*., 2013). Both conditions derivates in low energy and others nutriments intakes (Mitlöhner, *et. al*. 2002; Duff and Galyean, 2007; Barajas *et al.*, 2014), affecting negatively the performance and immunity of feedlot calves (Mader *et al*., 1999; Mitlohener *et al.*, 2002;). Several researches have shown the benefices pen-shade use on dry matter intake (Mitlohener *et al.*, 2001; Barajas *et al*., 2013). The effect of transport and handling on feed intake of calves during first days in feedlot has been measured previously (Fluharty and Loerch, 1996; Duff and Galyean, 2007; Barajas *et al.*, 2014). However, there is no enough information regarded the influence of pen-shade feed intake of newly arrived lightweight feedlot-calves when are subjected to a hot environment. This research was conducted to determine the influence of pen-shade on performance of calves during their first 21 days in confinement under hot weather conditions.

MATERIAL AND METHODS

The experiment was conducted from June to July, 2016 at Experimental Station for Beef Cattle located inside of the commercial feedlot yard "Ganadera Los Migueles, S.A. de C.V." in Culiacan, Mexico (24º 51' N. and 107º 26' W.), 57 m a.s.l.; mean temperature 25 ºC, and rainfall 645 mm.

All calves in the experiment were managed according to the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010). Sixty four Brahman cross bull calves 213.3 ± 5.8 kg were used. At arriving to the feedlot calves were fed a 40% roughage diet and had free access to alfalfa hay and fresh water. After 72 h, they were individually weighed, ear tagged, vaccinated against *Mannhemia haemolytica* (One Shot ®; Zoetis, Lincoln NE, USA), *Clostridia* and *Histophylus somni* (Utrabac®-Somnubac; Zoetis, Lincoln NE, USA); implanted with Component® TE with Tylan (ELANCO Animal Health, Indianapolis IN, USA), treated for internal parasites with albendazole (Albendaphorte® 10% Co; Lab Salud y Bienestar Animal, Mexico) and also injected with A, D and E vitamins (Vitafluid®, Lab Virbac, Carros cedex, France).

Based on the initial body weight, the animals were grouped in four blocks of 16 calves each; and in a randomized complete block design, in groups of four were assigned to two types of allotment: 1) dirt-floor pen (6 x 12 m; 18 m^2/calf), with 2.4 m of feed bunk and 0.6 m of drinker without ceiling (No Shade); or 2) pen as described but fitted with a center metal ceiling that provided 4.12 m^2 shade/calf (Shade). Calves were weighed at the start of experiment and 21 days later when concluded. Calves were feed *ad libitum* (105% of DMI of day before) twice a day (0700 and 1600 h) with the diet shown in Table 1. Orts were removed and weighed at 0630 h. Samples of diet and orts were taken and oven dried (65 °C; 48 h) for DM determination (AOAC, 1995). Air temperature (t) and relative humidity (RH) were recorded daily. THI was calculated using the formula: THI = [0.8 x t] + [(% RH/100) x (t – 14.4)] + 46.4 (Mader *et al.*, 2006).

Table 1. Composition of diet feed to bull-calves

Ingredients	Proportion in dietary dry matter, %
Corn silage without grain	36.0
Corn straw	30.0
Steam flaked corn	12.5
Soybean meal	17.0
Vitamin and Mineral Premix [1]	3.0
Buffer blend [2]	1.5
Total	100%
Calculated analyses (Dry matter basis)†	
Crude protein, %	15.09
Net energy for maintenance, Mcal kg^{-1}	1.340
Net energy for gain, Mcal kg^{-1}	0.774

† Calculated from tabular values (NASEM, 2016).

Statistics. Data were analyzed by ANOVA as a mixed model, for a randomized complete block design (Hicks, 1973); with a model that include variables as follows: block (random), day (random), and Shade (fixed). Pen was the experimental unit, and an alpha value of 0.10 was considered to acceptance statistical difference. Calculations were performed with the Version 9 of the software Statistix (2007).

RESULTS

Results of the influence of pen-shade on feedlot performance of calves are shown in Table 2. During the first five days, Shade did not modify DMI ($P > 0.10$), while spent 7 days to induces an increment in DMI as % of BW ($P = 0.10$). Cumulative DMI during days 1 to 7 was increased 18% ($P < 0.01$) by shade provision. Over complete experiment Shade increased ($P < 0.01$) in 7% the DMI, 55% weight gain ($P = 0.06$) and 45% feed efficiency of calves, respectively.

Table 2. Influence of pen-shade on their dry matter intake of bull-calves during their first days in confinement.

.Variable	Treatments		SEM[1]	*P-value*
	No Shade	Shade		
Bull-calves, n	32	32		
Pens, replicates, n	8	8		
Days in trial, n	21	21		
Weight day 1, kg	213.25	213.34	5.759	0.99
Weight day 21, kg	220.97	225.41	1.492	0.06
Average daily gain, kg	0.368	0.574	0.071	0.06
Dry matter intake, kg/day	4.50	4.82	0.026	< 0.01
Gain/DMI, kg	0.081	0.118	0.014	0.10
Dry matter intake by periods:				
Day 1, kg/day	1.91	2.08	0.072	0.13
% of BW [2]	0.90	0.98	0.034	0.15
Day 2, kg/day	2.26	2.46	0.085	0.13
% of BW [2]	1.06	1.15	0.040	0.15
Day 3, kg/day	2.84	3.08	0.107	0.13
% of BW [2]	1.33	1.44	0.050	0.15
Day 4, kg/day	3.36	3.66	0.127	0.13
% of BW [2]	1.58	1.70	0.060	0.16
Day 5, kg/day	3.70	3.87	0.085	0.17
% of BW [2]	1.73	1.80	0.040	0.24
Day 6, kg/day	3.93	4.12	0.071	0.09
% of BW [2]	1.84	1.91	0.033	0.16
Day 7, kg/day	4.10	4.35	0.083	0.06
% of BW [2]	1.91	2.01	0.041	0.10
Days 1 to 7, kg/day	3.16	3.74	0.031	< 0.01
% of BW [2]	1.48	1.57	0.015	< 0.01
Days 8 to 14, kg/day	4.82	5.09	0.034	< 0.01
% of BW [2]	2.22	2.33	0.015	< 0.01
Days 15 to 21, kg/day	5.53	6.00	0.051	< 0.01
% of BW [2]	2.52	2.68	0.021	< 0.01
Days 1 to 21, kg/day	4.50	4.82	0.026	< 0.01
% of BW [2]	2.08	2.19	0.010	< 0.01

[1] Standard error of the mean

[2] Dry matter intakes expressed as percentage of the body weight, the mean body weight at each day was calculated adding to body weight of day 1 the mean ADG (Days 1 to 21) by corresponding days in confinement.

DISCUSSION

During experiment the climatic conditions were: air temperature 30.8 °C (23.1 to 42.1°C); relative humidity 59.9% (45.3 to 69.9%), THI 81 (71 to 93), and wind speed 2.2 km/h; it implies that calves were exposed to severe hot environment conditions. Agree with THI code: Normal < 74; Alert 75 to 78; Danger 79 to 83; and Emergency THI > 84 (Mader *et al.*, 2006). Across the experiment calves were exposed to danger conditions (THI = 81) and during the hottest hour of day were in status of

emergency, reaching up to THI = 93. The mean DMI as % of BW for days 1 to 7 in both treatments of 1.53% BW is agreeing with the 1.55% BW expected for healthy newly arrived calves by NASEM (2016), and corroborates the low DMI reported previously for newly arriving calves (Fluharty and Loerch, 1996; Duff and Galyean, 2007; Barajas *et al.*, 2014). The 18% of increment in the cumulative DMI during first seven days as consequence of shade availability ($P < 0.01$) gives a strong support that under hot weather, shade helps to improve animal welfare and to ameliorate the negative impact on feed intake as was suggested by experiments with finishing cattle (Mitlöhner, *et al*., 2002; Gaughan *et al*., 2010). The augment in 7% over 21 days DMI is close to 7.5% observed both for Mader *et al*. (1999) as by Mitlöhner, *et al*. (2001) in longer experiment with finishing steers. The observed increments in weight gain and feed efficiency ($P < 0.01$) corroborates the benefices of pen-shade appreciated previously in growing and finishing cattle in high ambient temperature (Gaughan *et al*., 2010; Barajas *et al.*, 2013). It is concluded that pen-shade provision it is a profitable tool, that could contribute to improve the welfare of calves and can helps them to cope adverse hot climate condition, even during the critic period of early days in the feedlot.

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THE WELFARE QUALITY® ASSESSMENT PROTOCOL - HOW CAN IT BE ADAPTED TO FAMILY FARMING DUAL PURPOSE CATTLE RAISED UNDER EXTENSIVE SYSTEMS IN TROPICAL CONDITIONS?

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SUMMARY. Family farming is still the main source of income for many people in the tropical regions of the world. At the same time, the modern society is quickly becoming more aware about the welfare of animals for human consumption. The main objective of this study was to illustrate the need to modify some aspects of the original WQ® protocols developed by the EU-funded WQ® project, under the conditions of small community farmers in the tropics. Thirty-four dual purpose farms in the State of Chiapas, Mexico, whose main production focus is milk but where beef production is also of significant value, were evaluated utilising a merged version of the WQ® protocols for dairy and beef cattle. Based on their average score, the farms obtained at least an acceptable level in each indicator of welfare. However, after merging indicators from the dairy and beef cattle protocols of WQ® to adjust it to the prevailing conditions in the tropics, some sections are not applicable. This is true especially for the section related to good housing, where most of the items do not apply due to the absence of infrastructure; the farms obtained a very high score in this section but further studies to verify if this reflects a correct assessment of the welfare status should be performed. In general, the approach of the WQ® protocol was useful, however some aspects are quite different from the conventional intensive farming systems predominantly used in Europe and there is a need to implement some modifications.

Key words: Animal welfare, dual-purpose cows, tropics.

INTRODUCTION

In emerging economies, family farming is still the main source of income for a large part of the population, particularly in the tropical regions of the world (González-García et al 2012). Research is necessary to ensure the sustainability of such farms. At the same time, consumers are becoming more aware about the environment and the ethical treatment of animals, requiring information on the origin and quality of food produced from them (Smith & Brower 2012). One important aspect of direct concern to the quality of animal products is farm animal welfare, which is indeed becoming a great concern to society in both developed and developing countries.

Society concerns and increasing consumer awareness of animal welfare were the main driver behind the EU-funded Welfare Quality® project in 2004. Within this project, a set of predominantly animal-based on-farm animal welfare assessment protocols were developed. The cattle welfare assessment protocol has been used in a number of scientific studies (Knierim et al 2009; Popescu et al 2013) under intensive conditions. Nevertheless, the Welfare Quality® standards were mostly designed for

indoor/partial indoor systems, which inevitably tend to congregate animals in certain parts of the farm in scenarios that are not typical for animals raised under tropical conditions (Absalón-Medina et al 2011; Corro et al 1999). Hence, partly different indicators may have to be used to correctly evaluate the animal welfare status under these conditions.

The main aim of this study was to illustrate the necessity of modifying some aspects of the original WQ® protocols for dairy and beef cattle to accurately evaluate animal welfare under the conditions prevailing in small community farming in the tropics.

MATERIAL AND METHODS

This study was performed in 34 dual purpose farms whose main production focus is milk, sold to a local cheese factory. Male calves are sold for finishing, and old cows with subpar milk production are slaughtered for beef production.

The farms were located in the municipality of Villa Corzo in the state of Chiapas, Mexico. The climate in this region is hot and sub-humid with summer rainfall and an average precipitation of 1247 mm. The study was performed during the summer at an average temperature of max 31°C and min 20°C, with an average humidity of 86%. The size of the farms ranged between four to fifteen hectares while the herd size ranged from 7 to 90 cows, with approximately 2/3 of the farms ranged between 15 and 35 cows. Herds were mostly composed of crossbred animals (*Bos taurus* x *Bos indicus*). The farms in the study worked under the same system; a 24/7 pasture system with milking in the morning and after milking, cows were released to pasture, where they stayed for the rest of the day and night. Calves were kept together with the cows night and day but usually separated and left in a paddock near the milking parlour during milking. The herds had one or two bulls staying 24/7 with the cows.

Utilising the Welfare Quality (WQ®) protocols for dairy and beef cattle (Welfare Quality® Assessment protocol for cattle 2009); the indicators that could be applicable for an all-year-around grazing-based dual-purpose systems were selected.

Due to the prevailing conditions of the farms in this study, some features were evaluated during the milking sessions when the animals were gathered in the milking parlour and it was feasible to perform the observations at individual level. These observations covered the whole herd including cows, calves and bulls when present.

The calculation of scores was performed according to the calculations included in the WQ® protocol (WQ® 2009). The final result is represented by a number from 0 to 100 and the farms are divided in four categories according to their final score in each category, as follows: Excellent - 80.1 – 100; Improved - 60.1 – 80; Acceptable - 20.1 – 60; and Not classified - 0 – 20.

RESULTS

A total of 9 farms reached a level above the minimum scores to be considered acceptable in all the categories; hence 74% of the assessed farms scored below acceptability in one or more indicator categories of animal welfare. Based on the average score, the farms in the study area obtained an acceptable level with respect to each indicator of animal welfare considered in the protocol. Absence of prolonged hunger and absence of pain induced by management procedures represented a major weakness for the dual purpose farms in the region. Ease of movement, as well as expression of other

behaviours obtained the highest score; this may be directly related to the grazing conditions of the system. Good human-animal relationship also obtained the top score, indicating that even though being at pasture the most part of the time, cows were still used to human contact. The percentage of farms per each indicator of animal welfare and per category of classification are displayed in figure 1.

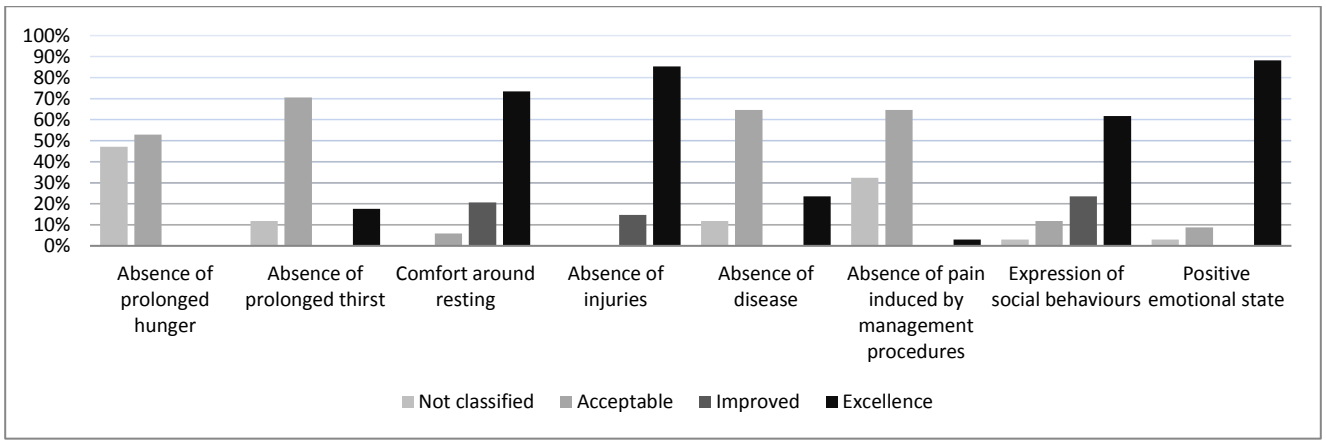

Figure 1. Percentage of farms per category of classification and separated by each indicator of animal welfare according to the WQ® protocol. Ease of movement, expression of other behaviours and good human-animal relationship are not shown in the graph since 100% of the farms reached the category of excellence.

In this study, three different sources of water were observed: troughs (artificial container intended to provide water to animals), rivers (natural flowing watercourse) and ponds (natural or artificial pit in the ground). Some farms presented combinations of two different sources. This is displayed in figure 2.

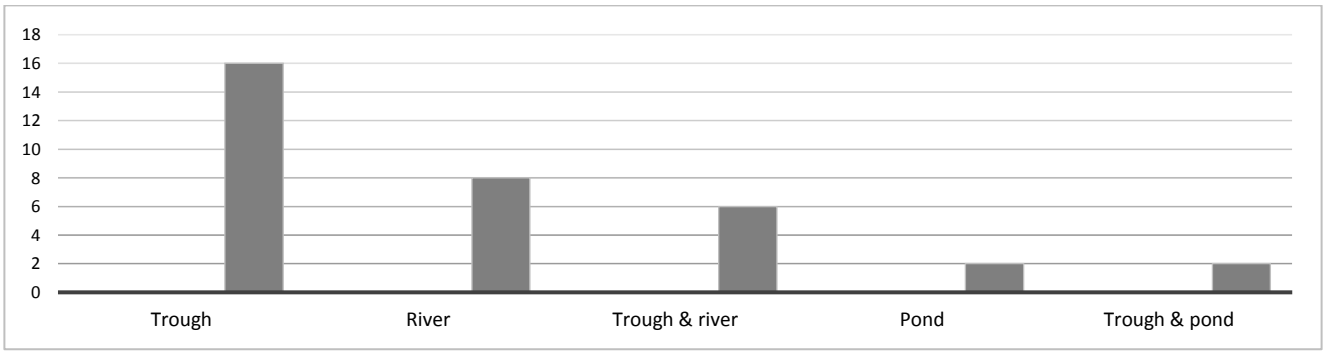

Figure 2. Different sources of water available for the cattle herds in this study (n=34). Three different sources of water were observed (Troughs, rivers and ponds), as well as two different combinations of these, trough + river and trough + pond.

DISCUSSION

Since the Welfare Quality® protocol was designed to assess intensive farms with a clear objective of high production; it does not necessarily correspond to the characteristics observed under the system implemented in grazing dual purpose farming in the tropics. This study aimed to evaluate if the protocol could still be a useful tool assessing animal welfare under the mentioned system. However, after merging indicators from the dairy and beef cattle protocols of WQ® to adjust it to the conditions in the tropics, some sections were found not to be applicable to these farms. This is true especially for

the part related to good housing, as most of the items do not apply due to the absence of infrastructure (i.e. no indoor housing); the farms did obtain a very high score in this section but further studies to verify if this reflects a correct assessment of the cows' welfare should be performed.

The section "animals colliding with housing equipment" does not apply to the conditions prevailing in this study area; instead a better approach might be to assess the stocking density, i.e. the number of animals/m^2 in the area where the cows were kept during the night and the site for milking, i.e. the area around the milking parlour (Waiblinger et al 2001; Schneider 2010).

In relation to absence of pain induced by management procedures, it should be mentioned that the farmers in this region do not practice tail docking or castration, but other procedures that may cause pain but which are not considered in the original protocol were identified. For example, ear tagging, hot iron branding, and cows routinely injected with oxytocin intramuscularly at each milking. Additionally, the absence of a livestock crush or any other area designed for performing these procedures, as well as other common features such as deworming or vaccination, could be major causes of stress (Orihuela & Solano 1994).

Social interaction between cows might be lower when compared to intensive farms, especially when calves and bulls are kept with the cows and the available pasture area is large and stocking density hence very low. Additionally, cows under pasture conditions spend most of the time in activities such as grazing and ruminating, and then not interacting among themselves. During this study, interactions with other types of animals than cows, including calves and bulls, but occasionally other species such as horses, poultry and wild fauna, were commonly observed. Hence, the importance of these events should also be taken in consideration while assessing this type of farms in order to obtain more information about expression of social behaviour (Masahiko et al 2013).

One of the main problems observed was the limited access to water, i.e. the shortage or absence of water sources either at pasture or in the milking parlour. Most farms only have water sources in one area, which results in animals spending long periods without drinking water. This can be a potential welfare problem especially in the hot dry season (Ahmed and El Hag 2003).

Family farming in the tropics generally involves a limited number of animals, sometimes even less than 10 cows. When this type of animal welfare assessment is carried out on a low number of animals, indicators evaluated as percentages, e.g. health related problems (i.e. diarrhoea not always caused by an infection) and body condition will considerably influence the total score given to that farm, since one single animal will then constitute a significant proportion of the herd, but without stating that the whole herd is affected or at risk. It is noticeable that the size of the herds can also benefit the approach and utility that the WQ® protocol might have in an extensive system since the difficulties and limitations observed in this study differed from the ones found by Huertas et al (2009) when applying the same protocol in extensive larger farms in Latin America. In that study the size of the herd and the lack of a routine gathering of the animals were the major problems related to the assessment of features such as disease indicatives and avoidance distance.

Finally, new features could be developed to accurately improve animal welfare in farms using these systems. Important aspects as the quality of feed and water should be some of the main targets to attend to. The evaluation of cleanliness of water sources, assessed in accordance with the original protocol in this study, should be improved in order to provide more useful information. In case of natural water

sources evaluating water aspects such as odour and colour and whether it is still or running water should be considered, and even further studies involving taking water samples for the analysis of water quality should be a research priority in future endeavours.

In conclusion, the approach of the Welfare Quality® protocol was useful also under the conditions prevailing in this study. However, as some aspects, such as absence of prolonged thirst, animals colliding with housing equipment and social interaction, are quite different from the conventional intensive farming systems predominantly used in Europe and there is a need to implement some modifications.

ACKNOWLEDGMENTS

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EFFECT OF *Brucella abortus* ANTIBODIES ON DAYS OPEN AND CALVING INTERVAL IN COWS

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SUMMARY

Brucellosis is an important disease in dairy production in some areas of Mexico, because it affects fertility rates of cows, bringing economic losses. The aim of this study was to evaluate the effect of antibodies to *Brucella abortus* on calving interval, open days and milk production of dairy production unit by analyzing and interpreting information from the records. A sample of 100 cows from a herd of dairy region of Tizayuca, Hidalgo, Mexico was used. The presence of *Brucella abortus* was performed using both specific card test and Pink-Bengal-Rivanol test. Laboratory results indicated that 11 cows were positive to infection by *Brucella abortus* using the specific test card criteria, but only two were positive when Pink-Bengal-Rivanol test was used over same samples. In the same herd analyses from the records indicates an incidence of aborts rate 13.04% some close to 11% of seropositive cows found. Abortions rate was negatively associated with reproductive performance of cows. Seropositive cows had longer calving interval, and open days in relationship to seronegative dairy cows. Results indicates that more than 10% of dairy cows in the area of Tizayuca, Mexico could be affected by infection due to *Brucella abortus*, this fact represent a high sanitary risk for dairy cows in the region, for the farm-workers, as well by the persons that could have contact with those cows or its food products, becomes in an public health item.

Key words: Photoperiod, sheep, synchronized.

INTRODUCTION

A very important factor affecting the fertility of the farm is the fact that it can have a large number of cyclical cows, free of reproductive diseases, to incorporate them into the breeding program. The physiological and hormonal parameters associated with the repair of the estrus and with the restoration of the ovarian and uterine functions are critical for the establishment of gestation (Risco and Archibald, 2005). The aim of this study was to evaluate the effect of antibodies to *Brucella abortus* on calving interval, open days and milk production of dairy production unit by analyzing and interpreting information from the records.

MATERIAL AND METHODS

He study was carried out in the facilities of the Autonomous Metropolitan University unit Xochimilco located in bone road 1100 colony Villa Quietud. Delegation Coyoacán and in the basin of Tizayuca-Hidalgo located 25 kilometers from Mexico City, along the Mexico-Laredo highway.

Information from 100 individual reproductive records of a dairy unit was used. Reproductive parameters (open days and interval between deliveries) were determined. In each year the cows were grouped according to the corresponding calving number (first calving, second calving, third calving and fourth calving).

Subsequently, the means of reproductive parameters collected each year and the percentage of annual abortions were obtained.

Blood samples were then collected and then refrigerated at 4 ° C for preservation and continued with the treatment at the Immunology Laboratory of the Autonomous Metropolitan University, performing the card test and rivanol.

RESULTS

Results of average reproductive parameters are shown in Table 1.

DISCUSSION

In the obtained results an incidence of abortions of 13.04% was found which alters the reproductive parameters and consequently the productive parameters of the herd, authors mention that the incidence of herd abortions should not exceed 1 to 4% in order not to affect reproductive parameters. The percentage of brucella abortus seropositive animals with the rivanol test was 16% of a total of 100 animals, the authors conclude that the overall prevalence of brucella abortus found in dairy herds is moderate, but at the herd level it is higher (Aparicio *et al.,* 2003).

Table 1. Averages of reproductive parameters of 3 years

Year 2011	Number of cows	Number of services	Open days	Interval between births	Abortions
1° Birth	21	2.80	196.85	401.85	8
2° Birth	11	2	111.18	358.54	2
3° Birth	10	3.2	211.7	458.1	6
4° Birth	6	4.5	348.5	429.2	0
Average	12	3.12	217.05	411.92	16
Year 2012	Number of cows	Number of services	Open days	Interval between births	Abortions
1° Birth	22	3.09	157.57	413.06	5
2° Birth	27	3.3	192.48	405.59	2

3° Birth	10	4	191.87	537	1
4° Birth	7	3.42	163.42	372.81	0
Average	66	13.81	176.33	432.11	8
Year 2013	Number of cows	Number of services	Open days	Interval between births	Abortions
1° Birth	11	3.45	126.62	0	3
2° Birth	19	2.89	142.89	383	1
3° Birth	25	2.42	106.22	412	5
4° Birth	11	3	117.66	311	2
Average	16.5	2.94	123.34	368.66	11

With regard to open days, the average in Mexico is 144.7; However, in the herd of Tizayuca, up to a maximum of 348 on average, this prolongation of open days is cause to pathologies as is the case of brucellosis, as well as different physiological, environmental and zootechnical factors. In the case of interval between deliveries we have a maximum of 15 months, knowing that the optimal level is 13 months (Meléndez, 2010). The high abortion rate in this study (13.04%) has the effect of affecting the reproductive parameters, such as the open days and the interval between prolonged births that were found in the test positive cows; In addition this incidence increases more because it is not possible to establish a program of elimination of seropositive animals because of the high economic cost to the producer. In conclusion, 10% of dairy cows in the Tizayuca area of Mexico are affected by the infection due to Brucella abortus, which represents a high sanitary risk for dairy cows in the region that may have contact with these cows or their products Becomes a public health article.

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PRESENCE OF SUBCLINICAL MASTITIS IN HOLSTEIN COWS AT THE TIME OF MILKING

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SUMMARY. Mastitis is one of the most important diseases that globally affect the dairy industry. It is considered the most costly infectious disease of dairy cows because it induces a decrease in production of about 4 to 30% in milk yield, and also to obtain milk of low quality. The aim of this study was to evaluate the effect of management during milking on the presence of subclinical mastitis in Holstein Friesian cows. Four samples were taken at days 1, 5, 7, and 15 from each other in 24 Holstein Friesian cows. The study was developed in the municipality of Amecameca, State of México, México, and the management system was traditional from rural areas. For diagnosis, California test was performed and for taking samples order was followed in handling milkers at the time of milking. It was found that 100% of the samples showed some degree of subclinical mastitis, increasing progressively as milking progressed. In conclusion, milking management directly impact on the degree of subclinical mastitis in Holsteins Friesian cows observed, presenting a greater degree of subclinical mastitis in the last pregnant cows to be milked. The findings of this trial focus the high prevalence of sub-clinical mastitis, indicating the potential economic implications related to the disease in smallholder dairy sector of this rural area of México.

Key words: Holstein Friesian, California test, subclinical mastitis.

INTRODUCTION

Bovine mastitis is an inflammatory response of the mammary gland to an aggression. It is characterized by the entry of somatic cells, mainly polymorphonuclear neutrophils, into the mammary gland and by an increase in the protease content in milk. This disease can be classified according to the degree of inflammation and local lesions, systemic implications in the cow. (Fernández et al., 2012)

It can occur clinically and subclinically. Subclinical mastitis is long-lasting and much more common than clinical mastitis. Among the methods most frequently used at the field level to diagnose clinical mastitis are the method of observation and palpation of the udder, and physical tests such as the milking bowl test, black cloth test and cup Tester Chemical tests, such as the electrical conductivity test of milk, mastitis indicator paper and Whiteside test that also serves to diagnose clinical and subclinical mastitis. Biological tests, such as the California mastitis test, the Wisconsin test, the bacteriological diagnosis by methods of isolation, culture, staining, biochemical testing and identification and counting of somatic cells by direct microcopy and somaticell. Mastitis detection methods are a tool that allows identifying the type of clinical or subclinical infection that can occur within a dairy herd, so that the

method chosen to determine the tests will be essential for a more accurate diagnosis. (Bedolla et al., 2007)

Mastitis is a highly prevalent disease in dairy cattle and is one of the most important diseases worldwide affecting the dairy industry, considered the most costly infectious disease of dairy cows because it induces a decrease in production from 4 to 30% milk and low in quality, as well as increasing costs of herd health care and premature disposal of genetically improved animals. As a result, it has been recognized for some time as the most expensive disease in dairy farms. (Bedolla and Ponce, 2008).

The objective of this work is to evaluate the effect of handling during milking on the degree of presence of subclinical mastitis in Holstein Friesian cows.

MATERIAL AND METHODS

A total of 84 individual room milk samples were processed from 24 dairy cows from Holstein friesian cows, from a traditional production located in Edo. Mexico.

In each sampling the dark background test described by Martínez et al., 2011 was used first, which allows to detect lumps in the milk (tolondrón) directing the first jets through a black mesh or in this case in some bowls , Which were moved rhythmically in circles to see their characteristics and whether or not there were foreign bodies. This was followed by the California Mastitis Test (CMT), which Fernández *et al.*, 2012, is the most widely used at the field level for the diagnosis of mastitis in dairy cattle. This provides an indication of the number of somatic cells in milk. CMT will only give rise to a visible reaction with a concentration of 400,000 cells / ml or more.

The classification obtained from subclinical mastitis was made 84 individual milk samples were processed from 24 dairy cows from Holstein friesian cows, from a traditional production located in Edo. México.

In each sampling the dark background test described by Martinez et al., 2011 was used first, which allows to detect lumps in the milk (tolondrón) directing the first jets through a black mesh or in this case in some bowls , Which were moved rhythmically in circles to see their characteristics and whether or not there were foreign bodies. This was followed by the California Mastitis Test (CMT), which Fernández et al., 2012, is the most widely used at the field level for the diagnosis of mastitis in dairy cattle. This provides an indication of the number of somatic cells in milk. CMT will only give rise to a visible reaction with a concentration of 400,000 cells / ml or more.

Table 1. The classification obtained from subclinical mastitis

Score	Meaning	Description of the reaction	Interpretation (Rcs/ MI)
N	Negative	Homogeneous	0-200.000
T	Trace	There is some thickening. The reaction is reversible and the viscosity observed for the first time has to disappear	150.000-500.000
1	Slightly positive	The mixture thickens, there is no gel formation in the middle of the vane and the viscosity observed has to persist. The mixture	400.000-1-500-000

		falls little by Little	
2	Positive	Gel will form in the center of the blade during the rotating movement. The gel will accumulate at the bottom of the blade when rotating movement is interrupted. When it converts the gelatinous mass falls and can leave some liquid in the well.	800.000-5.000.000
3	Very positive	Gel will form in the center of the vane and stick to the bottom of the well, but not to one side. When the mixture is poured, it is dropped to leave liquid behind.	>5.000.000

Bolaños *et al*., 2012.

RESULTS

Among the results obtained we can highlight that the four samples taken from milker 1, where "0" is negative, "0.5" is equivalent to trace, 1, 2 and 3 are the degrees of mastitis found. The grades of subclinical mastitis increase progressively as the milker progressed, and even in the third and fourth sampling, only 6 cows were observed since one of them was withdrawn from production because it did not produce the liters of milk that had to Give newspapers.

The four our taken from milker 2, where "0" is negative, "0.5" is equivalent to trace, 1, 2 and 3 are the degrees of mastitis found. It should be noted that, as in milker 1, the degrees of subclinical mastitis rise as the milking progresses.

DISCUSSION

As mentioned by Bedolla, 2008, subclinical mastitis is a highly prevalent disease in dairy cattle, this can be observed in all samples of the 3 milkers (graph 1,2,3,4,5,6,7,8, 9,10,11 and 12) since in these it was observed that there was always a degree of subclinical mastitis.

According to "Hans 2001" the poor management of the milkers by malfunctioning the milking machine causes subclinical mastitis, which can be observed in each of the pictures, since the first cows assigned to the milkers were well managed and sanitary , Which can be observed in cows 1, 2 and 3 of each milker, however as they progressed, they did not continue to take the same care in cleaning the udder, work equipment and milkers, which is reflected Of cows 4 in front of milkers, since it can be observed how the degree of subclinical mastitis goes up as the milking progresses. All this coinciding with Santivañez et al., 2013, which indicates that subclinical mastitis can be caused by poor disinfection of the udders at milking, poorly used milking machines, poor postharvest sealing, poor bedding status; All this predisposing to the mammary gland to the entrance of pathogenic microorganisms for this. And supported by Pizón et al., 2009, who mention that the increase in the prevalence of subclinical mastitis is due to inappropriate practices in the milking routine, which leads to an increase in the spread of disease in the herd.

It should be noted that as suggested by Hans, 2001, a manual of milking procedures and cleaning and disinfection of the equipment should be carried out in production, as well as teaching the correct

application of the milking procedure to milkers, which in production There was no increase in the risk of mismanagement at the time of milking and giving results like those obtained in this study.

With the above, it can be concluded that the management of milking directly affects the presence of the degree of subclinical mastitis in Hosstein Friesian cows, with the highest degree of subclinical mastitis being the last cows to be milked.

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COGNITIVE BIAS TEST AS A TOOL FOR ACCESSING WELFARE OF FISH

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SUMMARY

Studies prove that fish are conscious and sentient beings. They can feel not only pain, but also more complex emotions both negative and positive. Innovative approach to welfare studies is an idea to measure animals' affective states as an ultimate indicator of their quality of life. The aim of the presented research was to develop a cognitive bias test based on the assessment of ambiguous stimuli that would allow for assessing the valence of emotional state in fish and to use this test for investigating the influence of structural enrichment on welfare of Zebrafish. 100 Danio rerio were divided into two groups: BT (barren tank) and ET (tank with environmental enrichment). Previously conditioned fish were tested in a five arm, radial shaped chamber. Each arm had the back wall painted with a certain proportion of green colour (conditioned as positive stimulus) and red colour (conditioned as negative stimulus). This allowed to crate zones with different intensity of the two stimuli: the far left side representing the positive stimulus "A" (100% of green colour), the far right representing the negative stimulus "E" (100% of red colour). The three middle arms were representing ambiguous stimuli with the different intensities of red and green colours. During the test behaviour of each fish was recorded and analysed for activity in each zone using computer software. The fish from the ET group showed more overall activity and explored more of the ambiguous stimuli arms than the fish from the BT group. This suggests that environmental enrichment had a positive influence on the valence of affective state in fish from the ET tank. The presented research showed that the cognitive bias test is a promising tool for assessing welfare and affective states in fish. Results suggest that environmental enrichment had a positive effect on the emotional state and therefore the welfare of fish.

Key words: fish welfare, cognitive bias, zebrafish

INTRODUCTION

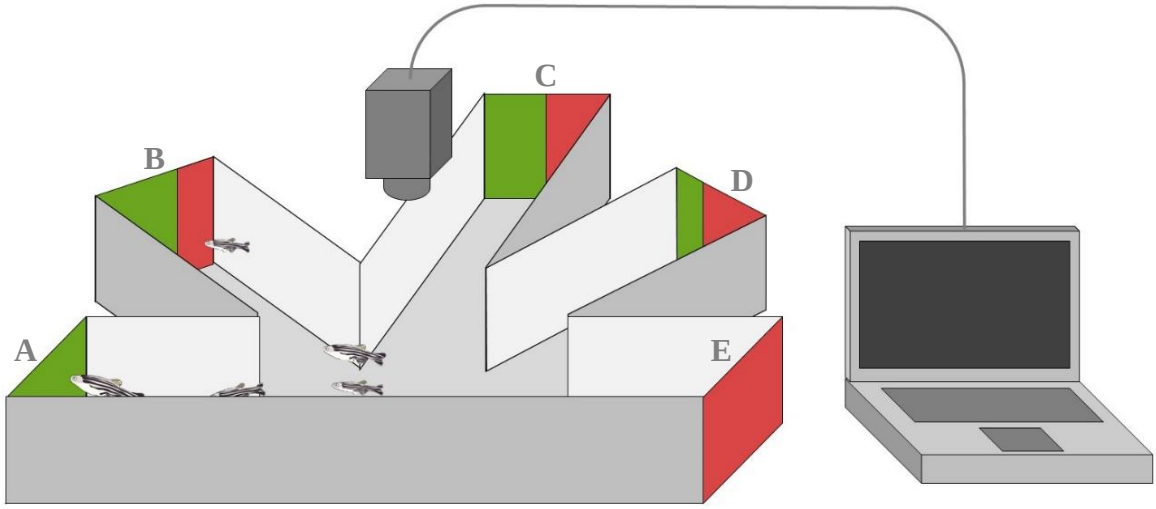
The most recent studies prove that fish similar to mammals are conscious and sentient beings. They can feel not only pain, but also more complex emotions both negative and positive (Brown, 2015). Due to dynamic growth of aquaculture sector and intensification of the production methods the number of threats for farmed fish welfare is constantly growing. Fish in aquaculture are often kept in barren tanks or cages deprived of any structural elements. However the research shows that structural enrichment has a positive influence on fish health, aggression level and growth rate (Naslund at al., 2016). Therefore it is important to develop methods that allow for accessing fish welfare. Innovative approach to this subject is an idea to measure animals' affective states as an ultimate indicator of their quality of life.

The aim of the presented research was to develop a cognitive bias test based on the assessment of ambiguous stimuli that would allow for assessing the valence of emotional state in fish and to use this test for investigating the influence of structural enrichment on welfare of Zebrafish.

MATERIAL AND METHODS

In the research there were 100 adult Danio rerio from line ABxTL used, selected for the size and body weight. The fish were divided into two groups: group BT (Barren tank): the fish in this group were kept in a tank devoid of any elements (completely empty tank) and group ET (Enriched tank): the fish in this group were kept in a tank equipped with hides and synthetic plants. The fish were conditioned for two stimuli, positive (A) identified with green colour and negative (E) identified with red colour. Conditioned fish were tested in a radial shaped cognitive bias chamber designed for this experiment. The chamber consisted of five arms positioned at an angle of 45o relative to each other. Each arm had the back wall painted with a certain proportion of green colour (conditioned as positive stimulus) and red colour (conditioned as negative stimulus). This allowed to crate zones with different intensity of the two stimuli: the far left side representing the positive stimulus "A" (100% of green colour), the far right representing the negative stimulus "E" (100% of red colour). The three middle arms were representing ambiguous stimuli with the different intensities of red and green colours (from left to right: arm "B" 75% green and 25% red; arm "C": 50% green and 50% red and arm "D": 25% green, and 75% red) *(Figure 1*). During the final test in each trial a group of five fish was placed in the middle of the testing chamber. Their behaviour was recorded for 5 minutes right after the doors for all arms were opened. Afterwards the recording was analysed for fish activity in each zone using computer software.

Figure 1. Five-arm radial testing chamber.



RESULTS

The fish from the ET group showed more overall activity and explored more of the ambiguous stimuli arms in the test chamber than the fish from the BT group. Fish coming from enriched tank (ET group) during the cognitive bias test trials spend 57,4% of overall test time on exploring arms with ambiguous clues. Most activity was presented in zone D2 (an average of 1,14 fish visiting this zone during the test time), A2 (0,81), N (0,67) and C2 (0,69). Least activity was presented in zone E2 (0,11). Fish coming from barren tank (BT group) on the other hand spend most of the overall test time (69,48%) in the zone A2 (arm associated with positive stimuli). Fish in BT group spend only 14,8% of overall test time on exploring arms with ambiguous clues. Most activity was presented in zone A2 (3,47) and there was no activity in zone E2 (0,00) associated with negative stimuli (*Figure 2 and 3*).

Figure 2. Fish activity in each zone of testing chamber.

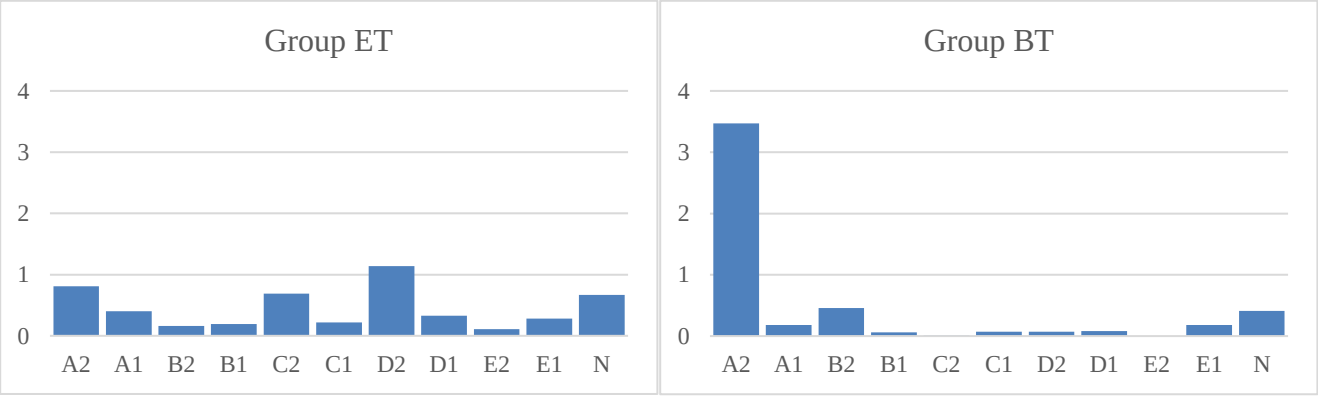
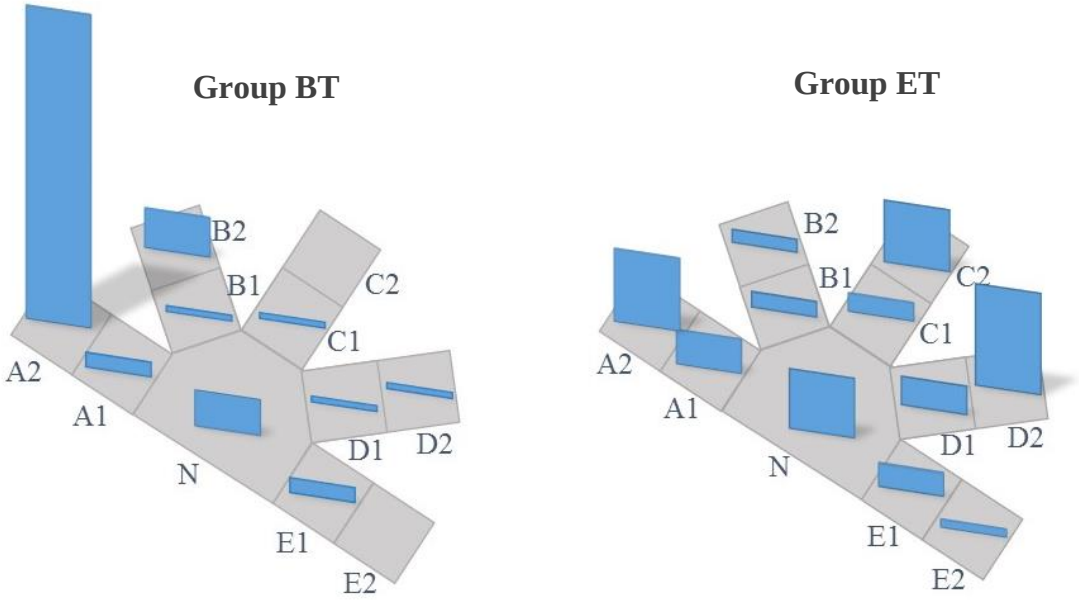


Figure 3. Graphical representation of fish activity in each zone of testing chambe



DISCUSSION

The presented research showed that the cognitive bias test is a promising tool for assessing welfare and affective states in fish. Results suggests that fish with a higher welfare (enriched tank providing better living conditions) were more positive biased than fish with a lower welfare (barren tank devoid of any enrichment). This was emphasised by higher activity in arms with ambiguous stimuli during the cognitive bias test. As literature concerning cognitive bias in fish is very limited or non-existent it is necessary to

look at research done on other groups of animals. Results from studies done on macaques (*Macaca mulatta*) (Bethell et al., 2007), dogs (Casey et al., 2008), pigs (Douglas et al., 2012), chickens (Salmeto et al., 2011), starlings (*Sturnus vulgaris*) (Matheson et al., 2008; Bateson and Matheson, 2007) and rats (Burman et al., 2008; Burman et al., 2009) suggests that higher exploration of ambiguous clues can be associated with more optimistic cognitive bias and higher welfare.

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MOBILE HOUSES FOR LAYING HENS – BOTH CHANCE AND CHALLENGE

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SUMMARY. Since the early 2000s, mobile houses have emerged as alternative housing systems for laying hens in Germany. Besides increasing consumer interest in animal friendly farming procedures, the objective was to alleviate common problems of static free-range units, such as an excessive use of the outdoor run near the stable, resulting in a loss of vegetation, and an accumulation of nitrogen and infectious agents. At present, a variety of mobile houses, from partially mobile polytunnels on skids to fully mobile trailer-like systems, is commercially available. The interior varies from single tier systems with manual feed hoppers and front roll away nest boxes to aviaries with automatic chain feeders and egg belts. Most units offer space for 200-2000 hens, and investment costs amount to 50-160 €/animal. However, the legal situation in Germany is complicated, as it usually depends on the respective competent authority whether or not a mobile house is exempt from a building permit. The frequency with which mobile houses are moved ranges between once a week and four times a year, largely depending on the respective housing system and local conditions. Whereas frequent site changes affect the nitrogen and pathogen load of the outdoor run positively, the skids or wheels of the barn and tractor may cause damage to the turf. Another challenge is the cleaning and disinfection of mobile houses. In contrast to static barns connected to the sewage system, the potentially contaminated wastewater is often directly carried into the soil. From an economical point of view, the investment costs as well as the high labour demands, especially for small units with less automation, must not be underestimated. To operate profitably, minimum prices of about 30 cents/egg have to be realised. The use of mobile houses for laying hens can be an animal friendly and environmentally sound alternative to static free-range systems. However, for developing detailed recommendations for farmers, veterinarians and the competent authorities, present gaps in knowledge should be filled through further, interdisciplinary research.

Key words: Poultry; Sustainable farming concepts; Animal welfare

INTRODUCTION

Mobile poultry houses, in the form of simple carriages, were already common at the beginning of the past century. They were mainly used to bring pullets to the stubble fields for "grazing" in order to save valuable feed (Van der Linde, 2015). Since the early 2000s, mobile houses for laying hens have re-emerged in Germany, especially in the organic sector. The objective now was to alleviate common problems of static free-range units, such as an excessive use of the outdoor run near the stable, resulting in a loss of vegetation, and an accumulation of nitrogen and infectious agents. In addition, consumer interest in farm animal welfare has grown over the past years (European Commission, 2007), and free-range housing systems are perceived as particularly animal friendly (Sossidou et al., 2011). Thus, mobile housing systems became also attractive for an increasing number of conventional poultry farms, especially for smaller units offering farm gate sales. At present, 23.7-43.5% of the owners manage their mobile houses conventionally (Trei et al., 2015). This paper provides an overview of the variety of mobile houses used for keeping laying hens. In this context, challenges and risks, possible solutions in the field and present gaps in knowledge are discussed.

COMMON MOBILE HOUSING SYSTEMS

In principle, a distinction is made between partially and fully mobile housing systems for laying hens. Partially mobile houses are polytunnels with open bottom on galvanised skids, which are mainly towed in the longitudinal direction by means of a tractor. It is also possible to move these stables between few paved locations with fixed grid and sewage system connection. In addition, a fully insulated wintergarden can be attached to the side of the stable. At present, partially mobile houses are available for 190-2000 laying hens. Depending on flock size and management practice, investment costs for partially mobile housing systems equipped as standard range between 50-128 €/hen (Van der Linde, 2015). Fully mobile trailer-like systems are particularly purchased for smaller flocks of about 225 hens (Trei et al., 2015). They mostly consist of tanks and photovoltaic systems or generators for an independent supply of water and energy. Furthermore, they have a chassis whose axes and wheels are either fixed or exchangeable. Commercial available fully mobile houses offer space for 225-1400 laying hens, and investment costs amount to 109-160 €/animal (Van der Linde, 2015). The interior of both partially and fully mobile houses varies from simple single tier systems to multilevel aviaries with manure belts. The degree of automation also differs: some houses feature for instance automatic chain feeders, egg belts and stable computers which send alerts to the owner's mobile phone, whereas in other houses feed hoppers and frontal rollaway nest boxes are operated manually. Besides commercially available systems for laying hens, some providers also offer mobile houses for broiler chickens, turkeys and domestic waterfowl. Moreover, a variety of self-constructed mobile housing systems, such as movable huts, old caravans or construction trailers, can be found in the field.

CHANCES, CHALLENGES AND REMAINING GAPS

LEGAL FRAMEWORK

At present, there is no consensus in Germany on how building law refers to mobile houses. It usually depends on the respective competent authority whether or not a mobile house is exempt from a building permit (Van der Linde, 2016). The legal situation is further complicated by mobile systems which are actually considered as a vehicle with trailer status. In the European Union, the Council Directive 1999/74/EC (1999) laying down minimum standards for the protection of laying hens and its national implementations provide detailed requirements for keeping laying hens. It must be borne in mind that these regulations also apply to commercially run mobile houses, whether available as standard or self-constructed. For organic farming, the corresponding European and national legal requirements have to be complied with.

RUN MANAGEMENT AND MOBILITY

A common problem of static free-range units is the excessive use of the outdoor run near the stable. This often results in a loss of vegetation and permanent turf destruction involving puddles and muddy areas with an accumulation of nitrogen and infectious agents (Elbe et al., 2005; Knierim, 2006). The advantage of mobile systems is that changing sites can alleviate these problems. The frequency with which mobile houses should be moved depends on the local conditions, the type of mobile house and the management of the outdoor run. In general, it is recommended to move partially mobile houses about four times a year, and fully mobile houses weekly to monthly (Deerberg, 2014). Whereas frequent site changes affect the nitrogen and pathogen load of the outdoor run positively, the skids or wheels of the barn and tractor may cause damage to the turf, especially in the times of low vegetation. To avoid unreasonably frequent site changes of the whole mobile house, a rotation of outdoor runs is useful. This can be realised, for instance, by providing the hens with the areas next to the right side of the house first, and then with the left-side areas. In case of mobile housing systems with open bottom or after longer periods at the same site, further measures to regenerate and vegetate the area, such as mowing, mulching, reseeding and pre- and post-grazing of other animal species, may be necessary.

In 99.6% of all examined laying hens from stationary free-range units, Kaufmann et al. (2011) found helminths. It can be assumed that changing sites regularly avoids an excessive accumulation of parasites in the outdoor run. However, if the actual prevalence of endo-parasites is lower in hens kept in mobile houses, remains to be studied.

Local accumulations of nitrogen (2086 kg N/ha) were found in the areas next to the barn of a stationary free-range unit when the distribution of the hens in the outdoor run was uneven (Elbe et al., 2005). In contrast, nitrogen loads were within the allowable range (172 kg N/ha) when the hens frequented the whole area equally. Fürmetz et al. (2005) showed that moving a fully mobile house even in winter, when vegetation was low, had positive effects of the nitrogen load. When sites were changed after six weeks instead of three months, the nitrogen load decreased from 37.4 mg N/kg soil to 24.7 mg N/kg soil. However, due to the experimental period of one year no conclusions can be drawn concerning long time effects of accumulation. Furthermore, there is little information on the nutrient load under the mobile house (systems with open bottom) and under different local conditions. According to a survey by Fuhrmann et al. (2011), farmers did not analyse the nutrient load of the outdoor areas routinely.

Another challenge is the cleaning and disinfection of mobile houses. In contrast to static barns connected to the sewage system, the potentially contaminated wastewater and disinfectants are often directly carried into the soil. At present, there are no studies on possible hygienic and environmental effects of this practice.

PREDATORS

Similar to static free-range units, predators are one of the main reasons for losses in mobile housing systems. Investigations by Andersson and Kaufmann (2016) showed that 15.9 to 17.7% of flock mortality was caused by a fox. Common strategies against ground predators, such as buried fences or double and triple fencing are hardly feasible for mobile runs. Therefore, portable wire nettings with small mesh size and electric wire fences may be more suitable. It is also important to check and repair the fencing regularly. Since some birds of prey like hawks may use trees and hedges in the outdoor run as starting points for hunting, smaller bushes and shrubbery offer better shelter from aerial predators. However, they limit the alternative use of mobile outdoor runs. Thus, natural and artificial shelters or covers should be easily movable along with the mobile house. In addition, smaller outdoor runs can be fully covered by protection nets for chickens. Further recommended measures, such as adding cocks to the flock, scarecrows or shiny objects are often not effective in the field.

ECONOMIC ASPECTS

An advantage of mobile houses is that farmers can start in the egg production sector with small flock sizes. However, the investment costs per hen as well as the high labour demands, especially for small units with less automation, must not be underestimated. Owners of mobile houses reported daily work times ranging between nine minutes and four hours (Trei et al., 2015). In addition, five minutes to 20 hours, and one minute to ten hours were needed for moving the houses and the fences, respectively. Since investment and running costs are relatively high, minimum prices of 34 cents/egg have to be realised for flocks with 200-300 hens (Böttcher, 2015). According to a survey by Böttcher (2015) 60% of the eggs produced in mobile houses were directly sold to the customer achieving prices of 30-50 cents/egg. Similar prices of 24-62 cents/egg in the organic sector were reported by Trei et al. (2015). However, with 15-28 cents/egg the proceeds for eggs produced in conventionally managed mobile houses were considerably lower (Trei et al., 2005).

CONCLUSION

With appropriate management, mobile houses for laying hens can be an animal friendly and environmentally sound alternative to static free-range systems. However, the investment costs as well as the high labour demands, especially for small units with less automation, must not be underestimated. In addition, present gaps in knowledge should be filled through further, interdisciplinary research. Building on that, detailed recommendations for farmers, veterinarians and the competent authorities should be developed.

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PROTOCOL TO ASSESS WELFARE IN DAIRY SHEEP AND DAIRY GOATS

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SUMMARY. It is of great importance in today's globalised society the growing concern of consumers for the bioethical implications of systems and ways of producing food from animal origin. The present research aims to generate an instrument to evaluate the animal welfare of dairy sheep and dairy goats in two different farms, A and B. The objectives of this study were: to evaluate the general management to determine the health status of the animals, to observe if the five welfare freedoms are fulfilled, and to determine if they have comfort in their respective facilities. The methodology used was the design and validation of a protocol to measure Animal Welfare in dairy sheep and dairy goats. The application was an effective tool for measurement of Animal Welfare, obtaining the following results: farm A reached 78 points against 85 obtained by farm B; the proposal goes beyond getting the animals to enjoy a state of health that can manifest all their productive potential and that allows them to face successfully reproductive events. These results aimed to provide farmers tools to measure animal welfare and thus obtain products in the best sanitary conditions, and conditions with favourable repercussions for their economy, and at the same time helps producers to have arguments that allow them to morally justify their strategies productive.

INTRODUCTION

Animal welfare is profitable because animals that are not afraid are more productive. When a dairy animal suffers abuse, an electric grip is applied to it or it is struck by what it is afraid, which causes an increase in the secretion of cortisol, a hormone associated with stress. This physiological events decrease the immune function, which makes animals more susceptible to the disease and also causes a drop in milk production (Grandin, 1993). Animals panic if they begin to slip. An anti-slip floor is essential for handling low-stress cattle, because quiet animals are handled more easily. Slips tend to be the main problem in small enclosure spaces, such as corrals, milking parlours and sleeves. Goats prefer dry sites. Therefore, the preferred habitats of goats are commonly found in dry and semi-humid areas. When the goats are forced to live in humid environments (which favour watering), the hoof tissue softens. The aim of this study is to validate a protocol to measure animal welfare in dairy goats and dairy sheep.

MATERIALS AND METHODS

The methodology used was the design and validation of a protocol to measure Animal Welfare in dairy sheep and dairy goats, taking as a reference point the protocol accepted in Europe. The protocol was divided into six areas: environmental, accommodation and facilities, milking parlour and anteroom, floor, food and water and animals. In addition, a protocol was elaborated with criteria of absence or presence of conditions in the farm that included lodging, feeders and drinking corridors, zone of descent among others. Individual behaviour and health was also observed in a sample of 10 animals.

Instruments, tools and materials:

- Tape measure (to measure cubicles, ships and feeders).
- Thermometer to measure body temperature in both species.
- Stethoscope to measure vital signs (respiration frequency and heart rate)
- Ethogram
- Stopwatch
- APA with the Animal Welfare Indicators
- Bio-productive and agro-meteorological records (ambient temperature, relative humidity, precipitation) from 2011 to 2015.

The thermohychrometric index (THI) was estimated using the following formula:

$THI = 0.81 \times A^t + Hr/100 \times (A^t - 14.4) + 46.4$, where:

THI = Thermohychrometric index

Hr = Relative Humidity

A^t = Ambient Temperature

The ambient temperature and relative humidity were averaged using 5 previous years (See Annexes) and its interpretation was done through the following table:

ITH	Interpretation	AW Scale Score
<72%	No risk of caloric stress	5
72 – 78 %	Light Stress	4
79 -89 %	Severe stress	3
90 -98%	Very severe stress	2
>98 %	Death by heat stroke	1

THI Tutorials, Accommodation - Installation, Health and Behaviour were applied, each of which had a scale with the categories Bad, Fair and Good. All data captured were entered into a computer program in an Excel data base, once installed the tutorials were evaluated by each indicator.

RESULTS AND DISCUSSION

Todaro et al. (2015) mention that poor ventilation, bed type and surface hygiene may be a limitation factors on the yield of the animals and their health. The first step in solving plant problems is to distinguish between serious design errors and easily corrected faults. Adequate ventilation, easy access to both food and water, and the ability to properly observe animals in confinement are also mandatory.

In farm A is carried out the breakdown, which is a good practice mainly in times of rain, as it favours the prevention of lameness problems which were not observed during the evaluation. In farm B this practice is not carried out due to the large number of animals that exist, despite this, the animals do not

suffer lameness. The application was an effective tool for the measurement of Animal Welfare obtaining the following results: farm A reached 78 points against 85 obtained by farm B out of 100 total points.; the proposal goes beyond getting the animals to enjoy a state of health that can manifest all their productive potential and that allows them to face successfully the reproduction.

In the environmental criteria only the temperature was rated very good within five indicators. In the criteria of facilities one indicator was optimal and two good indicators within seventeen indicators. The room and before milking room two indicators were good, an optimal indicator of a total of six. Regarding the floor had one indicator as optimal and another indicator as very good out of a total of six. Regarding the criteria of absence or presence of conditions on the farm of a total of 32 indicators, only in four aspects was the absence of desirable conditions detected and the presence of mastitis was detected.

Although ambient temperatures are low on farm B and high on farm A, the THI (Temperature and Humidity Index) is optimal and this is reflected in the body temperatures recorded in the samplings of the animals, which are inside of the normal parameters. Low temperatures on farm B are likely to cause the respiratory signs (sneezing and coughing) observed in sheep.

CONCLUSION

These results aimed to provide farmers with tools to measure animal welfare and thus obtain products in the best sanitary conditions and conditions with favourable repercussions for their economy, and at the same time help producers to have arguments that allow them to morally justify their strategies productive. Currently there is not much information on animal welfare in sheep and dairy goats, so it would be advisable to continue investigating their biology and behaviour and not lose sight of assessing these two farms in a certain time, to make an improvement in the aspects that were deficient.

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INFLUENCE OF CONTINUOUS ENVIRONMENTAL ENRICHMENT ON AGGRESSIVE BEHAVIOUR OF PIGLETS

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SUMMARY. In conventional pig production, environmental enrichment is expected to have positive effects on behaviour and performance of pigs. Especially a continuous availability of enrichment from birth to the end of the rearing period may have a positive impact on piglets' behaviour. In order to confirm this assumption, in three batches the agonistic behaviour and lesion score of 141 piglets originating from 12 litters kept in two different environments (E) were investigated. For two litters per batch different enrichment materials were available in the farrowing pen. Ten piglets from these two litters were mixed after weaning in a flat-deck pen for rearing with the same enrichment materials (EE). At the same time, 10 piglets from two unenriched litters in the farrowing pen were mixed in another pen in the same flat-deck without increased enrichment (CE). Evaluation of the lesion score one day before weaning showed a lower score in EE piglets ($P < 0.05$). Behavioural analysis of the first three days after weaning showed a lower frequency of aggressive interactions in CE groups than in EE groups on day one after weaning ($P = 0.02$). While there was no difference on day two, more aggressive interactions occurred on the third day after weaning in the CE treatment ($P = 0.017$). Rank order fights between piglets immediately after weaning could not be reduced by continuously increased environmental enrichment. However, enrichment seems to be already useful for suckling piglets, and, when continuously available, it may also have positive impacts on aggressive behaviour in later piglet rearing.

Key words: environmental enrichment, aggressive behaviour, piglet

INTRODUCTION

Conventional housing systems for pig production are usually characterized by manageable pens with limited space, slatted or at least partially slatted floors without litter and restricted feeding systems. The barren environment with scant opportunities for playing and exploring often leads to unsatisfied behavioural needs. Manipulative behaviour towards penmates, behavioural disorders and cannibalism can be the results (Hoy, 2009). Especially piglets frequently show play and exploratory behaviour (van Putten, 1978; Newberry et al., 1988). Play behaviour seems to have an important influence on successful socio-cognitive development. For this, the availability of environmental stimuli is of great importance (Martin et al., 2015). Furthermore, grouping of unfamiliar pigs is a common practice in conventional pig farming. Especially after mixing of piglets at weaning, when litters recently were separated from the sows and faced with change in environment and diet, enormous stress and reduced well-being can arise (Dybkjaer, 1992). Frequent aggressive interactions to establish a new social hierarchy (Friend et al., 1983), occurring for at least three days (Fels et al., 2014) are also stressful for the animals. An approach to enhance animal welfare and to avoid undesirable behaviour is to provide sufficient environmental enrichment. Pigs raised in enriched environments (O'Connell and Beattie, 1999) and especially piglets after weaning show less aggressive behaviour (Schaefer et al., 1990; Blackshaw et al., 1997; Melotti et al., 2011; Ledergerber et al., 2015). Additionally, different studies showed a positive impact of environmental enrichment on behaviour and performance of suckling piglets as well (Oostindjer et al., 2010; Telkänranta et al., 2014). However, for suckling piglets often no manipulable material at all, and for weaners only scant enrichment

is available. In order to achieve long term positive effects on animal welfare, constant environmental enrichment should be offered over the entire rearing period (Martin et al., 2015). Hence, the aim of this study was to investigate the effects of continuous environmental enrichment for piglets from birth until the end of rearing on the skin lesion score before and after weaning and the aggressive behaviour after weaning.

MATERIAL AND METHODS

The study was conducted in three batches, using 141 piglets (BHZP) at the research farm of the University of Veterinary Medicine Hannover Foundation, Germany. Piglets were kept in conventional farrowing pens with crates before weaning and in fully slatted pens for rearing after weaning. For the study, piglets were raised in two different environments. Two litters per batch were exposed to different enrichment materials (straw tower, paper dispenser, chew-bone, ropes) in the farrowing pen (EE). At an age of 35 days, piglets were weaned and five piglets from each of the two litters were mixed for rearing into a group of 10 with same enrichment (EE). At the same time, a second group of 10 piglets was made from two conventionally kept unenriched litters (5 piglets from each litter) which served as control group with only one chain with plastic bite ring as enrichment (CE). After weaning, piglets were individually marked and continuously video recorded for the first 72 hours. Because of technical difficulties in the third batch, recording started at day two. Behavioural analyses were conducted daily between 06:00 h and 21:00 h by analysing aggressive interactions between penmates. Therefore, the total number of fights, the wins, the defeats and the undecided outcomes were noted for each piglet. An aggressive interaction was defined as a fight or a displacement with physical contact initiated by one individual including aggressive behaviour elements followed by any form of submission performed by the opponent (Fels et al., 2014). Furthermore, a skin lesion score was assigned one day before and 4 days after weaning according to methods described by Borberg and Hoy (2009). Thus, a score from 0 to 3 depending on severity, separately for different body regions on the right and left side was determined (head, ear, neck/shoulder, flank, cross, ham and tail). Score 0 was given for no lesions at all, score 1 for few, small scratches (< 5 lesions), score 2 for several, clearly visible scratches or wounds from biting (> 5 lesions) and score 3 for deep wounds or widespread lesions. A scoring index as the sum of all scores was calculated for each piglet ranging from 0 to 39. Statistical analysis was carried out using the software IBM SPSS Statistics, Version 22 for Windows. Data were tested for normal distribution using histograms. One-way ANOVA analysis was conducted in order to find significant differences between the tested parameters. In case of aggressive interactions between littermates and non-littermates t-tests were conducted.

RESULTS

While on the first day after weaning significantly less aggressive interactions occurred in CE groups than in EE groups (21.3 vs. 32.4 interactions per piglet; $P = 0.02$), no significant difference between the treatments could be observed on day two (30.9 vs. 27.9 interactions per piglet; $P = 0.52$). On the third day after weaning, CE piglets showed significantly more aggressive interactions than EE piglets (41.6 vs. 28.9 interactions per piglet; $P = 0.017$; Fig. 1). Results of the lesion score one day before weaning showed a significantly lower mean scoring index in EE piglets than in CE piglets (6.5 vs. 8.0; $P < 0.05$). Four days after weaning, no significant difference was found in scoring index between EE and CE piglets (12.8 vs. 14.3; $P > 0.05$).

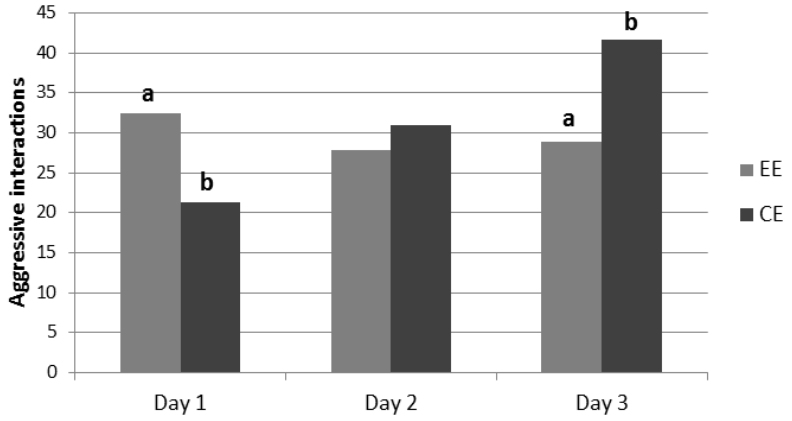

Fig. 1. Mean number of aggressive interactions between piglets from enriched (EE) and conventional (CE) flatdeck pens on day 1, 2 and 3 after weaning. Different letters indicate significant differences within the same day, $P < 0.05$.

DISCUSSION

The aim of the present study was to assess the impact of increased environmental enrichment, which was continuously available for suckling piglets as well as for weaners, on agonistic behaviour and skin lesion score. A lower lesion score in EE groups in the farrowing pen one day before weaning indicates that less manipulative behaviour occurred between piglets in enriched environment. In total, low lesion scores were detected which was expected one day before weaning. Usually, the rank order is already established within the first days of life and no violent fighting occurs afterwards (van Putten, 1978). Hence, injuries resulting from rank order fights were not expected one day before weaning. Previous studies already showed a positive impact of enrichment for suckling piglets by describing reduced manipulative behaviour towards other piglets in enriched environment (Telkänranta et al., 2014). In the present study, additionally a positive impact on aggressive interactions was observed on the third day after weaning, but not on the first two days. Different results were described by Ledergerber et al. (2015). They showed less agonistic behaviour in pens with enrichment within the first 12 hours after weaning. Similar to the results of the present study, Melotti et al. (2011) showed that an enriched environment had a positive impact on aggressive behaviour not immediately after mixing of piglets at weaning but one day later. Blackshaw et al. (1997) observed a positive impact of enrichment on aggressive behaviour over a three week period after weaning, and Schaefer et al. (1990) detected a reduction of aggressive behaviour by enrichment in the second and third, and in the fifth and sixth week after weaning. According to Martin et al. (2015) only a long-term availability of enrichment leads to positive effects on animal welfare. Especially the time immediately after weaning is characterized by frequent aggressive interactions, because unknown piglets are mixed together and a new rank order has to be established (Friend et al., 1983). Fels et al. (2014) showed that particularly the first three days after mixing are important to establish a new social hierarchy. The present investigations suggest that in this sensitive period aggressive interactions cannot be reduced by increased enrichment. On the contrary, the results showed more conflicts on the first day after weaning in EE groups. Possibly, piglets were not noticeably distracted by enrichment known from suckling period and started rapidly the establishment of a new rank order. Meanwhile the CE piglets may be first distracted by the poor enrichment which is still unknown until weaning. Further, it may be that the increased enrichment in EE groups led to more conflicts because of competition. Usually the advantage of a social hierarchy is to reduce aggressive behaviour in competition of resources (Richards, 1974). As the hierarchy was not established yet on the first day after weaning, it might be that the enrichment represented a defensible resource leading to competitive fights. Overall, more behavioural analyses are needed in order to confirm these assumptions.

In conclusion, the results of the present study suggest that a continuous enrichment during the lactation phase and after weaning can be suitable to reduce skin lesions before and aggressive interactions at later times after weaning. This effect can become obvious, especially when the social rank order is largely established after mixing. In which way the materials can distract from aggressive behaviour or, if the rank order is not yet established lead to competitive situations, should be clarified in further studies.

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ABOMASAL SECRETION IN THE MILK-FED CALVES WITH DIARRHEA

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SUMMARY. Digestive disorders in calves in the form of diarrhea are often recorded in the period of mass calving. We declare that the main cause of diarrheas is technological stresses. As a result of infringement of technology and feeding calves, the animal organism meets the stress response. Activation of the sympathetic-adrenal system of the body takes place in the phase of anxiety, resulting in inhibition of gastric secretion. Gastric acid is the natural barrier for environmental microflora entering the digestive tract of young animal per so and prevents dysbacteriosis. The body expels foreign elements beyond. This phenomenon is commonly referred to diarrhea. The aim of this study was to confirm our hypothesis studying the role of abomasal parietal cells in causing diarrheas of newborn calves. 12 milk-fed calves were fitted isolated Pavlov pouches and abomasal fistulas at 10-12 days of age and used within 3-4 months. All animals were fed only with the milk from their dams twice a day and kept in specially designed cages. Diarrhea was induced artificially (feeding cold milk). Aqueous solutions of various substances were administered through fistula and secretory response was observed. Each experiment lasted 5 hours (1h - registration of basic indicators, 2h - instillation of the solutions, 4h – feeding). Abomasal fluid was collected every 20 minutes. The volume, pH, content of HCl and proteolytic activity were measured in hour samples. Three sets of trials were performed: 1) saline in the healthy calves, 2) saline in the diarrheic calves, and 3) acetic acid in diarrheic calves. Experiments have shown occurrence of diarrhea to be closely related to the HCl concentration in abomasal fluid. It appears as a result of technological stresses. 2% solution of acetic acid is a dramatic stimulant of secretory activity of abomasal parietal cells in the preruminant calves after a short latency period.

Key words: calves, diarrhea, abomasum

INTRODUCTION

Digestion of nutrients in the preruminant calves occurs in the abomasum and small intestine. Digestive disorders in calves in the form of diarrhea are often recorded in the period of mass calving. We declare that the main cause of diarrheas is technological stresses. As a result of infringement of technology and feeding calves, the animal organism meets the stress response. Activation of the sympathetic-adrenal system of the body takes place in the phase of anxiety. This results in inhibition of the parietal glands' secretion. It is well known that gastric acid (HCl) is the natural barrier for environmental microflora entering the digestive tract of young animal per os. In the absence of this barrier the gates for environmental microflora opens resulting in insemination of the abomasum and small intestine. These non-typical microorganisms are the cause of dysbacteriosis. It is natural that macroorganism reflex expels foreign elements from the digestive tract beyond. This phenomenon is commonly referred to diarrhea. Therefore, the aim of this study was to confirm our hypothesis studying the role of the parietal cells of the abomasum in causing mass diarrheas of newborn calves. We state the main cause of the mass diarrheas to be non-typical environmental micro flora. These microorganisms enter the GI tract of new-born animal per os and normally all of them are subjected with gastric acid. Thus the secretion of abomasal parietal cells is the natural barrier for environmental micro flora. In the case of any stress occur, the sympatho-adrenal system of the organism excites resulting in the phenomenon of

achlorhydria. So, the "gates for the environmental micro flora" to be open and the latter provokes diarrheic response. That is why it is very important strictly to follow zoohygienic rules of feeding and keeping of new-born animals in the farms. In order to stop the possible consequences of stresses, we offer stimulation of HCl secretion with ecologically pure substances. Moreover, we have carried out the search for possible cures for this disease.

MATERIALS AND METHODS

Trials were performed on 12 milk-fed calves with isolated Pavlov pouches and abomasal fistulas. Calves were surgically prepared at 10-12 days of age and used within 3-4 months. All animals were fed only with the milk from their dams twice a day. During the experiment, animals were kept in specially designed cages. Diarrhea was induced artificially (feeding cold milk). The calf was used once again only in a week after the whole restore. Aqueous solutions of various substances were administered through fistula and secretory response (from the Pavlov pouch) was observed. Each experiment lasted 5 hours (1h - registration of basic indicators, 2h - instillation of the solutions, 4h - feeding with the milk of their dams from pails). It's 7 hours 1+2+4! Abomasal fluid was collected every 20 minutes. The volume, pH, content of HCl and proteolytic activity were measured according to Anson (1932) method on hemoglobin substrate (pH 1.5) during hour samples. To get the empty abomasum one evening feeding before the experiment was missed. The data obtained were expressed in % from basic parameters. 500 ml of 2% aqueous acetic acid was tested as a means of restoring normal secretion in the diarrheic calves. For the experimental design, three sets of trials were performed: 1) saline in the healthy calves, 2) saline in the diarrheic calves, and 3) acetic acid in diarrheic calves. Animal experimental procedures for this study followed the National Institutes of Health guidelines, and the Animal Care and Use Protocol approved by All Russian Institute for Physiology, Biochemistry and Nutrition of Farm Animals.

RESULTS

After the feeding the cold milk to animals, all the calves had symptoms of diarrhea. Moreover, all animals had shown absence of HCl in their abomasal juice. These evidences supported our hypothesis. In healthy calves immediately after the instillation of saline there was an increase of HCl concentration in the abomasal fluid. In the next hour its contents in the secretion of fundic glands increased by 67.1% ($P < 0.07$). Then a slight decline of the parietal cells' secretory activity was noted. But the average content of free HCl does not fall below 13.8 mM. After next meal, a sharp increase of oxyntic glands secretion was registered. In an hour after feeding it rose by 337.7% ($P < 0.001$). Thus, in these sets of trials we showed the typical work of the secretory apparatus in the healthy animals. After instillation of saline into the abomasum of diarrheic calf the HCl concentration decreased on average by 82.3% ($P < 0.05$), and by the fourth hour it was absent in the abomasal fluid. Even feeding the milk to sick animals did not cause its appearance in secretion. Thus an hour after feeding proteolytic activity increased by 98.0% over the previous hour ($P < 0.001$). In the next series of experiments, the secretory response of parietal cells of the abomasum in response to instillation of acetic acid has been studied. In diarrheic calves during the administration of 2% acetic acid the parietal cells' secretory activity was reduced by an average of 19,3% ($P < 0.05$). Later there was a steady increase of abomasal HCl secretion. Thus, in the 3rd hour oxyntic gland secretion increased by 34.1% ($P < 0.05$), and on the fourth hour - by 182.5% ($P < 0,001$) in comparison to the previous hour. However, after feeding the

acidity of the fluid was reduced by 54.6% ($P < 0.05$). Moreover, all the sick animals will recover from the diarrhea in 12 – 18 hours.

We have shown that the occurrence of diarrhea was closely related to the concentration of HCl in abomasal fluid. It appears as a result of technological stresses. 2% aqueous solution of acetic acid is a dramatic stimulant of secretory activity of abomasal parietal cells in the preruminant calves after a short latency period. Such an action of acetate-ion does not preclude the synthesis and release hormone ghrelin. Nevertheless we confirm our hypothesis, further research is necessary. These methods and data are patented in the Russian Federation (Patent of RF N 2228171, Patent of RF N 2335282).

DISCUSSION

The influence of various solutions on the abomasal secretion and emptying abomasal digest into duodenum were studied by many researchers from different countries. All of scientists got similar results, but they differently explained the mode of actions of the therapeutic agents. Zhirkov (1998a, b) showed the dramatic stimulation of abomasal secretion (especially HCl) in the preruminant calves thanks to synaptic action of 0,1M glycine on the wall of abomasum. As for action of the aqueous solutions of sodium acetate, it seems involving the gastrointestinal hormones. Smith et al. (2012) think that phenomenon is due to alkalization. Recommendations based on the results of in vitro studies that bicarbonate- or citrate-containing oral rehydration therapy (ORT) solutions should not be fed concurrently with cow's milk do not appear to be relevant to in vivo conditions when 2 L of a low-bicarbonate (25 mmol/L), low-citrate (12 mmol/L) ORT solution is fed (Constable et al, 2009), as Zhirkov (2001a, b) recommended 2-3 % aqueous solutions to be very effective. Moreover, we suppose the GI hormones are involved in the changes of abomasal secretions, pH of the digest and emptying into duodenum. However, findings of Goodell et al. (2012) support the concept that milk should continue to be fed to diarrheic calves that are being administered an ORT solution in order to maintain growth. More research is necessary to confirm these results.

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USE OF ECOLOGICAL PURE SUBSTANCE IN TREATMENT THE DIARRHEAS OF PRERUMINANT LAMBS

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SUMMARY. It is well known that basic feed digestion in new-born ruminants takes place in the abomasum and small intestine. Technological stresses during the first weeks of life provoke dysfunction of parietal glands in abomasal lumen resulting in achlorhydria. The natural barrier against environmental micro-flora is destroyed and animals get sick with symptoms of dysbacteriosis. Which latter results in diarrhoea and low body weight gain. To prevent diarrhoea, it was decided to stimulate HCl secretion with ecologically pure preparation of sodium acetate. Trials have been conducted in "Khanata" farm (Kalmyk Republic) during the lambing period. Sixty new-born diarrheic lambs of Soviet Merinos breed were divided into two groups (experimental [EG] and control [CG]) and given either 3 % sodium acetate aqueous solution [SAAS] or saline. All medicines (5.0 ml) were administrated orally every morning by the syringe cannula during 7 days. Animals were kept in neighbour pens with their dams according to traditional Kalmykian standard. Body weight (BW) of lambs was measured before and after treating. Animals of EG were ill approximately 3 time less, and gained 32.36 % more against the lambs of CG. It is hypostasized that these beneficiary effects are due to stimulation of the abomasum parietal glands. Days of illness 1.3 ± 0.2 and 3.8 ± 1.1; BW before treating 5.50 ± 0.42 and 6.01 ± 0.41 kg; BW after treating 9.49 ± 1.33 and 8.90 ± 1.76 kg; BW gain 3.09 ± 0.65 and 2.09 ± 0.87 kg for EG and CG, respectively. Many of ghrelin influences as well as vagal effects are similar to the action of acetate–containing preparations. Based in these facts it was hypothesized that 3 % SAAS secretagogue phenomenon involves above mentioned neuroendocrine complex. Sodium acetate at 3% aqueous solution is a viable alternative to stimulate HCL secretion in rearing lambs as its beneficiary influence on animal welfare by reducing illness days and improving performance.

Key words: lambs, rearing, diarrhoea

INTRODUCTION

It's well known that basic feed digestion in new-born ruminants takes place in the abomasum and small intestine. Technological stresses during the first weeks of life provoke dysfunction of parietal glands in abomasal lumen resulting in achlorhydria (Popov, 1932). The natural barrier against environmental micro flora destroyed and animals get sick with symptoms of dysbacteriosis. The latter results in diarrhoea and low body weight gain. The similar processes were shown in preruminant calves (Constable, 2004). To prevent diarrhoea and its consequences we decided to stimulate HCl secretion with ecologically pure preparation of sodium acetate (Khimprom JS). Similar methods of rearing new-born calves (Zhirkov, 2004a) and weaned piglets (Zhirkov, 2004b) are wide spread in Russia.

MATERIALS AND METHODS

Trials have been conducted in "Khanata" farm (Kalmyk Republic) during the lambing period. Sixty new-born diarrheic lambs of Soviet Merinos breed. Treatments included 1) 3 % sodium acetate aqueous solution (SAAS; EG); and 2) saline (CG. Doses (5.0 ml) were administrated orally every morning by a syringe cannula during 7 days. Animals were kept in neighbouring pens with their dams

according to traditional Kamykian standard. Lam body weight (BW) was measured before and after treating. Results are expressed as the means ± S.D. Statistical analysis was performed using Student's *t*–test. Differences between paired values were considered significant at $P < 0.05$.

RESULTS

Animals of EG were ill approximately 3 less times ($P < 0.05$), and gained 32.36 % more weight against the lambs of CG ($P < 0.05$). The corresponding observed mean values were: Days of illness 1.3 ± 0.2 and 3.8 ± 1.1; BW before treating 5.50 ± 0.42 and 6.01 ± 0.41 kg; BW after treating 9.49 ± 1.33 and 8.90 ± 1.76; BW gain, 3.09 ± 0.65 and 2.09 ± 0.87 kg for EG and CG respectively.

DISCUSSION

The better response on health and weight gain of lambs that received acetic acid treatments suggest that acetate containing preparation supplementation could be a stimulation effect of the abomasum parietal glands. Physiological experiments on operated animals showed that acetic acid (as well as 3 % SAAS) have a dramatic stimulant effect on abomasal HCl secretion both in adult sheep (Popov, 1932) as in preruminant calves (Zhirkov, 2007). Moreover, as it was shown before in diarrheic milk-fed calves, pH of abomasal juice was not affected by the infusion of 3 % SAAS. In contrast pH was increased 4.6 times with saline infusion (Zhirkov, 2006). Xu et al. (2016) conducted an experiment to compare the feed intake, digestibility and metabolism in lambs fed low-quality roughage versus lambs fed normal roughage from an early stage of their life; the lower thyroid hormone concentrations observed in low quality roughage suggest an adaptive change occurred in lambs to have a lower basal metabolic rate. Recent progress in the field of energy homeostasis was triggered by the discovery of the hormone leptin and revealed a complex regulatory neuroendocrine network; a late addition is the novel stomach hormone ghrelin, which is the motilin–related family of regulatory peptides (Inui et al., 2004). In addition, ghrelin stimulates appetite and induces positive energy balance leading to body weight gain (Akio et al., 2004). The central nervous system undertakes the homeostatic role of sensing nutrient intake and body reserves, integrating the information, and regulating energy intake and/or energy expenditure. Few tasks regulated by the brain hold greater survival value, particularly important in farmed ruminant species, where the demands of pregnancy, lactation and/or growth are not easily met by often bulky plant-based and sometimes nutrient-sparse diets. Information regarding metabolic state can be transmitted to the appetite control centres of the brain by a diverse array of signals, such as stimulation of the vagus nerve, or metabolic 'feedback' factors derived from the pituitary gland, adipose tissue, stomach/abomasum, intestine, pancreas and/or muscle. These signals act directly on the neurons located in the arcuate nucleus of the medio-basal hypothalamus, a key integration, and hunger (orexigenic) and satiety (anorexigenic) control centre of the brain. Interest in human obesity and associated disorders has fuelled considerable research effort in this area, resulting in increased understanding of chronic and acute factors influencing feed intake. In recent years, research has demonstrated that these results have relevance to animal production, with genetic selection for production found to affect orexigenic hormones, feeding found to reduce the concentration of acute controllers of orexigenic signals, and exogenous administration of orexigenic hormones (i.e. growth hormone or ghrelin) reportedly increasing DM intake in ruminant animals as well as single-stomached species ([Roche et al. 2008). Many of ghrelin influences as well as vagal effects are similar to the action of acetate–contained preparations. Result suggest that HCL secretion could be stimulated by acetate containing preparations (Popov, 1932), contributing to digestive tract stabilisation to reduce the diarrhoea events permits an appropriate nutrient absorption and stimulating intake. Increase in weight gain is explained by an appropriate nutrient abosptions and high dry matter intake. These facts made us to assume hypothesis that 3 % SAAS secretagogue phenomenon involves above mentioned

neuroendocrine complex. 3 % SAAS might be recommended in practical using in rearing lambs as its beneficiary influence on animal welfare.

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STUDY OF DIFFERENT LABORATORY METHODS FOR DIAGNOSIS OF BOVINE LEPTOSPIROSIS

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SUMMARY. Leptospirosis is a bacterial disease caused by pathogenic microorganisms of the genus Leptospira. It is a zoonotic disease of worldwide distribution. The objectives of this study were to determine the analytical sensitivity (SeA) of the direct immunofluorescence (DIF) test and bacteriological cultures as well as to compare the microscopic microagglutination test (MAT) with the DIF. To measure the SeA, negative bovines housed in the DILAVE were selected and sample. Urine and blood samples were taken from 10 animals. Each of the samples was seeded in EMJH medium with and without 5-fluorouracil and inoculated with Leptospiras reference strain from the DILAVE, in addition 7 serial dilutions were performed on base 10. The DIF was performed at all dilutions. Blood and urine to perform MAT and DIF, were taken from seropositive herds with history of abortion and leptospirosis. The SeA was analyzed from regression "probit". To measure the agreement between the MAT and DIF, the Kappa index was used. Experimentally inoculated leptospires grew in both culture media however the SeA was higher with the selective culture medium. The sensitivity for the DIF was 0.70. There was a "poor" agreement between MAT and IFD (kappa = 0.17). To be detected by the DIF animals must be eliminating high concentrations of leptospira in urine of at least $1x10^5$ leptospiras/ml. At the time of isolation, the selective medium is recommended, it has greater sensitivity and less contamination, the leptospiras are difficult to grow and the cultures must be incubated for long periods of time.

INTRODUCTION:

Leptospirosis is caused by different pathogenic strains of *Leptospira spp*. It is one of the most widely distributed zoonotic diseases. (Adler & de la Pena Moctezuma, 2010).

Different surveys show its growing impact on both human and animal health. Problems associated with its diagnosis in severe cases presently tend to underestimate its prevalence, which is officially situated in about 500.000 annual cases of human leptospirosis with lethal rates that exceed 10% (WHO, 1999)

The highest occurrence of leptospirosis is in those countries or areas with tropical or subtropical weather, especially due to the high humidity conditions that are favorable for the survival of the germ.

The infection is transmitted to the human being by direct contact with infected animals (urine) or indirectly through contact with contaminated water, soil (Gil & Samartino, 2000). While it is true that the disease is endemic in several countries, it is often presented as outbreaks, causing severe diseases, in humans as well as in animals, and it might be fatal. All species of domestic animals are susceptible to have leptospirosis. The presence of asymptomatic animals of the disease, domestic animal or a wild one, is necessary for its transmission. In bovines, the main symptoms are abortions or births of weak calves, a decrease in the production of milk and death in young animals. The aborted fetuses do not present pathognomonic signs, even though there is a wide autolysis. All ages and categories are susceptible of the disease. The clinical symptoms are fever during 4 to 5 days, anorexia, conjunctivitis and alteration of the milk secretion among others. Abortions occur between the first and third post-infection week, especially in the last third of the gestation period. There is placental retention in up to 20 % of the aborted animals. (Faine & Adler, 1999).

The genus Leptospira has been divided in 20 different species according to genetic criteria (Brenner et al, 1999). These species include saprophyte, pathogenic or intermediate ways. Different strains of 8 out of these 20 species are considered as the main etiologic agents of the disease, with a strong prevalence of L. *interrogans* and *L. borgpetersenii*. (Ahmed *et al.*, 2010). Presently, over 200 pathogenic serovars are recognized, in groups of 24 serogroups (Cerqueira & Picardeau, 2009), with permanent expansion, as long as the methodic classification studies extend to new regions and/or outbreaks.

In Latin America, the predominant serovars are closely related to the affected species, for example, in bovines, are *Pomona y Hardjo, follow* by Canicola e *Icterohaemorrhagiae*, with geographical variations. In Uruguay, *Pomona* y *Hardjo* (*L. interrogans)* are the serovars that more prevalent in our herds: 53 % of the samples that arrive to the official laboratory of the Ministry of Livestock, are positive for MAT a leptospira. (Suanes & Gil, 2013). Serological techniques are used for the diagnosis of leptospirosis, each one with different sensitivities and specificities. (Postic *et al.*, 2000). However, the MAT developed by Martin & Petit (1918), remains being the reference method (Borg-Petersen, 1949; Borg-Petersen & Fagraeus, 1949; Watt *et al.*, 1988; Postic *et al.*, 2000), and measures antibodies against leptospirosis in the blood serum. While it is true that it is the "gold standard" technique due to its high specificity (99%), it shows a low sensitivity that may range according to the state of the disease. On one hand, it may be useful for the diagnosis of leptospirosis in certain situations (severe cases) but, on the other hand it might make it difficult in other cases for example in the chronic and subclinical disease. The Direct Immunofluorescence Test in the urine of bovines has been used for the diagnosis of *Leptospita hardjo,*with a sensitivity of between 89% and 93% (**Error! Reference source not found.** The animals with subclinical disease are the hardest to diagnose and the big spreaders of the disease.

Repiso et al (2001) performed a research related to reproductive diseases that affect beef cattle, with a seroprevalence of 38.5% in beef cattle. In 2003, Gil et al performed a monitoring of animal health in the south of Uruguay (San José, Colonia and Florida), finding that seroprevalence varied according to the geographical area from 11-50%.

MATERIAL AND METHODS:

During 2015-2016 urine and blood samples were taken from 10 bovines negative to MAT, with no antecedents of the disease, which belonged to the Dirección de Laboratorios Veterinarios (DILAVE). Each urine sample was culture in EMJH medium with and without 5- fluorouracilo (selective and non-selective media respectively). Was prepared a mix of leptospiras obtained from the strains stocks of the DILAVE (Leptospira Canicola, Grippotyphosa, Hardjo prajitno, Icterohaemorrhagiae, Pomona) and also 7 serial dilutions on base 10 were performed. To formulate the mix, a counting of Leptospiras in the Petroff Hausser Counting chamber was made. They started from a concentration of inoculation of Leptospiras of $1x10^{7}$ leptospiras/ml. Likewise, two tubes of media EMJH and the Leptopsiras are prepared with a final dilution of 1/50, tubes A and B respectively. The tubes were incubated at 29ºC and were weekly observed under the dark field microscope at 40X in order to detect the growth of leptospiras in each dilution and the level of contamination. . Both variables were classified in a scale from 1 to 3, the tubes were discharged once they reached the level 3 of growth and contamination.

The urine samples were also used to make samples for DIF which was performed in all the dilutions. The inoculated urine samples were put in a special slide together with a positive (mix of leptospiras half diluted) and negative (sterile PBS solution) control. The samples were incubated for 30 minutes at 37ªC, then were fixed with acetone for 10 minutes. Two washings of 10 minutes each were made with PBS pH 7, 4 and finally add the conjugate provided by the USDA (Antibodies united by **Fluorescein**

isothiocyanate (**FITC**). After that, they were incubated 1 hour at 37ºC in humid chamber, the washing procedure was repeated and the slides were let to dry at room temperature avoiding light. Finally they were mounted in glycerin, the coverslip was set, and they were observed in a fluorescence microscope with a 60X objective lense. Once the DIF protocol was validated with the experimentally inoculated samples, it was also validated in the field. Urine and blood samples of 96 animals from establishments with backgrounds of leptospirosis and abortions were taken. Urine samples for DIF and blood samples for MAT technique were taken. The analytic sensitivity of the DIF technique and the bacteriological culturing, was analyzed from the "probit" regression. On the other hand, to measure the agreement between the MAT and the DIF, the Kappa index of Cohen was used.

RESULTS:

It was observed that the experimentally inoculated leptospiras grew in both media culture, however, the analytic sensitivity was higher in the selective culturing media (EMJH + 5FU). Inside the selective medium, tube B (more diluted) showed better analytical sensitivity than tube A. The analytical sensitivity in dilution 3 ($1x10^5$ leptospiras/ml) was 0.72 for tube A and 0.92 for tube B.

The sensitivity for DIF was 0,70 (dilution 3). There was "poor" agreement between MAT and DIF (kappa = 0,17). In order to be detected by IFD, animals should eliminate high concentrations of leptospira in the urine of at least $1x10^5$ leptospiras / ml, which could make diagnosis difficult to make in poor eliminating animals.

It is concluded that, in the moment of isolation, the selective medium is recommended, since it has more sensitivity. A factor that may have affected the isolated of leptospiras was the level of contamination. For this reason, the use of selective medium and the dilution of the sample was better. The leptospiras are difficult to growth and the cultures must be incubated for long periods of time, therefore, the selective media with inhibitors will decrease contamination improving the growth of leptospiras. The DIF technique showed low sensitivity, with the drawback of the presence of false negative animals that may be shedding low concentrations of microorganisms. This technique might be used as a complement of the MAT technique and performing a sampling of the animal population to a farm level and not as an individual diagnosis.

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TURMERIC AS AN ANTHELMINTIC ALTERNATIVE IN BACKYARD GOATS

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SUMMARY. Gastrointestinal parasitic infections in animals have a great impact in small ruminants. The misuse of antiparasitic agents has caused resistance towards them. *C. longa* powder, which is better known as turmeric, has been proven to have antiparasitic, antibacterial, antivirus and antifungal effects. The aim of this study was to demonstrate the antiparasitic efficacy of turmeric after its administration to backyard goats that were naturally-infected with gastrointestinal parasites. Ten goats with a mean weight of 30 kg, were given 50 mg/kg of live weight of turmeric during four consecutive days. Animals were sampled on days 0, 30 and 60. The flotation fecal and McMaster techniques were carried out to found and quantified eggs, were we found strongylid, *Strongyloides papillosus, Moniezia* spp., *Nematodirus* and *Trichuris ovis* eggs. In order to determine differences throughout time, a Huynh-Feldt test was used, and the Tukey analysis was performed for multiple comparisons. Regarding nematodes, *Haemonchus contortus, Oesophagostomum* spp., *Chabertia ovina, Tricostrongylus spp., Cooperia* spp. and *Nematodirus* spp. larvae were identified. Results demonstrated that the administration of turmeric at a dose of 50 mg/kg is a reliable alternative to reduce the shedding of strongylid eggs in backyard goats.

Key words: Turmeric, *Curcuma longa*, Nematodes, Goats

INTRODUCTION

Infections produced by gastrointestinal parasites, such as helminths and protozoans in goats, is one of the main causes of economic losses (González-Garduño et al., 2003; Molento, 2009) as they reduce meat, milk, or wool production (Alberti et al., 2014; Blackburn et al., 1991). Animals are therefore affected because parasites damage the abomasal and intestinal mucosal tissue, hence decreasing the nutrient absorption. In severe cases, anorexia, weight loss, diarrhoea and anaemia are observed (Hoste, 2001). This causes a decrease in overall performance and directly affects producing costs (Githiori et al., 2006).

The most used drugs with activities against gastrointestinal parasites are: macrocyclic lactones, ionophores, sulphonamides, quinolones, symetric triazinones and benzimidazoles (Ruiz et al., 2012; Sangster, 2001; Taylor, 2002). Currently, an increasing resistance towards anthelmintics and anticoccidials has been resported, mainly due to the inappropriate use of these drugs (Sangster et al., 2009). Wardhaugh *et. al* (2001) have reported that drugs accumulate in the host edible tissues as well as in the environment. As a consequence, alternative methods have been studied to control gastrointestinal parasites in goats (Chartier et al., 2000; Torres-Acosta et al., 2012; Torres-Acosta and Hoste, 2008).

During the last years, the use of *C. longa* as a feed additive has been researched to decrease the deleterious effects caused by coccidia in aninmals, essentially in poultry (Kim et. al, 2013; Rajput et. al, 2014). The main component of *C. longa* is curcumin, which is found in the rhizome and is a low-

molecular weight yellow-colored polyphenol. Curcumin is commonly used as a spice in the Asian culture due to its organoleptic and therapeutic properties, particularly as a cutaneous and hepatic protector (Del Prete et al., 2012; Oner-İyidoğan et al., 2013). The main pharmacological activities of curcumin are the antibacterial effects against Gram-positive bacteria (Lutomski *et al.* 1974), anti-inflammatory and antioxidant properties (Cho and Park, 2015; Mesa et al., 2000; Subramanian *et al.* 1994). Cervantes-Valencia et al. (2015, 2016) demonstrated that the administration of *C. longa* decreased *Eimeria* spp. oocyst shedding in naturally-infected rabbits and sheep. The antinematodic effects of curcumin have been described for *Toxocara canis* (Kiuchi et al., 1993), *Setaria cervi* (Nayak et. al, 2012) and *Schistosoma mansoni* (Allam, 2009).

In order to study a natural, non-toxic, biodegradable and economic alternative to control gastrointestinal parasites in goats, the present study was carried out using *C. longa* powder.

MATERIAL AND METHODS

The study was carried out in the community of San José del Progreso, Oaxaca, México, in the region of Valles Centrales (96º 41´ long W, 16º 41´ lat N) at 1580 meters over sea level. This is a temperate-dry to semi-warm weather with a mean annual temperature of 21º C.

Thirty mixed-breed 2-year old goats, with a mean weight of 30 kg were initially included in the experiment. Nevertheless, only 10 animals remained until the end of it, because the other 20 were sold. Diet consisted mainly of grazing, which was carried out in the adjacent zones of a dam named Bayito and on the mountains. At night time, the animals were confined in a pen of 6m x 8m, which contained soil and was covered with a 6m x 3m roof.

Before the experimental treatment began, each goat was sampled for feces directly from the rectum using a lubricated plastic bag. Samples were kept in refrigeration until further use. Two samples were performed, one after 30 days of the *C. longa* administration and the other 60 days after the ingestion of this spice.

Samples were examined using the flotation fecal technique to confirm the presence of parasites. Afterwards, eggs were counted using the McMaster technique (Gordon and Whitlock, 1939). The amount of eggs was expressed as eggs per gram of feces (EPG).

Helminth-positive samples were processed for generic identification of stage-3 larvae using the fecal culture technique (Hulínská, 1969; Liébano et al., 2011).

In order to ease the administration of *C. longa* powder to the goats, crackers were made with 50 mg/kg live weight *C. longa* powder, wheat flour, water, sugar cane distillate and a pineapple flavoring essence (Cervantes-Valencia et al., 2016). Goats were given these crackers during 4 consecutive days.

Data analysis: This study corresponded to a single-factor with three levels within subjects. A mixed univariated model was used. The Mauchly test was performed and no sphericity was rejected (natural logarithm of strongylid eggs, $x^2 = 2.8272$, $p = 0.2432$)

To determine differences throughout time, the adjusted test of Huynh-Feldt was used, and in case that a significant difference was found, the multiple-comparison Tukey test was carried out (Kuehl, 2001; Maxwell and Delaney, 1990). Prior to analysis, egg quantities were transformed to a natural logarithm (log [HPG+1]) to achieve normality of residuals. The approval for the use of animals in this study complied with the guidelines of the animal-care and use committee, namely, the Subcomité Institucional para el Cuidado y Uso de Animales Experimentados of the Universidad Nacional Autónoma de México.

RESULTS

In the fecal flotation technique, strongylid, *Strongyloides papillosus*, *Moniezia* spp., *Nematodirus* and*Trichuris ovi* eggs were found. Regarding nematode larvae, the following species were identified

after incubation of strongylid-egg positive faeces: *Haemonchus contortus, Oesophagostomum* spp., *Chabertia ovina, Trichostrongylus spp., Cooperia* spp. and *Nematodirus* spp. The percentage of strongylid genera of parasites collected on days 0, 30 and 60 are shown in Table 1. Statistical analysis was only performed for strongylid eggs, because scarce eggs were found for the other species.
Significant differences were found in the natural logarithm of the number of strongylid eggs throughout time (Univariate Huynh-Feldt test, $F = 27.89$ $p < 0.0001$). Means and standard errors are shown in Table 2. Tukey multiple comparison results indicated that differences were found in the mean natural logarithm of the number of strongylid eggs between days 60 and 0 and between days 60 and 30 after the ingestion of *C. longa* powder.

Table 1. *S*trongylid genera of parasites collected and mean results McMaster technique (HPG non-transformed) on days 0, 30, and 60 after administration of 50 mg/kg powdered *Curcuma longa* to naturally-infected goats.

	Parasite	Day 0 %	Day 30 %	Day 60 %
Parasite	*Haemonchus contortus*	40	50	50
	Oesophagostomum spp.	22	21	7
	Chabertia ovina	28	12	5
	Trichostrongylus spp.	10	0	5
	Strongyloides papilosus	0	2	6
	Cooperia spp.	0	15	23
	Nematodirus spp.	0	0	4
McMaster technique HPG	Mean ± SD	2825±1769	795±693	95±171

Table 3. Natural logarithm of strongylid eggs shed by goats treated with *C. longa* powder. Different letters within a column indicate significant differences ($p<0.05$)

Days	Mean ± S.E
0	7.5456 ± 0.5807^{a}
30	6.2467 ± 0.5807^{a}
60	1.7045 ± 0.5807^{b}

DISCUSSION

These results obtained are in agreement with findings reported by Kiuchi *et al.* (1993), who observed anthelmintic activity produced by the synergic action of curcuminoids against *Toxocara canis* stage-2 larvae. It is reasonable to speculate that the decrease in the parasitic load might have been due to the toxic effect of curcumin on parasite viability, which might have produce a dose-dependent effect, inhibiting egg production in adults. These alterations in parasite egg shedding were previously reported for *Schistosoma mansoni* (Magalhães *et al.,* 2009). Similar results were observed for *Pheretima posthuma,* in a study in which an alcoholic extract of *C. longa* administered at 10 mg/ml produced paralysis and death of the parasite (Singh *et al*., 2011). On the other hand, it has been previously

demonstrated that *ar*-turmerone, an isolated component of the rhizome of *C. longa*, displays its main activity in vitro, killing nematode larvae in 24 h at a concentration of 25 mg/ml (Valero et al., 2015). The mode of action of curcumin against nematodes has not yet been fully elucidated. Nonetheless, it has previously been suggested that curcumin perturbs microtubule function and assembly via tubulin binding, causing cell death and inhibiting cell proliferation (Gupta et al., 2006). The control for parasitic diseases relies mainly of the use of drugs. However, parasitic species tend to develop drug-resistant genotypes, particularly when these products are given at lower doses, administered for long periods of time or when a single chemical family of a drug is prescribed to animals in several successive productive cycles. Therefore, the search for alternative control measures is prompted, especially if natural and safe products for the host and environment are used. In the present study, *C. longa* powder produced an anthelmintic effect when it was given as in-feed medication in crackers.

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T. PISIFORMIS INDUCES HORMONAL AND BEHAVIORAL CHANGES ASSOCIATED WITH INFECTIVE DOSE

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SUMMARY

The interaction of a parasite with its host initiates processes requiring energy that can be provided by affecting other biological processes, which in turn, may result in behavioural changes. The relation between the extent of the behavioural changes and the magnitude of the infection has been scarcely studied, thus, the aim of this study was to evaluate the relationship between different doses of infection and the behavioural changes induced in the experimental T. *pisiformis taeniasis* in golden hamsters. Groups of nine hamsters were infected with three or six T. *pisiformis* metacestodes. Locomotive activity was measured in an open field test during 21 days after the infection; an anxiety test was performed in an elevated plus-maze with a dark/light area at 7, 14 and 21 days' post-infection, and serum cortisol levels were determined by radioimmunoassay before the infection and after day 22 of the infection. The experiment itself induced modifications on behaviour and cortisol levels in hamsters, with or without a successful infection (Taenia development). An experiment with three metacestodes produced a decrease in locomotive activity and an increase in anxiety in infected animals, while a higher and earlier decrease in locomotive activity and increased anxiety were observed when experimented with six cysticerci. When performing a necropsy, we found a 44% to 55% of implantation of Taenias in hamsters experimented with three cysticerci, and a 22-26% efficiency of implantation of Taenias when tested with six cysticerci. In conclusion, hamsters experimented with metacestodes showed changes in locomotive activity, anxiety behaviour, and serum cortisol level at any dose and whether the parasite is established in the host or not, while the extent of changes on the behaviour depends on the infective dose, but cortisol levels also differ depending on the establishment of the parasite.

Key words: T. *pisiformis*, behaviour, infective dose.

INTRODUCTION

How the parasites could modify the behaviour of their hosts is not fully understood. However, it has been suggested that it takes place through the interference with the neuro endocrine and immune systems using different chemical mediators that interact at genomic and epigenome level (Adamo, 2013; Hernández-Bello *et al.,* 2010). Different parasites produce behavioural and hormonal changes (Edwards., 1988; Hallal-Calleros *et al*., 2013). The relation between the infecting dose and behavioural changes in parasitosis remains unclear (Ramnath, 2009; Ozkan, 2011). The metacestodes of *T. pisiformis* experimentally infected in golden hamsters can originate the adult stage on the intestine

(Toral-Bastida *et al*., 2011). The main objective of this study was to determine the behavioural changes and cortisol levels that at different levels of infection induce, using hamsters as a model.

MATERIAL AND METHODS

All animal procedures followed the animal care and testing practices recommended by Mexican regulations (NOM-062-ZOO-1999). Thirty six nulliparous female golden hamsters (*Mesocricetus auratus*) were randomly assigned to four groups with 9 animals each, and kept in individual cages, fed with balanced rodent pellets (Nutricubos, Purina®, Mexico) and watered *ad libitum*. One group was inoculated with physiological saline; the second was immunosuppressed with a single dose of 4 mg of ethyl methyl prednisolone (MPA, Pfizer) intramuscularly (Flores-Pérez *et al.*, 2002); the third was orally infected with three metacestodes and immunosuppressed; and the fourth was orally infected with six metacestodes and immunosuppressed. Hamsters that were placed in an open field arena were recorded during ten minutes each day during 21 days after the infection; locomotive activity was measured by the number of times the hamster crossed one of the lines painted on the floor (Hughes, 1989). The anxiety test was performed in an elevated plus-maze (Lyte *et. al*., 1998; Moise, 2008) at days 7, 14 and 21 post-infection and measured by recording the time that each animal remained in a bright or dark corridor for a period of five minutes. Serum cortisol was measured individually from a blood sample collected before treatments and after a humanitarian sacrifice via intracardiac, using a commercial coated tube radioimmunoassay kits as indicated by the provider (Immunotech S.R.O.).

RESULTS

The experiment of hamsters with *T. pisiformis* metacestodes that decreased their locomotive activity (Fig. 1a) shows a significant decrease in the mean of the total locomotive activity in both groups of infected animals recorded during 21 days after infection. The decrease in locomotive activity was more noticeable in that hamsters experimented six cysticerci, rather than three. When the necropsy was performed, from all hamsters tested with three or six cysticerci each, some of them became infected (developed Taenia) and others didn't.In order to determine whether the observed changes in behaviour may have been induced by the experiment itself or the establishment of the tapeworm(s) in their intestines.The behaviour between those hamsters with taenia was compared with those without taenia. No differences in the daily locomotive activity were found between these two groups, then, the size of the experiment and not only the successfulness of the infection affect hamster's behaviour. Furthermore, we observed that the bigger the size of the experiment was, the more profoundly it affected their behaviour, given that hamsters tested with three metacestodes showed a decrease in locomotive activity since day 8 post-infection (Fig. 1b), while those tested with six cysticerci showed a higher diminution since the second day post-infection (Fig. 1c). These results are similar in infected animals whom developed taenias and those whom did not (Fig. 1ba and 1c). Similar results were observed in the elevated plus-maze test (Domínguez-Roldan *et al.*, 2016). Increased serum cortisol levels were found in all of the tested hamsters with some differences in magnitude, *i.e.*: an increase of 2.8 times in hamsters experimented with three cysticerci and who had or hadn't developed the infection, .In those experimented with 6 cysticerci but where no one was established; an increase of 4.8 times in the group of hamsters experimented with six metacestodes and whom developed taenias (Fig. 2).

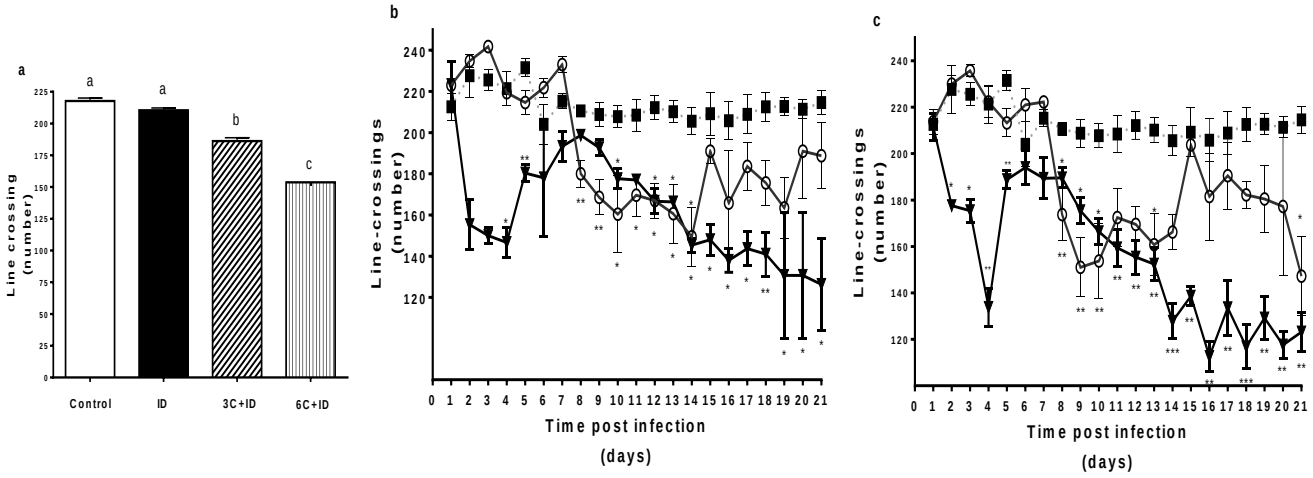

Figure 1. Total locomotive activity (mean±SE) during 21 days post m in all hamsters (a), in immunosuppressed and non-infected hamsters (filled squares), infected hamsters which developed taenia (filled triangles) or did not developed them (empty circles). Animals were infected with three (b) or six (c) cysticerci. Kuskal-Wallis test and Dunn's multiple comparisons post-test (*P≤0.05, **p≤0.01***P≤0.001).

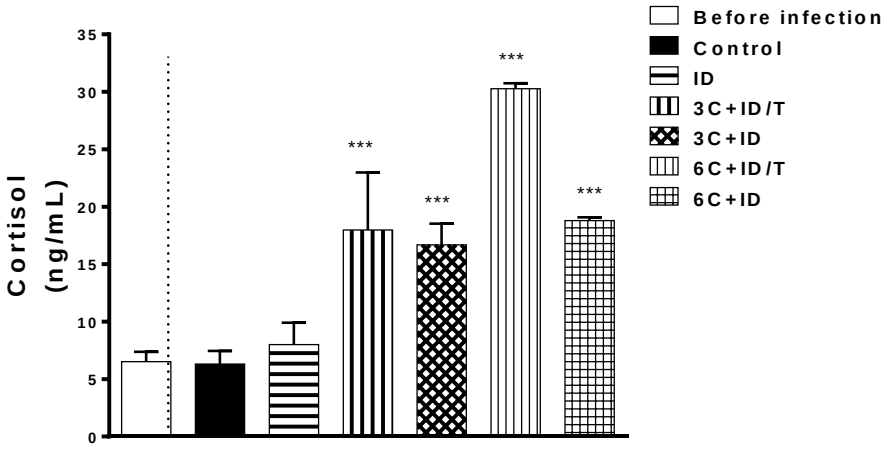

Figure 2. Serum cortisol levels (mean±EE) before infection and after 21 days post-infection. ANOVA and Tukey-Kramer tests (***P≤0.001 compared vs control). Empty bar=before infection, filled bar=control, horizontal lines=immunosuppressed, vertical lines=infected with three cysticerci and developed taenias, diamonds=infected with three cysticerci and did not develop taenias, filled bricks=infected with six cysticerci and developed taenias, empty bricks=infected with three cysticerci and did not develop taenias.

DISCUSSION

The effect of the parasitic challenge on the behaviour activities was observed. Groups of hamsters infected with three or six metacestodes that can develop or not to the tapeworm stage in their intestine exhibited a depressed locomotive activity, a coincident observation was referred previously in rats infected with *Trypanosoma brucei* (Grassi-Zucconi *et al*., 1995) The findings that reported herein are

consistent with changes in the behaviour only due to the experiment, but not necessarily due to the parasitoses itself. Locomotive activity was affected in individuals who developed the parasite and those where it was not established. An increase in serum cortisol levels in tested hamsters was observed when six rather than three cysticerci were employed (2.8 and 4.8 times, respectively). These results were consistent in rabbits observed with a chronic *Psoroptes cuniculi* infection, where at 33 days' post-infection a percentage of infected animals tended to increase compared to the controlled group (Hallal-Calleros *et al*., 2013). Finally, locomotive activity and serum cortisol levels are affected similarly in challenged hamsters, whether the parasite is established in the host or not. The extent of changes in the behaviour and cortisol levels differs depending on the infective dose.

ACKNOWLEDGMENTS

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FINDING OF *Libyostrongylus douglassii* IN OSTRICHES, THROUGH THE IDENTIFICATION OF L111 IN THE STATE OF MEXICO.

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SUMMARY. In Mexico there is little knowledge about diseases that affect ostriches (*Strutio camellus var. domesticus*), mainly parasitic. The most frequent gastrointestinal parasites reported in ostriches are the helminthes. Nematodes of the *Libyostrongylus* genus are small hematophagous worm found below the proventriculus membrane. These parasites are responsible for 50% mortality of juvenile ostriches, occasionally killing adults. The diagnosis of *Libyostrongylus spp.* includes clinical manifestations and finding morphology of typical *Trichostrongyllidae* eggs in feces, but this egg are similar to eggs of *Codiostomun struthionis*, a *Strongylidae* parasite of the cecum not considered pathogenic, so it's necessary make a coproculture to differentiate infective larvae. Previously to differentiate the species of *Lybyostrongylus* genus it was only possible by the characterization of adult parasites obtained from the proventriculus and gizzard during necropsy, but recently research have confirmed that it is possible to differentiate species based on morphology of the infective larva (L111). The aim of the present study was to identify gastrointestinal parasites in ostriches in a private collection. In this collection were sampled four ostriches. A total of 18 fresh fecal samples were collected between August and October 2016. Samples were examined for microscopy through the flotation technique with saturated saline solution. All samples were positive for strongyle egg, and were culture for obtained of infective larvae. A total of 100 larvae were measured and identified, presented mean total length of 845.0 +/- 9.18 μm, and a short sheath tail of 28.6625 +/- 0.8572 μm, all with acute termination and spiny knob at their larvae tail tip. All identified according to their morphology as *Libyostrongylus douglassii*. This is the first time this species has been identified in Mexico based on the morphology of the infective larva.

Key words: *Libyostrongylus douglassii,* L111, ostrich.

INTRODUCTION

Ostrich (*Struthio camelus var. domesticus*, Linnaeus 1758) raising or Ostriculture has been gaining importance in Mexico these last few years (Lozano *et al*, 2008). However, in Mexico there is little knowledge about diseases that affect them. The ostriches are birds belonging to the group of the ratites (Ederli and Oliveira, 2014). These birds originates from Africa, and currently, their commercial breeding has gained economic importance worldwide due to the ability of these birds to adapt to different environments and their lucrative agriculture potential (Tully and Shane, 1996). One of the most common problems in breeding ostriches in captivity is the control of parasitic diseases, particularly those caused by parasites that have a direct lifecycle (Huchzermeyer, 1998; Gomes, 2010). The parasites are responsible for economic losses in the ostrich, by reducing the production and productivity (Oliveira *et al*, 2012). The most frequent gastrointestinal parasites reported in ostriches are the helminthes, *Libyostrongylus spp*., *Houttuynia struthionis* and *Codiostomun struthionis* (Craig and Diamond, 1996; Ederli *et al* 2008a, b). Nematodes of the *Libyostongylus* genus are small hematophagous worm found below the proventriculus membrane of ostriches (McKeena, 2005). Parasitism by *Libyostrongylus spp*. may cause anemia, weight loss, anorexia and proventriculitus.

These parasites are responsible for 50% mortality of juvenile ostriches (Reinecke, 1983), occasionally killing adults (Sotiraki *et al*, 2001). *Libyostrongylus spp.* diagnosis includes clinical manifestations and finding eggs typical of the family *Strongylidae* in feces, but this egg are similar to egg of *C. struthionis*, a *Strongylidae* parasite of the cecum not considered pathogenic, there may be a misdiagnosis (Ederli *et al* 2008c). Genus definitive identification requires coproculture to obtain infective larvae that are morphologically distinguishable (Craig and Diamond, 1996; Ederli *et al*, 2008a,b,c). The genus *Libystrongylus* is actually comprised of 3 species: *L. douglassi, L. magnus and L. dentatus*. Of these, the most common species is *L. douglassii* (Ederli and Oliveira, 2014). *L. magnus* has been reported only in South Africa (Gilbert, 1937) and *L. dentatus* in the United States (Hoberg *et al*, 1995) and Brazil (Bonadiman *et al*, 2006; Ederli *et al*, 2008 a,c). Data about the lifecycle of the genus *Libyostrongylus* refer only to *L. douglassii*, which as a direct lifecycle typical of *Trichostrongylidae*, with a pre-patent period of approximately 36 day (Theiler and Robertson, 1915). These parasites appear to be specific to ostriches; however there was a single report of the occurrence of *Libyostrongylus* in emu (*Dromaius novaehollandiae,* Lathan 1790) in Sweden (Ponce Gordo et al, 2002). Previously to differentiate the species of *Lybyostrongylus* genus it was possible only by the characterization of adult parasites obtained from the proventriculus and gizzard during necropsy, but recently research have confirmed that it is possible to differentiate species based on morphology of the infective larva (Ederli et al, 2008c). *Libyostrongylus* control is curative or preventive similar to that other nematodes of production animals (Santos *et al*, 2010). Pereira *et al* have indicated that both *Libyostrongylus* species have acquired resistance to ivermectin. This also suggests that both species behave very similarly (Pereira *et al*, 2012). The aim of the present study was to identify gastrointestinal parasites in ostriches in a private collection.

MATERIAL AND METHODS

Samples were collected from one farm in Ayapango, State of Mexico. Ayapango is located in the eastern part of the State of Mexico, latitude 19°07′35″ north and longitude 98°48′10″ west; the municipality is at an altitude of 2,450 meters. This area has a semi-humid weather, with an average annual temperature between 12 and 18 ˚C and annual rainfall of 900mm. In this collection were sampled two group of ostriches, each group consists of a male and a female, all them adults. Each group of ostriches are in barnyard of 15 x 17 meters, they have water *ad libitum* and they are fed once a day with a mixture of vegetables with supplements. The samples were collected directly from the floor, in the morning, between August and October 2016, 3 samples for months, for each group. A total of 18 fresh fecal samples were collected. Samples were examined for light microscopy through the flotation technique with saturated saline solution. Those samples positive for strongyle egg, were applied the coproculture technique established by Corticelli and Lai (1963) for obtained of infective larvae. The infective larvae (LIII) were obtained through the Bearmann technique. A total of 100 larvae were obtained and examined, this last procedure was done with a ¨Leica¨ phase-contrast microscopy with 40x optical. Total infective larva and sheath tail length were measure under a light microscopy with the aid an eyepiece micrometers with 10x and 40x optical respectively.

RESULTS

All samples were positive for strongyle egg. The infective larvae obtained were measured, presented mean total length of 845.0 +/- 9.18 μm, and a short sheath tail of 28.6625 +/- 0.8572 μm, all with acute tail termination and the presence of a knob at their larvae tail tip (Figure 1). All the infective larvae obtained in this study were identified according to their morphology of the larvae tip tail and the morphometry of the sheath tail as *Libyostrongylus douglassii* (Table 1).

DISCUSSION

Infection by *Libyostrongylus* is a major parasitism of ostriches. These parasites are responsible for 50% mortality of juvenile ostriches (Reinecke, 1983), occasionally killing adults (Sotiraki *et al*, 2001). Previously to differentiate the species of *Lybyostrongylus* genus it was possible only by the characterization of adult parasites obtained from the proventriculus and gizzard during necropsy, but Ederli *et al* have confirmed that it is possible to differentiate species based on morphology of the infective larvae (Ederli *et al*, 2008c). The infective larvae of the genus *Libyostrongylus* are characterized by the presence of a knob in the larvae tip tail, also the infective larvae of *L. dentatus* had a long and filamentous sheath tail, while larvae from the *L. douglassii* had short sheath tail with an acute tail ending (Bonadiman et al, 2006; Ederli *et al* 2008 a,c; Ederli and Oliveira, 2014). However, the infective larvae of *C. struthionis* also have a long and filamentous sheath tail, but the tail of the larvae inside the cuticle has an acute termination, which is different from the genus *Libyostrongylus* (Ederli et al, 2008b). The total length of the infective larvae is not a good parameter to distinguish the species due to the similarity between nematodes (Ederli and Oliveira, 2014). Following this, it is possible to differentiate the genus and the two species of the genus *Libyostrongylus* by the morphology of the infective larvae tip tail and morphometry of the sheath tail recovered from fecal cultures (Bonadiman, 2006; Ederly *et al*, 2008 a,b,c; Ederli and Oliveira, 2014). The importance of this study is because it was no longer necessary to perform a necropsy to collect adult worm to obtain a specific diagnosis. This is the first time this species has been identified in Mexico based on the morphology of the infective larva.

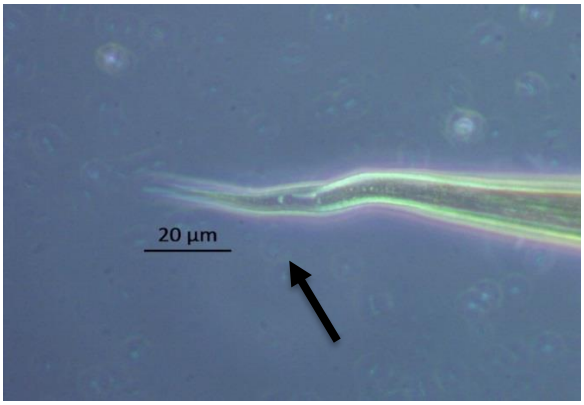

Figure 1. Posterior end of Infective larva. Phase-contrast microscopy; presence of a knob at their larva tail tip (Arrow).

Table 1. Total infective larvae and sheath tail length measurements (μm) and key to the identification of the infective larvae of nematodes of the genera *Libyostrongylus* and *Codiostomum*.

Parameters	Species			
	Study	*L. douglassii*	*L. dentatus*	*C. struthionis*
Total length (μm)	845.0 +/- 9.18 [d]	874 +/- 33.80 [a]	784.47 +/-43.63 [a]	**598.25 +/-25.15 [b]**
Sheath tail (μm)	28.6625 +/- 0.8572 [d]	29.52 +/- 4.11 [a]	61.20 +/- 2.17 [a]	110 +/- 13.46 [b]
Key to the identification of the LIII	Presence of a knob at the infective larva tip tail. Short sheath tail.	Presence of a knob at the infective larva tip tail. Short sheath tail [c]	Presence of a knob at the infective larva tip tail. Long sheath tail [c]	Absence of a knob at the infective larva tip tail. Long sheath tail [c]

[a] Ederli *et al*, 2008c. [b] Ederli *et al*, 2008b. [c] Ederli and Oliveira, 2014. [d] 100 infective larvae were measured. The total infective larvae and sheath tail length were calculated by *SPSS* program.

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EFFECTS OF STOCKING DENSITY AND GENOTYPE ON SOME BLOOD CHEMISTRY LEVELS OF HEAT STRESSED HEIFERS

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SUMMARY

The aim of this study was to determine the effect of two stocking densities on blood metabolite and electrolyte concentrations of feedlot heifers under heat stress conditions. Zebu, European heifers and its crosses (n=510) were randomly assigned to 6 pens, which were divided into two stocking densities: T1) 3 pens with 100 heifers/pen (9 $m^{2/}$animal); and T2) 3 pens with 70 heifers/pen (12.9 $m^{2/}$animal). Average initial body weight was 428±32 kg and blood samples were collected from 15 Zebu and 15 European type heifers from each pen three times (days 1, 28 and 66). Each sample was analysed for electrolytes (Na, Ca, K) and metabolites (cholesterol, glucose, urea, total protein, and triglycerides) using a blood auto-analyser of liquid phase. Data was analysed with a 2x3x3 factorial arrangement under a completely randomized design. The temperature-humidity index (THI) averaged 81.2 units during the study. Na levels of T1 were higher ($P<0.05$) than T2 in samplings 2 and 3 (135 vs 129 and 136 vs 133 mmol respectively). In sampling 1, Cl concentrations of T1 (108 mmol) were lower ($P<0.05$) than T2 (109 mmol), and during sampling 2, T1 (109 mmol) showed lower ($P<0.05$) Cl levels than T2 (111 mmol). In T1, Z and E presented similar K levels (5.06 and 5.00 mmol), but in T2 Z showed higher ($P<0.05$) K levels (5.05 mmol) than E (4.7 mmol) ($P<0.05$). At the beginning of the experiment, urea levels were lower in T1 (22.6 ml/dL) compared to T2 (20.1 ml/dL), however, during samplings 2 and 3 urea levels were similar in both treatments. Glucose and total protein were not altered by treatment, sampling or genotype. Under conditions of severe heat stress, reducing stocking density improved Na and Cl concentrations; while Zebu heifers showed better K concentrations.

Key words: heat stress, feedlot heifers, hematological parameters, electrolytes.

INTRODUCTION

In Mexico, the largest feedlot operations are located in northern states where grain production and cow-calf systems make this activity easier and more profitable. These regions are characterized by the presence of hot summers because they belong to arid and semi-arid zones (Avendaño-Reyes *et al.*, 2011). In the Mexicali valley, located in the north-western state of Baja California, the beef industry

has been historically one of the most profitable livestock activities, and the feedlots primarily fed the heifer's mates of the steers exported to the United States (Peel, 2005). Pen space has been proposed as an issue influencing stress in feedlot cattle, but stress is not easy to measure, especially when space available combines with hot weather in the pens; parameters such as blood serum components and electrolytes are useful indicators of stress (Gaughan *et al.,* 1994). Feedlot cattle under heat stress may fail to adjust physiologically resulting lower animal productivity and a tremendous economic loss for the beef industry (Hahn, 1999; Bernabucci *et al.,* 2010). Therefore, the purpose of this study was to determine the effect of two stocking densities on blood metabolite´s and electrolyte´s concentrations of Zebu and European feedlot heifers under heat stress conditions.

MATERIALS AND METHODS

The study was conducted in a commercial feedlot located in the Ejido Saltillo, Mexicali Valley, B.C., México. A total of 510 feedlot heifers in the finishing phase and weighing 432.05 ± 28 kg were used in this study of 66 d of duration in summer (July to September). Heifers Zebu, European and its crosses were randomly assigned to 6 pens, which were divided into two stocking densities: T1) 3 pens with 100 heifers/pen (area=9 $m^{2/}$animal); and T2) 3 pens with 70 heifers/pen (area=12.9 $m^{2/}$animal). Pen dimensions were 30 x 30 m and shades were oriented N-S. Also, T1 had a shade area of 2.8 m^2/heifer and T2 2.8 m^2/heifer. Each pen has a bunk line in the East side and an automatic waterer. Climatic variables were collected in the experimental site and were used to estimate the Temperature-Humidity Index (THI). Blood samples were collected on 30 heifers selected randomly from each treatment (10 per pen) on the days where weight was recorded (days 1, 30, and 66). From the sampling heifers, 15 were genotype *Bos taurus* and 15 from the genotype *Bos indicus*. Blood samples were collected in vacutainer tubes of 10 ml via venipuncture on the jugular vein before the morning feeding. Samples were centrifuged at 3500 X g for 15 min at 10º C; serum was separated by duplicate in vials of 2 ml and stored at -20 º C for further analysis in the Animal Physiology Laboratory of the ICA-UABC. Blood metabolites were analysed using a blood auto-analyser of liquid phase (Easyvet, KONTROLab, Morelia, Mich., México), and the electrolytes with an electrolyte analyser (LW E60A, Shenzen, China). Data was analysed with a 2x3x3 factorial arrangement of treatments in a completely randomized design. The factors were stocking rate (T1 and T2), genotype (European and Zebu), and sampling time (d 1, 30, and 66); level of error used was 5% and the analysis was performed with the SAS software (SAS, 2004).

RESULTS

The triple interaction was not significant for any response variable. Significant interactions ($P<0.05$) were: treatment x sampling time for Na and Cl (Figures 1 and 2); treatment x genotype for K (Figure 3); and for urea treatment x sampling time (Figure 6). Finally, for cholesterol and triglycerides the sampling time main effect was significant (Figures 4 and 5). Na levels of T1 were higher ($P<0.05$) than T2 in samplings 2 and 3 (135 vs 129 and 136 vs 133 mmol, respectively). In sampling 1, Cl concentrations of T1 (108 mmol) were lower ($P<0.05$) than T2 (109 mmol), and during sampling 2, T1 (109 mmol) showed lower ($P<0.05$) Cl levels than T2 (111 mmol). In T1, Z and E presented similar K levels (5.06 and 5.00 mmol), but in T2 Z showed higher ($P<0.05$) K levels (5.05 mmol) than E (4.7 mmol) ($P<0.05$). At the beginning of the experiment, urea levels were lower in T1 (22.6 ml/dL) compared to T2 (20.1 ml/dL), however, during samplings 2 and 3 urea levels were similar in both treatments. Glucose and total protein were not altered by treatment, sampling or genotype.

DISCUSSION

Heat stress is considered an external stressor caused by thermal environment. The THI obtained from the combination of ambient temperature and relative humidity was in average 81.2 units during the study. Silanikove (2000) indicates that a THI higher than 78 units may cause extreme distress in cattle and is unable to maintain normal body temperature. If tissues and body cells get too hot, metabolism increases. As metabolism rises, there is more metabolic heat production and as a result the metabolism goes even faster. The consequence is a situation called run-away hyperthermia which will conduct to death (Beatty, 2014). In general, serum concentrations of electrolyte and metabolites were within the levels considered normal for cattle (Wood and Quiroz-Rocha, 2010). K, Na, and Cl are cations and anions implicated in the maintenance of acid-base balance (Ronchi *et al.*, 1999). When cattle are exposed to hot conditions, blood concentrations of K and Na tend to decrease and Cl level tends to increase.

RESULTS

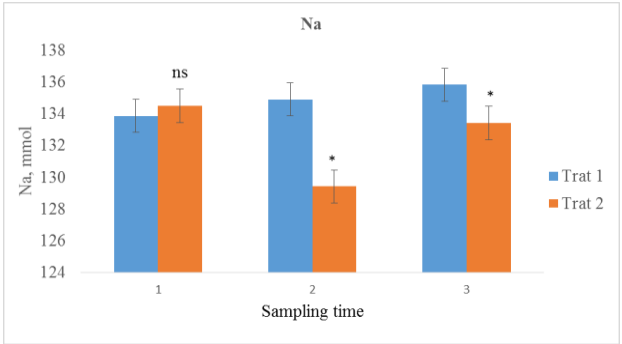
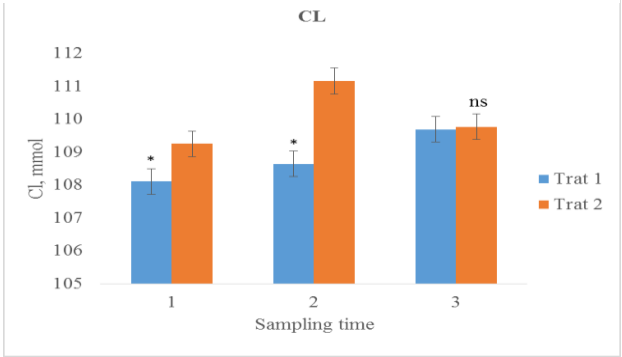


Figure 1. Sodium levels by stocking rate (Trat 1 and Trat 2) on each sampling time (ns=no significant difference; * significant difference P<0.05).



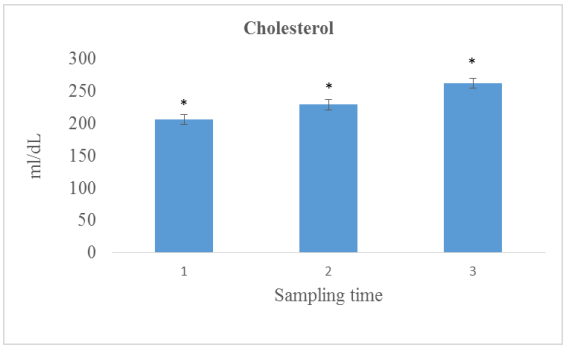
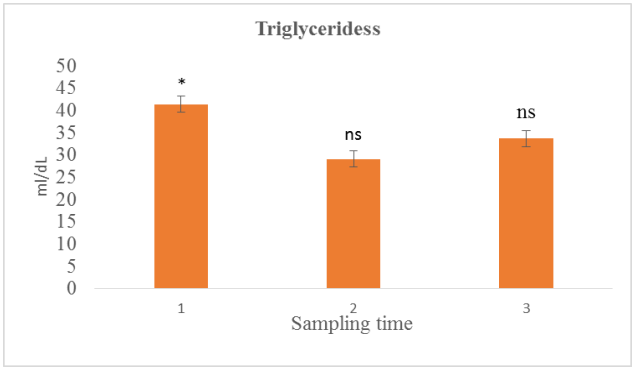
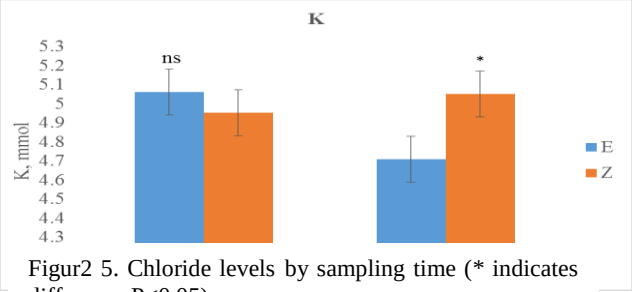


Figure 4. Cholesterol levels by sampling time (* indicates difference P<0.05).





Figur2 5. Chloride levels by sampling time (* indicates difference P<0.05).

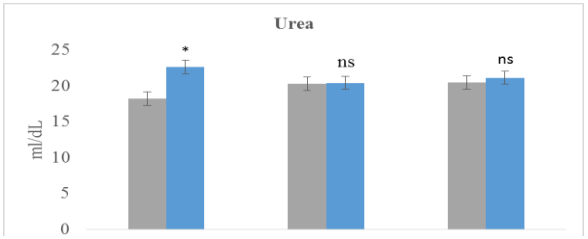


Figure 5. Triglycerides levels by sampling time (* indicates difference P<0.05).

Figure 3. Potassium levels by genotype (European and Zebu) by stocking rate (Trat 1 and Trat 2) (ns=no significant difference; * significant difference P<0.05).

Figure 6. Urea levels by stocking rate (Trat 1 and Trat 2) by sampling time (ns=no significant difference; * significant difference P<0.05).

Thus, cattle exposed to severe heat load may experience acid-base disorders as a result of respiratory alkalosis from intense panting (Schneider *et al.*, 1984). Johnson (1970) exposed experimentally *Bos taurus* and *Bos indicus* cows to heat stress and observed that *Bos taurus* cows show higher sweating rates than *Bos indicus* cows; however, the electrolyte content in secretions from the skin was similar between genotypes. The nature of the responses of heifers to heat stress in this experiment looked similar between *Bos taurus* and *Bos indicus*, but the magnitude of the changes was less intense in the *Bos indicus*, making it a more thermotolerant species. These results agree with Scharf et al. (2014), who evaluated the thermoregulatory capacity of two *Bos taurus* breeds and found that K concentrations were not affected by heat stress (temperatures=night: 26°C, and day:36°C). Under heat stress, panting is associated with a decrease in blood carbon dioxide levels, indicating excessive alveolar ventilation (Mitlöehner et al., 2002). Increases in cholesterol concentrations in heifers of the present study are related to increase in ambient temperature since as time passes heat stress increases.

CONCLUSION

In conclusion, there were no important changes in blood electrolytes or blood metabolites by effect of stocking rate or genotype of beef heifers. It is possible that heat stress was not intense enough to affect these blood components in feedlot heifers.

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EFFECTS OF SHADE ON GROWTH PERFORMANCE OF HAIRSHEEP EWES IN SEVERE HEAT STRESS CONDITIONS

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SUMMARY. The objective was to determine the effects of providing shade to hairsheep ewes during severe heat stress conditions. Twenty Dorper x Pelibuey ewes were individually allotted and randomly assigned to 2 treatments: 10 ewes with shade covered permanently (SH); and 2) 10 ewes with no access to shade at all (NSH). Shade was made of galvanized sheet and installed at 2.5 m height. Ewes were weighed at the start and end of the study; feed and water intake were registered daily, and daily weight gain (DWG) and feed efficiency (FE) were estimated. Respiration frequency (RF) and rectal temperature (RT) were collected at 06:00, 12:00 and 18:00 h every 3 days. Data was analyzed with a randomized block design and a repeated measurements design. While maximum THI in the NSH and SH groups were 92.8 and 88.4 units respectively, average THI were 82.2 and 81.2 for the same groups. Feed intake (1087 vs 964 gr/d), DWG (150 vs 180 gr), and FE (0.112 vs 0.185 units) were similar between SH and NSH ewes, respectively. In contrast, water intake was higher ($P<0.05$) in NSH group (4.5 L/ewe) than the SH group (3.8 L/ewe). At 06:00 h, RF was higher in S (58.0) than NSH ewes (44.7), but at 12:00 (193.2) and 18:00 h (202.6) NSH ewes had higher ($P<0.05$) RF than SH ewes (138.9 and 155 breaths per min). Same results were observed in RT since NS (39.1°C) ewes had lower ($P<0.05$) average than SH (39.6°C) ewes at 06:00 h, while at 12:00 and 18:00 h NSH ewes had higher ($P<0.05$) RT than SH ewes (40.2 and 40.3°C vs 40°C at both times). Productive performance was similar between SH and NSH ewes, but NSH ewes drank more water. Providing permanent shade to individually allotted ewes did not improve their productive performance.

Key words: Hot weather, growth performance, physiological constants.

INTRODUCTION

Global Warming is having a detrimental effect on the farm animal sector because world-wide temperatures are increasing every year. Indeed, average global temperatures have risen markedly so the IPCC have predicted increases of about 1.8 to 3.9 °C by year 2100 (Koneswaran and Nierenberg, 2008). The climate in northwestern México is characterized by high radiant temperatures during 6 months of the year, which directly impact thermoregulation mechanisms of ruminants. Studies of extreme temperatures in Mexicali, state of Baja California, México, have reported that the number of heat waves increased 2.3 times after 1970´s compared to before 1970´s, with greater intensity and duration of those latest heat waves (García-Cueto et al., 2010). This scenario confirms the severity of the Global Warming problem in this specific region of México. Stress from hot environments lowers productive efficiency and welfare of farm animals, which is especially true in confined ruminants so that shade structures play an important role to reduce the great heat load, generated through radiation during the day (Nardone et al., 2006). Among ruminants, hairsheep breeds are considered as tolerant

specie to heat stress compared to dairy and beef cattle because they originated in tropical countries (Marai et al., 2007). However, in the arid region of the Mexicali valley the use of shade for hairsheep has not been tested scientifically. Therefore, the purpose of this study is to characterize the presence of shade using hairsheep ewes evaluating their growth performance and some physiological parameters under severe heat stress conditions.

MATERIALS AND METHODS

The study was conducted at the Sheep Experimental Unit of the Instituto de Ciencias Agrícolas, which belongs to the Universidad Autónoma de Baja California, located in the Mexicali Valley, B. C., México (32.8º N, 114.6º W). Twenty Dorper x Pelibuey ewes of about 4 months of age and 30.4 ± 3.14 kg of weight were individually allotted, blocked by weight and randomly assigned to one of two treatments: 1) 10 ewes with shade covered permanently (SH); and 2) 10 ewes with no access to shade at all (NSH). The individual cages had dimensions of 1.0 x 1.5 m. Shade was made of galvanized sheet and was installed at 2.5 m height. The shaded pen was totally covered with shade cloth in the sides, so no direct sunlight was received by these animals. Diet offered to ewes of both treatments was the same and contained 66% wheat grain, 12% alfalfa hay, 11% wheat straw, 8% soybean meal, 1% limestone, 1% dicalcium phosphate, and 0.5% common salt, which provided 14% PC and 2.8 MCal/kg DM. Ambient temperature and relative humidity were collected with hygrometers placed in the experimental site to obtain the Temperature-Humidity Index. Ewes were weighed at the start and end of the study; feed and water intake were registered daily, so that daily weight gain (DWG) and feed efficiency (FE) were estimated. In addition, water consumption was obtained during the day (06:00 – 18:00 h) and night (18:00 – 06:00 h). Respiration frequency (RF) and rectal temperature (RT) were collected three times, at 06:00, 12:00 and 18:00 h every 3 days. Information was analyzed by period: a) 1 to 7 d; b) 8 to 16 d; and c) 1 to 16 d, which was the complete experimental period. Data on production variables were analyzed with a randomized block design and data related to physiological variables were analyzed with a repeated measurements design including the effects of block, treatment, time, and treatment x time interaction. All the analyses were performed with SAS (SAS, 2004) using GLM and MIXED procedures and the significance level used was 5%.

RESULTS

Table 1 shows the descriptive statistics for the climatic variables. Ambient temperature reached 50°C in the NSH group, while in SH group the maximum temperature was 45°C. The average THI was higher than 80 units for both treatments, but NSH ewes were subjected to a THI > 90 units. Even though relative humidity ranged from 8 to 93%, the average was lower than 50%, which is typical for arid zones. Table 2 shows the average of performance traits by period. Feed intake was similar ($P>0.05$) between SH and NSH ewes in the 3 periods. The DWG was higher ($P<0.05$) for NSH ewes in the first period, but in the second one SH ewes gained more weight ($P<0.05$) in average than SH ewes. However, there was no difference in the total period ($P>0.05$). Water intake was higher ($P<0.05$) in NSH ewes during the 3 periods. However, during the night SH ewes drank more water ($P<0.05$) than NSH ewes, but during the day it was the opposite ($P<0.05$). The same situation was observed for FR and RT (Figure 1) since interaction treatment x time was significant ($P<0.05$). That is, SH ewes had higher ($P<0.05$) RF and RT at 6:00 h, but at 12:00 and 18:00 h NSH ewes had higher ($P<0.05$) RF and RT than SH ewes. In addition, feed efficiency was similar for both treatments ($P>0.05$).

Table 1. Climatic variables collected during the 16 d of the study (July 22 to August 7).

Variables	Shaded	Non shaded

Temperature (°C)		
Average	34.1	35.3
Maximum	45	50
Minimum	20.4	18.6
Relative humidity (%)		
Average	42.7	42.7
Maximum	93.4	93.4
Minimum	8.7	8.7
THI (units)		
Average	81.24	82.24
Maximum	88.39	92.77
Minimum	67.16	65.34

THI = Temperature-humidity index

Table 2. Average of performance traits in shaded and non-shaded ewes by period during the study.

Items	Shaded	Non-shaded	S.E.	P-value
Feed Intake (g/d)				
1 – 7	1064	975	33.6	>0.05
8 – 16	1110	953	76.4	>0.05
1 - 16	1087	964	52.9	>0.05
Water Intake (L/d)				
1 – 7	3.5	4.1	0.16	<0.05
8 – 16	4.2	4.9	0.32	<0.05
1 – 16	3.8	4.5	0.21	<0.05
DWG (Kg/d)				
1 – 7	0.094	0.23	0.05	<0.05
8 – 16	0.21	0.12	0.04	<0.05
1 - 16	0.15	0.18	0.03	>0.05
Feed efficiency (units)				
1 - 7	0.04	0.24	0.06	<0.05
8 – 16	0.20	0.12	0.04	>0.05
1 – 16	0.12	0.18	0.03	>0.05
Water intake (day or night; L)				
Day (6-18 h)	2.8	3.6	0.19	<0.05
Night (18-6 h)	0.95	0.56	0.08	<0.05

S.E.=Standard error; DWG=Daily weight gain;

DISCUSSION

According to Neves et al. (2009), hairsheep exhibit signs of heat stress at THI of 79 units and at temperature of 30°C. The climatic variables of the present study were above these values, so ewes are exposed to a severe heat stress conditions during summer in this region. Early responses to heat stress are considered homeostatic mechanisms and consist of greater respiration and sweating rates as well as water intake, and also reduced feed intake and heart rate (Horowitz, 2002). When heat stress is prolonged, acclimatory homeostasis takes place by reducing catecholamine, growth hormone, and glucocorticoids, endocrine alterations that conduct to reduction in thyroid hormones (T3 and T4), which in turn lowers the basal metabolic rate, and therefore heat production (Bernaboucci et al., 2010).

Ewe´s respiratory frequency in both treatments increased notably during the evening in an attempt to dissipate the excess of heat load, which was also indicated by higher rectal temperatures registered at this time. However, in the morning, shaded ewes increased these same mechanisms because they did not lose heat load gained during the day since radiation could not took place. Water consumption was higher during the night in shaded ewes, but during the day non-shaded ewes consumed more water than shaded ewes. So providing a complete shaded structure did not allow ewes to lose heat by radiation. This is the possible reason why productive performance was not improved in shaded ewes. A similar feedlot performance was reported by Gesualdi et al. (2014) in the finishing phase of male lambs in a partial confinement system.

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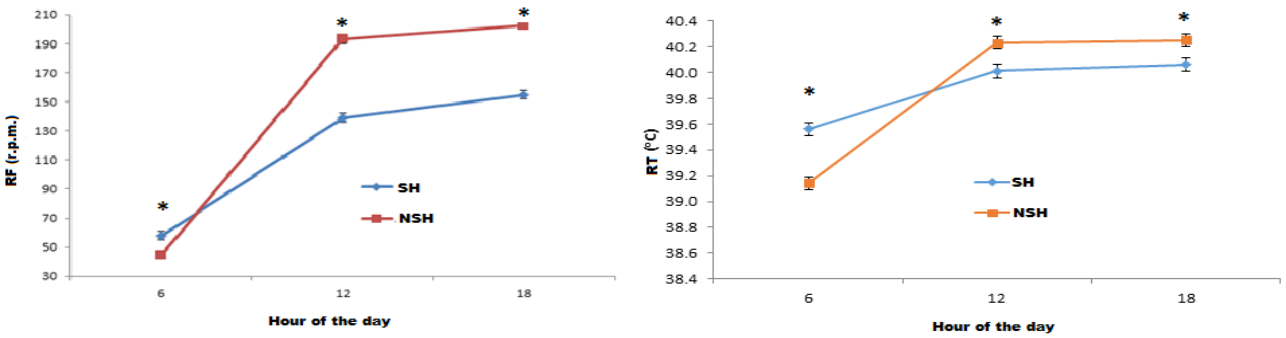

Figure 1. Respiratory frequency (RF) and rectal temperature (RT) by hour of the day in shaded (SH) and non-shaded (NSH) ewes subjected to heat stress during summer (* Indicates statistical difference at 5% error level).

THE USE OF AN ALGAE-BASED COMPLEMENTARY FEED HELPS LIMITING PEDV DAMAGE ON SUCKLING PIGLETS

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SUMMARY. The porcine epidemic diarrhea virus (PEDv) is an enteric disease of swine. Once ingested, the virus rapidly causes enterocyte lysis, leading to decreased nutrient absorption capacity resulting in watery diarrhea followed by a rapid dehydration, anorexia, and, in some cases, mortality. Managing symptoms and initiating a plan for barn inoculation is the best way to control a localized infection. In support to these measures, the use of a complementary feed capable of protecting the intestinal mucosa may be beneficial. The aim of this study was to test the efficacy of such complementary feed, based on algae extracts and montmorillonite clay, on piglet performance in a context of PEDv infection. The trial was performed in a farrow-to-finish farm of 450 gestating sows located in the center of Mexico. Twenty-two litters from the same batch and placed in the same room were involved in the trial. Eleven litters (127 piglets) constituted the control group, which did not receive any complementary feed, and eleven litters (120 piglets) constituted the test group which received the algae-based complementary feed from day one to weaning (21 days) at the dose of 50g/litter/day. The farm was diagnosed positive for PEDv at day eleven of the trial. Results showed that despite a lower birth weight (-160g, $P<0.05$), piglets from the test group had a higher growth rate than control (respectively 187 and 150 g/day, $P<0.05$). In addition, the use of the complementary feed tended to reduce the mortality rate from 26.8% to 14.2% ($P>0.05$). In summary, the use of an algae-based complementary feed in this farm, which was positive for PEDv, helped to improve growth rate and tended to reduce mortality. During the six months following the trial, the farm decided to extend the use of this complementary feed to all litters in order to support their control plan for PEDv.

Key words: Porcine Epidemic Diarrhea virus (PEDv), algae, clay

INTRODUCTION

The porcine epidemic diarrhea (PED) is a diarrheal disease of swine caused by porcine epidemic diarrhea virus (PEDv) (Pensaert, 1999). Once ingested, the virus rapidly causes enterocyte lysis (Song and Park, 2012), leading to decreased nutrient absorption capacity (Madsen *et al.*, 2014) resulting in watery diarrhea followed by a rapid dehydration, anorexia, and, in some cases, mortality (Shweer, 2015). Controlling PEDv is challenging as the virus is resistant to a wide range of humidity and temperatures (Morrison and Geode, 2014). Moreover, though vaccination has been used for many years in several Asian countries and is an option in North America, PEDv is highly recombinant and evolves quickly, making efficient vaccine development difficult. Thus, managing symptoms and initiating a plan for barn inoculation is the best way to control a localized infection. In support to these measures, the use of a complementary feed capable of protecting the intestinal mucosa may be beneficial. This study was implemented in the frame of PEDv outbreaks that have been affecting Mexican pig production since 2013. The aim of the study was to test the efficacy of such complementary feed, on piglet performance in a context of PEDv infection.

MATERIAL AND METHODS

The tested product (Ecopiglet) is a complementary feed containing algae extracts from *Ulva sp*, micronized clay (montmorillonite) and micronized clinoptilolite. The trial was performed in a farrow-to-finish farm of 450 gestating sows located in the Center of Mexico. Twenty-two randomly selected litters from the same batch and placed in the same room were involved in the trial. Eleven litters (127 piglets) constituted the control group, which did not receive any complementary feed, and eleven litters (120 piglets) constituted the test group which received the algae-based complementary feed from day one to weaning (21 days) at the dose of 50g/litter/day. On the first day, the complementary feed was administered individually in the mouth. On the following days, it was placed on the floor in the nesting area of piglets. The farm was diagnosed positive for PEDv at day eleven of the trial. Statistical analysis was performed on the data with a risk $\alpha = 0.05$. The litter constituted the experimental unit.

RESULTS

Results shown in Table 1 demonstrate that despite a lower birth weight (-160g, $P<0.05$), piglets from the test group had a higher growth rate than control (respectively 187 and 150 g/day, $P<0.05$). In addition, the mortality rate tended to be lower in the test group compared to control (respectively 26.8% and 14.2%).

Table 1. Performance of piglets exposed to PEDv and supplemented (Test) or not (Control) with an algae-based complementary feed.

Parameters	Control	Test	Difference	P-value
Number of litters	11	11	/	/
Average number of born piglets / litter	11.5	10.9	-0.6	0.57
Average number of weaned piglets / litter	8.5	9.4	+0.9	0.40
Average age at weaning (days)	20.5	21.5	+1	0.05
Average weight at birth (kg)	1.61	1.45	-0.16	0.03
Average weight at weaning (kg)	4.70	5.47	+0.77	0.04
Average daily gain, ADG (g/d)	150	187	+37	0.03
Mortality (%)	26.8	14.2	-47%	0.25

DISCUSSION

The results of this study show that the use of the algae-based complementary feed in this farm, which was positive for PEDv, helped to improve growth rate and tended to reduce mortality. These results are in accordance with previous results obtained with the same complementary feed on performance and diarrhea occurrence of suckling piglets (Cano Lopez *et al.*, 2014). Cano Lopez *et al.* also demonstrated the capacity of the algae-based complementary feed to increase villi length of weak suckling piglets. The evidenced effect on villi length is likely associated with the capacity of Montmorillonite clay to protect the intestinal tract (Subramanian and Kim, 2015), as well as the capacity of *Ulva sp* polysaccharides to stimulate the excretion of mucin protein (Barcelo *et al.*, 2000), thus increasing the protective mucus layer along the intestinal mucosa.

CONCLUSION

In conclusion, the tested complementary feed seems to be effective in reducing the effect of PEDv in maternity. During the six months following the trial, the farm decided to extend the use of this complementary feed to all litters in order to support their control plan for PEDv.

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SEROLOGICAL DETECTION OF *Ehrlichia canis* IN CANINES FROM CULIACAN, MEXICO

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SUMMARY. *Ehrlichia canis* is a gram-negative intracellular obligated bacteria, it is recognized as the causative agent of Canine Monocytic Ehrlichiosis (CME), which is transmitted by the bite of previously infected ticks, and its main vector is *Rhipicephalus sanguineus*. In the last decade it has been considered a potential zoonotic pathogen especially in the area of veterinary medicine. The aim of this study was detection of *Ehrlichia canis* in canine from Culiacan, Mexico with the use of the ELISA technique. The study was done in the parasitology lab of the Facultad de Medicina Veterinaria y Zootecnia- UAS. Blood from 81 canines, with or without clinical signs of disease and presence of ticks was collected in EDTA tubes from the cephalic vein, the bacteria was detected by blood smear, after each sample was dyed with Wrigth solution and observed under light microscopy (100 X); the serological study was done with ELISA (IDEXX® 4Dx). 11 samples were detected positives for *E. canis* showing a frequency of 13.5% by opticalmicroscopy, after serological test were carried out, 14 samples reacted to the presence of the specific antibodies against the bacteria, presenting a frequency of 17.2%. The presence of *Ehrlichia canis* in canines to Culiacan, Mexico, suggests a risk factor to public health due to the close contact with canines as pets and presence of the vectors, making pets a factor for the dissemination of this zoonotic pathogen.

Key words: *Ehrlichia canis*, Canines, ELISA.

INTRODUCTION

Ehrlichia canis is a intracelular obligated gram-negative bacteria, its target cell are the neutrophils of canines, they reproduce and give to the formation of morulae inside the vacuoles (Chen *et al*., 1997). its distribution is worldwide. (Romero *et al.*, 2011; Gal *et al.*, 2008). The Bacteria is classified in order *Rickettsial*, In 2001 it was reclassified, based on phylogenetic analyzes of the sequence of the 16S rRNA and groESL genes, as a result the family now includes the genus *Ehrlichia* spp. (Allison y Little *et al.*, 2013; Rikihisa *et al.*, 2011). Ticks are an important cause of morbidity and mortality around the world, *Riphicephalus sanguineus* (brown dog tick) is implicated as a vector of various infectious agents including *Anaplasma platys, Babesia gibsoni, Ehrlichia canis,* Groups of spotted fever, *Rickettsia* spp. And *Hepatozoon canis*(de Oliveira *et al*., 2011*).* The disease caused by *E. canis* is called canine monocytic Ehrlichiosis (CME), which involves three phases; an acute, subclinical, and sometimes chronic phase (Jimenez-Coello *et al.*, 2009). The clinical diagnosis is based on anamnesis, clinical signs and blood tests (Harrus y Waner, 2011). The diagnosis by optical microscopy of *Ehrlichia* spp. is observed under an optical microscope with a blood smear, inside monocytes (*E. canis* y *E. chaffeensis*) or granulocytes (*E. ewingii*), the detection of antibodies can be performed by immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA), but cross-reactivity between antibodies against *Ehrlichia* species is possible. Serological studies in dogs exposed to pathogens transmitted by ticks, are used to identify areas where people and dogs are at risk of acquiring the infection (Beall *et al.*, 2012). The seroprevalence of *E. canis* has been described in canines of Mexico, presenting its self in 2003 with 33.1%, in Yucatan, 2005 with 44.1%, Mexicali in 2007 with 49.3% and Sinaloa in 2013 with 74.5% (Núñez *et al.*, 2003; Rodríguez-Vivas *et al.*, 2005; Tinoco *et al.*, 2007; Sosa-Gutiérrez *et*

al., 2013). The aim of this study was detection of *Ehrlichia canis* in canine from Culiacan, Mexico with the use of the ELISA technique.

MATERIAL AND METHODS

The study was carried out in the Laboratory of Parasitology of the Facultad de Medicina Veterinaria y Zootecnia-UAS. Samples were obtained from dogs with or without ticks or signs suggestive of the disease CME. A total of 81 canines were taken with 3 mL of blood in EDTA tubes. The size of the sample was determined by intentional selection or convenience sampling, which is a non-probabilistic sampling technique (Thrusfield *et al*., 1990).

Sample analysis: Each sample was smeared with Wright stained to observe by microscopy in search of Ehrlichia canis morula on neutrophils observed under light microscopy (100 X Carl Zeiss® model Axiostar) And were performed by ELISA serological test (Snap 4Dx) discarding negatives. The data obtained were analyzed using frequency tables.

RESULTS

Of the 81 blood samples collected from canines, 11 of them were positive by microscopy, the characteristic canines of *E. canis* were observed in neutrophils obtaining a frequency of 13.5% and by ELISA 14 resulted seropositive to *E. canis* showing a frequency of 17.2%.

DISCUSSION

The results of this study confirm the presence of Ehrlichia canis in the municipality of Culiacán with a seroprevalence of 17.2%, these results are low compared to a study conducted in Sinaloa in 2013 with 152 samples, These results are low compared to a study carried out in Sinaloa in 2013 with 152 samples, in which 40.1% (61/152) of positive cases were obtained with microscopy and 74.5% (113/152) of seroprevalence (Sosa-Gutiérrez *et al*., 2014); in another study carried out in Yucatan in 2005 with 120 samples where 5% (6/120) were positive to microscopy and 44.1% (53/120) of seroprevalence (Rodríguez-Vivas *et al*., 2005). The aforementioned studies present a high percentage compared to that found in this study, this can be explained by the inclusion criteria used in both studies for sample taking, which was taking into account clinical sign of disease caused by *Ehrlichia canis a*nd the presence of ticks in the animal, while in this study animals with or without ticks or clinical signs were used for sampling.

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IDENTIFICATION OF *Anaplasma marginale* IN CALVES FROM CULIACAN, MEXICO

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SUMARY

The *Anaplasma marginale* is an intracelular obligated Rickettsia that invades the erythrocytes of ruminants and the hemolinfa of ticks, it is found in tropical and subtropical areas around the world, causing bovine anaplasmosis disease, which induces low milk production, infertility, reduced daily weight gain and retarded growth of calves, leading to economic losses in livestock. The aim of this study was to identify *Anaplasma marginale* in blood of calves from two production systems in the municipality of Culiacan, Mexico. The samples were collected from farms with a history of clinical problems of bovine anaplasmosis. The sample size was determined by simple random sampling. Fifty-nine blood samples were obtained from calves under 6 months of age, from the intensive and semi-extensive production systems. In the lab DNA was extracted using the phenol-chloroform technique and the msp5 gene of *A. marginale* was amplified by PCR. Identification of *A. marginale* was demonstrated by a band of approximately 500 bp in 22 samples (37.28%); which indicates its presence in calves of Culiacan, Mexico, making it a high risk factor for development of this disease in cattle of this region, it is necessary to continue research anaplasmosis in calves to achieve effective control for said microorganism.

KEY WORDS: *Anaplasma marginale*, Calves, PCR.

INTRODUCTION

Anaplasma marginale is a gram-negative bacteria, that invades mature erythrocytes of ruminants, causing the disease called anaplasmosis (Díaz *et al*., 2003, Rymaszewska and Grenda, 2008). It is found on the periphery and forms inclusion bodies (Kocan *et al*., 2007). Bovine anaplasmosis is an infectious disease that causes large economic losses due to decreased milk production, weight gain, delayed growth, abortions and deaths due to treatment costs in developing countries, noticeably in tropical and subtropical areas. The geographical distribution of *A. marginale* is broad due to the large number of vectors that transmit it, practically all the hematophagous arthropods such as ticks of several genus, like *Rhipicephalus (Boophilus*), *Amblyomma* and *Dermacentor*, as well as mosquitoes and flies. Man also collaborates in its transmission, when employing unhygienic zootechnical practices where contact with bovine blood is involved (Olguín, 2007). Diagnosis of anaplasmosis is difficult in asymptomatic carriers and when inclusion bodies within the red blood cells are not numerous enough to be detected by traditional methods (Corona *et al*., 2014). The amplification of the *msp*5 gene by PCR has been shown to be useful in the identification of *A. marginale* in asymptomatic bovines of different ages (Corona and Martínez, 2011). In the Punjab state of India the *msp*5 gene was used for the detection of *A. marginale* in cattle and a prevalence of 42% was obtained. In Mexico, serological studies and molecular studies have demonstrated the presence of the disease in more than 50% of the sampled cattle located in tropical and subtropical areas that includes the entire Gulf of Mexico, the Yucatan peninsula and a large part of the California peninsula (Rodríguez *et al*., 2009). In Culiacan,

Sinaloa *Anaplasma marginale* was identified by nested PCR in bovines using the 16S rRNA gene, obtaining a prevalence of 40% (Mariscal *et al*., 2013).

MATERIAL AND METHODS

The present work was carried out in the laboratory of Parasitology of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autonoma de Sinaloa, located in the city of Culiacan, Sinaloa, Mexico; The location coordinates of the cooperating farm were: Extensive farm: 24 °, 33 ', 53.4' 'north and 107 °, 27', 46.0 " west. Semi-extensive farm: 24 °, 25', 11.8" north, 107 °, 19', 40.3" west. Sample size was determined by simple random sampling (Jaramillo and Martínez, 2010). 59 blood samples from calves under 6 months of age, of either *Bos indicus* and *Bos taurus* and their crossbreed *Bos indicus* x *Bos Taurus* males and females were taken. 27 samples from the intensive system and 32 from the semi-extensive were collected including herds suspected of having anaplasmosis. Blood samples were obtained by puncture of the jugular or coccygeal vein. 3 mL of blood were collected in vacuum suction tubes with anticoagulant EDTA, each labeled with corresponding data from each animal. The samples were placed in a container with refrigerants at 4 ° C and transported to the laboratory. DNA was obtained by the phenol-chloroform technique (Sambrook *et al.*, 1989). PCR was performed with the amplification of the *msp*5 gene of *A. marginale* with oligonucleotides; Internal: F: 5'-GCATAGCCTCCGCGTCTTTC-3' and R: 5'-CACGAAACTGTACCACTGCC-3'. The mixture was run at a final reaction volume of 25 µl (1 µl of DNA, 25 ng of each oligonucleotide, 2 mM MgCl 2, 0.2 mM of each dNTP, 10 µl of reaction buffer, 1.25 U of DNA polymerase). The reaction was carried out in a thermocycler (BIORAD T100^{MR}) for 35 cycles. The process was carried out by preheating the mixture for 3 min at 94 ° C, the denaturation temperatures are 94 °C for 30 s the alignment at 54 °C for 30 s and the extension at 72 °C for 1 min with a Final extension of 72 °C for 10 min (Torioni de Echaide *et al*., 1998). DNA and the PCR product were observed on a 1% agar gel stained with gel network (Huang *et al*., 2010), in ultraviolet light, by looking at the amplified fragment size (500 bp), by comparison with the control Positive (bovine blood DNA experimentally infected with *A. marginale*, and size markers (1 Kb DNA ladder).

RESULTS

The DNA was observed intact, with no proteins present, however traces of RNA were present. This DNA was used to identify the presence of *A. marginale* in calves of the production systems, with the amplification being observed in a band of approximately 500 bp. By type of production system 11 positive animals were obtained from 27 in the intensive system obtaining a frequency 37.0% and of the semi-extensive of 32 animals 12 were positive, which is a frequency of 37.5%. These data show a general frequency in both production systems of 37.7% for *A. marginale* ($P \geq 0.6$). Likewise, the variables sex and racial type were verified by logistic regression analysis, where no statistical difference was obtained. Of 21 males, a frequency of 38.1% was obtained and 38 females 36.9% ($P \leq 0.99$). However, a trend was observed in pure breeds over native breeds (2:1), due to the frequency in pure breeds of 47.1% and crosses of 24.0% ($P \geq 0.06$).

DISCUSSION

The frequencies of 37% of *A. marginale* found in calves of both production systems are similar to those of Añez *et al*. In 2010, in the state of Zulia, Venezuela in which they worked with 35 calves and obtained positive results of 34.29% for *A. marginale*; which similar to the results found in the present study, differing only in 2.99%, despite the fact that in their study they used the *msp*1 gene of *A.*

marginale, and in the present study the *msp*5 gene of *A. marginale*, both genes of the majority surface proteins. Maldonado *et al*. In 2012, in Yaracuy, Venezuela, detected the presence of *A. marginale* in calves with less than 24 h of birth, using the *msp*5 gene of the pathogen, they worked with 29 animals, obtaining only 6 positive which equals 20.7%, this differs from the present study by 16.6%, perhaps because the study was done with calves less than 6 months of age, which had interacted with the environment for much longer that study. Based on the sex relation, no significant differences were obtained, as in the study shown by Mercado *et al*. In 2011, in Bolivia, where they describe that the sex of the animal does not influence the presence of the causative agent of anaplamosis. Regarding the races, the results shown here indicate a trend of purebred breeds (2:1), similar to that described by Bock *et al*., 1999, in southwestern Queensland, Australia, where *Bos indicus* has a greater tendency to the presence of the bacteria than in its crosses with *Bos taurus* (1.5:1). Florio *et al*. in 2012, identified A. marginale in 12% of adult cattle in a semi-extensive production system with ticks and hematophagous flies, and Bolivar et al. in 2014, in Merida, Venezuela, detected *A. marginale* in 27.7%, of cattle similar to results shown here. De la Fuente *et al*. (2005), in Sicily, Italy, worked with 160 adult cattle where they detected *A. marginale* in 55 cattle belonging to different production systems, presenting a prevalence of 13.7% in intensive system, 10% in semi-extensive and 10.6% in extensive system , Without showing significant difference, like what was found in this research.

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AIR FILTRATION SYSTEMS TO PREVENT AIRBORNE INFECTIONS IN PIG FACILITIES UNDER FIELD CONDITIONS

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SUMMARY. Air filtration can prevent infections by airborne pathogens in pig facilities. Therefore, three different air filter techniques were implemented in identical barns in order to evaluate the air quality and animal health compared to a barn without air filtration. In summary, only marginal differences were found in stables with and without filtration systems.

Key words: air filtration, air quality, pig health

INTRODUCTION

Air filtration systems have already been shown to decrease the risk of Porcine reproductive and respiratory syndrome virus (PRRSV) outbreaks in pigs (Dee et al, 2015; Dee et al., 2010; Spronk et al., 2010). Own studies on different air filter types using PRRSV, *Staphylococcus* (*S.*) *aureus*, and *Actinobacillus pleuropneumoniae* (APP) revealed high filtration efficiencies under laboratory conditions. Systems for air filtration of incoming and circulating air composed of these tested filter matters were implemented in a commercially pig plant. The objective was to determine whether air filtration has an impact on air quality, animal health, and pig fattening.

MATERIAL AND METHODS

The facility had four identical barns with approximately 960 pigs each. Three barns were equipped with air filtration: two for incoming air and the third for circulating air. The remaining was used as a reference without any air filtration. During three fattening periods, animal health, temperature, relative humidity, dust, CO_2 and ammonia were monitored. Air samples were collected to quantify the total amount of bacteria, methicillin-resistant *S. aureus*, *Escherichia coli* and coliform bacteria. One year after implementation of the filter systems samples were taken from the filter matter and examined for bacteria and PRRSV. Blood samples were collected from 15 pigs/barn at the beginning and at the end of each fattening period. At slaughter, pigs were examined with respect to respiratory diseases. Antibodies against PRRSV, H1N1, H3N2 and APP are currently measured.

RESULTS

Regarding the amount of total and target bacteria, dust particles, CO_2 and ammonia only marginal differences were seen between barns with and without air filtration. In addition, no differences were seen at slaughter so far. After one year of implementation, mainly environmental bacteria up to 10^3 colony-forming units/ml were found in the filter matters.

DISCUSSION

The efficacy of air filtration regarding air quality and animal health under field conditions was not proven in this study. Possible reasons and troubleshooting will be discussed at the presentation.

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Spronk, G., S. Otake, and S. Dee. 2010. Prevention of PRRSV infection in large breeding herds using air filtration. 166: 758-759.

ANIMAL WELFARE INDICATORS AND BODY WEIGHT OF BEEF CATTLE IN SILVOPASTORAL SYSTEMS OF URUGUAY

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Animal production systems that combine trees, shrubs or pasture are considered silvopastoral systems (SPS), although there are many alternatives within them. Studies performed mainly in Central America, conclude that SPS technologies have the potential to improve animal productivity, increasing the biological and economical efficiency of livestock farms while contributing to the ecological sustainability of these systems. Despite having several types of SPS and these beneficial impacts on the welfare and productive performance of beef cattle, most of the results so far are obtained from tropical regions and with *Bos taurus indicus* breeds. In Uruguay, most producers let animals graze within the system, as SPS, with the perception that this is relatively profitable. Aiming to clarify the existence of these benefits in temperate regions, where European breeds are more common, research was conducted on two extensive beef farms which raise *Bos taurus taurus* (Hereford breed), allocating animals to SPS and Natural Grassland (NG). Two groups (n= 20) were allocated to 20 hectares NG paddocks and two groups (n=20) allocated to 20 hectares SPS paddocks to compare good health indicators and body weight during the spring-fall period, October to May 2015-2016. Both farms were visited every 45 days. Good health indicators were individually recorded according to the Welfare Quality® Assessment protocol for cattle in the farm facilities prior to the entrance on the scale where each animal was weighted. A mixed model was used to analyse weight as response variable. Categorical variables were compared with Fisher´s exact test. Significant differences were found between farms and visits ($p<0.01$), but no differences were found between treatments ($p=0.176$). No differences for good health indicators were found ($p>0.05$). Therefore these results show that in temperate regions and under this specific SPS and NG, the SPS did not show inferior results to the NG.

THE EFFECT OF AMBIENT TEMPERATURE ON JOINTS IN THE DISTAL FORELIMBS OF HEALTHY RACEHORSES

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INTRODUCTION: The aim of the study was to describe the dependence on ambient temperature of distal joint temperature at the forelimbs of racehorses. The study also investigated the influence of ambient temperature on the temperature difference between joints: this was measured ipsilaterally (i.e. between the carpal and fetlock joints along each forelimb) and contralaterally (i.e. between the same joints of the left and right forelimbs).

MATERIAL AND METHODS: Sixty-four healthy racehorses were monitored in 13 imaging sessions over 10 months. At each session, three thermographic images were taken of the dorsal, lateral and medial aspects of the distal forelimbs. Temperature measurements were made from regions of interest (ROIs) covering the carpal and fetlock joints, allowing the average temperature differences to be ascertained between the joints using the dorsal, lateral and medial aspects.

RESULTS: There was a strong correlation between ambient temperature and absolute joint temperature at all ROIs. There was also observed a moderate correlation between ambient temperature and the ipsilateral temperature differences between joints when measured from the medial and lateral aspects. No significant correlation was noted when measured dorsally. The mean contralateral temperature differences between joints were all close to 0°C, confirming an overall symmetry of joint temperatures. The range of contralateral differences was wide, however, with one horse exhibiting a 14 °C difference between the fetlock joints when viewed medially. Weak correlations between ambient temperature and contralateral temperature differences were noted at the carpal joints when viewed laterally, and at the fetlock joint when viewed medially.

CONCLUSSIONS: Data support previous reports that the temperature distribution between the forelimbs of the healthy equine is symmetric, although some horses differ markedly from the average findings. The dorsal aspect is the thermographic view of choice, since we have demonstrated no dependence on ambient temperature of any difference between joints using this view.

OCTENIDINE HYDROCHLORIDE: DISINFECTION EFFICACY AGAINST MRSA OF DIFFERENT ORIGIN

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SUMMARY. Due to the capability of bacteria to generate multiple antibiotic and biocidal resistances constituting hazards in hospital settings, specific disinfection measures may be required. Octenidine hydrochloride (OH) is a frequently applied antimicrobial active substance used for skin, mucous membranes and wound disinfection in hospitals. The aim of the study was to determine OH's efficacy against 10 *Staphylococcus aureus* strains. The results showed that a sufficient effect for disinfection can be expected when using ready-to-use products according to the manufacturer's instructions.

Key words: octenidine hydrochloride, methicillin-resistant *Staphylococcus aureus*, disinfection efficacy

INTRODUCTION

Octenidine hydrochloride (OH) is a common used skin and mucous membrane disinfectant. Due to rising hazards of resistant bacteria in hospital settings, especially MRSA, it is questioned if these micro-organisms can develop biocidal resistances, too. This study investigated the efficacy of OH eliminating MRSA in the presence and absence of BSA.

MATERIAL AND METHODS

Disinfectant testing was performed according to the guidelines of the Association for Applied Hygiene (VAH) with 10 *Staphylococcus aureus* strains (9 MRSA, 1 MSSA, human/animal origin) in comparison to 2 reference strains. To evaluate OHs efficacy, minimum inhibitory concentrations (MICs) were determined using the broth dilution technique. Once an adequate neutralizer was identified, qualitative and quantitative suspension tests (ST) with and without organic load (3.0 g/l BSA) were carried out at 5 and 3 different contact times, respectively.

RESULTS

MICs were identical for all bacteria (0.0002%) and in agreement to the literature (Rebert, 2014). For all strains, qualitative ST revealed a 4x higher OH concentration after 1 min exposure but were identical to MIC after 60 min of contact time. However, strain differences were seen at other incubation times. The required reduction ≥ 5 $\log_{10}$ was confirmed in quantitative ST using concentrations of OH similar to the qualitative ST. With organic soiling higher concentrations were needed to inactivate bacteria.

DISCUSSION

OH was found to be equally effective against all tested strains in concentrations lower than the commonly used concentration (e.g. octenisept® containing 0.1% OH). MRSA strains tested were not

less susceptible to OH than non-resistant strains. With organic soiling higher OH concentrations were required for full inactivation. Nevertheless, a sufficient effect for skin and mucosal disinfection can be expected when using ready-to-use products according to the manufacturer's instructions.

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EFFICIENCY OF DIFFERENT AIR FILTER TYPES AT LABORATORY SCALE

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SUMMARY. We evaluated four different air filter types for their filter efficacy regarding different viruses and bacteria at laboratory scale. Depending on filter matter and pathogen, a reduction of up to 3 log_{10}-steps was achieved by the air filters. Nonetheless, infectious particles were able to pass the filters. Within the filter material, viruses tested survived up to 24 h whereas bacteria remained infectious up to four weeks.

Key words: air filter efficacy, viruses, bacteria.

INTRODUCTION

Air filtration is known to reduce pathogens in incoming air (Dee et al, 2015; Dee et al., 2010; Spronk et al., 2010). Aiming at the reduction of pathogens below a certain infectious dose in incoming as well as in circulating air, we determined the retention efficiency of different air filter types at laboratory scale.

MATERIAL AND METHODS

Four filter prototypes were tested; two consisted of a pre- and a secondary filter. Equine Arteritis Virus (EAV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Bovine Enterovirus (BEV), *Actinobacillus pleuropneumoniae* (APP), *Staphylococcus aureus* (SA), and *Mycoplasma hyorhinis* (MH) were chosen as test candidates. With each pathogen, five trails per filter were conducted. Depending on the prototype, the airflow was set at 1800 m^3/h or 80 m^3/h. Pathogens were atomized and inoculated into the test chamber. Air samples were collected prior to and behind the filter and the filter retention efficiency was calculated. Cell culture, quantitative real-time PCR, and the spread-plate method were used to grow and quantify microorganisms from collected air samples. Furthermore, survivability of APP, SA and PRRSV over time in the filter material was examined.

RESULTS

Using the two-part filter system, a 96.0%, 97.5% and 98.0% reduction was determined for BEV, EAV, and PRRSV, respectively. With SA and APP 99.1% and 95.2% were achieved. A prototype composed of filter wool with a central glass fiber reached 99.9% (SA, APP), 98.7% (BEV, EAV), and 92% (PRRSV). Using MH, no sufficient bacterial count in front of the filter could be reached although MH cultures revealed 10^9 colony-forming units/ml. In the filter matter, APP and PRRSV remained viable 4 h and 24 h, respectively, whereas SA survived for four weeks.

DISCUSSION

Air filter efficiently reduced airborne pathogens at laboratory scale. Depending on filter matter and pathogen, a reduction of up to 3 log10-steps was achieved and pathogen viability faded over time. Currently, further evaluation under field conditions in a commercial swine system is conducted.

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Spronk, G., S. Otake, and S. Dee. 2010. Prevention of PRRSV infection in large breeding herds using air filtration. 166: 758-759.

EVALUATION OF ANIMAL WELFARE IN PIGS DURING DISCHARGE, PENS STAYING AND STUNNING EFFECTIVENESS

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SUMMARY. The present study aimed to evaluate the animal welfare of the pigs during the discharge, the pens staying and the effectiveness of the stunning to the slaughter at the slaughterhouse. The discharge from six pig transport trucks was evaluated, waiting conditions in eight pens, and 64 animals at the moment of stunning. The parameters were evaluated by making adaptations of the Welfare Quality® protocol at slaughterhouse for pigs. In general, the conditions during the discharge were acceptable, observing only in greater incidence the percentage of slips and falls with 11.47% and 21.03% respectively, the animals were appropriately arranged in the holding pens. The discharge time was considered acceptable with an average of 23.5 minutes, but the conduction of the animals to the area of stunning was performed in an inadequate manner, observing 54.68% percentages in continuous vocalizations. The most striking variables were head and neck elevation with a 23.43%, the attempt to rejoin with 12.5% and ocular movements occurred in 9.3% of the animals, indicators of deficient stunting. In general, the animal welfa re of the pigs during the discharge was good. Staying in pens was acceptable, although the waiting time for some animals was excessive; this is because the slaughterhouse does not have a specific discharge shift for each client, and some leave animals in existence for more than 48 hrs. The move to stunning is deficient due to the mismanagement on the part of the operators. The stunning was considered deficient due to the high presence of three indicators.

Key words: Animal welfare, pigs, slaughterhouse

INTRODUCTION

The welfare can be defining as the "status of an individual in relation to their attempts to cope with their environment" (Mota, 2012). An animal is in good health if it is healthy, comfortable, well fed, safe, can express innate forms of behaviour and if it does not suffer unpleasant sensations of pain, fear or restlessness (Méndez et al., 2013). It is important to promote the evaluation of animal welfare, especially in those intended to produce meat for human consumption (Gallo-Stegmaier, 2010). Because of its importance, the OIE recommends establishing animal welfare principles (Rojas et al., 2005). The discharge evaluates actions such as slips, falls, vocalizations and thermoregulation problems. Another aspect that should be considered is the use of waiting pens on the trail and finally the quality of sensitization that presented the pig after the stunning. That is why animal welfare assessments are necessary and allow us to improve their conditions to obtain better quality in the meat and lower economic losses. Given the increasing importance of animal welfare, the objective of this work is to

evaluate the methods used during landing, retention in pens and the efficacy of stunning based on the Welfare Quality® protocol for pigs, thus verifying the level of animal welfare with which they count.

MATERIAL AND METHODS

The evaluation of the process was according to the Welfare Quality protocol® (Grandin, 2010). Six trucks were evaluated at the time of landing. To analyze the variables of retention in pens, eight pens were evaluated, and 64 animals were divided into three groups for stunning efficacy. Landings and lorry space, transport time and the percentage of animals that slipped and those that fell were assessed at the landing; the presence of lameness, thermoregulation problems, animals unable to move and dead in transport. In the waiting pens, the availability of space, the provision of food and water, the type of soil and the presence of tremors, panting and piling were evaluated. When the animals were transferred to the CO_2 chamber, the vocalizations were evaluated, taking into account two types: the continuous ones and the snapshots according to the methodology proposed by (González et al., 2012). The following physiological indicators of consciousness observed were the presence of rhythmic breathing, incorporation reflex, presence of corneal reflex and vocalisations immediately after exit of the CO_2 chamber. The results were analyzed by descriptive statistics, thus obtaining a global evaluation of animal welfare in the slaughter unit.

RESULTS

During the transport, the animals presented an average density of 0.44 m^2 / animal, located within the limits allowed according to (NOM-051-ZOO-1995). The time of transfer in none of the cases was greater at 8 h and the time of discharge had an average of 23.5 minutes. Figure 1 shows the percentage of animals showing falls, slips, lameness, fallen and dead animals in the slaughterhouse.

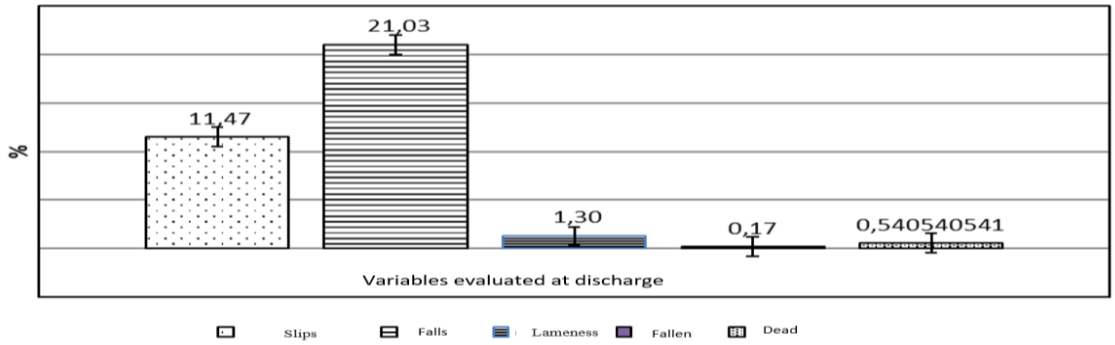

Figure 1. Percentage of animals exhibiting falls, slips, lameness, fallen and dead animals in the slaughter facility.

Seventy-five percent of the pens presented acceptable density; in two, overcrowding was observed with 0.44 and 0.5 m^2 / pig. The type of soil was acceptable in 75% of the evaluated pens, two of them present mostly some unevenness that could cause some injury in the animals. A pen presented more than 20% of animals with tremors, while for none presented a percentage greater than 20% of gasps. It was observed that more than 50% of the animals presented continuous vocalizations during their

transfer from the waiting pen to the stunner, while almost 10% presented instant vocalizations. The percentage of animals that presented stunning physiological reflexes are shown in Figure 2.

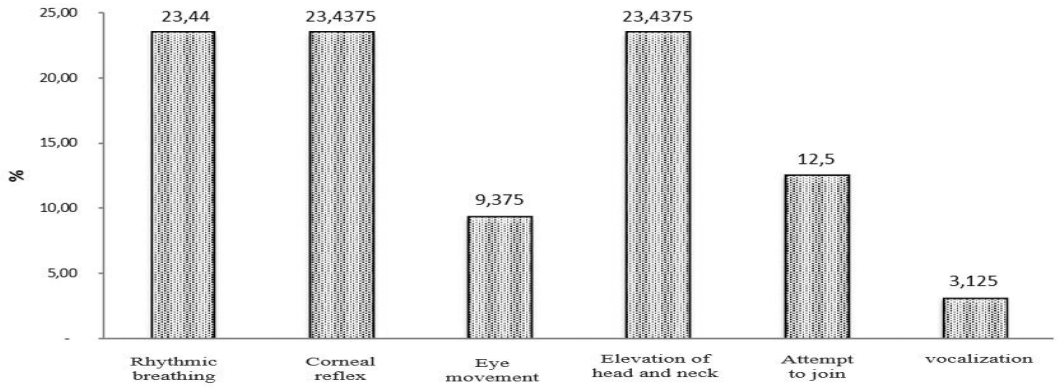

Figure 2. Percentage of animals exhibiting positive physiological reflexes to stunning

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DISCUSSION

The percentage of slips and falls is considerable, because the landing ramp was wet and muddy due to lack of roof in the area and the presence of rains on some days of evaluation. The percentage of lameness was very low and is attributed to the poor positioning of the animal during the transfer from the farm to the slaughterhouse. It was observed that there was crowding in pens where there was overcrowding and in those in which the waiting time of the pigs was more prolonged, which should not occur, since according to (Méndez et al, 2013) causes discomfort in the animal and has repercussions on the quality of the meat. The stunning indicated a high level of stress of the animals in this period, the previous one may be due to the handling by the operators of the trail, which mobilized the animals with shouts, whistles and blows of sticks with the handling sleeve, In this respect Miranda de la Lama (2013) these actions create a moment of tension that causes the animals to delay their way, so that operators manipulate incorrectly, hitting the pigs. In this study the percentage of rhythmic breathing was less than 25%, if we take this factor into account only, it could be considered that an actual stunning is performed. However, Peréz et al. (2013) mentioned that animals with ocular movement show a degree of return to sensitivity, in this case, although the percentage was low and could have the consequence of negatively affecting the effectiveness of the stunning. In addition, unconscious animals should not attempt to reintroduce themselves or vocalizations as they may be indicative of poor stunting (Grandin, 2010). For this reason it is considered that the presence of eye movement, head and neck elevation and the attempt to incorporate observed indicated that there are deficiencies in the stunning that must be repaired. Such deficiencies in stunning are due to several causes, the first could be the decrease in the percentage of concentration of gas between groups and the time of exposure to gas, which is not very varied, and the number of animals introduced per basket. It is worth mentioning that on some occasions the staff introduced up to three animals per compartment, resulting in a lower concentration per animal. Due to the above, it was observed that the deficiencies in the stunning were due to the management by the personnel.

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COMPARISON BETWEEN HAIR COAT THERMAL INSULATION OF ALPACAS AND MERINOS

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SUMMARY. Introduction: The aim of the study was to evaluate and compare hair coat thermal insulation of alpacas and merinos, and collect comparative temperature data for the coat surface of both species using infrared thermography.
Materials and Methods: The study included 13 clinically healthy huacaya alpacas and 14 Polish merino sheep. Coat samples were taken from each animal to measure coat fibre length and average fibre diameter. The insulating properties of each sample were determined using a calorimetric method. In addition, thermographic examination of the trunk (lateral and medial aspects) was performed for both species in an indoor environment. Six regions of interests (ROIs) were determined from each thermographic image (corresponding to the coat sample sites) and the mean temperature within each ROI was calculated. All statistical analysis was performed using STATISTICA v. 10.
Results: Merinos had significantly ($p < 0.01$) longer and thicker hair than alpacas, but less efficient insulation at all sample sites. Merinos had significantly higher coat surface temperature than alpacas at all ROIs .
Conclusion: It was found that alpacas have greater thermal insulation than merino sheep. Infrared thermography also provides valuable information about coat temperatures in vivo, which can be used to validate models of heat exchange in homoetherms.

Animal hygiene and herd health.

A LONGITUDINAL STUDY TO ASSESS THE HYGIENIC QUALITY OF DISINFECTION MEASURES ON PIG FARMS

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SUMMARY. Hygienic measures in terms of biosecurity are essentiell to the health, welfare, and efficient production of animals. Biosecurity includes preventive measure to reduce the risk of transmission of infectious and zoonotic diseases. An important tool for the biosecurity in livestock farm management is the cleaning and disinfection of surfaces. Although the importance of cleaning and disinfection on pig farms is known from the literature, information on the quality of hygienic regimes are limited. The aim of the present study was to contribute to a more detailed understanding of the quality of cleaning regime. In order to gain an overview of the quality of cleaning and disinfection regimes on pig farms, data were generated from December 2013 to July 2016. Contact plates (Trypticase soy agar) with sample area of 25 cm2 were used for monitoring total aerobic counts (TAC) on surfaces after cleaning and disinfection. In total, 740 samples from 60 pig farms located in north-west Germany were analyzed. The number of samples showing high growth of bacteria (18.2%) was higher than the number of samples showing no growth at all (10.8%). This dataset exceeded the guideline value according to IKB (< 1.5) most frequently (45%). Additionally, a questionnaire for gathering information about the cleaning regime on pig farms was done in 2016. In total four questions were answered by 187 agriculturist and analysed. The present study highlights the disparity of hygienic measures and the possible role of poor cleaning and disinfection regime quality as a potential source of disease transmission and indicates the need for further monitoring and hygienic measures to optimize the cleaning and disinfection regime on pig farms.
Key words: Disinfection, Biosecurity, Pig Farm

INTRODUCTION

The increasing demand for animal products such as meat has resulted in structural change in farming practice. The increase in stocking densities has led to more disease problems and advanced management requirements introducing preventive measures to reduce the risk of transmission of infectious diseases. Therefore, biosecurity has become a significant importance in livestock housing facilities. Biosecurity includes all measures that prevent pathogens from entering a herd (external biosecurity) as well as reducing the spread of pathogens within the herd (internal biosecurity) (Amass and Clark, 1999). In pig herds, biosecurity is an important aspect of preventing the transmission of diseases, thus improving health and reducing the need for antimicrobials (Laanen, 2013). Prevention and control of disease relies not only on quarantine, vaccination, diagnostic and treatment, but also on a healthy environment. Therefore, hygienic measures such as cleaning and disinfection of surfaces are still essential tools for reducing infection pressure on pig farms (Luyckx et al., 2016). Although the importance of cleaning and disinfection on pig farms is known from the literature, information on the quality of hygienic regimes is limited. The aim of the present study was to contribute to a more detailed understanding of the quality of cleaning regime in livestock housing by monitoring total aerobic counts on surfaces after cleaning and disinfection. Further, a questionnaire gave additional information about individual disinfection practices on pig farms.

MATERIAL AND METHODS

In order to gain an overview of the quality of preventive disinfection on pig farms, data were generated from December 2013 to July 2016. In total, 740 samples from 60 pig farms located in north-west Germany were analysed. Contact plates (Trypticase soy agar) with sample area of 25 cm2 were used for monitoring total aerobic counts (TAC) on surfaces after cleaning and disinfection. Sampling was performed 12-24 h after individual cleaning and disinfection protocol. The agar medium was pressed against selected surfaces. After sampling the plates were incubated at 37°C for 48 hours. Additionally, we surveyed German swine producers to describe their attitude and disinfection practices. Our written questionnaire with four questions regarding internal biosecurity (preventive disinfection) was handed out during a symposium in November 2016.

RESULTS

After disinfection, there was a significant difference in reduction of bacteria between pig farms. The number of samples showing high growth of bacteria (18.2%) was higher than the number of samples showing no growth at all (10.8%). This dataset exceeded the guideline value according to IKB (<1.5) most frequently (45%).

Table 1. Contact plates (n = 740) were taken after cleaning and disinfection of surfaces.

Bacterial growth	Rating	Number	Percent, %
0 CFU	0	80	10.8
1-40 CFU	1	359	48.5
41-120 CFU	2	166	22.4
> 121 CFU	3	135	18.2

In total four questions concerning the disinfection regime on livestock farms were answered by 187 agriculturists and analysed. The results of the questionnaire are shown in table 2.

Table 2. Questionnaire (n = 187) for gathering information about the disinfection regime on pig farms.

Questions	Yes (%)	No (%)	Not stated (%)
Do you think cleaning and disinfection is important?	179 (95.7)	5 (2.7)	3 (1.6)
Do you use detergents for your cleaning regime?	134 (71.7	47 (25.1)	6 (3.2)
Do you let dry the surface before disinfection?	138 (73.8)	41 (21.9)	8 (4.3)
Have you ever monitored the disinfection by contact plates?	28 (15.0)	146 (78.1)	13 (7.0)

DISCUSSION

In livestock housing pathogens and the risk of disease outbreaks can be reduced by cleaning and disinfection (Davies and Breslin, 2003; Mannion et al., 2007). However, the so-called ‘preventive’ disinfection which is performed during the vacant interval from one to the next group of animal is advantageous only if it is accurately and continuously carried out. Therefore, the proper application is also a crucial factor for the effectiveness of disinfectants. The present study was conducted to give

information about the hygienic quality of cleaning and disinfection regime on pig farms. Studies describing biosecurity measures in pig herds have been published before (Backhans et al., 2015). However, there is no detailed description about the hygienic quality of preventive disinfection measures in Germany. During this study differential results of contact plates taken after disinfection of pig facilities were documented. A various number of contact plates (40.6%) showed a high bacterial growth (> 41 CFU).

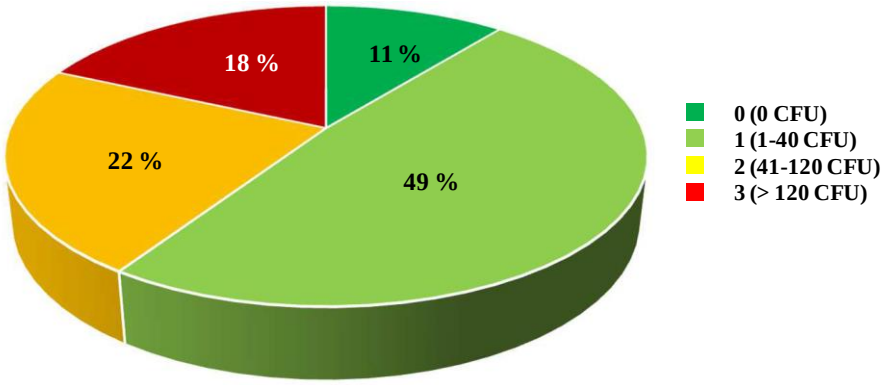

Figure 1. Chart showing the distribution of bacterial load after disinfection measures on pig farms.

The intention of cleaning and disinfection is to remove organic matter from surfaces and to kill remaining micro-organisms. It is generally accepted that in farm environment cleaning alone removes approximately 90% of bacteria and disinfection further 6-7% of bacteria (Fotheringham, 1995). The survival time of a microorganism in the environment is increased by the presence of organic material. Furthermore can the level of organic matter on surfaces impact the effectiveness of chemical disinfectants (Hancox et al., 2013). A previous study has even shown the importance of thorough cleaning with detergent before disinfection to reduce viral contamination on the farm (Chandler-Bostock and Mellits, 2015). Removal of this organic matter is therefore an essential and previous element in the disinfection process. According to the questionnaire most farms used detergents for cleaning (71.7%). However, a certain number of farms (25.1%) do not use detergents giving evidence that disinfectant may not be effective.
According to the farmers cleaning and disinfection protocols were similar on all farms and disinfectants were used equal to the manufacturer's recommendation. However, cleaning procedure was variable and information about the required demand is lacking. Few farmers (21.9%) did not allow the building to dry before disinfection. The finding that not all farms achieved an adequate disinfection may be associated with remaining organic material or moisture on the surface. Although most farms consider biosecurity measures such as cleaning and disinfection of surfaces an important preventive measure (95.7%), few have monitored the disinfection by contact plates (15%). Methods for testing the efficacy of disinfection under field conditions have already been described in the past (Tamasi, 1995). Contact plates with tryptic soy agar seem to be an appropriate method to monitor the efficacy of disinfection procedures on pig farms. It allows fast, easy screening of solid surfaces and is therefore recommended for use in quantitative environmental testing.
The present study highlights the disparity of hygienic measures and the possible role of poor cleaning and disinfection regime quality as a potential source of disease transmission and indicates the need for

further monitoring and hygienic measures to optimize the cleaning and disinfection regime in pig farms. Regular monitoring of hygienic regime measures and the implementation of regular control mechanism are highly recommended. Further investigations are required in order to determine suitability, efficacy, limitation of disinfectants and procedures. This includes surveys on cleaning and disinfection of surfaces and its effects on economic interests as well as animal health.

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FACTORS ASSOCIATED WITH THE AGE-TIME TO PRRSV SEROCONVERSION IN SWINE INFECTED HERDS

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SUMMARY. The study was carried out in 58 farrow-to-finish French pig herds. Tracheo-bronchial mucus and blood were taken from a random sample of 4, 10, 16 and at least 22 week-old pigs (45 pigs/herd). Sera were tested by ELISA for porcine reproductive and respiratory syndrome virus (PRRSV) antibodies. Infection by *Mycoplasma hyopneumoniae* (Mhp), *Actinobacillus pleuropneumoniae*, swine influenza viruses H1N1 and H1N2 and porcine circovirus of type 2 (PCV2) were detected by specific serological or PCR tests. Data related to husbandry, biosecurity, management and housing conditions were collected by a questionnaire. The outcome in the statistical process was the age-time to PRRSV seroconversion. The within-batch frequencies of seropositive pigs were used to estimate the time-interval during which seroconversion was deemed to occur in each herd. Factors related to the age-time to PRRSV seroconversion were identified and quantified by a Cox proportional hazards model. A common housing for the gilts and the sows during lactation (Hazard Ratio [HR]=3.0; IC95%[2.0-4.3]), large sized nursery pens (HR=2.9; IC95%[2.0-4.1]), a small number of pens per fattening room (HR=2.5; IC95%[1.7-3.6]) and the lack of all-in all-out management in the fattening section (HR=2.5; IC95%[1.8-3.4]) were significantly related to an early age-time to seroconversion. A small range of temperatures controlling ventilation in the nursery room was also associated with early PRRSV seroconversion (HR=3.9; IC95%[2.8-5.4]). Infection by Mhp (HR=3.2; IC95%[2.3-4.5]) and a high PCV2 infection pressure (HR=4.6; IC95%[3.1-6.9]) also increased the odds of early seroconversion. These results suggest associating management practices minimizing direct and indirect contacts between animals within batches and from different batches whilst providing the pigs with favourable climatic conditions for a better control of within-herd PRRSV infection dynamics.

Keywords: PRRSV, seroconversion, risk factors

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) is widespread in pigs reared in large and confined populations. PRRSV impairs swine health in many pig-producing countries worldwide. Several infection patterns are encountered in growing-finishing pigs in the field. The age at infection time was found to be associated with the type of respiratory outcome in finishing pigs (Fablet et al., 2012b). However, the factors associated with the age at PRRSV infection-time have not been investigated to date. Knowledge of PRRS-related risk factors is important to design and implement

cost-effective control measures. Hence, the present study aimed at exploring the factors associated with PRRSV age-time to seroconversion in PRRSV-infected farrow-to-finish herds.

MATERIAL AND METHODS

Data and sera used were collected in 58 French pig herds infected by PRRSV without PRRS vaccination in the growing pigs. Blood was taken from a random sample of 4, 10, 16 and at least 22 week-old pigs (15 pigs/batch). Sera from all batches were tested for PRRSV antibodies (ELISA test 2XR-IDEXX). Sera from the oldest pigs were tested for *Mycoplasma hyopneumoniae* (Mhp) and *Actinobacillus pleuropneumoniae* serotype 2 and serogroup 1-9-11 antibodies (DAKO ELISA-Kitvia, Swinecheck App2-Biovet and Swinecheck App1911-Biovet, respectively). Antibodies against European swine influenza A viruses (SwIAV) H1N1 and H1N2 were searched in sera from the oldest pigs by a haemagglutination inhibition test (Kyriakis et al., 2013). The porcine circovirus type 2 (PCV2) genome load in sera from batches of 4, 10 and 16 week-old pigs was quantified by real-time PCR (Grasland et al., 2005). Tracheo-bronchial swabs were taken from 10 of the 15 pigs selected from the batches of 4, 10 and 16 week-old animals and placed in 2 ml of buffered peptone water broth. Mhp DNA was searched in all swabs by modified nested-PCR (Marois et al., 2010). Data related to husbandry, biosecurity, management and housing conditions were collected by a questionnaire. The outcome in the statistical process was the age-time to PRRSV seroconversion. The within-batch frequencies of seropositive pigs were used to estimate the time-interval during which seroconversion was deemed to occur in each herd. Factors related to the age-time to PRRSV seroconversion were identified and quantified by a Cox proportional hazards model.

RESULTS

The mean age-time to seroconversion was 101 days (standard deviation= 3.9). Factors retained in the final model are presented Table 1.

Table 1: Final Cox regression model for factors associated with age-time seroconversion to porcine reproductive and respiratory syndrome virus (PRRSV) in infected herds (58 farrow-to-finish herds, hazard ratio (HR) with 95% Confidence Interval (CI))

Variable	HR	95% CI	p
Highest PCV2 genome load from four batches (4 to 22 weeks old, copies of genome)			<0.01
$\leq 4.5.10^6$	1	-	
$> 4.5.10^6$	4.6	3.1-6.9	
***Mycoplasma hyopneumoniae* infection status of 16-week-old pigs** (by n-PCR)			<0.01
Non-infected	1	-	
Infected	3.2	2.3-4.5	
Gilts and sows housed in the same farrowing room			<0.01
No	1	-	
Yes	3.0	2.0-4.3	
Range of temperature values for ventilation control in the nursery room (°C)			<0.01
≤ 5	3.9	2.8-5.4	
>5	1	-	

Number of pigs per pen in the nursery room			<0.01
< 28	1	-	
≥ 28	2.9	2.0-4.1	
All-in all-out in the finishing room			<0.01
No	2.5	1.8-3.4	
Yes	1	-	
Number of pens in the finishing room			0.02
≤ 12	2.5	1.7-3.6	
> 12	1	-	

DISCUSSION

Housing gilts in separate farrowing premises from the sows was associated with delayed PRRSV seroconversion in infected herds, a finding in line with previous reports (Freese and Joo, 1994; Dee et al., 1995). This may be related to the breeding herd's heterogeneous PRRSV-related immune and infection status, which could be responsible for instability and active infection of the litter. These results emphasise the need to consider the breeding herd when tackling diseases in the grower-finisher population, especially in endemically infected farrow-to-finish herds.

Mixing different batches in the same finishing room was associated with a shorter age-time to seroconversion. In continuous flow system, there is a greater likelihood of direct contact between older and younger pigs at pen level than in all-in/all-out management. These practices are more likely to lead to persisting circulation of PRRSV and to faster viral transmission between pigs at various stages of infection and immunity soon after entry into the room.

Large nursery pens and few pens in the finishing room significantly shortened the age-time to PRRSV seroconversion. The pen number was negatively correlated with the pen size in our dataset. In large pens, it is inevitable that pigs of different immune and infectious status will mix, thus fostering the transmission of infectious pathogens. Mixing of pigs unfamiliar with each other can create stressful conditions which might enhance their susceptibility to infections (de Groot et al., 2001; Merlot, 2004). Taken altogether, these conditions may therefore have a positive effect on viral persistence at the herd level with different age-time to seroconversion following viral exposure. The earlier the pigs are exposed to these situations, the greater the risk of the virus spreading in the first stages of their life.

A small range of temperatures controlling the ventilation rate in the nursery room reduced the age-time to PRRSV seroconversion. Relationships have previously been found between indoor climatic parameters and their management with respect to respiratory diseases and swIAV infection (Fablet et al., 2012a; Fablet et al., 2013). One putative explanation could be exposure to climatic stress due to the cold and draughty conditions created by such settings. This situation may affect the pig's immune system and enhance its susceptibility to infections.

A high PCV2 infection pressure and Mhp infection were characteristics of infected herds with an early PRRSV seroconversion pattern. However, results must be interpreted with caution as the study design did not allow the time sequence of events to be established. Experimental dual co-infection studies have previously shown that PRRSV interacts with those pathogens (Opriessnig et al., 2012). The mechanisms underlying the interactions are not yet fully understood (Dobrescu et al., 2014), but PRRSV is able to compromise the innate and adaptive immune functions thus predisposing to further infections. A prospective observational study where herds are followed with short intervals to determine when each infection occurred would clarify temporality and causal links. These findings

might also indicate that PRRSV and other pathogens share common risk factors which promote their introduction and persistence within a herd.

Several non-infectious and infectious factors are associated with PRRSV infection. Recommended measures aimed at a better control of within-herd PRRSV infection dynamics would include management practices minimizing direct and indirect contacts between animals within batches and from different batches whilst providing the pigs with favourable climatic conditions.

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RUSTIC MODEL OF MILK PRODUCTION AND QUALITY AND THE PREVALENCE OF PATHOGENIC BACTERIA IN BOVINE MASTITIS IN JALISCO, MEXICO

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SUMMARY. The primary milk production in Mexico is characterized by being heterogeneity, productive and economic. Being in the same region systems with high genetic and biotechnological development, computerized production systems and extensive development of markets in coexistence with numerous family production units. Characterized by uneven technological development and little market development. The aim of this study is to determine causative agents of bovine mastitis in relation to rustic dairy production systems in the municipality of Tlajomulco de Zuniga. The study was conducted during a period of two years, in the municipality of Tlajomulco, Jalisco State in western Mexico. Jalisco has the highest milk production (18% of national production). Rustic systems of dairy production (operated units familiar way, less or no technological development, low number of cows in operation manual milking, lower quality forage, etc.) were evaluated in five villages of the municipality, determining prevalence and incidence of bovine mastitis by California mastitis test (CMT), somatic cell count and bacteriological cultures. 884 udder quarters were analyzed and 258 quarters were affected with mastitis. We isolated *Staphylococcus aureus* and *Enterobacteria* in respectively 45 and 30% of milk samples. The most frequently pathogens isolated were infectious (*S.aureus* and *Enterobacteria*) and environmental respectively.

Key words: Bovine mastitis, *Enterobacteria, Staphylococcus aureus*, small farms.

INTRODUCTION

The diversity of conditions and ways of producing milk in Mexico, reflects a marked contradictory position. The conditions of integration and modernization have been relevant in terms of the performance of the livestock. These producers have also been affected by changes in international milk prices. (Villar 2005). Another factor in the last 10 years in the performance of the domestic dairy farming, was the reduction in the incorporation of replacements, many of them were imported from Canada cattle since the first half of 2003 and from USA, since early 2004, so it had to find alternative countries to import dairy replacement heifers, this problem has been solved by opening imports of heifers from USA (Villar 2005). Bovine mastitis usually results in intramammary infection by bacteria that can produce clinical and subclinical disease (Field, 2003). It may be accompanied by clinical signs or not. Intramammary inflammation is associated with an increase in somatic cell count in milk. However, the magnitude of increase in somatic cell count (SCC) varies according to the bacteria involved in intramammary infection. (Djabri, 2002). Over 137 different microorganisms have been isolated from mammary glands affected by bovine mastitis. *Staphylococcus aureus (S. aureus*) is one of the prevalent and contagious pathogens responsible for subclinical and clinical infections. These bacteria can cause permanent damage to the glandular tissue by affecting the synthesis and secretory

capacity of the mammary gland. It can also play a very important role in the transmission of pathogenic microorganisms' paper, insects, rodents, dirt and mud. They are very important for the transmission of coliforms bacteria environmental of external source (Wolter et al., 2004). The wide variety of environmental pathogens *Escherichia coli* and *Klebsiella pneumoniae* are the most frequently isolated from intramammary infections and are the main causes of clinical mastitis (Barkeman et al., 1999; Lin et al., 1999). Mastitis control is important not only because of the economic losses of the producers and the dairy industry, but also from the consumers perspective, taking into account the deterioration of the nutritional and hygienic quality of milk (Kruze, 1998). The programs of the FAO (United Nations Food and Agriculture) are focusing increasingly on an approach from the farm to the table as an effective means to reduce hazards foodborne include all activities. They are carried out to ensure the quality, safety and honest presentation of food at all stages, from primary production, through processing and storage, to marketing and consumption (FAO, 2004). The aim of the study was to evaluate the prevalence and etiology of bovine mastitis in relation to rustic dairy production systems in the municipality of Tlajomulco, Jalisco.

MATERIAL AND METHODS

The study was conducted in the municipality of Tlajomulco, Jalisco, a State of Mexico, where there are rustic systems of dairy production (operated units in a familiar way, less or no technological development, and low number of dairy cows). They were evaluated in operation manual milking, lower quality forage, etc.), determining the prevalence and incidence of bovine mastitis by California test (CMT), somatic cell count and bacteriological cultures 884 quarters/222 cows in production of different race in five villages of the municipality: Lomas de Tejeda, Tlajomulco, Tejeda, Tecolote, and Cajititlan. The processing of the milk samples was conducted in the laboratory of Mastitis and Molecular Diagnostics, at the University Center for Biological and Agricultural Sciences of the University of Guadalajara, as well as in the laboratories of Food Science Institute of Technology Tlajomulco de Zuniga Jalisco (ITTJ).

RESULTS

The bacterial cultures of *Staphylococcus aureus* were isolated in 45% of samples, *Streptococcus spp.* in 24% and *Enterobacteriaceae* in 30% of the total of 884 samples (Figure 1).

In California mastitis test, values were obtained in the range of trace (150,000 to 500,000 somatic cells/ml) in greater proportion with 151 quarters from the total of 258 affected (Table 1).

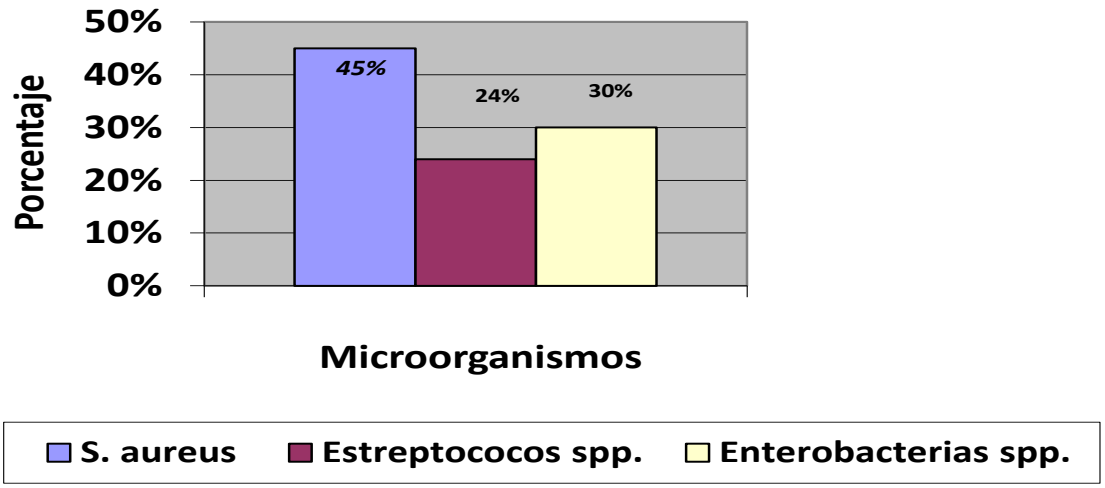

Figure 1. Results in percentage of infectious microorganisms

Table 1. Results of the relationship of the affected quarters

Level of infection	R. R.	R. L.	F. R.	F. L.	TOTAL	%
Negatives	146	154	151	159	610	64.0
Trace	47	35	38	31	151	20.4
1	14	13	16	11	54	7.3
2	10	15	12	16	53	6
Blind Quarters	5	3	4	4	16	2
Affected quarters					258	31
Total quarters					**884**	

DISCUSSION

Other authors in different research studies have already observed the problem of pathogenic bacteria isolated from cow's milk in rural production models where poor hygiene at milking, as well as a high number of somatic cells in milk are frequent (Lin et al., 1999; Wolter et al., 2004; Kruze, 1998; Castañeda, 2010; Castañeda et al., 2004). It is necessary to educate and teach milk producers, through government support programs to improve the quality of milk in rural areas.

CONCLUSION

It is important to issue measures of basic sanitary hygiene as sealing teats, general cleaning of the stable and milkers, for improved quality and hygiene of milk regardless of the hardiness of the production system model with a culture of craft dairy production that reflects local needs in a highly productive State within a global environment in relation to bovine mastitis in Mexico.

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When All-In/All-Out is not 'AIAO': a technical note on its consequences for pig health

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SUMMARY: The objective of this study was to track animal movements in a farrow-to-finish commercial pig farm with self-declared AIAO management and to investigate possible associations with animal health. A batch of 1,050 pigs born within one week were individually tagged at birth and followed to slaughter. Management on this farm after weaning at 28d included nursery, growing and finishing stages of 8, 4 and 8 weeks, respectively. Animal management was as per usual practice in the farm and weekly movement of animals was tracked. Pigs were initially allocated to 17 pens. Four weeks post-weaning, tagged pigs were found in 29 different pens. It took 5 extra weeks than planned to clear the nursery of tagged pigs. By 9 weeks post-weaning, there were 8 pens of tagged pigs in the nursery, 25 pens in the grower and 22 pens in the finisher houses. All animals were slaughtered within 1 week at approximately 20 weeks post-weaning and were retrospectively classified into three production flows (F) according to the time spent in each stage (F1 = normal, F2 = delayed 1 week and F3 = delayed >1 week). Tail lesions (TL), pleurisy, enzootic pneumonia lesions (EP), pericarditis and heart condemnations were recorded at slaughter and analysed using logistic regression. There was no difference between flows in the likelihood of TL or EP ($P > 0.05$). Pigs in F2 were 2.86 times at greater risk of pericarditis and 2.82 times at greater risk of lameness compared with pigs in F1 ($P < 0.05$). Similar results were observed between F3 and F1 pigs ($P < 0.10$). The failure to, inadvertently, adhere to the stated AIAO policy of this farm was associated with negative consequences for the health of the animals. An 'all-forward' policy might be more easily adhered to on Irish pig farms.

Key words: All-in/all-out, pig health, production flow

INTRODUCTION

All-In/All-Out (AIAO) production has several advantages for pig production systems such as reduced disease transmission and improved management and growth performance (Scheidt, et al., 1995; Owsley et al., 2013). In a true AIAO system, groups are move together to the next production stage so that the facility is completely emptied before the next group arrives. This however, represents a challenge for the management of slow growing pigs. In an AIAO system, slow growing pigs should only be allowed to accumulate off-site (Owsley et al., 2013) but this might not happen in practice. To our knowledge, there is limited information regarding the management of slow growing pigs in commercial farm that declare to follow an AIAO production system and its possible implications on pig health. Therefore,

the objective of this study was to track animal movements in a farrow-to-finish commercial pig farm with self-declared AIAO management and to investigate possible associations with animal health.

MATERIAL AND METHODS

The study received ethical approval from the Teagasc Animal Ethics Committee (TAEC 40/2013). The study was conducted on a 1,500 sow farrow-to-finish commercial farm in Co. Limerick, Ireland. A total of 1,050 pigs born within one week were individually tagged at birth and followed to slaughter. Gender, number of piglets born alive, sow parity, number of times each piglet was cross-fostered and lactation length were recorded. Management on this farm after weaning at 28d included nursery, growing and finishing stages of 8, 4 and 8 weeks, respectively. Animal management was done as per usual practice in the farm (for more details please refer to Calderón Díaz et al.; *submmitted*) and the weekly movement of animals was tracked. Pigs were initially allocated to 17 pens (Figure 1a). Four weeks post-weaning, tagged pigs were found in 29 different pens (Figure 1b) in different rooms of the nursery. It took 5 extra weeks than planned to clear the nursery of tagged pigs. By 9 weeks post-weaning, there were 8 pens with tagged pigs in the nursery, 25 pens in the grower and 22 pens in the finisher houses (Figure 1c). In total, it took 15 weeks post-weaning to move all the pigs into the finisher stage.

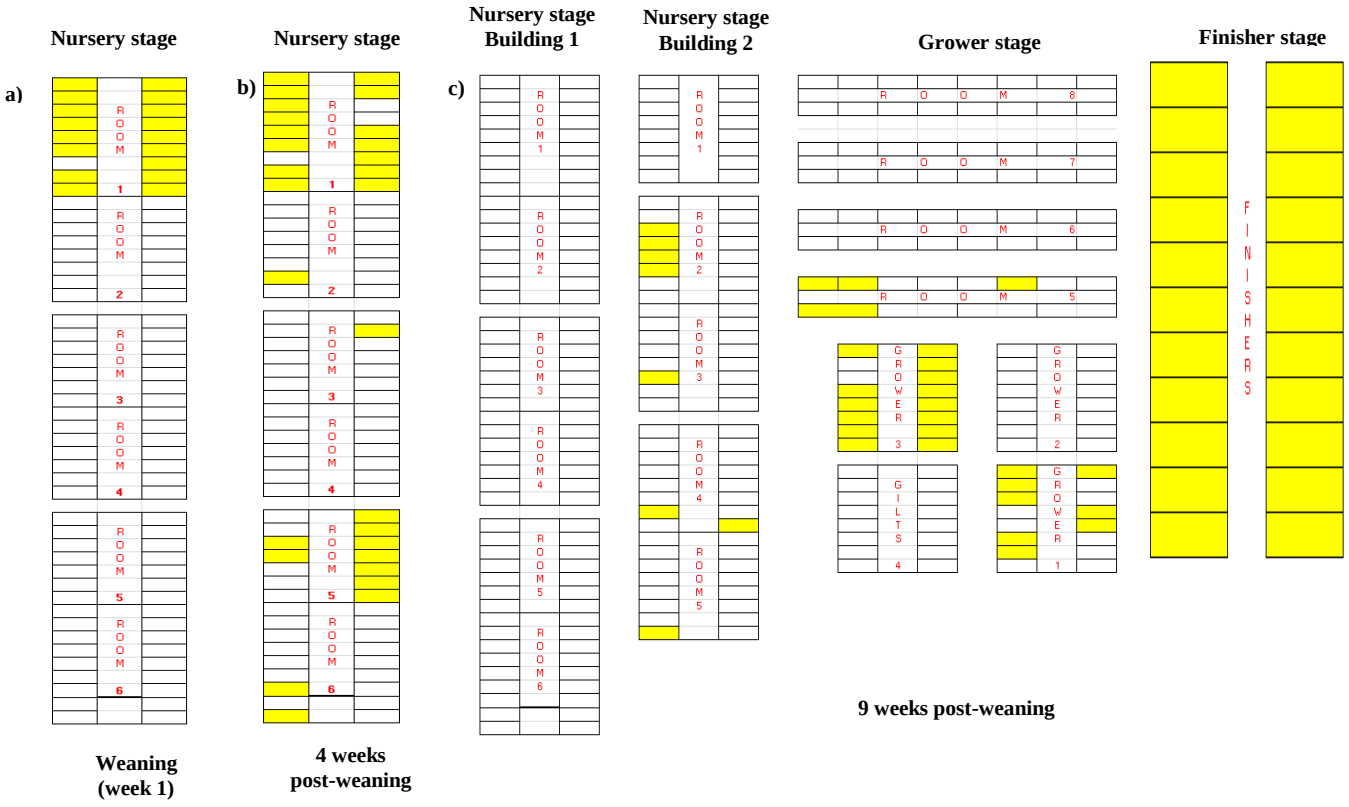

Figure 1. Layout of the pig facilities on a commercial farm with self-declared AIAO management. Pens in yellow a) denote pens were pigs were moved at weaning; b) denote the pigs' locations 4 weeks post-weaning and c) denote the pigs' locations 9 weeks post-weaning.

Eight-hundred-and-twenty-four pigs reached slaughter age/weight and they were slaughtered within 1 week at approximately 20 weeks post-weaning. Pigs were retrospectively classified into three

production flows according to the extra time they required to be moved to the next production stage [i.e. flow 1 = normal (n =620 pigs), flow 2 = delayed 1 week (n = 111 pigs) and flow 3 = delayed >1 week (n = 93)]. Prior to slaughter, pigs were scored for lameness by a single trained observer on a 3-point scale where 1 = non lame; 2 = mildly lame and 3 = severely lame. At slaughter, tail lesions were scored after scalding and dehairing by one trained observed as per Harley et al. (2012). Pleurisy was scored using the Slaughterhouse Pleurisy Evaluation System (SPES; Dottori et al., 2007) and Enzootic pneumonia (EP) like lesions were scored according to the BPEX Pig Health Scheme (BPHS; 2016) by a trained observer. Additionally, presence or absent of pericarditis and where organs such as the heart and liver were condemned was also recorded.

Statistical analysis. Each pig was considered as the experimental unit. As only one pig was scored as severely lame, lameness was classified as non-lame and lame. Only 15 pigs had their liver condemned, therefore, liver condemnations were not analysed. Tail lesions, pleurisy and EP were re-classified as present or absent. Univariable logistic regression models (PROC GENMOD; SAS Inst. Inc., Cary, NC), with flow as predictor variable, were used to investigate the relationship between flow and the recorded variables. Alpha level for determination of significance and trends were 0.05 and 0.10, respectively.

RESULTS

Table 1 shows the odds ratios for the likelihood of presenting the lameness, tail lesions, pleurisy, EP, pericarditis and heart condemnations by flow. Pigs in flow 2 had at greater risk of lameness and pericarditis compared with pigs in flow 1 ($P < 0.05$). Pigs in flow 3 had a greater risk of lameness, pleurisy, pericarditis and heart condemnations compared with pigs in flow 1 ($P < 0.05$). Additionally, pigs in flow 3 had a greater risk of pleurisy and tended to have a greater risk of heart condemnations compared with pigs in flow 2 ($P < 0.05$).

Table 1. Univariable logistic regression models of the risk in three different production flows[1] associated with lameness prior to slaughter, tail lesions, pleurisy, enzootic pneumonia (EP), pericarditis and heart condemnations in 854 finisher pigs followed from birth to slaughter in a commercial farm with self-declared AIAO management.

	Flow 1 vs Flow 2			Flow 1 vs Flow 3			Flow 2 vs. Flow 3		
		95% CI[3]			95% CI			95% CI	
Health conditions	**OR[2]**	**Lower**	**Upper**	**OR**	**Lower**	**Upper**	**OR**	**Lower**	**Upper**
Lameness[4]	2.82[a]	1.73	4.58	3.74[a]	2.27	6.18	1.33	0.72	2.43
Tail lesions[5]	0.98	0.64	1.51	0.69	0.44	1.08	0.7	0.4	1.24
Pleurisy[6]	1.34	0.82	2.17	3.31[a]	2.08	5.27	2.48[a]	1.35	4.56
Enzootic Pneumonia[7]	1.16	0.77	1.74	1.21	0.77	1.89	1.04	0.6	1.83
Pericarditis	2.86[a]	1.54	5.29	4.97[a]	2.77	8.92	1.74	0.86	3.53
Heart condemnations	1.60	0.84	3.08	3.18[a]	1.77	5.73	1.98[(a)]	0.92	4.28

[1]All animals were slaughtered within 1 week at approximately 20 weeks post-weaning and were retrospectively classified into three production flows according to the extra time they required to be moved to the next production stage (i.e. flow 1 = normal, flow 2 = delayed 1 week and flow 3 = delayed >1 week); [2] Odds ratios; [3]95% confidence interval

[4]Scored prior to slaughter on a 3-point scale were 1 = non lame; 2 = mildly lame and 3 = severely lame; [5]Scored after scalding and dehairing by one trained observed as per Harley et al. (2012); [6]Scored using the Slaughterhouse Pleurisy Evaluation System (SPES; Dottori et al., 2007); [7]Scored according to the BPEX Pig Health Scheme (BPHS; 2016).

DISCUSSION

The farm where this study was conducted did not follow an AIAO production system but the farmer and personnel were not aware about it. This problem has been observed by our team in many other farms. Many factors such as staff rotation, disease outbreaks or economic decisions might influence the adherence to an AIAO policy. The production system followed in this particular farm more resembled a continuous flow rather than an AIAO system. Rooms and /or pens did not have any sort of identification (i.e. age group, weaning date, date on arrival to that particular stage, etc.) making the adherence to the AIAO policy more challenging. By anecdotal accounts, in this particular farm, some staff thought that the smaller pigs were in fact younger pigs and thus, they had to stay for some extra time in that particular stage. Others thought that retaining slow growing pigs for longer periods of time in each stage would allow them to catch up and reach adequate slaughter weights. However, as noted by Owsley et al. (2013), slow growing pigs are usually affected by disease, are less efficient to convert feed into weight gain and may never reach an acceptable slaughter weight. In this study, pigs that did not follow the normal production flow were at greater risk of diseases supporting the theory that delaying pigs in the different production stages is associated with re-circulation of disease. For instance, lung infection could act as a port of entry of colonization of mycoplasmas (especially *M. Hyopneumoniae*) of the pericardium (Coelho et al., 2014) and pericarditis has been reported as one of the reasons for carcass condemnations in slow growing pigs (Martínez et al., 2007). Nonetheless, further analysis is needed to elucidate whether the greater risk of diseases in delayed pigs are causative or explanatory. For example, it is possible that lame animals were delayed to allow them to recover but it is also likely that animals that were delayed were also remixed several times increasing the likelihood of lameness (Spoolder et al., 2009).

In conclusion, the failure to, inadvertently, adhere to the stated AIAO policy of this farm was associated with negative consequences for pigs' health. It is possible that farm staff is not completely clear in what an AIAO encompasses and thus, it is important to develop teaching methods (i.e. workshops, discussion groups, newsletters, etc.) that would help to clarify the AIAO policy and to identify the best practices to implement it on farm. An 'all-forward' policy (i.e. no pig is left behind from stage to stage but rather use a split marketing approach when sending pigs to slaughter) might be more easily adhered to on Irish pig farms.

ACKNOWLEDGMENTS

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EFFECT OF WINTERING SYSTEM ON THE BEHAVIOUR OF YEARLING DAIRY HEIFERS

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SUMMARY. Determining appropriate wintering systems for replacement heifers is vital in ensuring optimal lifetime performance and longevity in low-cost systems of milk production. The aim of this study was to evaluate the effect of three wintering systems on heifer behaviour. Ninety heifers (age= 270 d, weight= 172 kg) were blocked according to breed and age and transferred from pasture in nine groups of ten animals to: 1) fully-slatted (FS) pens indoors (n=3); 2) woodchip pads outdoors (WC, n=3) and 3) rubber covered concrete pads outdoors (RC, n=3) from November 15, 2010 until February 11, 2011. All heifers had *ad libitum* access to grass silage (70% DMD) mixed with maize and concentrates for a weight gain of 0.5 kg/d. Behaviour of heifers in each group was observed directly by two observers during four observations lasting 5mins each on 3d/week between 1030 and 1600 h. All occurrences of comfort, social and locomotory play behaviour as well as slips and falls were recorded. Additionally, 18 dataloggers (TinyTag) were attached to the mid tarsal part of the right hind leg of six heifers per group during two 24hr periods/group. Total time lying, number of lying bouts and lying bout duration were calculated from uploaded data. All data were analyzed using PROC MIXED of SAS program. WC and RC performed more ($P<0.001$) play behaviours than FS heifers. FS heifers performed more ($P<0.01$) grooming behaviour compared to WC and RC heifers. There was no difference in time spent lying between treatments ($P>0.05$). FS heifers had longer ($P<0.001$) lying bouts durations and fewer ($P<0.001$) lying bouts than RC and WC heifers. Shorter lying bout length combined with higher rates of posture changing reflects comfort lying on woodchip and rubber surfaces relative to concrete slats.

Key words: Dairy heifers, Behavior, Welfare

INTRODUCTION

Appropriate rearing and management of replacement dairy heifers is vital to their future productivity and longevity in grass based systems of milk production. In temperate climates such animals are typically housed during the winter period. In spite of their obvious importance in determining future production levels, replacement dairy heifers are often assigned the poorest housing conditions (Gardner, 2003; O'Connell et al., 1993). In Ireland, replacement dairy heifers are often kept indoors on slatted floors which are associated with welfare problems ((Tuyttens, 2005; Platz et al., 2008). Boyle et al. (2008) reported behavioural improvements in yearling heifers out-wintered on a wood chip pad compared to animals kept indoors in a free-stall system (solid concrete floors). Out-wintering on a rubber surface is potentially a more economical and less labour intensive alternative. Indeed rubber covered concrete is associated with advantages to animal behaviour and welfare compared to uncovered concrete (Boyle et al. 2007; Platz et al., 2008). Out-wintering systems also conform to the low cost approach which is crucial to the profitability of grass based systems of milk production

particularly in expanding herds. This study compared the behaviour of replacement dairy heifers out-wintered on either a wood chip or rubber covered out-wintering pad with that of animals kept indoors on concrete slats. The aim was primarily to determine potential differences in lying behaviour which is an important indicator of comfort and therefore animal welfare (Tuyttens, 2005).

MATERIAL AND METHODS

The study was conducted at Teagasc "Ballydague" research farm, which is part of the Animal and Grassland Research and Innovation Centre at Moorepark (50°072N; 8°162 W) between November 17th 2010 (d0) and February 11th 2011 (d91). Ninety heifers (*Bos taurus*), born in the spring of 2010, were blocked according to breed (Jersey, HF, Norwegian Red and crosses of same) and age (average age 271d at start) into nine groups (10 heifers/group). The heifers were assigned to 1) Wood chip (WC) covered out-wintering pads (OWP) (n=3); 2) Rubber covered (RC) (MaxGrip flushing mat™ EasyFix™) OWP (n=3); or 3) Fully slatted (FS) concrete pens indoors (n=3). On WC and RC the space allowance for lying was 5.65 m^2 /head and heifers fed via a neck rail barrier from an adjacent concrete area (2.31 m^2/head). Indoors the space allowance was 2.51 m^2 per head.

The heifers outdoors on the OWPs had access to feed from a concrete area adjacent to the pad. *Ad lib* grass silage of 70% DMD mixed with maize and concentrates (targeted to gain 0.5kg/heifer/day) was fed through a standard neck rail feed barrier. FS heifers had access to the same silage through a standard neck rail feed barrier. All heifers were fed once daily by a diet feeder at approximately 11.00h. Enough forage was offered to each group to ensure that some was left over to enable group feed intakes to be recorded. Heifers in all groups had continuous access to water from a trough.

The WC OWP was constructed of layers of graded stones and a deep layer of wood-chip (approximately 100 kg/m2) placed on top as described by Hickey et al. (2003). Fresh wood-chips were used on the OWP at the start of the study and the dirty layer of woodchips was replenished during the first week in January. The RC OWP was constructed with concrete which was covered with EasyFix™ max grip flushing mats. The mats were flushed once a day with water stored in a tank adjacent to the pad and released from 12 tubes simultaneously. The heifers indoors were kept in three square fully slatted concrete pens separated by steel bars. The slats were 11cm wide and the openings between the slats were 2.8 cm wide. Two trained observers observed each group of heifers directly for four five min long (20mins/day in total) observations for 3d/wk during 11 of the 12 weeks of the study. Observations were conducted between 1030 and 1600h each day. All occurrences of comfort, social, agonistic, play, locomotory, sexual and other behaviours were recorded (see Table 1 for ethogram).
TinyTag™ data loggers were used to measure lying behaviour during 2×24hr periods/heifer during the experimental period. Eighteen data loggers were available which were rotated between the animals in the groups across the treatments according to a balanced schedule. They were attached to the right hind leg after being wrapped in cotton wool using a bandage. After 24h of recording the dataloggers were removed and the data were uploaded to a computer with the program Tinytag explorer 4.4™. The time spent lying, the number of lying bouts and the duration of each lying bout was calculated and the mean of the two 24h periods was used in the analysis. Data were analysed using Proc Mixed of SAS taking repeated measures into account and using Tukey's test for multiple comparisons.

Table 1. Ethogram used to evaluate the effects of wintering systems for dairy heifers on behavior

Behavior	Definition
Comfort behaviour	
Stretch	Straighten out limbs
Lick costal arc	Stand with one hind leg lifted and a front leg positioned diagonally
Self-groom/groom	Lick part of body while standing or lying/a heifer licks another while
Agonistic behaviour	
Threat/butt	Head lowered, back may be arched/blow with forehead
Chase	Animal pursues another with head lowered
Displace	Animal displaced from lying area or feed face owing the agonistic
Play/locomotory behaviour	
Head play	Face-to-face contact associated with pushing and rubbing
Play chase	One yearling runs after another, may involve bucking
Buck/canter	Two back legs kicked up/fast four beat gait
Other	
Trip, slip or fall	Animal stumbles or slips, may or may not thereafter fall to the ground
Sex mount	Mounting another animal

RESULTS

There was no difference in time spent lying between treatments (P>0.05). FS heifers had longer (P<0.001) lying bouts durations and fewer (P<0.001) lying bouts than RC and WC heifers. FS heifers performed more self-grooming behaviors than the WC and RC heifers (Table 2). There was no treatment effect on the frequency of agonistic behavior but over time, levels of agonistic behaviour reduced in all treatments (P<0.001). RC and WC heifers performed more locomotory and other play behaviors than FS heifers.

Table 2**.** Effect of winter accommodation on (selected) behaviours of yearling dairy heifers during a 12 week trial

Behavior	WC OWP	RC OWP	FS	s.e.	T	Time	T*T
Self groom (no./20min.)	24.47[a]	21.03[a]	31.22[b]	0.915	0.01	0.001	0.001
Agonistic (no./20min.)	6.67	6.61	7.81	0.755	0.231	0.001	0.089
Play (no./20 min.)	1.81[a]	2.03[a]	0.47[b]	0.077	0.001	0.001	0.001
Total time lying (hh:mm:ss)	13:54:09	13:28:26	13:35:32	00:10:34	ns	0.001	0.001
Lying bout dur. (hh:mm:ss)	00:52:48[a]	00:53:42[a]	01:01:53[b]	00:01:25	0.001	0.001	0.001
No. lying bouts (hh:mm:ss)	16.23[a]	15.56[a]	13.51[b]	0.527	0.001	0.001	0.05

T = Treatment; WC OWP = Wood chip out wintering pad; RC OWP = Rubber covered OWP; FS = Fully slatted floor indoors; ns=non- significant; Differences between treatment means with different superscripts [a,b,c] are significantly different

DISCUSSION

In agreement with the findings of Boyle et al. (2008) RC and WC heifers performed more play behavior than the housed heifers which is a positive welfare indicator (Oliveira et al., 2010). This was

because of the larger space allowances outdoors which facilitated all types of play behaviour but particularly locomotory play. The surer surfaces underfoot probably also played a role (Platz et al., 2008). Over time, levels of agonistic behaviour reduced in all treatments reflecting establishment of the dominance hierarchy. FS heifers indoors self-groomed more than the WC and RS heifers. Self-grooming in the form of licking is a positive welfare indicator but in the case of the heifers indoors, self-grooming mostly took the form of scratching. This could reflect more problems with parasites in the more closely confined animals indoors although all animals were treated for ectoparasites at the start of the experiment. Alternatively scratching may have been more easily facilitated by the pen fixtures indoors. There was no difference in overall daily lying time, but FS heifers had longer, and hence fewer, lying bouts than WC or RC animals. This behavioural pattern is consistent with a reluctance to make postural changes while lying on concrete and hence is a reflection of discomfort while lying (Andreae and Smidt, 1982). In conclusion the behavioural patterns of the heifers indoors suggest that aspects of their welfare was poorer than that of the out wintered animals under the specific circumstances of this trial.

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AIRBORNE DETECTION OF SWINE INFLUENZA A VIRUS AND *MYCOPLASMA HYOPNEUMONIAE* IN FRENCH SWINE FARMS

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SUMMARY. The detection in aerosols of three pathogens involved in porcine respiratory disease complex (PRDC), namely swine influenza A virus (swIAV), porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (Mhp), was evaluated in three French commercial pig farms. The herds were known to be affected by PRDC involving those three pathogens. Aerosols were collected using a wet cyclone technology (Coriolis®µ air Sampler). In all herds, air samples were taken in rooms housing pigs and in the corridors (inside or between buildings) without pigs. The loading area with slaughter-aged pigs waiting for the slaughterhouse delivery was sampled in two herds. Aerosols were also collected in the attic under the roof of one building. Specific real time RT-PCR or PCR tests were used to detect swIAV, PRRSV and Mhp genomes, respectively. SwIAV and Mhp were found in air samples of all herds whereas PRRSV was not detected in any sample. SwIAV was the most frequently detected pathogen (10/12 samples) followed by Mhp (8/12 samples). Both pathogens were found in the rooms housing pigs (4/6 rooms for swIAV and 4/6 rooms for Mhp), the corridors of the building or between two buildings (3/3 corridors for swIAV and 2/3 corridors for Mhp), the loading areas (2/2 areas for swIAV and 1/2 area for Mhp) and even the attic under the building roof. Although the infectivity potential of the pathogens detected in air samples was not evaluated, the results suggest that swIAV and Mhp can be present in detectable concentrations in aerosols and might be airborne transmitted within commercial swine confinement building environments. The significance of this finding from an epidemiological point of view will need further investigation.

Key words: bioaerosols, swine buildings, respiratory pathogens

INTRODUCTION

Porcine respiratory disease complex (PRDC) is one of the most common and costly production diseases affecting growing finishing pigs reared in large groups and confined conditions worldwide. PRDC is multifactorial in nature and results from infection with various combinations of primary and secondary bacterial and viral respiratory pathogens (Opriessnig et al., 2012). Swine influenza A virus (swIAV),

porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (Mhp) are among the main acknowledge contributors to PRDC. Due to their particular tropism for the respiratory tract, exposure of susceptible pigs to infectious aerosols produced by infected pigs is a main infection route. Depending on the building design, airborne transmission might occur within a room, between rooms within a facility or between facilities of a farm or between herds. Microbial air quality is a respiratory health factor in confined buildings that should be taken into consideration in the understanding of respiratory diseases outcome and for control and preventive purposes. PRRSV and Mhp have been detected in aerosols collected up to 9km from a shedding source farm or inside building and swIAV in room housing acutely infected pigs and the exhaust air in the USA or Korea (Otake et al., 2010; Corzo et al., 2013; Damte et al., 2014). However, little is known about the airborne detection of these three pathogens in piggeries under European conditions. Thus, the aim of this study was to explore the airborne detection of swIAV, PRRSV and Mhp under field conditions in PRDC affected farms.

MATERIAL AND METHODS

The study was conducted in three French commercial pig farms (herds 01, 02 and 03) known to be affected by PRDC involving swIAV, PRRSV and Mhp and kindly selected by swine veterinarians. Aerosols were collected using a wet cyclone technology (Coriolis®µ air Sampler, Bertin Technologies, St-Quentin en Yvelines, France) processing 300L/min of air. Briefly 15 mL of 0.005% Triton solution were added to the liquid cyclonic collector collection vessel for viral pathogen detection. For Mhp collection, 10 mL of buffered peptone water broth were used. The collector was run for 10 minutes allowing airborne particles to be mixed with the collection media. Once air sampling was completed, the vessel was closed and identified. The device was then cleaned and dried between consecutive air samplings.

In all herds, air samples were taken in rooms housing pigs as well as in the corridors (inside or between buildings) without pigs. The loading area with slaughter-aged pigs waiting for the slaughterhouse delivery was also sampled in herds 02 and 03. In addition, aerosols were collected in the attic of one building (herd 03). For swIAV and PRRSV genome detection, the air samples were concentrated by centrifugation for 30 min at 3900 × g using Amicon® Ultra-15 centrifugal filter units 30K (Merck Millipore, Billerica, MA, USA). Then, viral RNA purification was performed using the NucleoSpin RNA virus kit (Macherey-Nagel, Düren, Germany) and specific real time RT-PCR kits were used to detect swIAV and PRRSV (in air samples), i.e. Adiavet™ SIV RealTime and Adiavet™ PRRS RealTime, respectively (Adiagene, Saint-Brieuc, France). Mhp genome was detected in the samples and quantified by a qPCR (Marois et al., 2010).

RESULTS

SwIAV and Mhp were found in air samples of all herds whereas PRRSV was not detected in any sample. SwIAV was the most frequently detected pathogen (10/12 samples) followed by Mhp (8/12

samples). Both pathogens were found in the rooms housing pigs (4/6 rooms for swIAV and 4/6 rooms for Mhp), the corridors of the building or between two buildings (3/3 corridors for swIAV and 2/3 corridors for Mhp), the loading areas (2/2 areas for swIAV and 1/2 area for Mhp) and even the attic under the building roof. Based on cycle threshold (Ct) values, the swIAV genome was detected at non quantifiable loads. Mhp genome load varied from $4.3.10^3$ (loading area with fatteners coughing) to $1.9.10^5$ (room with piglets exhibiting respiratory clinical signs) fg of mycoplasma DNA per aerosol sample. Detailed results at the sample level are given Table 1.

DISCUSSION

The role the aerosol plays in the transmission and persistence of respiratory diseases within and between herds has not been fully elucidated. Our exploratory study showed that airborne detection of swIAV and Mhp genomes within and between buildings is possible in herds affected by PRDC. This is the first report on detection of both pathogens in aerosols under field conditions in French pig herds. Our results support previous findings where Mhp or influenza genetic materials were detected in the air and downwind from experimentally infected or acutely respiratory affected swine populations (Stärk et al., 1998; Otake et al., 2010; Corzo et al., 2013; Damte et al., 2014) or upon influenza challenge under experimental conditions (Cador et al., 2016). Conversely to an earlier study, PRRSV genome was not detected in the air inside building housing pigs showing respiratory signs (Otake et al., 2010). Putative explanations for the lack of PRRSV detection in our study may be linked to the absence or low number of PRRSV infected animals and/or the low viral load excreted by infective animals when present. Further studies combining individual sampling of the pigs and air collection inside the room housing those animals, in the different parts of the building and in the exhausted air would be required to provide evidence that the infectious agents detected in aerosols originated from the pigs.

An interesting finding relies on the detection of two respiratory pathogens from the air sampled in the attic under the building roof and from the corridors. This clearly shows that air flow should be taken into account when understanding the persistence of respiratory diseases on a farm.

Although the infectivity potential of the pathogens detected in air samples was not evaluated, the results suggest that swIAV and Mhp can be present in detectable concentrations in aerosols and might be airborne transmitted within commercial swine confined building environments. The significance of this finding from an epidemiological point of view will need further investigation.

Table 1: Detection of swine influenza A virus (swIAV), porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (Mhp) by PCR tests in air samples under field conditions (3 pig herds – 12 samples)

			Infectious pathogen		
Herd	***Sample***	**Sampling site**	**swIAV**	**PRRSV**	**Mhp**
01	1	Pregnancy room (96 sows)	nd*	nd	**$1.3x10^4$ fg of Mhp DNA/ml of air sample**
	2	Pregnancy room (93 sows)	nd	nd	**$3.4x10^4$ fg of Mhp DNA/ml of air sample**
	3	Corridor between pregnancy and nursery rooms (outside)	**Detected**	nd	**$9.4x10^4$ fg of Mhp DNA/ml of air sample**
	4	Nursery room 228 4-week-old piglets	**Detected**	nd	**$1.9x10^5$ fg of Mhp DNA/ml of air sample**
	5	Nursery room 274 5-week-old piglets	**Detected**	nd	nd
02	1	Corridor of the fattening building	**Detected**	nd	**$6.9x10^4$ fg of Mhp DNA/ml of air sample**
	2	Fattening room 132 pigs -14 weeks of age	**Detected**	nd	**$1.5x10^4$ fg of Mhp DNA/ml of air sample**
	3	Loading area 85 pigs – 25 weeks of age	**Detected**	nd	**$4.3x10^3$ fg of Mhp DNA/ml of air sample**
03	1	Loading area 112 pigs – 23 weeks of age	**Detected**	nd	Nd
	2	Attic under the building roof	**Detected**	nd	**$1.6x10^4$ fg of Mhp DNA/ml of air sample**
	3	Nursery room 166 8-week-old piglets	**Detected**	nd	Nd
	4	Corridor of the nursery and fattening building	**Detected**	nd	Nd

* nd: not detected by PCR or RT-PCR

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Mastitis caused by *Mycoplasma bovis* in Brazil

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SUMMARY. Bovine mastitis is the cause of one of the biggest losses in milk production and, in 90% of the cases, the origin of this disease is associated with bacterial infections. The mastitis caused by *Mycoplasma bovis* are described in the literature as relatively common in American dairy herds, causing an estimated $108 million dollar a year economic loss. This pathogen, however, is still underestimated, especially in Brazil because of the selective environment needs and the unique conditions associated with its isolation. The objective of this study was to investigate the presence of *Mycoplasma* spp. and *Mycoplasma bovis* in the aetiology of clinical mastitis from Brazil. Five hundred and sixty-one (561) milk samples were evaluated of animals with clinical mastitis from the following states: Ceará, Goiás, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul and São Paulo. The samples were grown on Hayflick broth added of thallium acetate 0.01%, incubated in microaerophilic atmosphere at 5% CO2. The samples were also subject to molecular evidence polymerase chain reaction (PCR) for the detection of *Mycoplasma* spp. and *Mycoplasma bovis*. Colonies of *Mycoplasma* spp were isolated in 11 (1.96%) samples. The 561 milk samples included in this study were subjected to molecular detection for Mollicutes family, from which 17 samples were positive. All identified as positive were reassessed for the molecular diagnosis for *Mycoplasma bovis*. Among these 17 were positive for *M. bovis*, 29.4% (n = 5) from the state of São Paulo, 17.64% (n = 3) of Minas Gerais, 17.64 % (n = 3) of Goiás and 35.29 (n = 6) of Paraná. It can be concluded with the results of this research, the presence of *Mycoplasma bovis* in dairy herds evaluated, but with a lower incidence of this pathogen when compared to other countries, such as the United States.

Key words: micoplasmosis, clinical mastitis, PCR

INTRODUCTION

Brazil is one of the largest milk producers in the world, and its production is essential to the Brazilian economy (USDA, 2011). Bovine mastitis is the cause of one of the biggest losses in milk production and, in 90% of the cases, the origin of this disease is associated with bacterial infections (PHILPOT & NICKERSON, 1991).

Mycoplasma spp., has a worldwide distribution and can cause severe pneumonia in calves and mastitis in lactating cows (QUINN et al., 2005 a), keratoconjunctivitis (KIRBY & NICHOLAS, 1996). And when were experimentally infected also induced endometritis, salpingitis, otitis, miscarriage and seminal vesiculitis (RUHNKE, 1994).

The mastitis caused by *Mycoplasma bovis* are described in the literature as relatively common in big dairy herds (QUINN et al., 2005 b), causing an estimated $108 million dollar a year economic loss (ROSENGARTEN & CITTI, 1999).

This pathogen, however, is still underestimated, especially in Brazil, where there are few reports of its occurrence as agent of mastitis due to a small number of laboratories that includes *M. bovis* in their routine analysis (NICHOLAS & AYLING, 2003), and because of the selective environment needs and the unique conditions associated with its isolation.

MATERIALS AND METHODS

Milk samples

Five hundred and sixty-one (561) milk samples were evaluated of animals with clinical mastitis from the following states: Ceará, Goiás, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul and São Paulo (Image 1). The samples were analysed at the Centre for Research in Mastitis (NUPEMAS), Collage of Veterinary Medicine and Animal Science (FMVZ), UNESP, Botucatu, São Paulo.

Mycoplasma spp. microbial culture:

The samples were received frozen and after thawed were grown on plates containing Hayflick broth (WHITFORD et al., 1994) added of thallium acetate 0.01%, incubated in microaerophilic atmosphere at 5% CO2 for 15 days. The analysis of its isolation was performed by visualization of the colonies in inverted microscope, and selecting those that presented the characteristic feature of a "fried-egg", starting from the third day of culture and then every three days.

DNA extraction

The extraction of direct DNA from the raw milk was performed using the commercial kit Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare), with some adaptations previously standardized in the Laboratory of Molecular Biology Applied to the Diagnosis of Zoonoses - UNESP-Botucatu-SP.

Amplification of nucleic acid (PCR)

The samples were also subject to molecular evidence polymerase chain reaction (PCR) for the detection of *Mycoplasma* spp. and *Mycoplasma bovis*. For amplification of DNA mollicutes, the primers MGSO (5'TGC ACC ATC TGT CAC TCT GTT AAC CTC 3') and GPO-3 (5'GGG AGC AAA CAG GAT TAG ATA CCC 3') were used, common product of 270 pairs from the basis. When PCR were positive with generic primers, amplification of the DNA for *Mycoplasma bovis* with the specific primers MBOR (5'- GCC AGG TCA CTA TAG CAT CAT TTC 3'T) and MBOf (5'CCT AGA TTT TTG TAG CGG GGA ATG 3'), with 360 base pair product, were performed.

RESULTS

Colonies suggestive of Mycoplasma spp were isolated in 11 (1.96%) samples. The 561 milk samples included in this study were subjected to molecular detection for mollicutes family, from which 17 samples were positive. All identified as positive were reassessed for the molecular diagnosis for *Mycoplasma bovis*. Among these 17 (3.03%) were positive for *M. bovis*, 29.4% (n = 5) from the state of São Paulo, 17.64% (n = 3) of Minas Gerais, 17.64 % (n = 3) of Goiás and 35.29 (n = 6) of Paraná.

DISCUSSION

The present study found a prevalence of 3.03% of M. bovis as a pathogen in clinical mastitis in cattle. There is a variation in the prevalence results of the mastitis occurrence by this pathogen between Brazilian states and around the world. Thomas et al. (1981) gives as main hypothesis of this variation the size of the herds, being the risk of a herd being positive for *Mycoplasma* spp. 15 times greater in large herds (> 350 animals) when compared to smaller herds. This fact can be explained by a combination of several incorrectly applied property management factors in large dairy farms, especially with regard to hygienic management established before and after milking.

It can be concluded with the results of this research, the presence of Mycoplasma bovis in dairy herds evaluated, but with a lower incidence of this pathogen when compared to other countries, such as the United States. Emphasis was added in the importance of further studies to include a larger number of flocks in different locations in the country and evaluate samples of expanding properties tanks,

including cultivation and PCR, to further document the role of this pathogen in the aetiology of mastitis in this country.

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EPIDEMIOLOGY OF GASTROINTESTINAL PARASITES IN EWES FROM MEXICO

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SUMMARY. Sheep are susceptible to infections by gastrointestinal parasites causing considerable losses in production and even deaths of lambs. Local information is needed for effective control. The aim of this study was to determine the frequency and intensity of parasites in sheep from Temascaltepec, State of Mexico. 90 sheep females, most pregnant ewes were employed: 50 with grazing in the forest, 30 implanted with grazing pastures and 10 in permanent pens. Sampling was conducted since December 2015 until May 2016. Faecal samples were collected from the rectum, Mc Master technique was performed, the morphological characteristics were observed, were counted oocysts and eggs per gram of faeces (opg and epg), the prevalence frequency and intensity was obtained. With positive samples, stool culture was performed and genera were identified. The parasites were present in 100% of samples, their frequency and intensity varied due to management and physiological state of animals. Pregnant ewes in the last month of gestation and lactation had high parasite loads (up to 20,000 epg). In all ewes, *Eimeria*, *Moniezia* and nematodes were identified. Genera recorded were *Haemonchus*, *Trichostrongylus*, *Cooperia*, *Nematodirus* and *Trichuris ovis*. In flocks, a frequent deworming is done without previous diagnosis, sometimes without ewes require it. Therefore it is necessary to conduct studies on population and parasite dynamics for selective and integral control to produce a higher yield in animal, achieve an environmental benefit and economy producer and final consumer.

Key words: gastrointestinal parasites, epidemiology, ewes, control.

INTRODUCTION

Gastrointestinal parasites cause production losses, in the U.S. It has been estimated that parasite infection in ruminants is one of the main problems and losses amount to more than 3 billion dollars per year, approximately 60% corresponds to sheep, in New Zealand only 23 million New Zealand dollars in anthelmintics per year (seven treatments / animal / year) (Luna-Palomera et al., 2010; Figueroa and Acevedo, 2011). The effect of gastroenteric parasites varies in severity, decreases sheep productivity, delayed growth and puberty, reduced weight gain by up to 50%; (Knox et al., 2006), and mortality rates between 20 and 50% (Torres et al., 2006). Gastrointestinal nematodes are the most common parasites of ruminants worldwide, especially in tropical or humid temperate regions.

In most productive units, treatment, prevention and control are based on the use of anthelmintics such as benzimidazoles, imidazothiazoles and macrocyclic lactones. Repeated application, underdosing and, due to the rare occurrence of a previous diagnosis to detect the parasitic prevalence and to establish an adequate treatment, have led to the selection of nematode populations resistant to anthelmintics, becoming an emerging problem (Figueroa and Acevedo, 2011).

For this reason, it is essential to know the population and parasite variation in order to establish effective control measures that promote a decrease in the administration of drugs, be profitable for the producers and also reduce the side effects in the animals and the environment.
With the purpose of having local information to achieve a comprehensive control to reduce the negative impact of parasites, improve animal life, produce quality animal protein, with less environmental damage and promote better nutrition and human health , It is necessary to identify the animals with high parasite load, for which it is necessary to make a diagnosis and thus to develop selective deworming strategies that allow the increase of the parasite populations susceptible to anthelmintics, as well as the reduction of costs by treatment and (Torres-Acosta and Hosté, 2008). However, it is important to note that in the control strategy, the anti-parasitic treatments are used exclusively for the animals that really need them.
Sheep production has economic relevance in the State of Mexico, since it is the main producer of Mexico (SIAP, 2014). To reduce the negative impact of gastrointestinal parasites, it is necessary to know the behavior of parasitosis in order to establish comprehensive control programs. The objective was to determine the frequency and intensity of parasites in sheep of Temascaltepec, State of Mexico during the dry season (December 2015 - May 2016). Ninety female sheep were used, most of them pregnant; 50 with grazing in the forest, 30 with grazing in implanted prairies and 10 in permanent pens.

MATERIAL AND METHODS

The study was carried out in localities of the municipality of Temascaltepec, State of Mexico, at an altitude of 2,250 m, with temperature 16º-20º C and rainfall of 800 to 1,200 mm. It is located on the mountainous extension chain of the volcano Nevado de Toluca, with pine forest vegetation.
Five flocks of adult females between 1 and 5 years old were studied, most of them were pregnant and reared. Two flocks with extensive grazing in the forest and nocturnal housing: flock 1 of 18 sheep; The flock 2 of 25 sheep. Two flocks with pasture in implanted prairies, nocturnal housing and with food supplementation: the flock 3 conformed by 18 sheep, the flock 4, with 7 sheep. A flock with permanent housing. Fecal samples were collected directly from the rectum in plastic bags individually each month, during the dry season (December 2015 to May 2016). The samples were labeled and transferred to the Parasitology laboratory of FMVZ-UNAM. They were kept at 4 ° C until processing. The Mc Master technique was performed, the intestinal parasites were identified and counting was performed. With stools positive to gastrointestinal nematodes, they were homogenized with sterile sawdust in plastic containers, the mixture was moistened without excess water, incubated at 27 ° C for 10 days and oxygenated daily (Liébano, 1989). Third larvae (L_3) were collected by means of the larval migration technique for 24 hours. The larvae migrated from the faeces and, through gravity, passed through a sieve, sedimented at the bottom of the funnel and the first drops were taken (Thienpont et al., 1979). A drop of the collected liquid was taken from the Baermann funnel and a drop of lugol was placed to fix the L_3. By means of the microscopic observation the taxonomic identification of the different genera of NGI was realized (Niec, 1968, Vega and Romero, 1983; van Wyk, et al., 2004).

Statistical analysis: Oocyst and egg counts per gram of faeces (opg and epg), frequency (percentage of positive samples) and mean intensity were obtained: mean oocysts or eggs of the positive samples (Eckert et al., 1984). From the mean intensity in egg removal, the standard deviation was obtained. The percentage of identified genera or species was obtained

RESULTS

In the study period, gastrointestinal parasites were identified in all flocks. The protozoa *Eimeria spp*. They had a frequency of 0 to 68%, the frequency was higher in the flock 3, on the contrary, the flock with less frequency was in the flock 1. The intensity was 0 to 686 opg. *Moniezia* cestode had a frequency of 0 to 42%, with an intensity of 0 to 3617 epg, the flock 1 had frequency and greater intensity, while the flock 5 had less frequency and intensity.

The nematodes were represented by tricostrongylides, the frequency was 100% in all flocks and an intensity of 285 to 9500 epg. Flock 4 had the highest intensity, while flock 5 had the lowest intensity (Figure 1). The nematodes identified were *Haemonchus, Trichostrongylus* and *Cooperia*. *Chabertia* was identified in flocks 1, 2, and 3. The genus *Oesophagostomum* was identified in flocks 1 and 3 in March and April respectively; *Bunostomum* and *Teladorsagia* were recorded only in flock 2 in January and April respectively (figure 2). Flock 2 had the greatest diversity with six genera.
In addition, nematode eggs of the genus *Nematodirus* and *Trichuris ovis* were identified in all flocks.

DISCUSSION

Gastrointestinal parasites are common in sheep, so local studies are necessary to provide local solutions. In this study, protozoa, cestodes and nematodes were identified, even in the flocks in housing, the largest group was nematodes, since they were identified in 100% of the sheep and the parasite load was higher. For example, when considering trichostrongylide nematodes, the phenomenon of peripartum increase occurs, and was noticeable in all flocks, since most births occurred between January and March, months with a higher nematode egg intensity. Among the genera identified, *Haemonchus* was the most frequent, followed by *Trichostrongylus* and *Cooperia*; however, genera *Bunostomun*, Oesophagostomum, *Trichuris ovis* and others that are common in temperate regions such as *Teladorsagia, Chabertia* and *Nematodirus* were also identified.
Although the flocks are located in the same geographical region, the parasitic loads were different, which is directly related to the management, for example, the flocks in grazing in the forest have little food supplement and are only dewormed when some technician recommends it without making a previous diagnosis. In flock 3 there was a decrease in egg removal in March, because at the end of February ivermectin was administered because of a problem of mange that was present, so it also had an effect on the gastrointestinal nematodes, so that the Number of eggs decreased by almost 75%. Flock 4 had the highest intensity, although they had a good quality food supplement, sheep graze daily in the same pasture, so they are constantly infected. On the other hand, although flock 5 is permanently in housing, it was recorded that 100% of the ewes were positive for trichostrongylide nematodes although its intensity was less than 1000 epg.
Due to the information gathered, it is concluded that it is necessary to carry out previous diagnostic studies before deworming, in order to carry out an integral parasitic control, including parasitic management and load, which will affect the health of the animal and is expected in its production.

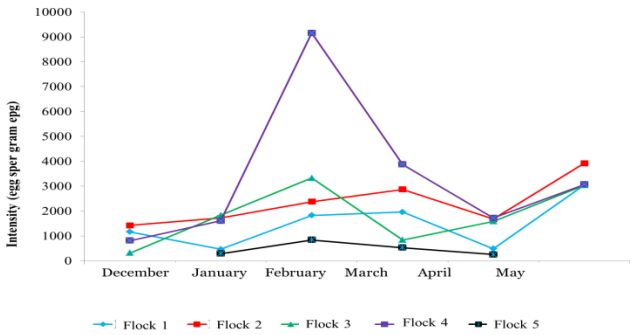

Figure 1. Intensity of trichostrongylid nematodes of sheep from Temascaltepec, State of Mexico.

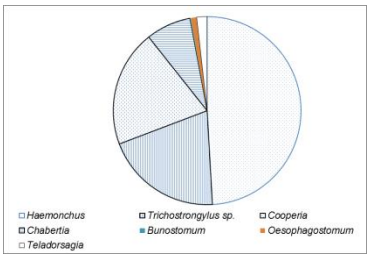

Figure 2. Genus of trichostrongylide nematodes identified through larvae 3 of sheep from Temascaltepec, State of Mexico.

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MICROBIAL LOAD OF DUST SAMPLES FROM LAYING HENS FLOCKS IN EGYPT: FIRST RESULTS

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Summary. Poultry houses are highly contaminated with airborne dust which can be harmful to animals, humans and the environment. Investigations and a better understanding of the dust helps to estimate the health risks for poultry, poultry workers and peoples living in the vicinity of poultry farms. Airborne dust contains a mixture of bio-aerosol such as bacteria, fungi and endotoxin. Due to the potential hazardous effects of dust and the lack of knowledge about the composition and amount of bio-aerosol in dust from laying hen houses in Egypt, a study was conducted to evaluate microbial load of dust from 28 laying hen houses at Dakahlia governorate, Egypt. Furthermore, to raise awareness of human in and around the poultry farms to health risks of dust and the need for control. Pooled settled dust samples were obtained from 10 different locations within the hen house and examined for total bacterial count (CFU/g), total fungal count (CFU/g) and *E.coli* concentration (CFU/g). Findings showed that the examined dust contains high load of bacteria, fungi and *E.coli*. In conclusion, laying hens dusty environment contain high level of bacteria, fungi and *E.coli* which may impair the health of birds, farm workers and peoples living near the farms.

Keu words: Laying hens, settled dust, bacteria, fungi

INTRODUCTION

Organic dust is considered one of the major challenges affecting production in poultry farms. Bio-aerosols are a component of organic dust including whole bacterial cells, fungi, spores, viruses and their by-products, endotoxins and mycotoxins. The emitted amount of organic dust in poultry farms depends on the type of birds, manner of housing, stocking density, growth stage, humidity, feeding system and adoption of hygienic standards (Oppliger *et al.*, 2008). Battery cages represent the lowest levels of dust production in comparison to the raising of chicken in floor housed operations (Ellen *et al.*, 2000). The approximated ratio of dust concentration in littered floors to battery cages was 1.6 (Vaicionis *et al.*, 2006). Knowing the microbial and fungal load of poultry farm dust is of great concern. The predominated bacteria within bio-aerosols in poultry farms are gram positive bacteria that include *Bacillus* spp. and *Staphylococcus* spp. Gram negative species especially *Escherichia coli (E. coli)* constituted 7-17% of bio-aerosols in poultry farms (Whyte, 1993). The prevalent airborne fungi enclosed in dust of poultry are *Aspergillus, Penicillium, Cladosporium* and *Fusarium* (Lee *et al.*, 2006). Zoonotic potential of bio-aerosols in poultry farms have been associated with the respiratory affections in farm workers (Hartung and Saleh, 2007) and the survival of foodborne pathogens in the environment with the possibility of its transmission to humans either directly or indirectly. There is a dearth of information about the dust composition in laying farms with its potential effect on the birds, environment and humans in Egypt. The overall aim of this study is to evaluate the degree of microbial dust contamination among different laying farms in Dakahlia Governorate, Egypt.

MATERIAL AND METHODS

Poultry Farms and dust collection:

The studies were conducted on 28 different lying hen barns in Dakahlia Province, Egypt, that were housed in deep litter system during the period between July 2015 to August 2016. From each farm, dust sample was taken from only one flock that representing the whole farm. Sampling date, number and age of birds are listed in Table 1.

Dust was collected from 10 different points of the house from the floors and several horizontal surfaces. All dust samples were collected in the morning between 9:00 - 10:00 am, using a sterile shovel and brush and were placed in sterile plastic containers and stored at 4°C until used for examination. The examination was carried out for all dust samples at the same time.

Quantification of total viable mesophilic bacteria, *E.coli* and total fungi:

Total viable mesophilic bacteria (TVMB), *E.coli* and total fungi (TF) count were determined 0.1 g dust was shaken for 30 min in a water bath at 25°C. Subsequently, the suspension was vortexed for 4 min with a VORTEX-2 GENIETM (Scientific Industries Inc., Bohemia, NY, USA).TVMB and *E.coli* counting were carried out by spreading 0.1 ml aliquots in triplicate on Tryptone Soya Agar plates and MacConkey agar plates (Oxoid Ltd., Basingstoke, UK), Respectively. Plates were incubated at 36°C for 48h. Furthermore, the TF count was performed by plating 0.1 ml aliquots in triplicate on DG-18agar plates (Oxoid Ltd., Basingstoke, UK). Plates were incubated at 25°C for 2-5 days. After incubation, the recovered colonies and suspected *E.coli* colonies grow on MacConkey agar were counted and 5 colonies at least were confirmed using API 20E (bioMérieux, France), and the results were expressed in CFU/g of dust.

RESULTS

All dust samples showed growth of mesophilic bacteria. The TVMB count varied between $9.07 \times 10^6$and 1.41×10^{10}CFU/g dust in flocks No.7 and 26, respectively (Figure 1). Table 1 and Figure 1showed that, there was seasonal influence on the TVMB recovered from dust, samples collected during summer season showed numerically higher numbers of bacteria than other samples collected in other season. Furthermore, all dust samples indicate the presence of fungi. The total fungal count ranged from 9.84×10^4 to 3.67×10^7 CFU/g dust in flocks 26 and 2, respectively (Figure 1). The higher TF count occurred mainly in summer and fall season (flocks 2, 8 and 9) except for flock 26 that showed the lowest TF count. While, the lower TF count was predominant in winter and spring.

On the contrary, only 16 flocks out of 28 contained *E.coli* in the dust samples. Flocks 1, 10, 12, 13, 15, 17, 18, 19, 22, 23, 24 and 25 were negative for *E.coli*. Regarding the dust sampling time, *E.coli* was detected in older dust (10 out of 14) than new dust (6 out of 14).

Table 1 Sampling time, bird age and season

Sample Nr.	Sampling time	Bird age (Week)	Season
1	11.07.2015	25	Summer
2	25.07.2015	34	Summer

3	05.08.2015	54	Summer
4	16.08.2015	55	Summer
5	29.08.2015	59	Summer
6	02.09.2015	68	Fall
7	21.09.2015	39	Fall
8	07.10.2015	29	Fall
9	25.10.2015	24	Fall
10	01.11.2015	42	Fall
11	25.11.2015	58	Fall
12	01.12.2015	69	winter
13	18.12.2015	51	winter
14	08.01.2016	67	winter
15	03.02.2016	28	winter
16	04.03.2016	33	winter
17	21.03.2016	70	winter
18	05.04.2016	30	Spring
19	19.04.2016	50	Spring
20	06.05.2016	63	Spring
21	23.05.2016	27	Spring
22	10.06.2016	40	Spring
23	26.06.2016	36	Spring
24	05.07.2016	44	Summer
25	18.07.2016	52	Summer
26	30.07.2016	26	Summer
27	08.08.2016	64	Summer
28	30.08.2016	33	Summer

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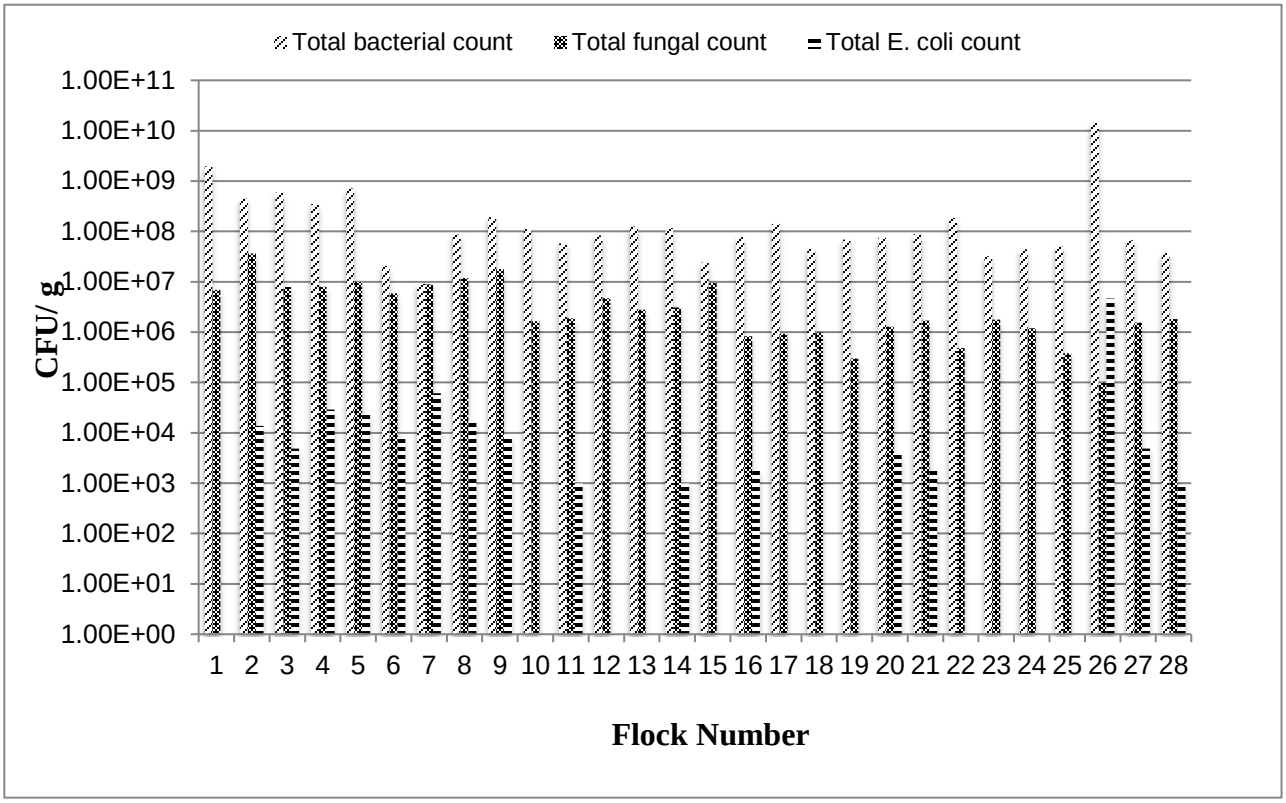

Figure 1. Total viable mesophilic bacteria, total fungi (TF) and *E.coli* count per gm dust

DISCUSSION

In floor housed poultry farms, litter is considered a favourite media for the growth of certain pathogenic bacteria and fungi. In our study, TVMB count varied between 9.07×10^6 and 1.41×10^{10} CFU/variable isolation rates of aerobic mesophilic bacteria from the settled poultry dust and litter were determined previously by Witkowska *et al.* (2010) (10^7-10^9 CFU/g) and Skóra *et al.* (2016) **(**3.2 **x** 10^9CFU/g).Likewise, the total fungal count varied among different studies as reported by Okiki and Ogbimi (2011) (3.5-42 x 10^6 CFU/g) and Skóra *et al.* (2016**), (**1.2**x** 10^6 CFU/g).The difference of total bacterial and fungal count from settled dust in poultry farms has been linked to many factors such as housing system, age of birds, type of production, ventilation, seasonal variation, dynamic of litter exchange and method of sampling collection. Recent investigations were able to isolate *E.coli* from dust with level reached to 1**x** 10^5CFU/g (Schulz *et al.*, 2016), the difference between our finding and this research may be due to the dryness of dust and the different management system in the animal house in the two studies.

It is clearly obvious that the total bacterial and fungal count showed increased values in summer season than other seasons. This was in consistent with results from Plewa and Lonc (2011) and Lawniczek-Walczyk *et al.* (2013). The reason may be attributed to the higher temperature associated with rise in relative humidity that provided the suitable climate for the growth of mesophilic bacteria and fungi. With increasing ventilation in the floor housed farm during hot season, the dust emissions increase

(Ellen *et al.*, 2000) and consequently the risk of spreading bio-aerosols with potentially adverse health effects increases (Hartung and Saleh, 2007).

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SEASONAL VARIATION OF SUBCLINICAL MASTITIS DURING THE SUMMER-AUTUMN PERIOD IN A DAIRY HERD OF FAMILY PRODUCTION

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SUMMARY. Bovine mastitis affects the economics of dairy herds, animal welfare and milk quality. This disease is characterized by an increase in the level of somatic cells in milk when intraglandular infection is associated with the conditions of the production environment and the possible influence of climatic factors. The objective of the study was to determine the seasonal variation of subclinical mastitis (SCM) in dairy cows during the summer-autumn period. In a dairy herd, 30 cows were studied in the production line during the months of July-October, to perform the California mastitis test (MTC) and to obtain milk samples to estimate the somatic cell level, considering reaction 1 and 2 rating as an indicator. The results were evaluated by comparing the months related to the seasonal period Summer (July-August) and Autumn (September-October) using the contingency tables and the Chi-square test ($P < 0.05$). The results obtained were an average frequency of SCM in the herd of 60%, at the beginning of the study, in contrast to 5% of frequency in the same period. However, at the end of the study period, there was a downward trend in cases of CSM cows with a frequency of 45%, whereas during the same period the negative cases had a frequency of 21.25%. During the study period, there was a variation in the presentation of important SCM, which may be related both to production conditions such as hygiene and handling in the dairy herd and also by environmental issues such as the seasonal period in the study was performed.

Keywords: Seasonal variation, subclinical mastitis, summer-autumn period

INTRODUCTION

Mastitis causes great economic losses as it affects animal welfare, milk quality and has become a disease of great importance in public health. The poor management and hygiene that is commonly performed on dairy farms during the milking process, allow the persistence of the disease, affecting the mammary gland (GM) and causing affectation in the quality and safety of milk and its products (Rodriguez 2006). This disease is characterized by increased somatic cell count (SCC) (Peters, 2002). The use of SCC to assess udder health has been shown to be a useful tool for the detection and control of mastitis (Kehrli and Shuster, 1994). Among the existing tests for estimating CCS, the California test (CMT) is the most widely used in the field. The reaction is classified on a scale that includes traces and positive results from 1 to 3 (Aguado, 2001). Mastitis can be classified as clinical, subclinical or chronic depending on its severity (Peters, 2002). While clinical mastitis can be diagnosed for its observable clinical signs in the udder, subclinical mastitis is not detectable by the naked eye. SCM is long-lasting, difficult to detect and predominates more in the herd than the clinical form to which it generally precedes. One of the major changes that occur during SCM, and that serves to detect it, is the increase in CCS (Philpot and Nickerson, 1991, Peters, 2002). The presence of subclinical mastitis in the herd is influenced by different factors such as handling, hygiene and environmental changes that affect the comfort status of cows. Different authors have found variations in CCS associated with the months and

seasons of the year (Whitaker et al., 2004; Olde et al., 2007). In temperate climates, CCS are generally lower in the winter and higher during the summer, which coincides with the increase in the incidence of mastitis during the summer months (Olde et al., 2007). Hogan and Smith (1997) suggest that stress caused by high temperature and humidity may increase susceptibility to infections. In addition to this other factors such as rainfall usually favor the presentation of mastitis being the months of greater precipitation in which cases of mastitis usually increase. Therefore, the objective of this study was to determine the seasonal variation of SCM in dairy cows during the summer-autumn period. In a dairy herd of 30 milking cows during the months of July to October, performing the MTC test on milk samples obtained from cows to estimate the somatic cell level, considering the reactions rated 1 and 2 as indicator.

MATERIAL AND METHODS

The MCT test was performed monthly on 30 dairy cows in production line, during the period from July to October. In order to determine the seasonal variation of the SCM presence for the Summer (July and August) and Autumn (September and October) periods, the inclusion criterion for integrating the study group was the production stage (≥3 and 3 At 6 months) and cows with SCM were those that showed positive reactions to the MCT test (reactions 1-2). Likewise, the percentage of cows negative to the MCT test was obtained. Results were assessed using the Chi-square test ($p > 0.05$).

RESULTS

From the total of the cows sampled, a high percentage of positive calves were tested in the California test (reaction 1-3) in the cows of the present study during the first three samplings; With an average frequency of SCM (reactions 1 and 2) in the 60% herd at baseline, in contrast to 5% of negative cows during the same period. However, at the end of the study period, there was a downward trend in cases of SCM cows with a frequency of 45%, while in the same period the negative cases had a frequency of 21.25% (Table 1).

When comparing the results in relation to the seasonal period Summer (July and August) and Autumn (September and October). No significant differences were observed in the mean percentages of MSC presentation per seasonal period compared to those obtained in the monthly analyzes. However, the statistical analysis shows that if there are statistical differences between the presentation of MSC by seasonal period and also also there are statistically significant differences between the data obtained for the negative cows to the MTC in the periods of study (P0.05) (Table 2).

DISCUSSION

A percentage of 60% of tits with SCM were observed in the cows evaluated at the beginning of the study, with a downward trend during the end of the study, these data are in agreement with those obtained by Rodriguez (2006), in which 57.4% Of cases of mastitis and a high CCS during the Summer season, attributed to fluctuations in temperature that cause stress in cattle. Ferreira and De Vries (2015), studied the CCS in different regions of Florida (USA) obtaining a clear seasonal pattern with increase of the CCS in the summer these results are to the caloric stress that the animals suffer in that region. Similarly, Hogan and Smith (1997) report that the high levels of CCS in milk during the summer are caused by the stress caused in animals by high temperatures and humidity, increasing the susceptibility to infections as well as the number of pathogens To which the cow is exposed, affecting milk production directly.

Table 1: Results obtained in the California test (Negative tits, Trace reactions, reactions rated 1, 2, 3) and percentages of positive tits reactions 1-2 (MSC).

Month of sampling	Negative Tits	Trace reaction	Reaction 1	Reaction 2	Reaction 3	Tits with clinical mastitis	Percentage of positive tits reaction 1-2
July	6	22	37	35	17	3	60%
August	8	19	57	29	4	3	71.7%
September	11	24	40	30	12	3	58.3%
October	34	27	22	16	17	4	45.8%
Average	15	23	41	27	13	3	55.4%

Table 2: Results of SCM and negative tits obtained when performing MTC and percentages of tits with SCM in the seasonal periods of Summer-Autumn.

Periodo estacional	Negative tits	Trace reaction	Reaction 1	Reaction 2	Reaction 3	Percentage oftits tetas with SCM
Verano	14	41	94	64	21	65.83%
Otoño	45	51	62	46	29	45%

During the study period, there was a significant variation in MSC presentation, which may be related to both production conditions and hygiene and management in dairy cattle and also by environmental issues such as the seasonal period in the study.

In the present work a clear decrease was obtained in the number of cows with subclinical mastitis in the fall period (September and October), as well as the number of negative cows to the CMT test increased from 6% in Summer to 19% in Autumn, This may be due to the fact that the greater number of negative cows to the CMT test corresponds to the time when rainfall is lower or absent. These results agree with those obtained by Valero-Leal et al., (2010), in cows of Maracaibo Venezuela, which had a higher frequency of subclinical mastitis during the rainy months (Summer), decreasing the frequency of this disease (Rodriguez, 2006) reports that the highest frequency of outbreaks of subclinical mastitis occurs during the summer months, when the monthly precipitation is slightly above 100 mm.
In the present study, the presence of SCM is clearly influenced by the seasonal period in which the sampling was performed, although a longer study is necessary to establish a more solid opinion about the influence that the seasonal period can have on the Presentation of MSC in dairy herds of low-scale production.

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EFFICACY OF SODIUM HYPOCHLORITE AGAINST MULTI-RESISTANT GRAMNEGATIVE BACTERIA

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SUMMARY. Sodium hypochlorite (NaOCl) is the most commonly used disinfectant in water treatment aiming elimination of pathogenic bacteria in order to protect health of both, human and animals. The purpose of this study was to examine the susceptibility of 21 multi-resistant gramnegative bacteria to NaOCl. The results showed a sufficient germicidal effectiveness against all tested strains. Chlorination is a measure to retain microbial safety of drinking water and therefore prevents from waterborne diseases.

Key words: sodium hypochlorite, multi-resistant bacteria, disinfection efficacy

INTRODUCTION

Pathogenic bacteria in drinking water constitute a potential hazard to animal health. Sodium hypochlorite (NaOCl) is often used for water treatment in animal settings. This study aims to determine its efficacy against multi-resistant gramnegative bacteria.

MATERIAL AND METHODS

Tests were performed according to the guidelines of the Association for Applied Hygiene (VAH) using *Pseudomonas aeruginosa* (n=3), *Acinetobacter* (*A.*) *baumannii* (n=3), *A. pittii* (n=2), *Klebsiella* (*K.*) *pneumoniae* (n=6), and *K. oxytoca* (KO, n=2) in comparison to reference strains. To evaluate NaOCl efficacy, minimum inhibitory concentrations (MICs) were determined. Once an adequate neutralizer was identified, qualitative and quantitative suspension tests (ST) with and without organic load (0,3 g/l BSA) were carried out at 5 and 3 different contact times, respectively.

RESULTS

MICs were similar for all strains (0.1% and 0.2%). Referring to the qualitative ST, species- and strain-specific differences were noticed. Generally, concentrations up to 400x lower the MIC were sufficient to inactivate the bacteria. Quantitative ST results revealed bactericidal concentrations 25x less than MIC for most strains (i.e. 0.004%, i.e. 4 mg/l free chlorine) without organic load but were equal to MIC with organic load. In both tests, contact time had an only marginal effect.

DISCUSSION

NaOCl was found to be effective against all tested strains and the multi-resistant bacteria did not require higher concentrations for complete inactivation. For water disinfection, concentrations up to 6 mg/l can be used according to the German Drinking Water Regulation. For KO, the concentrations determined by quantitative ST without organic load in this study lay below that value. Hence, treatment with NaOCl is an effective and inexpensive procedure to eliminate pathogenic and also biofilm forming bacteria in water pipelines.

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DO ESBL- /AMPC-PRODUCING ENTEROBACTERIACEAE SURVIVE DISINFECTION MEASURES IN BROILER FARMS?

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Background and Objectves:

Poultry farms are known as reservoirs of extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC β-lactamase-(AmpC) producing Enterobacteriaceae. Therefore, cleaning and disinfection (C&D) between the fattening periods are crucial for an effective elimination of these microorganisms. However, the general procedure of C&D has never been questioned and studies investigating the possibility of transmission of ESBL- /AmpC-producing Enterobacteriaceae between fattening runs are missing. We therefore deeply surveyed broiler farms after C&D to identify possible niches for bacterial survival on broiler fattening farms.

Materials and Methods:

For the study we investigated a conventional broiler fattening farm in Germany. First, we identified ESBL- /AmpC- positive flocks based on boot swabs and pooled faeces samples from chicken at end of their fattening period. Following, we systematically sampled four of ESBL- /AmpC carriage positive barns after C&D. The disinfection was done twice with at first a formaldehyde fumigation followed by application of liquid disinfectants. Environmental samples *i.e.* gauze swabs as well as boot swabs from inside and outside the barn were taken and suspicious enterobacteria were isolated after cultivation (using MacConkey agar with 1 mg/l cefotaxime). Species identification was performed using MALDI-TOF, affiliation to ESBL- /AmpC was performed by real time PCR for resistance genes followed by sequencing. As an indicator for faecal contamination all samples were furthermore analysed for Enterococci using a selective agar (bile esculin azide agar).

Results:

Our results indicate that both microorganisms investigated can survive the process of C&D: on average 42,1% of the pre-enriched samples in the barns (n=38) were positive for Enterococci^{A} and 3,9% positive for ESBL- /AmpC-producing Enterobacteriaceae^{B}. While the anteroom of the barn (n=22-23, no C&D) was much less contaminated (26,5%^{A} vs. 1,1%^{B}), the surrounding areas (n=7-9, no C&D) showed highest contamination at all: 87,4%^{A} vs. 9,8%^{B}.

Thereby, ESBL- /AmpC-producing Enterobacteria were only found in samples simultaneously contaminated with
Enterococci. In comparison to the initial sampling at the end of the fattening period, the isolates of our ESBL-/AmpC- producing Enterobacteria comprise identical resistance genes.

Summary and Conclusion:

The so far analysed stables are pre-selected by an initial screening of the ESBL-/ AmpC- status of the previous broiler flock at the end of the fattening periode. Only stables with positive flocks are included in our study. Our preliminary results from four out of five analysed stables demonstrate that there is a survival of resistant bacteria in cleaned and disinfected broiler fattening stables. Sequence analyses will follow to extensively compare the isolates before and after the process of C&D. Though, our data give evidences for a possible transmission of ESBL- /AmpC-producing Enterobacteriaceae from the former chicken flock to the newly arriving chicken. ESBL- /AmpC-producing Enterobacteria survive the process of C&D especially via hygienically critical niches such as scratches in the ground. Theses niches were also positive for *Enterococci* indicating a faecal contamination – a sign for an inadequate cleaning and disinfection. This knowledge is necessary to develop a concept for an optimisation of C&D especially according to these hotspots. An effective C&D is crucial to reduce the spread of ESBL-/AmpC- producing Enterobacteria in poultry farming.

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TRANSMISSION OF ESBL-/AMPC-PRODUCING ENTEROBACTERIACAE IN THE BROILER PRODUCTION

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INTRODUCTION. Extended-spectrum beta-lactamase- (ESBL-) and AmpC beta-lactamase enzymes reduce the effectiveness of specific antibiotics. Therefore, ESBL-/AmpC-producing *Enterobacteriacae* represent an increasing problem, both in human and veterinary medicine. The occurrence of these resistant bacteria in broiler fattening farms is known, however, there are only a few transmission investigations. To elucidate possible transmission routes, we investigated seven different broiler fattening flocks starting with the parent flocks, followed by investigations in the hatchery, the fattening farms and in the slaughterhouse.

MATERIAL AND METHODS. Parent flocks were screened for ESBL-/AmpC-producing *Enterobacteriacae* as only flocks from positive parents were included. The hatchery as a putative bottleneck for bacterial transmission was investigated for the occurrence of the resistant bacteria on/in the hatching eggs themselves as well as in the environment. After the chick's hatching the animals of the respective flocks were tracked during the fattening period and finally the carcasses were investigated in the slaughterhouse.
ESBL-/AmpC-producing *Enterobacteriacae* were isolated and examined for their species, phylogroup and resistance genes. Possible epidemiological relationships of the isolates from the different stages of the production chain will be verified in this ongoing study by pulsed-field gel electrophoresis or whole genome analyses.

RESULTS AND DISCUSSION. In the hatchery, only 0.6% of the investigated samples (n=1,571; eggs and environment) were tested positive for ESBL-/AmpC-producing *Enterobacteriaceae*. Despite this low incidence at the early stage of the broiler production chain the resistant bacteria occurred in all seven broiler fattening flocks and in the slaughterhouse samples. Molecular analyses showed that the vertical transmission from the hatchery into the broiler fattening farms has a low impact on the spread of ESBL-/AmpC-producing *Enterobacteriaceae* along the broiler production chain. However, there are indications for horizontal transmission routes of the resistant bacteria through contaminated farm environment. These results will be presented.

EVALUATION OF THE HYGIENE MANAGEMENT IN AN EQUINE SURGERY CLINIC

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SUMMARY. Clinical settings for horses are challenging environments regarding hygiene management and infection control. A hygiene plan at such premises ensures a targeted hygiene management with clear responsibilities. In our study on hygiene management in an equine surgery clinic, multi-drug resistant bacteria that might lead to intensive and prolonged medical care, higher treatment costs and risk of nosocomial spread were not detected.

Key words: equine clinic, hygiene management, bacteria

INTRODUCTION

Nosocomial infections with multi-drug resistant pathogens are of rising importance in veterinary hospitals. Most often, patients are already colonized by these pathogens at admission (Walther et al., 2014). A proper hygiene management is needed to eradicate infectious agents from clinical settings and to prevent nosocomial outbreaks. To get an overview regarding the infection pressure at various hospital sites and to elucidate potential infection sources, the Large Animal Clinic for Equine Surgery at our faculty was surveyed over a period of one year.

MATERIAL AND METHODS

A questionnaire was compiled to record routine hygiene management. Bacterial investigations were carried out on swab samples taken at all premises of the equine hospital including stables and also equipment. In addition, air sampling was performed using the wet cyclone technique (Coriolis® µ Air Sampler, 10 min intervals, flow rate 300 l/min). Total bacterial counts were determined and samples were screened for *E. coli*, coliform bacteria, extended spectrum beta lactamase-producing (ESBL-) bacteria, *Salmonella*, *Acinetobacter baumannii* (AB), MRSA, *Rhodococcus equi* (RE), *Streptococcus equi* ssp. *equi* (SEE) and *zooepidemicus* (SEZ), additionally. Colonies suggestive of MRSA were confirmed by *spa*- and *mecA*-PCR (Harmsen et al., 2003; Strommenger et al., 2003). Antibiotic susceptibility testing and *Acinetobacter* species typing was done at an external laboratory.

RESULTS

At the Large Animal Clinic for Equine Surgery a few standard operating procedures were available but a hygiene plan was not implemented. Bacterial counts varied at the different premises and were lowest at the operation room and highest at the stables. Most samples revealed coliform bacteria and *E. coli* whereas MRSA was isolated sporadically and SEZ only once. ESBL-bacteria, *Salmonella*, AB, SEE, and RE were not detected in any sample. Total bacterial counts in air samples collected at the alley revealed up to 10^5 colony-forming units (cfu)/m^3 of air, which was slightly higher than reported in literature (Eckardt, 2008). At the operation room 10^2 cfu/m^3 were found.

DISCUSSION

A hygiene plan is required at facilities where animals are medically examined, treated or kept for care. It ensures a targeted hygiene management with clear responsibilities. In this study, a hygiene plan was not implemented, however, the clinic staff adhered to fundamental hygiene requirements and minor weaknesses in cleaning and disinfection did not lead to an increase in surgical site infections or nosocomial infections. Moreover, multi-drug resistant bacteria which might lead to intensive and prolonged medical care, higher treatment costs and risk of nosocomial spread were not detected. Nonetheless, a cleaning and disinfection scheme has been implemented and its realization and benefit is followed up.

ACKNOWLEDGMENTS

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PREVALENCE, CHARACTERISTIC AND RISK FACTORS FOR INFECTION OF ENTEROPATHOGENIC AND SHIGA TOXIN-PRODUCING *E. coli* IN CATTLE IN SOUTH-WESTERN POLAND

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SUMMARY. Shiga toxin-producing *E. coli* (STEC) and enteropathogenic strains *E. coli* (EPEC) is an important food-borne pathogen. The aim of this study was to investigate the prevalence, virulence genes, serotype, antibiotic resistance of the EPEC, STEC including *E. coli* O157 in cattle in south-western Poland. The study was performed in 251 cows from 27 farms located in western Polnad (from 2009 to 2013). All isolates were assessed for the presence of stx_1, stx_2, *eaeA*, and *hlyA*. EPEC, STEC and EHEC were cultured from 31.47%, 13.94% and 16.73% cattle, and mainly belonged to serogroup O25, O26, O111, O114 and O126. *E. coli* O157 was isolated from 4.78% (12 strains) samples, and the virulence genes *stx1*, *stx*2, *eaeA*, and *hlyA* were detected in 75.00%, 41.66%, 91.66%, and 33.33 % of strains, respectively. Other virulence genes, such as *saa, iha, espP, and* $lipA_{O113}$ were frequently detected in examined strains. Based on MIC results, tetracycline had the most common antimicrobial resistance (31.70%), followed by neomycin (24.39%), amoxycilin (19.51%), ampicillin (12.16%), chloramphenicol (6.08%), sulfonamide and cefotazidime (4.05%). Prevalence of pathogenic strains of *E. coli* in cattle was correlated with small traditional type of farms, feeding with grass, presence of domestic animals on farm (particularly dogs and cats).The results of our study suggested that cattle in Poland could be important reservoir for transmission of STEC and EPEC to humans.

BEHAVIOURAL AND NEUROHORMONAL ANALYSIS OF DEHORNING PROCEDURE IN CALVES

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SUMMARY. Dehorning is a standard procedure in dairy cattle and it is widely used in cattle production and dairy cattle holdings. The method is usually performed by chemical or thermal way. The chemical procedure involves removing of the buds with chemical reagents, while thermal procedure is based on burning the buds in temperature over 600 °C during at least 20 seconds. The stress reaction and animal's pain that occurs when carrying out those routine procedures is often marginalized by breeders, despite the fact that the perception of these stimuli in calves is not widely known.

The study presents an experimental design of behavioural and neurohormonal analysis in Polish Holstein-Friesian calves during dehorning procedure. On the basis of behavioural monitoring (Noldus, Netherlands) and biochemical analysis of blood (Pentra – 400, HORIBA ABX, Canada), the level of stress reactions in calves were investigated. The behavioural study covered primarily the locomotor and social interactions in calves before and after dehorning. The biochemical analysis of blood samples was focused on the hormone concentrations (cortisol, adrenaline) in both the physiological as well as in phase acute stress in calves. Bioelectrical activity of cerebral cortex (electroencephalography) was performed with the use of stationary and portable devices – EEG COMET AS 40, AURA respectively (Grass Technologies, USA) to define the clinical protocols of brain waves in calves during dehorning and to correlated the results with the behavioural observations and blood analysis.

Animal hygiene, food quality and food safety

THE IMPORTANCE OF MICROBIOLOGICAL ANALYSIS OF DRINKING WATER INTENDED FOR ANIMAL CONSUMPTION

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The waterborne pathogens potentially causing illness include: bacteria, viruses, parasites (protozoa and helminthes). Most waterborne pathogens are introduced into drinking water by its contamination with human and animal faeces, they do not grow in water and they induce infection in the gastrointestinal tract following the ingestion. Besides ingestion, other routes of transmission can include inhalation of aerosols, leading to infections of the respiratory tract (e.g. *Legionella*) and cutaneous contact by bathing, leading to infections of the skin and brain (e.g. *Naegleria fowleri*). In Romania, it is considered that the water intended for animal consumption must have the same quality as the drinking water, because there isn't a distinct quality standard for water intended for animal consumption. Therefore, in this study/paper, the indicator parameters for the microbiological contamination of the drinking water, such as: coliform bacteria, *Escherichia coli*, enterococci, colony formating micro-organisms at 22 °C and 37 °C, *Pseudomonas aeruginosa* and *Clostridium perfringens*, are defined and described. The importance of their analysis in the context of the appreciation of the quality of drinking water is marked, the standardized methods of analysis and the current European and Romanian legislation for the interpretation of the results of the analysis are also presented. A comparative study on the microbiological quality of the water intended for animal consumption, at the national level, performed in the period 2007-2015, is also presented. A marked decrease in the number of water samples was observed, as well as the necessity of monitoring the water quality as a major objective, in order to maintain the state of health and the level of animal production, to prevent outbreaks of transmissible diseases among animals and from animal to human, to protect the environment and, last but not at least, to protect the human health.

Key-words: drinking water, indicator parameter, microbiological analysis

INTRODUCTION

The waterborne pathogens potentially causing illness include: bacteria, viruses, parasites (protozoa and helminthes). Most waterborne pathogens are introduced into the drinking water by its contamination with human and animal faeces; they do not grow in water and they induce an infection in the gastrointestinal tract following the ingestion. Besides this route, other routes of transmission can include inhalation of aerosols, leading to infections of respiratory tract (e.g. *Legionella*) and cutaneous contact by bathing, leading to infections of the skin and brain (e.g. *Naegleria fowleri*).

In Romania, it is considered that the water intended for animal consumption must have the same quality as drinking water, because there isn't a distinct quality standard for the water intended for animal consumption (Decun, 2007). Therefore, the scope of this paper is to define and present the main indicator-parameters of the microbiological contamination of the drinking water and their importance for the assessment of the drinking water quality.

Coliform bacteria are Gram-negative, aerobic and facultatively anaerobic, non-spore-forming bacilli, capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose. This group include the genera with faecal origin (human and animal faeces) as: *Escherichia, Citrobacter, Klebsiella, Enterobacter, Serratia, Hafnia,* and environmental origin

(capable of multiplication in water and soil). Their presence indicate an inadequate treatment, biofilm formation through ingress of foreign material (soil, plants) in the distribution systems and water tanks.

Escherichia coli is a member of the thermotolerant coliforms group, capable of lactosis fermentation at higher temperature, but it is different from the coliforms by its ability to produce indole from triptophan and β-glucuronidase enzyme. It is present in an important quantity in human and animal faeces. *Escherichia coli* is considered the **most suitable indicator for faecal contamination**, its presence providing an evidence of recent faecal contamination, which imply actions of investigation of potential sources such as: inadequate treatment or breaches in the distribution systems integrity.

Enterococci are a subgroup of faecal streptococci represented by the following species: *Enterococcus faecalis, Enterococcus faecium, Enterococcus durans, Enterococcus hirae*. They are Gram-positive bacteria, relatively tolerant of sodium chloride solution (6,5%) and alkaline pH levels, facultatively anaerobic. Enterococci are relatively specific for faecal pollution because most species don't multiply in a water environment, but some can originate from other habitats, e.g. soil, in the absence of faecal contamination. They are considered the **indicators of recent faecal pollution** like *Escherichia coli*, but they tend to survive longer in a water environment, and are more resistant to drying and chlorination.

Micro-organisms formating colonies are represented by bacteria and fungi, capable of growing on rich growth media, without inhibitory or selective agents, over a specified incubation period and at a defined temperature (22 °C and 37 °C). Here are included: micro-organisms sensitive to the disinfection processes (coliform bacteria), micro-organisms resistant to disinfection (sporulated micro-organisms), microorganisms that rapidly proliferate in treated water in absence of residual disinfectants and opportunist pathogens such as: *Acinetobacter, Aeromonas, Flavobacterium, Klebsiella, Moraxella, Serratia, Pseudomonas, Xanthomonas*. There is no evidence of an association of any of these organisms with a gastrointestinal infection through the ingestion of drinking-water in the general population. The microorganisms that grow at 37 °C originate from human and animals with warm blood and those growing at 22 °C are of aquatic origin. Their number should be as low as possible, a big number providing an inadequate treatment, presence of biofilm and lack of integrity of distribution systems.

Pseudomonas aeruginosa is a member of *Enterobacteriaceae* Family, unsporulated, with a weak mobility. They synthesize pigments like: pyocianin, pyoverdin and pyorubin, and is considered a hygiene indicator.

Clostridium perfringens is a species of *Clostridium* Genus, represented by a group of Gram-pozitive, anaerobic, sporulated (spores are resistant in unfavorable conditions of aquatic habitat: UV irradiation temperature, pH extremes and chlorination), sulfite-reducing bacilli. It is considered an **indicator** with high specificity for **intermitent faecal pollution**, an indicator of protozoa and enteric viruses and a useful indicator for the effectiveness of the filtration, because the *Clostridium perfringens* spores are smaller than oocysts or cysts of protozoa.

MATERIAL AND METHODS

A comparative study concerning microbiological quality of the water intended for animal consumption, at the national level, was performed in the period 2007-2015, by collecting data from the County Sanitary Veterinary and Food Safety Laboratories and the Institute for Diagnosis and Animal Health Bucharest.

In Romania, the main microbiological parameters for the quality assessment of the drinking water intended for animal consumption are: coliform bacteria, *Escherichia coli*, enterococci and micro-organisms formatting colonies at 22 °C and 37 °C.

The specialists used the methods of analysis of microbiological parameters mentioned in the EU Directive 2015/1787 for the modification of EU Directive 98/83/EC on the quality of water intended for human consumption.

The evaluation of the number of colony formating micro-organisms at 22°C and 37°C /ml is made by the inoculation of 1 ml of sample, and another 1 ml of sample decimal dilution in Petri dishes, an operation performed two times, followed by the counting of colonies grown on culture medium – yeast extract agar – after aerobic incubation at 22±2 °C and 36±2 °C and the calculation of weighted average.

These are the steps for the detection and enumeration of *Escherichia coli* and coliform bacteria, by the membrane filtration method: filtration of 100 ml of samples; transfer of the membrane on selective medium – chromogenic coliform agar (CCA), with substrates for enzimes as: β-galactozidase (synthesized by coliforms) and β-glucuronidase (synthesized by *Escherichia coli*); incubation of the plate at 36 ± 2 °C, 21 ± 3 h; examination of the colonies grown on the plate; the dark blue-violet - β-galactozidase-positive and β-glucuronidase-positive colonies are considered *Escherichia coli* confirmed/plate and pink-red colonies - β-galactozidase-positive - coliform bacteria that are not *Escherichia coli* presumtive and their confirmation of the latter by oxidase test (negative).

The principle of the method for the detection and enumeration of intestinal enterococci is based on the filtration of 100 ml of sample, the incubation of the membrane on Slanetz-Bartley medium, that contains sodium azide (for inhibition of Gram-negative bacteria) and 2,3,5-triphenyltetrazolium chloride (colourless substance), that is reduced formazan (red) by enterococci. By the transfer of membrane with typical colonies on bile-aesculin-azide agar, preheated at 44^0C, the hydrolysis occurs in 2 hours (the final product, 6,7-dihidroxicumarin, combines with iron(III) ions in order to give a tan-coloured to black compound that diffused into the medium).

RESULTS

The number of colony formating micro-organisms at 22°C and 37°C /ml represents the weighted average of numbers of colonies/plates inoculated as mentioned above.

The number of coliform bacteria /100 ml represents the number of coliform bacteria /plate, expressed as sum between the number of *Escherichia coli* confirmed/plate (dark blue-violet colonies - β-galactozidase-positive and β-glucuronidase-positive) and the number of coliform bacteria that are not *Escherichia coli*, both presumtive (pink-red colonies - β-galactozidase-positive) and confirmed (oxidase-negative)/plate.

The number of intestinal enterococci /100 ml is the number of presumtive colonies that grow on Slanetz-Bartley medium (pink, red or maroon colonies) and confirm on bile-aesculin-azide agar (black colonies, with blackening of medium under the colonies).

The data on the quality of the drinking water intended for animal consumption in the period 2007-2015 were collected from the County Sanitary Veterinary and Food Safety Laboratories and the Institute of Diagnosis and Animal Health Bucharest and are presented in the Table 1.

Table 1: A comparative study on the microbiological quality of the water intended for animal consumption, at the national level, performed in the period 2007-2015

Year	Total number of analyzed samples	Total number of adequate samples	Percentage of adequate samples	Total number of inadequate samples	Percentage of inadequate samples
2007	15523	10682	68,81 %	4841	31,19 %
2008	5851	4060	69,38 %	1791	30,62 %

2014	**951**	**890**	**93,58 %**	**61**	**6,42 %**
2015	**843**	**801**	**95,01 %**	**42**	**4,99 %**

DISCUSSION

The interpretation of these microbiological parameters was performed according to the Law 311/2004 for the modification and completion of Law 458/2002 on the quality of potable water, transposed form the EU Directive 98/83/EC on the quality of water intended for human consumption.

In the period 2007-2015, a marked decrease of number of water samples from 15523 to 843 (94,95 %) was observed: a small difference between 2014 and 2015 (11,35 %), high difference between 2007 and 2008 (62,31%) and between 2008 and 2014 (83,74 %), which requires the necessity of monitoring the water quality by lab analysis (Figure 1).

It was observed also an increase in the percentage of adequate samples: 68,81% (2007), 69,38% (2008), 93,58% (2014) and 94,95% (2015), that is correlated with an improvement in the quality of the drinking water intended for animal consumption (Figure 2).

It is necessary to monitor the water quality in order to respect (observe) the animal welfare (principles), to preserve the health status and the level of animal production, to prevent outbreaks of transmissible diseases among animals and from animal to human, to protect the environment and, not at least, to protect human health.

Figure 1. Number of samples analysed in the period 2007-2015

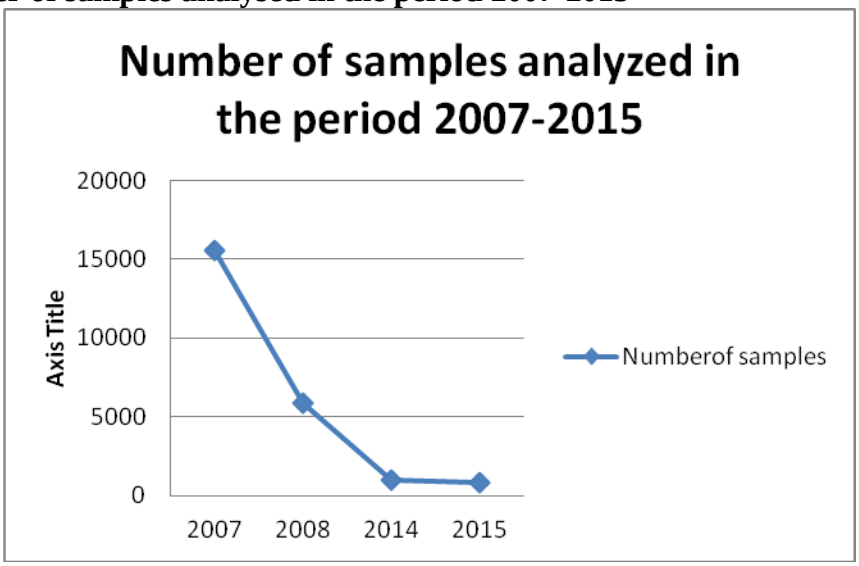
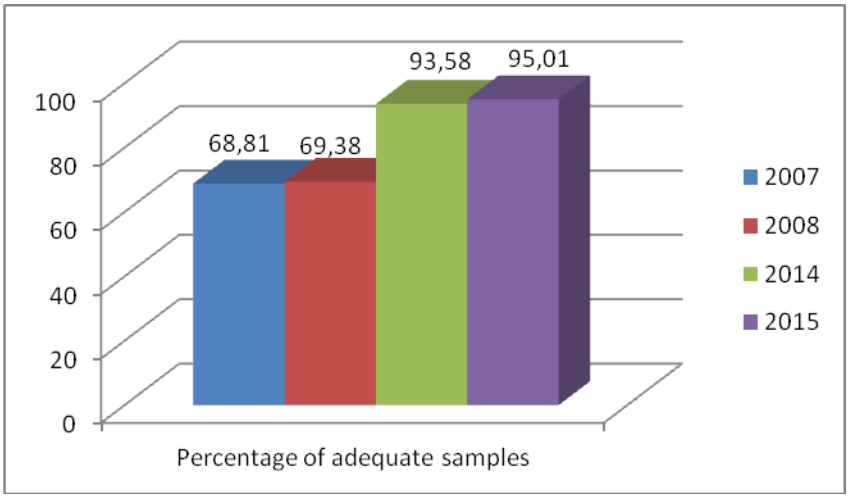


Figure 2. Percentage of adequate samples



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SR EN ISO 9308-1:2015 - Water quality – Detection and anumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method for waters with low bacterial background flora

EFFECT INOCULANT MIXED CULTURE ON THEOBROMINE COCOA POD SILAGE

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SUMMARY. The objective of this study was to identify the effect of *L. plantarum* and *S. cerevisiae* inoculated into cocoa pod silage on theobromine concentration. This experiment consists of four treatments: 1) 1 kg freshly harvested cocoa pods without inoculant as control (K); 2) inoculated with *L. plantarum* (KLp); 3) inoculated with *S. cerevisiae* (KSc); and 4) inoculated with L. *plantarum* and *S. cerevisiae* mixture (KLp+Sc), and adding cassava meal as additive. Each treatment was replicated 3 times, and then was fermented for 21 days. Variables observed were theobromine concentration. Collected data of theobromine concentration was analyzed by one-way analysis of variance and followed by Duncan's new multiple range test (DMRT), if there were any significant difference. Results showed that treatment effect was significant ($P<0.05$). Content of theobromine of cocoa pod freshly was 161.28 ppm. Cocoa pod silage inoculated with *L. plantarum* and *S. cerevisiae* mixed culture had lower ($P<0.05$) theobromine concentration than cocoa pod silage with no inoculant (3.88 ppm vs. 7.80 ppm, respectively). Based on this result we can conclude that inoculants *L. plantarum* and *S. cerevisiae* can reduce theobromine concentration on cocoa pod silage and that is safety when used to feed cattle.

Key words: Theobromine, Cocoa pod, Silage.

INTRODUCTION

Fermentation is a process to improve cocoa pod silage quality. Cocoa pod freshly have high concentration of theobromine and tannin which can negative impact to ruminal metabolism. Using specific microbes can reduce antinutritional factor on cocoa pod, and then it can be safety to feed cattle. Ashihara et al. (2011), showed that 0.6-0,7% of theobromine in cocoa pod can be toxic to livestock. McDonald et al. (2002), showed that theobromine in extracted cocoa bean meal can be toxic and induce died in poultry. Alexander et al. (2008), said that theobromine cocoa pod have negative effect, because can inhibit rumen microbes activity, decreasing its ability for fiber degradation.

Detoxification of cocoa pod from theobromine can be done through physical, chemical and biological treatment or its combination. Adamafio (2013), showed that added liquid and boiling treatment can reduce theobromine concentration 56% and 72%, that biological method such as inoculation of *Aspergillus niger* can reduce in the concentration of theobromine. Sukha (2003) affirm that heat treatment can reduce theobromine concentration. Munier (2012), found that the theobromine concentration in cocoa pod was lower (32.5 ppm) when was inoculated with *A. niger* and dried at 55 °C; compared with 44.0 ppm appreciated when cocoa pod was inoculated of *A. niger* and freeze dried. Traditional farmer usually use *L. plantarum* and *S. cerevisiae* to make cocoa pod silage, however it is no known the magnitude of the impact on theobromine concentration in the final silage. The objective of this study was to identify the effect of the inoculation with *L. plantarum* and *S. cerevisiae* into cocoa pod silage on theobromine concentration.

MATERIAL AND METHOD

Preparation of cacao pod sample and inoculum

Cacao pod for silage making was harvested and obtained from Gunungkidul District, Yogyakarta. Inoculant consisted of lactic acid bacteria (*L. plantarum*) and yeast (*S. cerevisiae*) obtained from Centre Trans University- Gadjah Mada University (PAU-UGM). Inoculant prepared by pre-culturing of *L. plantarum* on MRSB (Mann Rogosa Sharpe Broth) and yeast on MEB (Malt Extract Brooth).

Experimental procedure

One kg of cacao pod (45% DM) and 200 g of cassava meal (55% DM) were used for ensilage process. Prior to the ensilage process, the cacao pod sample was added with or without inoculum of *L. plantarum, S. cerevisiae*, or their mixed culture (1:1) as many as 0.1% DM. The treatments were as follows:

K0: cacao pod + cassava meal + water as control,
KLp: K0 + *L. plantarum* ,
KSc: K0 + *S. cerevisiae*,
KLp+Sc: K0 + *L. plantarum* + *S. cerevisiae*.

All cacao pod sample with or without inoculums was then fermented in the plastic fermenter (2 kg/pack) for 21 days at room temperature. Proximate analysis is shown in Table 1 (Zakariah et al., 2015). Theobromine analysis was performed by HPLC (European Food Safety Authority, 2008; Alexander et al., 2008), following the procedure described AOAC (2005), in LPPT Universitas Gadjah Mada Yogyakarta.

Table 1. Chemical composition of cocoa pod silage (Zakariah et al., 2015)

Parameters	Percentage based on DM (%)		
	cocoa pod	cassava meal	cocoa pod + cassava meal before ensilage
Dry matter (DM)	13,48	86,70	26,02
Organic matter (OM)	88,90	99,01	94, 03
Crude protein (CP)	4,06	0,89	2,51
Crude fiber (CF)	31,26	1,23	13,71
Extract ether (EE)	0,97	0,99	0,52
Nitrogen free extract (NFE)	52,61	95,90	77,29
Theobromine (ppm)	161,28		

Stastical analysis

Data of theobromine concentration was analyzed with analysis of variance (ANOVA) and followed by Duncan's new multiple range test (DMRT) if there were any significant differences (Steel et al., 1996). All of statistic calculated with software Statistical Product and Service Solution version 16.0 (Soleh, 2005).

RESULT

Result theobromine concentration is shown in Table 2.

Table 2. Theobromine concentration on cocoa pod + cassava meal ensilage without inoculant as control(K0), control + *L. plantarum* (KLp), control + *S. cerevisiae* (KSc), control + *L. plantarum* + *S. cerevisiae* (KLp+Sc)

Silage	Theobromine (ppm)
K0	7,80[c]
KLp	0,09[a]
KSc	22,05[d]
KLp+KSc	3,88[b]

[a, b, c] different superscripts at the same rows indicate significant difference (P<0,05)

DISCUSSION

Content theobromine of cocoa pod freshly is 161.28 ppm, generally ensilage have content theobromine lower than cocoa pod without ensilage. Cocoa pod silage was inoculated *L. plantarum* and *S. cerevisiae* mixed culture have theobromine concentration lower than uninoculated. Cocoa pod silage was inoculated *L. plantarum* have lowest theobromine concentration than other treatment. Wulandari et al. (2014), showed that inoculant mixed culture (lactic and cellulolytic microbes) can reduce theobromine concentration in cocoa pod. Lefeber et al. (2012), found that *Lactobacillus fermentum* and *Acetobacter pasteurianus* induces a lower theobromine concentration than *L. fermentum*, *A. pasteurianus* and *S. cerevisiae* mixed culture.

However, all cocoa pod silage treatments have lower than cocoa pod freshly. Aerobic phase produced heat increment; it will reduce theobromine in cocoa pod silage. Beside, raised content water of cocoa pod silage can reduce content theobromine. De Vuyst et al. (2010), showed that decline theobromine at ensilage because it is soluble in fluid cell till diffusion out.

Fermentation time cocoa pod silage in this research as long as 21 days is choice from many references, references showed that silage get stable condition at this time, and it can be storage long time. Fermentation time 21 days affected chemical composition and theobromine concentration. Brunetto et al. (2007), observed that increasing fermentation time beyond 21 days not induce a further decrement on theobromine concentration.

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CRYPTOSPORIDIUM IN PIGLETS AND CALVES OF RIVER BASINS IN KATHMANDU: AN ISSUE OF ANIMAL HYGIENE AND HEALTH

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SUMMARY. A cross sectional study was conducted in piglets and calves from the river basins of Kathmandu valley to investigate the prevalence and its associated risk factors of *Cryptosporidium*, a zoonotic protozoan parasite. Fresh faecal samples from calves below 6 months (n=50) and piglets below 4 months (n=50) of age were directly collected from farms located in the periphery of river basins of Kathmandu valley. The samples were examined using the Ziehl Neelsen staining technique after the modified Sheather concentration method with centrifugation, and the oocyst was observed using a high power microscope. The results indicate higher prevalence ($P<0.05$) in piglets (42%) than calves (32%). Higher prevalence ($P<0.05$) of parasite among piglets was observed in Manohara river basin (40.65%) than in Bagmati river basin (44.44%). There was significant difference in susceptibility among the age groups of piglets with one month old being most susceptible followed by two, three and four months old piglets ($P<0.05$). However, there was no significant difference in prevalence between piglets demonstrating clinical symptoms and subclinical cases. Among the samples from calves, Manohara (58.33%) had higher prevalence ($P< 0.05$) than Bagmati (23.68%) and the male calves (42.42%) had higher prevalence ($P< 0.05$) than female calves (11.76%). The parasite had higher prevalence in 4-6 months aged calves ($P< 0.05$). The results also demonstrated that there was an association between the shed hygiene and the prevalence of *Cryptosporidium* ($P < 0.05$). The distance of the farm from the river was another factor strongly associated with the prevalence of the parasite ($P < 0.05$). We concluded that the parasite is present in the animals in the river basin of the Kathmandu valley. The prevalence is found to be associated with age, sex, shed hygiene and the distance from rivers. Thus, considerations should be made on risk factors of the infection and the precautions should be taken to prevent the parasitic infection in the animal population.

Key words: Animal Health, Cryptosporidium, Nepal

INTRODUCTION

Infection with *Cryptosporidium* is important from livestock health and also public health perspectives. Economic loss due to immunosuppression and stunted growth following the diarrhoea in animals and opportunistic infection in immunocompromised humans is of special concern. Furthermore, *Cryptosporidium* oocysts remain viable in water for over 140 days and are found to be resistant to the most common disinfectants making them difficult to destroy by conventional water purification methods (Ramirez et al., 2004). In Nepal, the first report of Cryptosporidiosis was from a three years old boy with chronic diarrhoea at Kanti Children Hospital, Kathmandu brought in relation to Rotavirus infection (Shrestha et al., 1993). A very high prevalence of C. parvum was reported in the different parts of Nepal such as Jomsom (17%), Kathmandu valley (17.5%) and Chitwan (14.6%) (Sherchand and Shrestha, 1996). Among animals, the highest prevalence was observed in deer (71%) followed by

rhinos (25%). Calves and buffaloes were found to have 34 and 37% prevalence of the parasite, respectively (Karna, 2010). River water being the main source of transmission of the parasite and most of the animal operations being situated in the river banks of Kathmandu. The objective of this research is to investigate the prevalence of the pathogen in calves and piglets of the same river basin. This would establish river water as an epidemiological factor for the transmission and verify the potential for the cross transmission of the pathogen between species.

MATERIAL AND METHODS

A total of 50 piglets and 50 calves' fecal samples were obtained from the periphery of the river basins of Kathmandu valley by rectal collection of the fecal samples using sterile gloves. The samples were collected in sterilized zip-lock sample bags and transported in ice cooled sample cold box. The samples were refrigerated at 4°C until processing. The sampling was done purposively to include the calves below 6 months of age and piglets below 4 months age, all living in the same river basins. The samples were subjected to Ziehl-Nelson (ZN) staining technique after modified Sheather concentration technique using centrifugation (Zajac and Conboy, 2006; Zhang et al., 2012). An amount of 5 gr of the collected faeces was taken in a tube containing 10 ml of distilled water and was mixed up well. An amount 20 ml of the supersaturated solution of NaCl (393 gm/L) was added to the solution and centrifuged at 2000 rpm for 15 min after which the supernatant was taken. Distilled water was added to the supernatant to make 100 ml of final volume. This solution was then centrifuged at 5000 rpm for 15 min. The sediment was taken using glass rod and smear on the glass slide was prepared which was air dried. The air dried glass slide was fixed with methanol and set for staining. The air dried smear was fixed with methanol and set for staining. Smear was stained with unheated Carbol fuchsin for 7 min, decolorized with 3% acid alcohol and counter stained with 0.5% malachite green for 30 seconds. Finally, the smear was examined microscopically for oocysts using a low power magnification to detect the oocysts and the oil immersion objective to identify them. The positive samples were examined quantitatively as high or low severity. From about 50 fields observed in the slide, the samples were classified as high severity if more than five fields contain oocysts in them. Similarly, if the oocysts were found in the clusters in two or three fields, then the slide was also marked as high severity (SER). On the other hand, if the slide contains individual oocysts in just one or two fields, it was classified as low severity. The results obtained with their details were entered in a MS-Excel 2007, Office application software. Statistical analysis was done with PHSTAT2 version 3.07.

RESULTS

The overall test result demonstrated that 42% (n =21) of piglet samples (95% CI [Confidence Interval]: 28-55) and 32% (n=16) of the calves' samples (95% CI: 19 – 44) were positive for *Cryptosporidium*. From the two rivers, higher prevalence was reported in Manohara river (38.02%) than Bagmati river (25.05%), though not significantly different ($P > 0.05$). In calves, 58.3% of the samples from Manohara basins were positive and 23.7% of the samples from Bagmati basin were found to be positive (Table I). The difference in prevalence in the two river periphery was statistically significant ($P<0.05$). Within the calves, 11.8% of samples from females and 42.4% of the samples from males were positive. The males have higher chances of acquiring the parasitic infestation due to significantly different prevalence ($P<0.05$). The samples were found to be 6%, 12% and 14% positive from each age group of the calves, demonstrating the statistically significant difference according to the age ($P<0.05$). Although no statistically significant ($P > 0.05$), 66.7% of the depressed samples were positive, 50% of the diarrhoea samples and 28.9% of the asymptomatic samples were positive. In piglets, higher prevalence was observed in Manohara river (40.65%) than Bagmati river (44.44%, $P > 0.05$) (Table II). 61.5% of the one month old piglets, 66.67% of the two months old piglets, 21.42% of three months old piglets and

18.18% of four months old were found to be positive with highest prevalence in one and two months ($P < 0.05$). Female piglets (62%) were found to have higher prevalence ($P<0.05$) than males (57.14%). However, lethargic piglets (66.67%) had higher prevalence ($P<0.05$) than diarrhoeal (57.74%) and samples from asymptomatic (32.35%).

DISCUSSION

The results from calves are similar to those indicated by Karna (2010), who found a prevalence of 34.57% in the pre-weaned calves in Nepal. Swai and Schoonman (2010) also found a prevalence of 35%, which is very near to our finding. This results indicate that *Cryptosporidium* has been established in the calves from Nepal and are in correspondence with Santin et al. (2004) and Fayer et al. (2007) who suggested that the matured dairy cattle are at low risk of infection as compared to pre-weaned calves. No sex effect was observed in *Cryptosporidium* infection among calves, which agrees with Rehman et al. (1985) and Shobhamani (2005). However, in the present study was found that males were more infected when compared with female animals, which may be due to careless management of male calves in Nepal. This is the first report indicating the prevalence of *Cryptosporidium* in Nepali pigs. The prevalence of 42% in piglets is similar to Siwila and Mwape (2012), who found to be 44.2% in piglets of Zambia and can be regarded as an alarming situation. Chen et al. (2011) support our findings by concluding that *Cryptosporidium* was mainly found in piglets within 2 months' age. Siwila and Mwape (2012) also mention the higher prevalence of *Cryptosporidium* in pre-weaners as compared to other age groups. This can be due to stressed condition of young ones corresponding to growth and maturing immunity.

Table 1. Results of explanatory variables in calves

Variables	Labels	No of Samples (%)	Prevalence (% of n)	P value
Location	Manohara	24	14	0.025
	Bagmati	76	18	
Age group(months)	0-2	16	6	0.0014
	2-4	50	12	
	4-6	34	14	
Sex	Male	66	28	0.028
	Female	34	4	
Disease	Diarrhoea	4	2	0.21
	Lethargic	6	4	
	Asymptomatic	90	26	
Total in each Variable		**100**	**32**	

Table 2. Results of explanatory variables in Piglets

Variables	Labels	No of Samples (%)	Prevalence (% of n)	, P value
Location	Manohara	64	26	0.79
	Bagmati	36	16	
Age group(months)	0-1	26	16	0.018
	1-2	24	16	
	2-3	28	6	
	3-4	22	4	
Sex	Male	54	20	0.44
	Female	46	22	
Disease	Diarrhoea	14	8	, 0.122
	Lethargic	18	12	
	Asymptomatic	68	22	

Total in each variable	**100**	**42**

CONCLUSION

This study identified *Cryptosporidium* in the river basins of Kathmandu valley. This is the first research which establishes river as an epidemiological factor for the transmission of *Cryptosporidium* spp. in Nepal. Piglets, calves and humans lie in the epidemiological cycle. Furthermore, this research reports *Cryptosporidium* in piglets for the first time in Nepal.

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MELAMINE NEGATIVELY AFFACTES TESTOSTERONE SYNTHESUS IN MICE

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SUMMARY. Several studies have found that melamine causes damage to the testes, epididymis and sperm. However, few studies have investigated the effect of melamine on the synthesis of testosterone, which plays an import role in testicular development and spermatogenesis. In present study, mice were orally administrated with 2, 10 or 50mg/kg of melamine for 28 days. In these groups, various abnormalities were observed including disruption of the seminiferous tubule structure, an increased necrotic germ cells and sperm abnormalities, and a reduced sperm count. Melamine exposure also decreased the level of serum testosterone and levels of testicular StAR, P450scc and 17β-HSD. In addition, melamine exposure reduced the number of Leydig cells. Taken together, these results indicate that melamine exposure reduces the level of testosterone through down-regulation of StAR and testosterone synthetic enzyme expression and also a decreased number of Leydig cells. This may further affect testicular development and lead to sperm damage.

Key words: Melamine; Testosterone; Leydig cell

INTRODUCTION

Melamine is widely used in industry in the manufacture of plastic, fabrics, laminates, food contact materials and tableware products. Because of its high nitrogen content, melamine has been added illegally to human and animal food. Two high-profile examples are the 2007 pet food recall due to melamine contamination in the USA and the 2008 infant renal calculus cases in China, (Brown, 2007; Guan,2009) both of which led to our interest in melamine. In addition, due to its use in food contact products, melamine can leach into food, a process which is accelerated by high temperatures or when acetic acid is present (Clik, 2011; Lynch, 2015). It has been reported that consumption of foods contained in melamine bowls increased urinary melamine levels (Wu, 2013) and therefore the potential risk of melamine also should be considered. Since testosterone (T) is essential for normal testicular development and spermatogenesis, it is possible that these effects are due to the effect of melamine on testosterone production. Since there are currently few studies on the effect of melamine on testosterone synthesis, the present study investigates the change in testosterone level in mice after exposure to melamine. The production of testosterone in the Leydig cells is influenced by the steroidogenic acute regulatory (StAR) protein and testosterone synthetic enzymes including cytochrome P450 cholesterol

side-chain cleavage enzyme (P450scc), cytochrome P450 17α-hydroxysteroid dehydrogenase (P45017α) and 17β-hydroxysteroid dehydrogenase (17β-HSD); thus the number of Leydig cells and the change of StAR and testosterone synthetic enzymes were also studied.

MATERIALS AND METHODS

Forty ICR male mice were divided randomly into four groups of 10 mice per group. Three groups were given melamine (MA, Shanghai ANPEL Laboratory Technologies Inc., Shanghai, PR China) at doses of 2, 10 or 50 mg/kg/day (MA2, MA10, MA50, respectively), as previous studies.[9,10] These compounds were given as a suspension in edible oil once a day by oral gavage for 28 consecutive days. Control mice received edible oil only in the same manner. Clinical signs and body weights were recorded daily after administration with MA or edible oil. After the final day of treatment, blood samples were collected from the retro-orbital sinus before mice were euthanized by CO_2 inhalation in designated CO_2 chambers. The testes were collected and weighed, and the relative weights of the testes were calculated as a proportion of each animal's body weight. Ten right testes were fixed in 10% formaldehyde for histopathological examination and immunohistochemical analysis. Ten left testes were stored at -80°C for real-time PCR and Western blotting analysis. Epididymides were also collected for sperm morphological observation and abnormality counting. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Agricultural University.

RESULTS

Clinical observation and weight change of body and testes

The mice in the control, MA2 and MA10 groups grew steadily and no clinical changes were observed. However, the mice in the MA50 group showed a mild clinical change including depression and roach back. There was no significant difference in food and water intake (data not shown) or body weight (Fig. 1A) between the MA-treated and control groups ($P>0.05$). No mice died during the MA administration period. After euthanasia, no obvious gross lesions were found in the kidneys or testes, and there was no significant difference in testis weight ($P>0.05$, Fig.1B).

Change of sperm number and sperm abnormalities

The effect of melamine on sperm number and abnormalities are shown in Fig.1C and Fig.1D. The numbers of sperm in MA-treated groups were significantly lower than in the control group ($P<0.01$), and the rate of abnormal sperm in MA-treated groups were much higher than in the control group ($P<0.01$). The effect of melamine on both sperm count and abnormality rate was in a dose-dependent manner, indicating that the sperm number decreased and abnormal sperm number increased with increasing MA dose. The damage to sperm occurred mainly in the tail, including a fold or coil in the middle and end (data not shown). These results suggest that melamine has toxicity to sperm.

Testis morphological change

To investigate the effect of melamine toxicity on testes, they were analyzed for morphological changes under a light microscope (Fig. 2). The testes of control mice were histologically normal: there was a clear boundary between the seminiferous tubules and Leydig cells, the spermatogonia and different stages of spermatogenic cells in the basement membrane showed no signs of disorganization, and the sperm in the lumen were densely arranged. However, disruption of the seminiferous tubule structure, decreased spermatogenic cell series, nuclei pyknosis and decreased sperm number were found in the MA50 group. Slight lesions were observed in the MA10 group, but no obviously changes were found in MA2 group. These results indicate that MA can cause damage to testes, although no gross lesions were found in testes.

Serum testosterone change

The levels of serum testosterone are shown in Fig.3. Testosterone levels decreased with increasing MA dose, the level in the MA10 and MA50 groups were significantly lower than in the control group ($P<0.01$). These results suggest that MA affects testosterone production.

mRNA levels of StAR and testicular testosterone synthetic enzymes

The effect of MA on mRNA expression of StAR, P450scc, P450 17α and 17β-HSD is shown in Fig.4. The mRNA expression level of StAR in the MA10 and MA50 groups decreased significantly compared to the control ($P<0.05$). The mRNA expression levels of P450scc and 17β-HSD in the MA50 group was also lower than in the control group ($P<0.05$). However, there was no difference between expression of P450 17α mRNA between the MA-treated and control groups. These results indicate that MA decreased mRNA expression of StAR, P450scc and 17β-HSD, which contributed to the decrease of testosterone level.

Change in expression of proteins associated with testosterone synthesis

Based on the results of mRNA levels, the protein expression levels of StAR, P450scc, P450 17α and 17β-HSD were identified by Western blot (Fig.5). The expression of 17β-HSD in all MA-treated groups was significantly lower than the control ($P<0.01$) and expression of StAR and P450scc in the MA50 group was significantly lower than in the control group ($P<0.05$). However, there was no significant difference in P450 17α expression between the MA-treated and control groups ($P>0.05$). These results are consistent with the level of mRNA expression of these protein genes.

Change in Leydig cell number

Leydig cells were identified by staining for 3β-hydroxysteroid dehydrogenase (Fig.6A). The quantitative analysis showed that the number of Leydig cells was significantly lower in the MA10 and MA50 groups compared with the control ($P<0.01$). There was no difference between the MA2 and control groups (Fig. 6B). This result indicated that melamine was associated with a reduction in Leydig cell number, which in turn could contribute to the decrease in testosterone level.

DISCUSSION

Lesions were observed in the MA-treated mice, including tissue disorganization and necrosis of spermatogetic cells. MA-treated mice also had a reduced sperm count and a greater rate of sperm abnormality. Therefore, it is possible that melamine can directly cause abnormal sperm morphology and number. Blood testosterone level significantly decreased after mice were exposed to melamine, especially in the MA10 and MA50 groups. The low level of testosterone may contribute to abnormal sperm and testis lesions, so we can conclude that melamine also can also induce abnormality indirectly.

The mRNA and protein expression of StAR, P450scc and 17β-HSD significantly decreased in mice after exposure to melamine. These results indicate that the down-regulation of StAR, P450scc and 17β-HSD contributed to the decreased level of testosterone.

In conclusion, the present study showed that melamine down-regulates StAR and testosterone synthetic enzymes, and decreases the number of Leydig cells. These changes contributed to the decrease in blood testosterone, which further cause damage to testes and sperm.

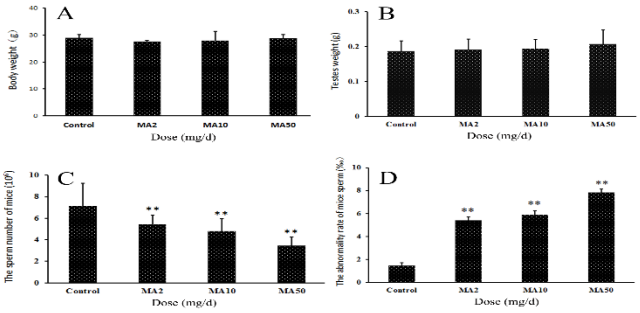

Figure 1 Effects of melamine on mouse body weight, testis weight, sperm count and sperm abnormality. Values are expressed as mean ± SD, n=10. *P<0.05, **P<0.01: melamine groups versus control group.

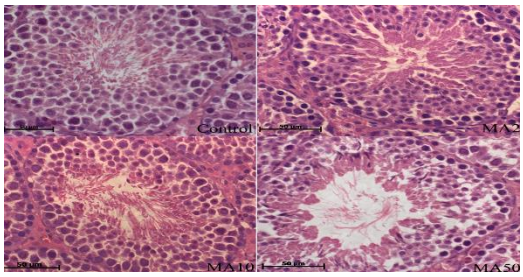

Figure 2 Morphological changes in mice after treatment with melamine. Hematoxylin-eosin

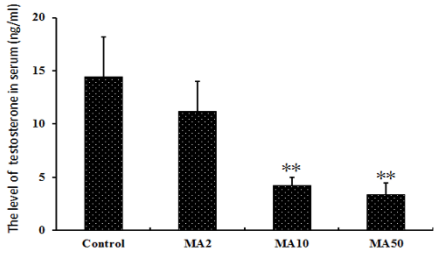

Figure 3 Serum testosterone changes in mice after treatment with melamine. Values are expressed as mean ± SD, n=10. *P<0.05, **P<0.01: melamine groups versus control group.

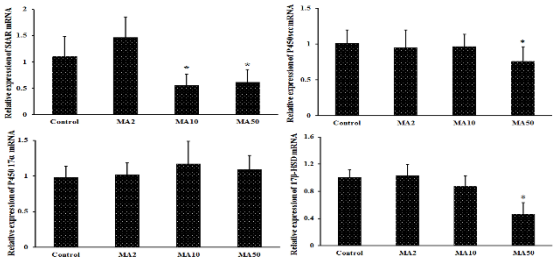

Figure 4 Change of mRNA levels of StAR and testicular testosterone synthetic enzymes in mice after treatment with melamine. Values are expressed as mean ± SD, n=10. *P<0.05, **P<0.01: melamine groups versus control group.

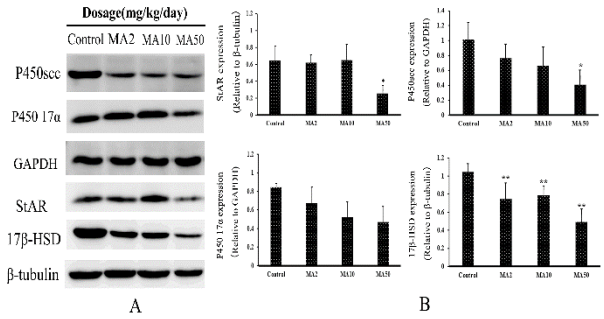

Figure 5 Changes of protein levels of StAR and testicular testosterone synthetic enzymes in mice after treatment with melamine. Values are expressed as mean ± SD, n=10. *P<0.05, **P<0.01:

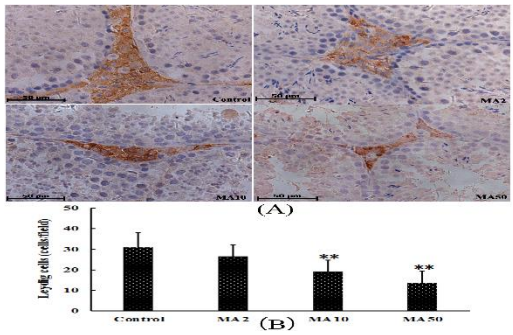

Figure 6 Changes in Leydig cell numbers in mice after treatment with melamine. Values are expressed as mean ± SD, n=10. *P<0.05, **P<0.01: melamine groups versus control groups.

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THE MICROBIOLOGICAL MONITORING OF LABORATORY ENVIRONMENT

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SUMMARY. Laboratory environment is defined as ensemble of physical (temperature, moisture), chemical and biological (particles, micro-organisms) factors of air from lab, that could influence the results of analysis by contamination of samples and present a risk to health status of personnel, who works in the lab, by non-respecting of bio-safety rules. The laboratory testing obligation for monitoring, control and register of environment conditions (microbiological sterility, particles, moisture and temperature) is mentioned in standard SR EN ISO/CEI 17025: 2005 – General requirements for the competence of testing and calibration laboratories, at the chapter 5.3 Accommodation and environment conditions.
The EURACHEM Guide – ACCREDITATION FOR MICROBIOLOGICAL LABORATORIES (Ed. 2, 2013), in APPENDIX D - Guidance on equipment validation and verification of performance, recommends the microbiological monitoring of laboratory environment and laminar air flow cabinets, by sampling of air (using air samplers and settle plates) and sanitation (using swabs and contact plates) to be performed weekly, for micro-organisms count (bacteria, yeasts and moulds). In this paper, a process description is performed for monitoring the efficacy of air and surfaces disinfection in the laboratories of the Institute of Diagnosis and Animal Health (I.D.A.H.), Bucharest, Romania, by following several steps, such as: elaboration of air and surfaces disinfection procedure, establish of Annual Programme for Monitoring of Efficacy of Disinfection in the I.D.A.H. Laboratories, where is mentioned the frequency of sampling, the mode of air and surfaces sampling, the election of sampling points, the establishing of parameters for analysis, the elaboration of Standard Operational Procedures for performing of analysis, the evaluation of the number of samples from the beginning of 2015 to the end of 2016, and the interpretation of results and measures taken in case of inadequate disinfection.
Key-words: laboratory environment, disinfection

INTRODUCTION

The laboratory environment is defined as ensemble of physical (temperature, moisture), chemical and biological (particles, micro-organisms) factors of air from lab, that may influence the results of the analysis by contamination of the samples and present a risk to health status of personnel who works in the lab. The obligation of any laboratory testing for monitoring, controlling and registering the environment conditions (microbiological sterility, particles, moisture and temperature) is mentioned in the standard SR EN ISO/CEI 17025: 2005 – General requirements for the competence of testing and calibration laboratories, at the chapter 5.3 Accommodation and environment conditions. The EURACHEM Guide – ACCREDITATION FOR MICROBIOLOGICAL LABORATORIES (Ed. 2, 2013), in APPENDIX D - Guidance on equipment validation and verification of performance recommends the microbiological monitoring of laboratory environment and laminar air flow cabinets, by sampling of air (using air samplers and settle plates) and sanitation (using swabs and contact plates) to be performed weekly, for micro-organisms count (bacteria, yeasts and moulds).

MATERIAL AND METHODS

This paper describes the activity for monitoring the efficacy of cleaning and disinfection in the laboratories of the Institute of Diagnosis and Animal Health (IDAH), Bucharest, Romania. This activity is documented by the elaboration of a general procedure for cleaning and disinfection (PG), a general instruction for verifying of disinfection in IDAH laboratories (IG), an Annual Programme for Monitoring of of Disinfection in IDAH laboratories and Standard Operational Procedures for performing of analysis (SOP). PG offers the following information: the classification of IDAH spaces in 3 categories: cleaned, low contaminated and high contaminated spaces from the risk point of view, the products and materials and rules for their use, the components of the equipment for the individual protection of the personnel, the steps for cleaning and disinfecting, the rules and frequency for carrying them out. IG refers to the development of an Annual Programme for Monitoring of Disinfection in IDAH laboratories and air and surfaces sampling by a specialist after the completion of The Request for the verification of the disinfection. In this form must be filled in: the name of applicant laboratory, the type of verifying of the surface, or air disinfection, or both, the number of rooms and the date of the sampling. After analysis, the specialist must report the results to the head of the applicant laboratory, by e-mail, in the shortest time possible. When the results exceed the admitted limit, the cleaning and disinfection action must be repeated.

In the Annual Programme for Monitoring the Disinfection in IDAH laboratories, it is mentioned the date and the day of the week or frequency of sampling for different labs, established by each lab on the basis of the data of the risk analysis. Depending on the risk index, the laboratories were classified in 3 groups: 1. High risk (e.g. morphopathology, virusology, bacteriology, molecular biology, animal health) 2. Medium risk (e.g. parasitology, mycology, toxicology, immunology) and 3. Low risk (e.g. haematology).

The number of samples for verifying the surface disinfection is minim 5 per room and the sampling points are: working tables, incubators, fridges, safety cabinets, laminar flow cabinets, centrifuges, balances and other lab equipment having a close contact with the samples analysed in lab. For sampling the operator wipes the surface to be tested, delimited by template with an area of 100 cm^2, by swab in 2 plans (transversal and longitudinally) and wash it in the tube with diluent (1 ml, product available commercially or diluent prepared in the lab, such as peptone water or peptone saline solution). The parameters for the verification of the surface disinfection are: the number of micro-organisms forming colonies/100 cm^2 and the number of moulds and yeasts/100 cm^2, and they are investigated according to the Standard Operational Procedures. The evaluation of the number of colony forming micro-organisms /100 cm^2 is made by inoculating 1 ml of sample in a Petri dishes (pour plate technique), followed by the counting of colonies grown on culture medium – plate count agar, nutritive agar or yeast extract agar – after aerobic incubation at 30±1°C and 72±3 h. The evaluation of the number of moulds and yeasts /100 cm^2 is made by inoculating 1 ml of sample in 3 Petri dishes with culture medium (surface inoculation), followed by the counting of colonies grown on the culture medium – dichloran rose Bengal chloramphenicol agar – after aerobic incubation at 25±1°C for 5 days.

The sampling places for the verification of the disinfection of the air are, especially, the laminar flow cabinets. For the sampling, the operator uses an air sampler, which aspirates 1 m^3 air and casts it on a surface of culture medium in a Petri dish. The evaluation of the number of colony forming micro-organisms/m^3 air and the number of moulds and yeasts /m^3 air is made by counting the colonies grown on the culture medium – plate count agar, nutritive agar or yeast extract agar / dichloran rose Bengal chloramphenicol agar or dichloran glycerin agar – after aerobic incubation at 30±1°C and 72±3 h / 25±1°C for 5 days.

An evaluation of the number of samples and their results from the beginning of 2015 to the end of 2016 is also performed.

RESULTS

The number of colony forming micro-organisms/100 cm^2, the number of colony forming micro-organisms/m^3 air or the number of moulds and yeasts/m^3 air represents the number of colonies counted on inoculated Petri dishes or the sum of the number of colonies counted on inoculated Petri dishes for the number of moulds and yeasts /100 cm^2 (Figure 1, 2).

The data resulted after the verification of the disinfection in IDAH laboratories in the period 2015-2016 were obtained in Hygiene Laboratory of the Institute of Diagnosis and Animal Health Bucharest and centralized in the Table 1, 2.

Table 1. The evaluation of the number of samples for the verification of the disinfection in the IDAH laboratories, performed in 2015

2015										
Laboratory	Surface disinfection						Air disinfection			
	No. of samplings	No. rooms with inadequate disinfection	No. samples analysed	No. negative samples	No. positive samples	No. of samplings	No. rooms with inadequate disinfection	No. samples analysed	No. negative samples	No. positive samples
Lab. A	12	0	62	61	1	0	0	0	0	0
Lab. B	4	0	20	18	2	0	0	0	0	0
Lab. C	58	1	290	269	21	11	1	11	10	1
Lab. D	7	0	35	35	0	1	0	1	1	0
Lab. E	7	1	35	33	2	2	0	2	2	0
Lab. F	7	0	35	34	1	0	0	0	0	0
Lab. G	4	0	55	54	1	0	0	0	0	0
Lab. H	11	0	56	55	1	5	1	5	4	1
Lab. I	19	2	95	88	7	0	0	0	0	0
Lab. J	6	0	30	30	0	1	0	1	1	0
Lab. K	2	0	10	10	0	0	0	0	0	0
Lab. L	18	0	90	84	6	2	0	2	2	0
TOTAL	**155**	**4**	**813**	**771**	**42**	**22**	**2**	**22**	**20**	**2**

Table 2. The evaluation of the **of samples for the verification of the disinfection in IDAH laboratories, performed in 2016**

2016										
Laboratory	Surface disinfection					Air disinfection				
	No. of samplings	No. rooms with inadequate disinfection	No. samples analysed	No. negative samples	No. positive samples	No. of samplings	No. rooms with inadequate disinfection	No. samples analysed	No. negative samples	No. positive samples
Lab. A	30	0	152	151	1	8	0	8	8	0
Lab. B	23	4	115	98	17	0	0	0	0	0
Lab. C	133	10	578	540	38	6	3	6	3	3
Lab. D	24	0	125	124	1	11	0	11	11	0
Lab. E	12	0	60	59	1	5	3	5	2	3
Lab. F	16	0	67	65	2	1	0	1	1	0
Lab. G	2	0	10	10	0	0	0	0	0	0
Lab. H	12	0	56	55	1	5	1	5	4	1
Lab. I	22	4	110	97	13	0	0	0	0	0
Lab. J	14	0	61	60	1	1	0	1	1	0
Lab. K	3	0	11	10	1	0	0	0	0	0
Lab. L	14	1	70	68	2	0	0	0	0	0
TOTAL	**305**	**19**	**1415**	**1337**	**78**	**37**	**7**	**37**	**30**	**7**

DISCUSSION

The interpretation of these microbiological parameters was performed according to admitted limits: a positive result means the value > 4 CFU/100 cm^2 or 10 CFU/m^3 and a negative result is correlated with the value ≤ 4 CFU/100 cm^2 or 10 CFU/m^3. The disinfection of each room was considered suitable when ≤30% of samples collected from that room had a positive or inadequate result – when the

percentage is more than 30%. After the analysis of the data presented in Table 1, 2, the following observation were made: an almost double increase of number of samplings (196,77 % for surface disinfection monitoring; 168,18 % for air disinfection monitoring) and, implicitly, an increase in the number of sanitation samples (174,04 %) and the number of air samples (168,18 %)., The percentage of rooms with adequate disinfection was high: 97,42 % in 2015 and 93,78% in 2016.

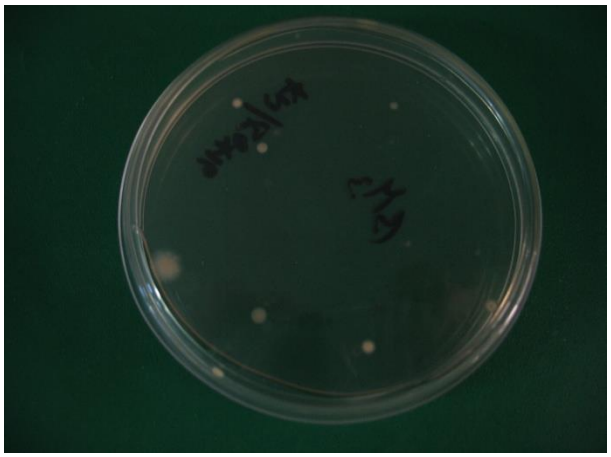

Figure 2. Colony Count/plate (original)

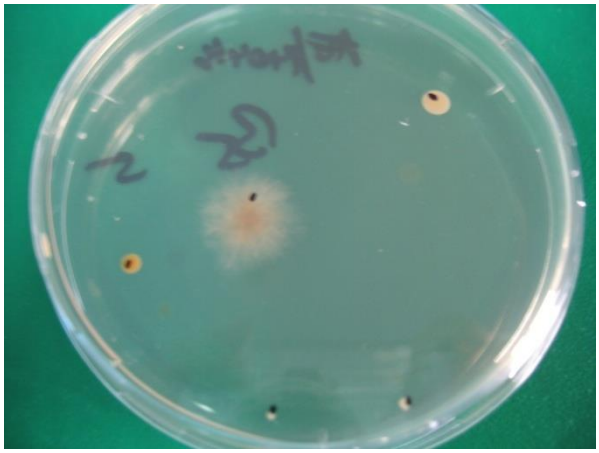

Figure 1. Colony Count/plate (original)

The causes of the positive results in some sanitation samples might be: the action performed incorrectly, insufficient personnel, as well as products and materials for cleaning and disinfection, and for air samples - incorrect settings of programmes of UV lamp for sterilization (1 h instead of 3 h) or UV lamp defects. The measures taken in case of inadequate disinfection consisted in: repeating the cleaning and disinfection action, the training of the personnel responsible for the cleaning and disinfection, the supply with products and materials for cleaning and disinfection and the assurance of the maintenance of the equipment. The microbiological monitoring of the laboratory environment is essential because it provides wide databases about the validation of cleaning and disinfection action, very important for assurance and quality control of analytical process.

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LASSA FEVER RISK PERCEPTION AND "ONE-HEALTH" CONSIDERATIONS ASSOCIATED WITH RODENT CONTROL PRACTICES IN A NIGERIAN UNIVERSITY

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SUMMARY

Lassa fever has an enormous public health impact in West Africa with thousands of human cases reported annually. The disease is both endemic and zoonotic in Nigeria and the Mano River Union countries of Sierra Leone, Guinea and Liberia in West Africa. The University of Benin, Benin City and its environs, are located within an epicentre of Lassa fever (LF) in the south-south region of Nigeria. Personal and public considerations about safety of agricultural products and the general living environment were investigated among students and staff within the university community. Selected socio-economic variables were evaluated. Results indicated that higher education was associated with animal hygiene related knowledge of LF (73.4%), compared to 37.3% of respondents with basic education who demonstrated poor knowledge of transmission of the disease ($p<0.0001$). High risk awareness was linked to one-health information dissemination on LF in media and campaigns (75.5%), in comparison with native oral transmission of information about the disease (24.5%). Risk perception was significantly associated with measures aimed at rat and various species of mice control amongst respondents ($p=0.022$). Ability of respondents to recognize rats, including *Mastomys natalensis complex,* the carrier of LF-virus within residential dwellings (71.1%) and contact of rats with human food (9.6%) elucidate the persistent public health threat in endemic areas. Cultural practices such as eating of rats (4.4%) and rat hunting practices (6.1%) further corroborates the value of a one-health agenda for LF control. This paper presents the control of rats and mice within the university campus as an animal hygiene and one-health agenda, which incorporates multiple socio-cultural factors for a more robust LF prevention model.

Key words: Animal hygiene, Lassa fever, One-Health

INTRODUCTION

Lassa fever (LF) is an acute and sometimes fatal disease caused by a single-stranded, negative-sensed RNA virus belonging to a diverse group of *Arenaviruses* of the family Arenaviridae. Other *Arenaviruses* which are known to be pathogenic in human causing hemorrhagic fevers include Lujo virus in Africa; and Junin, Machupo, Guanarito, Sabia and Chapare viruses in South America. LF is zoonotic and endemic in a known Lassa fever belt of West Africa comprising Nigeria, Sierra Leone, Guinea and Liberia (McCormick et al, 1987; Monath et al, 1973). The natural reservoir of Lassa fever virus (LASV) appears to be restricted to the single rodent species, *Mastomys natalensis* (Lecompte et al, 2006) which lives in close contact with humans. However, available evidence suggests LASV and other *Arenaviruses* may have adapted to other host during their evolution. For example, *Arenaviruses* has been detected in different African rodents but has not yet been linked to human diseases. *M. natalensis*, super order Euarchontoglires and order Rodentia,exhibit persistent, asymptomatic infection and profuse urinary virus excretion (Olugasa et al, 2014). Transmission to man occurs from exposure to secretions and excretions of infected rat (through ingestion of contaminated food or water) or patients. Like other haemorrhagic viruses, LF has an enormous social and public health impact in West Africa

with thousands of cases reported annually. The disease affects an estimated 500,000 individuals annually with a case fatality rate of up to 90% for pregnant women and 15-20% in symptomatic and hospitalized cases.

In Nigeria, the disease remains endemic with persistently high case fatality rates (Bond et al, 2012). The devastating effect of Lassa fever was demonstrated by the 2012 outbreak in Nigeria, acclaimed to be most severe, with 21 states affected including Federal Capital Territory (FCT), Abuja and 855 suspected cases and 136 laboratory confirmations were reported between January and April, 2012. During 2016 outbreak, the Federal Ministry of Health reported that 26 of the 36 states in the Country, including the FCT, were affected. This trend has remained unabated over the years. Retrospective data shows that Edo State is a LF endemic state, consistently reporting high figures of infection and mortality rate since 1989.Socioeconomic factors such as poverty, low educational level, deficiency in home technologies, high demographic density, and rural living have largely influenced the transmission and persistence of LASV and other infections. Poor housing quality, poor external hygiene sanitation and inadequate waste disposal facilities in urban and rural settings have been shown to increase the occurrence of rodents in human dwelling and risk of LF transmission in villages and camps (Fichet-Calvet and Rogers, 2009; Olugasa et al., 2014). Agriculture-related cultural practices such as bush burning, post-harvest grain storage density on residential dwellings, cassava (garri) processing have been suspected to influence M. natalensis breeding and transmission of LASV to human (Olugasa et al, 2014). Selected interventions designed to improve any of these situations may fail if they are applied in an isolated manner. Personal and public considerations about safety of agricultural products and the general living environment were investigated among students and staff within the university community. Selected socio-economic variables were evaluated. This paper presents the control of rats and mice within the university campus as an animal hygiene and one-health agenda, which incorporates multiple socio-cultural components for a more robust LF prevention model.

MATERIAL AND METHODS

Study location

The study was conducted at the two campuses (Ugbowo and Ekenhuan) of University of Benin (UNIBEN), Benin City, Edo state, Nigeria; located on Latitude $6^0 24^{'}$N and Longitude $5^0 36$'E. The university has eight (8) hostels at Ugbowo and two (2) hostels in Ekenwan campuses with a total population of about 15,000 resident students with combined staff (academic and non-academic) strength of about 8000. Other students live off-campus. It is a cross-sectional study.

Sample size

Sample size was determined using the method described by Cochran (1977) while adjusting for 10% non-response rate. Quantitative and qualitative data were collected using pretested structured questionnaire. Respondents were selected using stratified random sampling technique after obtaining their informed consent. A total 602 responded to the questionnaire survey. This comprised students (n=301) and staff (n=301) respectively. UNIBEN students who were squatters in the halls of residence, staying off campus or in staff quarters during the period of the study were excluded. Contract staff were similarly excluded from the study.

Data analyses

The questionnaire responses were screened and collated using the Microsoft Excel package (Microsoft Corporation, Redmond, Washington). Collated data were subjected to SPSS (Statistical Package for Social sciences version 22) for descriptive and inferential analyses. Descriptive and categorical analysis

of quantitative data was used to sort and analyse frequency distributions while inferential statistics using Chi-Square was used to test for at $p \leq 0.05$. Risk perception was evaluated based on behavioural response to persons who suffered from the disease or pragmatic steps taken to reduce risk of exposure.

Cranial morphometry

Killed rats around residential dwellings of students on campus were collected and measurements taken of specific external morphology. Bones of killed rats were prepared using warm water maceration according to standard procedure (Ekeolu and Ozegbe, 2012) and skull dimensions were obtained with digital venire calliper (Bruder Werkeuge. Art.-Nr.:823-160). Head and corporal parameters were analysed using ANOVA (SPANOVA) in SPSS 22.0 statistical software. Results of cranial morphometric were reviewed using findings in Tanzania (Breno et al., 2011) and 103 skulls of *Rattus rattus* in Tunisia (Ben Faleh et al, 2012) respectively. Congruent anatomical landmarks were matched and level of significance tested using one tail t-test ($p \leq 0.05$).

RESULTS

The age range of respondent students was between 15 and 44 years old. In contrast with staff whose ages ranged from 25 to 54 years old. Male respondents accounted for 46.2% of the total students while 53.2% were females. About 93.9% of the students earned less than thirty thousand naira monthly. The male and female respondents amongst staff were 43.2% and 50.8% respectively. More than 77% of staff earned at least seventy thousand naira monthly.

External morphometry suggests that all retrieved rodent samples were more closely related to the species *Rattus rattus.* However, cranio-dental analysis of captured rats shows variations from the mean of typical *Rattus rattus*

A significant ($p<0.05$) difference was found in income, level of education and living standard between staff and students of the University. Individual and group knowledge of Lassa fever amongst respondents was significantly associated with their level of education. Respondents with postgraduate education (73.4%, n=91) demonstrated a relatively balanced one-health knowledge conception of the disease, compared with 37.3% of respondents with basic education who demonstrated poor knowledge of the transmission of LF ($p<0.0001$). The knowledge of Lassa fever disease was higher amongst the older staff compared to younger staff but this was not statistically significant. High risk awareness was linked to one-health information dissemination media on LF campaigns (75.5%), in comparison with native oral transmission of information about the disease (24.5%). Risk education was found to be significantly associated with individual's perception of risk posed by Lassa fever disease ($p=0.015$). However, awareness of the disease from exposure through various mediums of dissemination had a higher impact on the knowledge of participants than age as a singular variable.

Students (70.6%) and staff (71.5%) acknowledged that they lived with rats and 51.6% (students) and 42.7% (staff) of this number sees rats at least daily, weekly or monthly. Amongst respondents, 21.1% of students and 7.3% of staff respectively, admitted to rat having contact with their food. Students were more likely to consume such likely contaminated food directly (4.3%) or simply rip out affect portion. This was perceptibly lower for staff who will readily dispose the entire food item (72.2%), although 25% of staff will cut off the eaten portion and 0.7% will consume the food item. The fact that 16.7%

and 13.7% of students and staff respectively have direct contact with faeces or urine of rats with 7.3% (students) and 4.8% (staff) still engages in rat hunting and preparation and 3.8% engaging in rat consumption, indicates that the risk of Lassa fever and other zoonotic diseases remains high. Despite the increase frequency with sightings of rats, 19.3% of the students are did not take any active measures to control the rat population within their rooms. Some 50.5% (n = 152) use rat poisons, 29.9% (n = 90) use traps, 1.3% (n = 4) use cats as rat control measures respectively. Amongst staff, the two most frequently used rodent control measures were rat poisons (46.2%) and traps (39.9%). Few staff prefers the exclusive use of cat (4.3%) and sanitation (1%) in rodent control respectively and 96.1% opined that the chosen control measures adopted was either very effective or effective.

DISCUSSION AND CONCLUSION

The study reveals that there remaining grey areas in knowledge about precise landmarks for identification of reservoirs of Lassa virus within the disease epicentre in Edo State, Nigeria. Study limitation includes, absence of current sero-prevalence of Lassa fever among the respondents, infectivity of the virus outside its reservoir host or in contaminated food items, and possible risk of exposure caused by infected dead rat. The best method of disposal of dead rats and how long the virus remains infectious even in infected dead reservoirs remains to be determined. This study of the effect of knowledge on the risk perception of staff and students of the University of Benin, towards Lassa fever disease offers a critical starting point for comprehensive animal hygiene evaluation and improvement of rodent control. A holistic approach to Lassa fever prevention has however commenced with advocating good hygiene and incorporating improved socio-economic conditions that hold a more effective strategy against the disease.

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Listeria monocytogenes PARTICIPATION IN THE PRODUCTION CHAIN OF FAMILIAR MILK HUSBANDRY AT BOTUCATU, SÃO PAULO, BRAZIL

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SUMMARY. In spite of milk nutritional qualities, it is considered an excellent mean for pathogenic development or microorganisms spoilage. Cooling is a method of raw material preservation, however, when raw milk is refrigerated for a long time, the quality deviations and some bacteria evolution may be verified. Among this group of microorganisms, more specifically, in pathogenic genera, remains *Listeria monocytogenes*. Its detection in food is important not just because of pathogenic effects on human and animal health, but also because it has economic importance. The main purpose of this study was to identify the presence of *Listeria monocytogenes* on milk samples from nineteen dairy farms of family husbandries, from Botucatu-SP. The data was collected on fifteen-days intervals with five collections per property by the time the milk was added to the bulk tank milk. Samples attainment was performed after homogenization of milk cans for each property and then preserved in cool boxes. Subsequently, microbiological analysis was performed by cultivation in PALCAM and ALOA agar and molecular analysis through polymerase chain reaction (PCR) *Listeria monocytogenes* detection. Results unveiled isolated *Listeria monocytogenes* among the ninety five samples cultured milk, despite the growth of blue colonies, suggesting the bacteria on agar ALOA and black colonies and also suggesting the presence of *Listeria monocytogenes* in PALCAM agar. The amount of contaminants in milk samples probably prevented the isolation of the searched agent. The PCR analysis of samples with primer inlA for pathogen detection was also negative. Although not detected *L. monocytogenes*, the data is important for the epidemiological study of the agent in the region. We suggest further studies with larger samples size and properties to evaluate their presence in smaller milk husbandries targeting to identification of risks to animal and public health.

Key words: raw milk, listeriosis, contamination, PCR

INTRODUCTION

Despite of the nutritional characteristics of milk, due to it is composition is considered an excellent environment for the growth of pathogenic or deteriorating microorganisms that can compromise the quality, altering its composition (MERUSSI et al., 2013). Making it a pathway of transmission of pathogens to humans, as well as their toxins (DE FREITAS GUIMARÃES et al., 2013). The bacterial contamination of raw milk can to occur in the animal or it may be related to the environment, with the manipulation of production line and handling of milk as final product. All these situations are directly related to hygienic milk production (SILVEIRA & BERTAGNOLLI, 2014). There are several aspects to be considered, such as the quality of the water used in the disinfection of milking and animal equipment, correct use of sanitizers, milking and plant hygiene (LANGONI, 2013). An efficient method for preserving raw milk is refrigeration. If refrigerated for long periods, raw milk may lose quality due to the growth of some bacteria (MARTINS & REIS, 2014). These microorganisms can develop at temperatures below 7 ° C, and their optimal development varies among different species of bacteria (SØRHOUG & STEPANIAK, 1997). Poor water quality, deficiency in hygiene procedures, and bovine mastitis favor their growth (JAY, 2000; ENEROTH et al., 2000;

MURPHY & BOOR, 2000). In addition, it is important to note that the use of these enzymes in the production of lipoproteins has been shown to reduce the economic value of milk, since its functional proteins and fat are altered by proteases, lipases and phospholipases (Chen et al., 2008). These microorganisms may also cause changes in the taste of various products and decrease in cheese yield (CHEN et al., 2003). It is important to carry out research that contributes to the clarification of factors related to the microorganisms that can compromise the quality of food. It is suggested that the pathogens present in the milk be essential for the development of the human being and indispensable for most of the population.

MATERIALS AND METHODS

Properties and obtaining samples

Samples were taken from a set of 19 family farms, which deposit milk in a community expansion tank, located in the city of Botucatu - SP. Immediately after the arrival of the samples in the laboratory, the direct culture for the detection of *Listeria monocytogenes* and the extraction of the DNA for molecular research were carried out. Next, the samples were kept under refrigeration temperature at 4 ° C for 48 hours, repeating the procedure for bacterial isolation and bacterial DNA screening of the agent, to evaluate the effect of refrigeration on the insulation.

Detection of the presence of Listeria spp. by microbial culture

The 25 ml of each sample were used, homogenizing in 225 ml of LEB (Listeria Enrichment Broth-Oxoid) broth, incubating at 30 ° C for 4 hours. Next, 0.5% nalidixic acid (1.8ml), 1% cycloheximide (1.15ml) and 0.5% acriflavine (0.455ml) were also added as selective agents, and reincubated at 30 ° C, for 48 hours. After 24 and 48 hours, with a nickel chromium loop, 10µl of the sample was cultivated on ALOA agar (Sigma) and PALCAM agar (Oxoid), incubated at 35ºC for up to 48 hours. After this period, up to five characteristic colonies (blue, on ALOA agar and black with black halos on PALCAM agar) were peeled into a tube with TSAE agar (TSA plus 0.6% yeast extract), incubated at 35 ° C /24 hours. At that time preliminary tests were carried out for identification, such as Gram staining (Gram positive rods), catalase test (catalase positive), esculin hydrolysis and sowing on agar motility for observation of "umbrella" growth.

DNA extraction

The extraction of direct DNA from the raw milk was performed using the commercial kit Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare), with some adaptations previously standardized in the Laboratory of Molecular Biology Applied to the Diagnosis of Zoonoses - UNESP-Botucatu-SP.

Amplification of nucleic acid (PCR)

The 25 µL volume was composed of 2.5 µl PCR Buffer 10x (Invitrogen), 0.75 µM Magnesium Chloride (Invitrogen), 200 µl of each dNTP, 1 U of Taq DNA Polymerase, 10 picomoles of each primer , Ultrapure autoclaved water (qsp) (Milli-Q Plus, Millipore) and 3 µL of the DNA sample. Incubation was performed on Gene Amp PCR System 9700 (AppliedBiosystem) using the initial cycle parameters at 94 ° C for 5 minutes for initial denaturation, followed by 35 cycles at 94 ° C / 30s, 60 ° C / 30s and 72 ° C / 30s. The final extension temperature was 72 ° C for 4 minutes. In all reactions, a negative control was used, replacing nucleic acid with ultrapure water. As a positive control, two standard strains of *Listeria monocytogenes* (ATCC 7644 and ATCC 16313) will be used.

RESULTS

The five samples were taken at intervals of fifteen days of milk from the set of properties at the moment when the producers reached the site of the community expansion tank with the brass. After enrichment in LEB medium, one hundred ninety-nine samples were cultured on ALOA and PALCAM

agar during the five collections, followed by the purpose of an immediate culture at the arrival of samples in the laboratory and another after forty-eight hours of milk cooling. It was not possible to isolate *Listeria monocytogenes* from any of the samples, despite the growth of blue colonies on ALOA agar and black colonies on PALCAM agar, suggestive of *Listeria monocytogenes*. The amount of contaminants in the samples probably prevented the isolation of the investigated agent. All samples, regardless of DNA extraction performed directly from raw milk or LEB broth, were negative in the PCR for *L. monocytogenes*. Positive controls were used for extraction and for PCR, and tests were performed, with the controls being cultured in LEB broth and raw milk prior to extraction, all with positive results in the visualization of the amplified products.

DISCUSSION

Although *L. monocytogenes* was not detected, data is important for the epidemiological study of the agent in the region. We suggest further studies with larger sample sizes and properties to evaluate their presence in smaller milk husbandries targeting the identification of risks to animal and public health.

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DEWORMING OR NOT? INDISCRIMINATE USE OF ANTIPARASITICS, ESPECIALLY IVERMECTIN

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SUMMARY. Treatment, prevention and parasite control is based on the use of anthelmintics. Macrocyclic lactones (LM), especially ivermectin, are widely known, recommended, distributed and managed by industry, veterinarians and producers due to their residual effect (30-150 days) and their broad spectrum of nematodes and arthropods such as ticks and flies. Their indiscriminate use in production animals due to the repetitive application or under dosing have led to the selection of resistant parasites, becoming an emerging problem. Besides toxicity in animals, no economic loss for the period of withdrawal of the meat and milk. WHO and FAO confirmed the impact of ivermectin on animal products and the environment. Ivermectin accumulates in meat or products, and prolonged ingestion produces toxic effects on the nervous system, coma and death. The LM is excreted in faeces and urine in its active form, and improper disposal of empty bottles pollutes the soil. Residues of ivermectin have been detected after 40 days of faecal deposition, and there has been decrease in soil invertebrates such as beetles and Diptera coprophagues, worms and other insects. It has also been determined as pollinator decline of biodiversity. Is also considered as water contamination with traces of ivermectin in aquatic ecosystems. The challenge is to transfer knowledge to students, veterinaries and producers to work together and implement strategies on parasitical integral control with a prudent and selective use of anti-parasitic, and combined with other alternative methods, depending on the circumstances, producing higher economic benefits and reducing damages to public and environmental health.

INTRODUCTION

Gastrointestinal parasites, specifically nematodes (NGI), cause production losses in the U.S. It has been estimated that parasite infection in ruminants is one of the main problems and losses may reach more than 3 billion dollars per year. In 1980-82, the Bureau of Economic Agriculture estimated the average costs for prevention and treatment for parasite infection in ruminants of 53 million dollars and losses in production of 253 million dollars per year; it has been estimated at 8.7% of annual production costs in sheep production. In the mid-1980s, it was estimated that one-third of sheep production units in New Zealand relied on chemicals to control NGI; For this purpose, 23 million New Zealand dollars were spent on anthelmintics per year (seven treatments / animal / year) (Luna-Palomera et al., 2010, Figueroa and Acevedo, 2011).

Parasitic control

Control of GIN is based on the use of a therapeutic chemical strategy. The periodic use of anthelmintics decreases the massive parasitism and prevents death to the most affected animals, but they do not prevent reinfections. Unfortunately, sometimes they are used perhaps by ignorance, as a preventive measure. The broad spectrum anthelmintics available against GIN are benzimidazoles, tetrahydropyrimidines, salicylanilides, organophosphates and macrocyclic lactones. However, the frequent use of anthelmintics, the irrational and indiscriminate use of drugs (many applications in a short time: up to 8 times a month especially in the hotter months (Martínez and Cruz, 2009), administration of short-term dosages and short-term alternations of drugs of different families lead to new parasites resistant to new antiparasitic agents (Coop et al., 2001; Torres et al., 2003). The first case

of thiabendazole resistance was described in 1968, levamisole in 1975, and ivermectins in 1985 (Gray, 1995). There are currently populations of *Haemonchus contortus* to a wide range of deworming agents (Aumont, 1995; Coronado et al., 2003), and Mexico is not the exception (Campos et al., 1992, Figueroa et al. Torres et al., 2003).

The use of ivermectins

In most productive units, treatment, prevention, and control are based on the use of anthelmintics such as benzimidazoles, imidazothiazoles and macrocyclic lactones. Currently, the most commonly used anthelmintics are from the family of the lacythes, specifically the ivermectin, widely known. This group has a wide acceptance because it has activity against nematodes and against arthropods such as insects and ticks and has a residual effect. For example, ivermectin given to sheep intravenously has a life of 40 hours, although life varies according to the form of administration, residual effect can be 10 to 12 weeks; its efficacy in adults and larvae is greater than 90% and for eggs it is between 50 and 74% (Sumano and Ocampo, 1997). Unfortunately, as a consequence of its indiscriminate use, the development of anthelmintic resistance was promoted, so that *Haemonchus*-resistant strains are already present (Coronado et al., 2003, Montalvo et al., 2003; Torres et al., 2003; Encalada et al., 2008; Torres-Acosta et al., 2012).

Ivermectin and public health

Although it has been commented that macrocyclic lactones do not accumulate in tissue, there is evidence that this happens. An important effect of the use of synthetic anthelmintics, in addition to toxicity in animals, is the indirect economic effect they cause, through the withdrawal period of meat and milk, and ecotoxicity (Beynon, 2012). WHO and FAO confirmed the impact of ivermectin on animal products and the environment. Ivermectin has total absorption at the site of application, in the intestine reaches high levels in a very short time, the maximum residues are in fat and liver and has wide distribution in body fluids. Another characteristic is that its persistence of residues in milk for a long time, although they are below the maximum limits and has great affinity for the adipose tissue that acts like deposit of the drug, in such a way that it has period of withdrawal in meat until of 122 days which in many cases is not respected which is demonstrated by the detention of ivermectin channels at levels higher than those adhered to. If the meat or by-products of animals treated with ivermectin become consumed by humans, it is usually a public health problem since prolonged ingestion can produce toxic effects related to the nervous system, coma and death (Yang, 2012).

Ivermectin and the environment

Periodic deworming causes environmental damage. Some macrocyclic lactones such as ivermectin, doramectin and abamectin cause damage to the environment through their direct excretion in the feces and urine of the treated animals, and by improper removal of empty bottles. They can remain active up to three months in the middle, and impact on populations of insect coprophagues, earthworms, pasture ecosystems and edaphic factors, even in aquaculture ecosystems (Webb et al., 2007; Martínez y Cruz, 2009; Aparicio-Medina et al., 2011; Basto et al., 2016; Lopez et al, 2016). By decreasing its population, it reduces the return of nutrients to the soil, the reduction of the vegetation cover, and thus the erosion of the soil. In addition, the residues of these deworming agents are washed away with rain until they reach bodies of fresh and salty water. In this way, they also cause water pollution and decrease the biodiversity of aquatic invertebrates. Therefore, the indiscriminate use of dewormer does have an effect on climate change.

MATERIAL AND METHODS

The challenge lies in first time in educating students, transmitting information to eradicate the old practice of de-worming all animals. Reaffirming ethical values, the practice of deworming is an economic income, however, we must think if it is really convenient to do it repeatedly and to all animals. At the same time, it is necessary to find control strategies that allow a combination of the prudent use of available antiparasitics, combined with non-chemical strategies, control alternatives such as herd management, breeding of pens and facilities (e.g. improving hygiene). It is proposed to give a seminar for students of Veterinary Medicine and Animal Husbandry in which the subject of ivermectin as anthelmintic and ectoparasitacid dewormer is addressed. It will also propose to emphasize the diagnostic clinic and laboratory prior to deworming and based on the results to make a selective deworming. It is fundamental to do a clinical diagnostic, combined with the use of the body condition and even the FAMACHA technique can be used to define animals that should be diagnosed in the laboratory. Once the laboratory results are obtained, carry out a selective deworming, in such a way that only deworming the animals that really require it. As alternative, known synthetic dewormer may be combined with plants of a nematicidal effect, e.g. rich in tannins, and even if grazing is managed, or on the contrary, if it is a herd, to analyse the conditions and take the better decisions before de-worming. On the other hand, it will be proposed to make information leaflets that can be given to producers to make diffusion on the irrational use of anthelmintics. It is essential to carry out extensionism, to work not only among doctors, but also to collaborate with producers, since they are the ones who manage their animals and who will benefit more, as well, collaborate with institutions and promote projects that make the diagnosis more available.

CONCLUSION

The challenge is to transfer knowledge to students, veterinaries doctors and producers to work together and implement strategies parasitical integral control with a prudent and selective use of antiparasitic and combined with other alternative methods depending on the circumstances and produce a higher yield economic benefits and reduce damages in public and environmental health.

It is essential to promote the practice of a professional ethic that allows the best decisions to be made and not only to favor economic interests.

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PRACTICES OF DEWORMING IN CATTLE

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SUMMARY: Parasites cause production losses for up to 4 billion USD annually, due to the decrease in the quality of life of animals and costs of anthelmintics, work and predisposition to other diseases. It is common to deworm the whole herd following programmes established without previous diagnoses, even without requiring it. The frequency and intensity of gastrointestinal parasites in cattle from Culiacan, Sinaloa in dry season and after rains was determined. Five groups of native females dual purpose grazing in rangeland were used, three groups had been previously dewormed with ivermectin and levamisole. The faecal samples were collected in the dry season and subsequent rains. The frequency and intensity of oocysts and eggs per gram of faeces (opg and epg) was obtained. *Eimeria* was identified in 18% (150 opg) and 3.1% (100 opg) in drought and rains, respectively. *Moniezia* was recorded in the rainy season with a frequency of 59% (103 epg). Nematodes' frequency was 20% (410 epg) and 56% (245 epg), respectively; single *Haemonchus* and *Strongyloides* were identified. In the groups by *Eimeria spp.* oocysts the deworming was identified. Groups without deworming, only 4 and 6% of cattle, had more than 750 epg who were candidates for deworming loads, which prove that it is not always necessary deworming all animals in the herd. Therefore, it is required to make prior diagnosis to implement a comprehensive and selective deworming, which will result in a benefit to animal health and welfare, the economy of the producer to reducer or avoid withdrawal periods and reduce the negative impact on the environment.

Key words: gastrointestinal parasites, deworming, cattle.

INTRODUCTION

Gastrointestinal parasites cause direct economic losses due to reduced production (meat and milk reduction and death of animals) and indirect costs due to control costs (anthelmintic (AH), labor, equipment), reduction in quality Of the canal and predisposition to other diseases. It is estimated that the annual worldwide expenditure to combat parasitic diseases is $ 1.7 billion USD, while if indirect costs are considered, it increases to $ 4 billion USD. In Mexico, there are few studies, however, it was determined that 48 million of kilograms of meat and 4.4 million liters of milk are lost annually due to gastrointestinal parasitism in cattle (Dominguez et al., 1993).

Most of the studies have been carried out on cattle raised in tropical climate, few studies have been done in other latitudes of the country as the dry tropic, in which also livestock grazing is done in agostaderos, reason why little information exists on The behavior of natural populations of parasites that affect cattle. In the state of Sinaloa, some studies have been carried out, such as those mentioned

by Gaxiola in 1999, which show the presence of parasites in faeces of slaughtered cattle (Quiroz, 2012).

Control is generally carried out by the periodic administration of dewormer to all animals; this strategy is based on a productivist concept of immediate maximum benefit, so it is common to desparasite without a previous diagnosis. On the other hand, failure to respect the withdrawal time of anthelmintics in animals destined for human consumption, becomes a public health problem, due to chemical residues in products of animal origin and damage to the environment (Luna- Palomera, et al., 2010), also promotes the emergence of resistance. One option is to do a selective deworming, in which the use of dewormer is diminished and animal welfare is favored and the producer in turn has an economic benefit by reducing the expenditure on deworming and the economic loss by the time of retirement, However, to do this, more data are required to relate the parasitic load to the time of year and to have elements that lead to the development of control measures.

The frequency and intensity of gastrointestinal parasites in cattle from Culiacan, Sinaloa in dry season and after rains was determined.

MATERIAL AND METHODS

The study was carried out in five herds of Culiacan Sinaloa of dual - purpose cows from 2 to 5 years of age grazing on natural rangelands, the vegetation is composed of pastures and low deciduous savanna. As part of the management, all animals are dewormed every six months without a previous diagnosis. Fecal samples were collected directly from the rectum in two different seasons: 1) in February during the dry season: one herd with 36 cows of 3-5 years old and one herd with 25 calfs, 6-12 months old and 25 cows, 3-5 years old. 2) After the rainy season, in November, three groups were used: one of 32 cows without having previously dewormed and one of ten cows with deworming with levamisole two days prior to sampling; Samples to another group of 30 dewormed cows with ivermectin 7 days before sampling without a previous diagnosis.
After the samples were collected in individually labeled plastic bags, they were transferred to the Parasitology laboratory, FMVZ-UNAM and stored at 4 ° C until processing. The Mc Master technique was performed, the intestinal parasites were identified and counting was performed. The faeces positive to *Eimeria* spp. were treated with 2% Potassium dichromate with electric stirrer for 5 days for sporulation of the cysts. Later, the taxonomic identification of cysts was performed (Besné et al., 2006). Gastrointestinal nematodes (GIN) positive faeces were homogenized with sterile sawdust in plastic containers, the mixture moistened without excess water, incubated at 27 ° C for 10 days, and oxygenated daily (Liébano, 1989) . The third stage larvae (L_3) were collected by the Baermann technique (Thienpont et al., 1979). It was observed under the microscope and the taxonomic identification of the different genera of NGI (Niec, 1968, Vega and Romero, 1983; Van Wyk, et al., 1997). Ectoparasites were collected and taxonomically identified.

Statistical analysis: Oocyst and egg counts per gram of feces (opgh and epg), frequency (percentage of positive samples) and mean intensity were obtained: mean oocysts or eggs of the positive samples

(Eckert et al., 1984). From the mean intensity in egg removal, the standard deviation was obtained. The percentage of identified genera or species was obtained.

RESULTS

Gastrointestinal parasites were present in the cattle from Culiacan, Sinaloa, after performing the coproparasitoscopic analysis, the frequency and intensity were obtained the following results that are shown in table 1.

Gastrointestinal parasites were present in both seasons although the cestodes of the genus *Moniezia* were recorded only during the rainy season.
During the rainy season the frequency of the three groups of parasites was higher in comparison with the dry season, however, the gastrointestinal nematode epg was higher in the dry season, which could be related to the physiological state of the cows or the seasonality of the same parasites.

In groups 1, 2 and 3 were more diversity because they were not previously dewormed. The genera identified in group 1, during the dry period *Strongyloides* eggs and 100% larvae corresponded to *Haemonchus*, in group 2 were recorded oocysts of *Eimeria* spp., *Strongyloides* eggs, and larvae 3 of *Haemonchus* (62%) and *Cooperia* (38%). In group 3, *Eimeria bukidonensis* was recorded, however, it was not possible to identify the nematodes.

In the case of dewormed cattle, there was no presence of cestodes or nematodes, however, if there were presence of protozoa of the genus *Eimeria*, since deworming is generally administered against cestodes, trematodes and nematodes, without considering the protozoa. According to the results, only 4% of group 2, and 6% of group 3 had parasitic loads higher than 750 hpg, which would be the candidates to deworming, the group 1, do not need dewormed

DISCUSSION

Cattle from Culiacan, Sinaloa, there were reported gastrointestinal parasites, which present a seasonal variation related to the dry or rainy season. In order to carry out an adequate and integral control it is necessary to make a diagnosis so that the deworming is correct, to be carried out against the present parasites and preferably to perform a selective deworming in which only the correct dewormer is administered to the cattle with high parasitic loads , its will reduce economic spending due to the investment in medicine and the period of withdrawal of meat and milk since they are dual purpose cattle, thus promoting a benefit to animal health and welfare and will have a positive impact on the economy Of the producer.

Table 1. Parasites identified in cattle of Culiacan Sinaloa, in two seasons.

		Eimeria spp.		*Moniezia*		Trichostrongylides	
		Frequency %	Intensity opg	Frequency %	Intensity epg	Frequency %	Intensity epg
Dry season							
Group1		0	0	0	0	13	60
Group 2	Calfs	36	150	0	0	0	0
	Cows	0	0	0	0	20	410
After rainy season							
Group 3		3.1	100	59	703	56	245
Group 4*		20	750	0	0	0	0
Group 5**		7	475	0	0	0	0

* Levamisole two days before to sampling.

** Ivermectin seven days before to sampling.

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RESEARCH ON *Yersinia enterocolitica* IN EXPANSION TANKS AT DAIRY FARMS IN SAO PAULO, BRAZIL

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The milk quality can be determined by its degree of contamination by psychrotrophic microorganisms, since it will serve as an indicator of the hygienic conditions on which milk was obtained and stored, since milking to its consumption, considering the dairy expansion tanks as the main contamination elements. The *Yersinia* bacteria are able to reproduce in temperatures below 7ºC (45ºF), which represents high risk to consumers. So as to determine the degree of contamination, 32 milk samples were collected from tanks of 32 different properties that distribute milk to dairies in center-west region of São Paulo State – Brazil. For the pre-enrichment of the samples, 25 mL (0,85 FL OZ) of each milk sample from the tank were added to 225 mL (7,6 FL OZ) of Buffered Peptone Water for pre-enrichment for 30 days under 4ºC (39ºF). After, it was added to PSTA, supplemented with ampicillin, incubated under 28ºC (82ºF) for 48 hours. After pre-enrichment, *MacConkey agar supplemented with calcium chloride was used. Selective Yersina agar and Hektoen agar with ampicillin (5mg/L), incubated under 28ºC (82ºF) for 48 hours. Small colonies were selected (1 to 2 millimeters), translucent or light pink or small light and punctiform rosy colonies to the Gram technique. For the execution of the biochemical tests, the suspect colonies were submitted initially to oxidase, catalase, sucrose, raffinose and citrate tests, to which no samples accused the typical biochemical structure of Yersinia enterocolitica* at this first selection. Aiming at the importance of proper and correct hygiene during the whole milking process, as well as with equipment and expansion tanks, even though primary results show no *Yersinia enterocolitica* on milk samples, new collections will be done, since it's an insufficiently studied kind of bacteria and highly important when it comes to public health.

Keywords: Enterobacteria, raw milk, contamination

INTRODUCTION

The increased demand for unpasteurized milk has emphasized the importance of the hygienic and microbiological quality of raw milk (RUUSUNEN et al., 2013). This increase in consumption is justified by taste, convenience, cost and high nutritional value (Kaylegian et al., 2008); however, milk is an ideal medium for the growth of a wide variety of pathogenic bacteria, behaving as an important substrate for transporting microorganisms from infected animals to humans.

In 2000, in North America, raw milk and its derivatives were associated with 45 outbreaks of foodborne diseases, with 687 people affected with 8 deaths. Already in 1999, in Europe, 100 outbreaks were recorded, 50 to 60% of which were caused by raw milk products (RUUSUNEN et al., 2013). Psychrotrophic bacteria are able to develop at low temperatures, thus being one of the main pathogenic and degrading agents present in refrigerated raw milk and its derivatives. *Yersinia enterocolitica (Y. enterocolitica)* is one of the possible psychrotrophic and pathogenic bacteria that may be present in

milk. Bacteria of the genus *Yersinia* can multiply at refrigeration temperatures below 7ºC (45ºF), representing a high risk for consumers, considering the consumption of raw or inadequately pasteurized milk, leading to outbreaks of enteric infections (SOLTAN-DALLAL et al., 2004).

Bacteria of the genus *Yersinia* are Gram-negative, facultative anaerobic, acid-producing, but non-gas-producing, catalase-positive, oxidase-negative, and measure 0.5 x 0.8µm wide by 1 to 3µm in length ; in young cultures at 25°C (77ºF) the coccoid forms predominate, in cultures at 37°C (98,6ºF) the bacilli tend to be pleomorphic. Although optimum in vitro multiplication conditions are observed at 25 (77ºF) and 28°C (82ºF), they are capable of multiplying at temperatures ranging from 0°C (32ºF) to 44°C (111,2ºF) and can survive in frozen foods, blood banks, vacuum packaging or in packaging with modified atmosphere (Thabulsi; Alterthum, 2015); as a result, it becomes a micro-organism of concern for public health.

Y. enterocolitica is transmitted to man almost exclusively by the ingestion of contaminated food and water (FALCÃO et al, 2006). This has already been isolated from raw milk and its derivatives, on ice used for human consumption, pork and its derivatives, poultry meat, as well as faecal material from humans and sick animals and the environment (VIDON,1981; FALCÃO et al., 2002; FALCÃO et al., 2006; RUUSUNEM et al., 2013; DARWISH et al. 2015).

Among the genus *Yersinia*, *Y. enterocolitica* is the most prevalent species linked to diseases in humans. It is an invasive enteropathogen that causes a series of intestinal clinical symptoms, with diarrhea being the most common (SILVA JR., 2014) and extra-intestinal, ranging from mild gastroenteritis to mesenteric lymphadenitis and acute abdominal pain, which mimics appendicitis and, in rare cases, may develop into septicemia. Infection caused by *Y. enterocolitica* has acquired relevance in clinical practice not only for its intestinal and extra-intestinal manifestations but also for its potential to lead to immunological sequelae including arthritis, erythema nodosum and glomerulonephritis (MALTEZ et al., 1993). It has a tropism for the lymphoid tissues and ability to resist the host's nonspecific immune response, in particular phagocytosis and death by macrophages and polymorphonuclear leukocytes (SALYERS AND WHITT, 2002).

Intestinal infections of *Y. enterocolitica* are normally self-limited and do not require specific antimicrobial treatment; however, in the case of immunosuppressed patients and patients with gastrointestinal complications and extraintestinal infections, the administration of doxycycline, trimethoprim-sulfamethoxazole, fluoroquinolones and cephalosporins of broad spectrum is recommended (FALCÃO et al., 2006).

Considering the importance of correct and adequate hygiene throughout the milking process, as well as equipment and bulk tanks, we propose the research and identification of *Yersinia enterocolitica* in milk samples from bulk tanks of different dairy properties of center-west region of São Paulo State – Brazil, because it is a bacterium little studied and of great importance in public health.

MATERIAL AND METHODS

Microbiological analyzes and biochemical identification were performed at the Animal Healthy Laboratory of Bauru - APTA / SAA – Paulista Agency of Agribusiness Technology- Center-West Pole.

To determine the degree of contamination, 32 milk samples were collected from tanks of 32 different properties that distribute milk to milk factories in the Center-west region of the State of São Paulo. Samples were collected directly from the individual bulk tanks, with a maximum temperature of 4°C (39,2ºF), and milking performed at most 48 hours (two days). Prior to collection, the tank shaker was switched on so that the milk was homogenized for at least 5 minutes, and after this period, with the aid of a previously sterilized stainless steel shell, the 250 mL volume was collected into a sterile flask,

which was placed in an isothermal box containing recyclable ice and then sent to the laboratory for the microbiological procedures described below, according to Soltan-Dallal et al. (2004).
Yersinia reference strains of the serological group 1B, 2, 3, 4 and 5 from the collection of cultures of the Department of Bacteriology from IOC / FIOCRUZ (Rio de Janeiro, RJ), were used to identify the colonies. Twenty-five (25) mL of each milk sample from the tank were added to 225 mL of Buffered Peptone Water pH 7.2 (Isofar®) for pre-enrichment for 30 days at 4°C (39,2ºF).
After 30 days, 1 mL of the pre-enrichment was added in 100 mL of sucrose, tris(hydroxymethyl)aminomethane, sodium azide, and ampicillin (PSTA) composed of 1 g peptone A (Acumedia®), 1 g sucrose (Synth®), 3 g of TRIS hydroxymethylaminomethane (Ludwig Biota®), 0.0125 g of Bright Green (Synth®), 0.192 g of Sodium Azide (Synth®) and distilled water qsp 1 L, supplemented with ampicillin (Sigma Aldrich®) at 5 mg/L - pH 8.3 and incubated at 28°C (82ºF) for 48 hours (VIDON; DELMAS, 1981).
Three culture media were used to attempt isolation after pre-enrichment: MacConkey Agar supplemented with 0.2g / calcium chloride and 10 mL/L Tween 80 (WAUTERS et al., 1987), Yersinia selective agar (WAUTERS et al., 1987), Hektoen agar with 5 mg / ampicillin (VIDON; DELMAS 1981). All plates were incubated at 28°C (82ºF) for 48 hours. Small colonies (1 to 2 mm in diameter), translucent or light pink staining, or small, punctiform light colonies were selected. All colonies were subjected to Gram staining and a pre-screening to the oxidase, catalase, sucrose, raffinose and citrate tests. If these colonies had characteristics of *Y. enterocolitica*, they would be submitted to the API 20E identification system (BioMerieux®).

RESULTS

Small colonies were selected for biochemical tests, but different results than expected for *Yersinia enterocolitica* were found, ie none of the samples were positive for this specie.

DISCUSSION

This paper presents preliminary data about isolation of *Yersinia enterocolitica* from bulk tank milk. Although a methodology directed to the isolation of *Yersinia enterocolitica*, with prior use of specific enrichment medium and subsequent attempt of isolation in selective medias, the negative results obtained may have occurred due to the methodological difficulties commonly found in the isolation of this bacterium. The microbiota of milk is rich in several microorganisms, and *Y.enterocolitica* presents a small number of strains in the samples, making it difficult to isolate and identify (DARWISH et al., 2015).
The detection of *Y. enterocolitica* in foods can be significantly improved by the molecular PCR technique, corroborated by several authors, that it would be the best method to confirm the identification of *Y. enterocolitica*, due to its greater sensitivity in relation to conventional techniques and due to less time demand for its execution and result (HANIFIAN et al., 2012; DARWISH et al., 2015).
To estimate a supposed prevalence of 7% (RUUSUNEM et al., 2013; DARWISH et al., 2015), with a margin of error of plus or minus 5%, it would be necessary to collect 101 samples from different expansion tanks calculated in the OpenEpi program (DEAN, 2015). Since the work is in progress, more samples will be collected, as well as the molecular PCR technique will be performed, associated to the microbiological diagnosis. Thus, although the results are negative at the moment for the studied samples, we expect to be possible to evaluate at the end of this research the importance of *Yersinia*

enterocolitica in milk samples from bulk tanks, since, once in this food, it can provide outbreaks of infection to consumers, denoting a major public health problem.

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FORAGE PRODUCTION OF THREE SUDAN HYBRIDS IN TWO LOCATIONS WITH RAINFED CONDITIONS IN SINALOA, MEXICO

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SUMMARY. Sweet Bite is a vigorous conventional forage hybrid obtained from crosses of Sorghum x Sudan grass. The objective of this study was to evaluate the fodder production of three Sudan varieties: Sweet Bite I, Sweet Bite II, and GW-300 in the state of Sinaloa, México. Two production environments with low pluvial precipitation and similar climatic, topographic and socioeconomic conditions were selected. In these two locations, the agricultural system is developed under rain fed conditions and the precipitation level oscillates between 350 and 550 mm. Soil preparation and agronomic management were performed including a seed density and nitrogen fertilization of 412,500 plants/ha and 150-52-00 kg/ha, respectively. For weed control pest products with the active ingredient (aminopyralid + 2, 4-D) and Lambda-cyhalothrin respectively were applied. A completely randomized block design was used with five replicates of 1 ha each. Data production was an average from 3 cuts applied to all replicates. The data was statistically analysed with the Statistix 9 software. Results showed that Sweet Bite II (8.62 ton/DM/ha) and GW-300 (7.93 ton/DM/ha) hybrids produced higher ($P < 0.05$) amount of forage than Sweet Bite I hybrid (7.15 ton/DM/ha). It can be concluded with these results that the genotypes Sweet Bite II and GW-300 showed better performance than the material Sweet Bite l, obtaining increments up to 20% in fodder yield under rain fed conditions of the state of Sinaloa, México.

Key words: Sweet Bite, forage, rain fed.

INTRODUCTION

The production of meat and milk under grazing conditions depends to a large extent on the availability of native grapes in Latin America or introduced to regions generally not suitable for intensive agricultural use. Agriculture continues its advance on the best cattle lands and, in turn, along with livestock advances to areas with undisturbed vegetation (Baruch, 2005). In Mexico, sorghum area (Sorghum bicolour L. Moench) in 2012 was approximately 2' 207, 844 ha, with a production of 6 million 429 thousand 311 T of grain and 3 million 937 thousand 931 T of green fodder. The state of Sinaloa occupies the second national place in surface planted of sorghum, after Tamaulipas; there are planted 337 thousand 321 ha. Regarding production, Sinaloa ranks third in production in Mexico: one million 284 thousand 874 T of grain and 286 thousand 488 T of sorghum green forage per year (SIAP, 2012). One of the main problems in the Sinaloan cattle breeding is the lack of forage in quantity and quality, for the feeding of bovine cattle throughout the year, mainly in the dry season. Traditionally the agricultural producers have depended on the harvest shearing (corn and sorghum), to feed their cattle in the dry season. This traditional system has the disadvantage of being subject to the great seasonal variability of forage production during the year, as well as that the grazing of sorghum forage, allows optimum use of 40% of the available fodder. In Sinaloa, the livestock population between beef cattle and milk on average is one million 540 thousand 885 head of cattle (SIAP, 2012).

In Sinaloa, agricultural activities are carried out mainly under temporary conditions, in dispersed areas, located in hills (with thin soils and slopes of 2 to 25%), together with poor distribution and rainfall 450 to 600 mm) means that the area devoted to agriculture is not suitable for the cost-effective production of grains. This situation has forced the producers to integrate agricultural and livestock activities. In the region, since 1993 a new production technology was started, based mainly on the sowing of forage sorghum and the use of forage conservation practices, such as hay and silage.

The main problems facing the cultivation of sorghum in Sinaloa are drought, caused by the erratic distribution of rainfall and the scarce use of conservation practices and the use of moisture. Considering that 70% of the sorghum is cultivated under temporary conditions, while 30% under irrigation, with yields of 1.35 t ha-1 and 6.83 t ha-1 of grain, respectively; While in forage sorghum they are 7.94 t ha-1 in temporal and 13.60 t ha-1 in irrigation (SIAP, 2012), which affects the obtaining of more efficient genotypes for the use of available soil moisture, in addition Biological cycle must be shorter or intermediate to fit the erratic distribution of rainfall (Hernández-Espinal et al., 2010). The use of grasses in improved grasslands is markedly higher in the humid tropics compared to dry tropical climate zones, but in general the use of introduced pastures has not been widely diffused among the different potential livestock areas (Eguiarte et al., 1987). The climatic conditions in the dry tropic regions determine the application of specific criteria for the management of production systems with grazing livestock, the most important being: selection of suitable pasture, fertilizer application and dosage, grazing system, loading Animal, establishment of programs of supplementation and finally the use of shears and legumes. Due to different factors currently facing the national stockbreeding among them, the poor quality of available seeds, with low purity, germination and high prices in the national market (Eguiarte 1984). Due to the above, the objective of this work is to determine the productive behaviour of three sudan hybrids under seasonal conditions in the state of Sinaloa.

MATERIALS AND METHODS

The present study was carried out in two locations of the temporary zone of the state of Sinaloa, located in the municipality of Mocorito, with a geographical location of 26 ° 32'19.23" N and 108 ° 20'33.41" W; and the other in Cosala with coordinates of 25 ° 34'33.97" N and 107 ° 41'19.48" W. Agricultural equipment used was: tractor, drawer type, hydraulic drawer, sprinkler, sprinkler, shovels, wheelbarrows, agricultural inputs (herbicides, insecticides, urea fertilizers 46-00-00, mono-ammonium phosphate 11-52-00). Sweet Bite I, Sweet Bite II and GW-300 varieties, 10 x 10 (100 m^2) plots were limited. They were fertilized and planted in July 2013, and these plots corresponded to cooperating local producers. The methodology mentioned in the previous paragraph was applied in agro-ecological areas (semi-arid tropics) with low productive potential that, due to their socio-economic and socio-climatic characteristics, allow for the development and adoption of sustainable agriculture technology. Specifically in those areas totally of temporary. Dry matter as response variable was collected 60-65 days after sowing for the Sudanese, and each sample was from an area of 100 m^2 making the cut at 7 cm height from the ground level and weighing the forage cut on a clock scale using a tripod at the place of the plot. For the statistical analyses, a completely randomized block design was used with five replicates per hybrid. The Statistix 9 package was used considering a level of 5% of error.

RESULTS AND DISCUSSIONS

From the statistical analysis for dry matter yield, no significant difference was found between Sweet Bite II and GW-300 hybrids, with yields of 8.27 t / MS / ha and 7.93 t / MS / ha respectively, the hybrid Sweet Bite I showed the lowest forage yield per variety with 7.15 t / MS / ha.

Table 1.- Analysis of variance of yield by forage treatment in two localities of the zone of temporary of the state of Sinaloa.

Hybrid	Yield (t/MS/ha)
Sweet Bite II	8.62[a]
GW-300	7.93[a]
Sweet Bitee I	7.15[b]

C.V= 4.18%, EEM= 0.1152, P= 0.01

For the variables, treatment by locality, no significant difference was found for the treatments GW-300 and Sweet Bite II in the locality of Kosala with 8.50 t / MS / ha and 8.45 t / MS / ha respectively, and Sweet Bite II in Mocorito with 8.10 t / MS / ha.

Table 2.- Analysis of variance of the yield by locality of the temporary zone of the state of Sinaloa.

Treatment	Location	Yield (t/MS/ha)
GW-300	Cósala	8.76[a]
Sweet Bite II	Cósala	8.71[a]
Sweet Bite II	Mocorito	8.53[ab]
Sweet Bite I	Mocorito	7.47[bc]
GW-300	Mocorito	7.1[bc]
Sweet Bite I	Cósala	6.83[c]
Sweet Bite I	Cósala	6.83[c]

C.V= 4.18%; EEM=0.1630 P =0.01.

CONCLUSIONS

According to the results obtained in the two localities, the GW-300 and Sweet Bite II treatments presented the best forage yield with 8.50 T / MS / ha and 8.45 T / MS / ha for the locality of Cosala and 8.10 T / MS / ha for Sweet Bite II in the town of Mocorito. These results were obtained under the weather conditions and precipitation of the months of July-September 2013, in the temporary area of the state of Sinaloa.

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WATER HYGIENE: AN IMPORTANT PARAMETER ON THE WAY TOWARDS IMPROVED ANIMAL HEALTH

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Drinking water represents the most important feed for livestock. According to Regulation (EC) No. 183/2005 Appendix 3 it has to be offered in sufficient volume and suitable quality at any time to the respective animal species. However, "suitable quality" lacks further definition. In Germany, guideline parameters for animal drinking water exist in accordance with legal regulations for human drinking water. At the dew ponds (nipple, trough) these microbiological criteria are often not achieved. Beside initial charges of the water source, all drinking systems are microbiologically challenged by biofilm development. The pipe material, low water flows and high temperatures are only a few factors influencing biofilm development. Biofilm can impact animal health by endotoxin release, and can also represent a source of obligate and facultative pathogens as well. Even though water quality as preventive tool has achieved more attention over the last years, the knowledge on quantitative and qualitative assessment of biofilms and method to reduce their growth is still poor. Therefore, in one study the use of removable duct segments (25 cm), ball valve closed and incorporated at the start and the end of a PVC-water pipe system, for biofilm analyses was tested in a pig rearing unit. Moreover, the effect of water disinfection on biofilm growth was tested. It was shown, that during the water disinfection, the total mesophile flora (36°C) was reduced to less than the critical value of 1,000 CFU/ml. In another study, the effect of a physical water treatment on water quality on turkey farms was evaluated. A significant reduction of bacteria, as detected by chemical treatment, was not observed. Both studies clearly show that water hygiene is one important factor that should be considered when the aim of high animal health and performance should be realised preventively without the extensive use of antibiotics.

EFFECTS OF GLYPHOSATE ON FARM ANIMAL-ASSOCIATED BACTERIA

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INTRODUCTION. Residues of glyphosate, the most used herbicide in the world, are commonly found in the environment and food supply chain. Recently, its effects on microorganisms and antibiotic resistance have been recognised, raising concerns about the effects of glyphosate in animal feed on microbiome.

MATERIAL AND METHODS. The objective of present study was to investigate the actual levels of resistance to glyphosate in diverse isolates of *Escherichia coli* and *Salmonella* spp., and its ability to induce resistance.
Minimal inhibitory concentrations (MIC) of glyphosate and the glyphosate containing herbicide product Roundup™ were determined using a broth microdilution method for 120 *Salmonella* spp. and 113 *E. coli* isolates. Two strains of *E. coli* (ESBL and non-ESBL) were further passaged daily in gradually increasing concentrations of glyphosate and Roundup.

RESULTS. The MIC values ranged within 40-80 mg/ml for glyphosate and Roundup in *Salmonella* spp. and 5-10 mg/ml for glyphosate and 20-40 mg/ml for Roundup in *E. coli*. Antibiotic resistance status, host species, geographic location, phylogroup or time of isolation relative to the introduction of glyphosate did not affect the levels of resistance. Resistance induction response for Roundup was similar in two *E. coli* strains, with early extinctions of bacterial populations at 2x MIC. However, there was an increased level of tolerance at 2x MIC to glyphosate in both strains.

DISCUSSION. Our results demonstrate differences in glyphosate sensitivity between *E. coli* and *Salmonella*, which were not affected by the antibiotic resistance status or isolate origin. Although glyphosate resistance does not occur easily, at least in our experimental setting, it is possible to select for increased tolerance. Further investigations are necessary to determine the ubiquity and mechanism of this phenomenon, as well as relevance to animal husbandry.

Zoonoses and emerging diseases

PREVALENCE AND RISK FACTORS ASSOCIATED WITH SEROVARS OF *Leptospira* IN DOGS, RELATED HUMAN SEROPOSITIVE

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SUMMARY. To determine the seroprevalence and the main risk factors associated with serovars of *Leptospira* in dogs from Culiacan, Sinaloa, we obtained serum from blood samples of 106 dogs, related humans seropositive (blood donors of public hospitals). The samples were stored at -40 °C until use, and were analyzed by the microbiology laboratory of the Centro Nacional de Sanidad Animal (CENASA) using the leptospirosis microscopic agglutination test (MAT). An epidemiological survey was conducted in order to identify risk factors. Statistical analyzes were done using the chi-square test. The risk (OR: odds ratio) and the confidence intervals were calculated using the logistic regression model. A value of $P < 0.05$ was considered statistically significant. The prevalence of Leptospira was 17% (18/106), and we identified eleven serovars and their respective frequency: Wolffi (66.6%), Bratislava (22.2%), Australis (16.6%), Canicola (11.1%), Grippotyphosa (11.1%), Pyrogenes (11.1%), Hardjo (5.5%) Icterohaemorragiae (5.5%), Pomona (5.5%), Hebdomadis (5.5%), Shermani (5.5%). The risk factors associated with the detection of antibodies to this bacteria, based on epidemiological surveys, include the gender of female pets ($P < 0.05$; OR= 2.9) and the dogs who stayed at home and had access to the street were marginally significant (P= 0.06).

Keywords: Leptospira, prevalence, dogs.

INTRODUCTION

Leptospirosis is the most widespread zoonotic disease in the world, with great economic and health importance. In Mexico, it is a mandatory notification disease. The World Health Organization (WHO) and the Pan American Health Organization (PAHO) classify *Leptospirosis icterohaemorrhagiae* with the key A 27.0 (OMS, 1992). The Official Mexican Standard NOM-029-SSA2-1999 establishes the mandatory character and the general procedures for monitoring cases of Leptospirosis. This disease is caused by a spirochete of pathogenic strains of the genus Leptospira, affecting wild and domestic animals, as well as humans. The diagnosis of leptospirosis is performed by various methods, however, plate microagglutination (MAT) is regarded as the gold standard. The reactions determine the presence of agglutinating antibodies against the tested serovars (OMS, 2008). The main source of infection for animals, especially dogs, is the urine of asymptomatic carrier animals (dog to dog), as well as vectors, rodents being a natural reservoir (Alton *et al*., 2009; Meeyam *et al*., 2006) The serovars icterohaemorrahgiae and pomona produce hemolysins, which are responsible for hemoglobinuria. In the case of the serovar icterohaemorrahgiae, it causes severe jaundice in dogs. In Mexico, serological studies have been carried out in dogs from various states of the country. Moles *et al*. (1990) analyzed sera from 218 dogs from the anti-rabies center of Culhuacán, Mexico City, and found that 28.4% were seropositive to one or more serovars; Leptospira canicola was the most prevalent with 22% seropositivity. Flores and Solana (1992) observed a frequency of seropositivity of 61.7% in all dogs studied. Garcia and Ibarra (1992) found 41.5% seropositivity in dogs from Toluca, in the State of Mexico. Luna (1993) analyzed 485 sera from dogs in the area of Naucalpan, State of Mexico, and found 48.4% seropositivity. The main source of infection for animals, and dogs in particular, is the urine of asymptomatic carrier animals, and vectors, of which the most important are rodents, due to their

capacity to act as natural reservoir for the bacteria. Dogs are considered the most important domestic species in the transmission of leptospirosis to man (Venkataraman and Nedunchelliyan, 1992; Luna, 1997; Brod *et al*., 2005). In the state of Sinaloa, this disease has been diagnosed in humans, ruminants and pigs (Luna *et al*., 2005). The state of Sinaloa is endemic zone of Leptospirosis, and occupies the first national place in mortality by this disease with 117 deaths in the period 2005-2014 (58% of lethality). As for morbidity, it occupies the third national place with 201 cases in the mentioned period below only the states of Tabasco and Veracruz (DGIS, 2016). Thus, the objective of this study was to determine the seroprevalence of the disease and to identify risk the factors associated with serovars of *Leptospira Interrogans* in dogs of Culiacan, Sinaloa.

MATERIALS AND METHODS

This study was conducted in the city of Culiacan, Sinaloa, Mexico, located at 24° 48' N and 107° 23' W, 60 meters above sea level; the climate of the region is classified as semi-dry, with a very warm average temperature of 25.5 ºC, maximum temperatures of 45 ºC in the months of July and August, minimum temperatures of 7 ºC in December and January, and an annual rainfall of 671.14 mm. Blood samples were collected from 106 canine from colonies where seropositive blood donors from public hospitals in Culiacan, Sinaloa were identified, and not vaccinated against leptospirosis. Before taking blood samples, we applied a questionnaire to pet owners in order to obtain their address and information related to the conditions and characteristics of the places where the dogs lived; the questions were directly related to the epidemiological variables that are determining factors in the transmission of leptospirosis. We also asked for their authorization to take blood samples through an informed consent form; samples (3 mL) were obtained by puncturing the jugular vein and blood was free of pollutants, not hemolyzed, and the serum samples thus obtained were frozen at -40 ºC in an ultra-freezer. Once collected, samples were taken to the laboratory of the National Animal Health Centre in Tecamac, State of Mexico, where they were processed using the Microscopic Agglutination Test (MAT), with specific reactions for each serovar. A panel that included the following serovars: Ballum, Canicola, Hardjo, Pomona, Pyogenes, Icterohaemorrhagiae, Bratislava, Wolffi, Australis, Grippotyphosa, Hebdomadis and Shermani was used. 106 samples were taken in vacutainer tubes without anticoagulant, were centrifuged for 5 min at 1008 g to obtain serum, and then frozen. The cut-off points of the tests considered titers of 1:100 or greater as positive (Goldstein *et al*., 2006). The questionnaire asked for: name of the owner, address, number of dogs per owner, sex, race, age, vaccinations applied, place of habitation, type of floor, presence of rodents, water supply in the house, presence of pools, open water containers, presence of drainage and number of residents per dwelling. A transversal analytical study was performed. An epidemiological survey was applied to the people responsible for the dogs in order to identify risk factors. The homogeneity of proportions was tested using chi-square statistic test; the risks (OR, odds ratio) and the confidence intervals were estimated using a logistic regression model. A value of $P < 0.05$ was considered statistically significant. All analyzes were performed using the statistical package Stata Intercooled V.13.1.

RESULTS

We studied 106 serological samples from dogs associated with seropositive blood donors from public hospitals of Culiacan, resulting in 18 canine positive cases. The descriptive analysis indicates the number of pets with owner was 96%, as well as 201 dogs related to sampled animals and 444 humans. The age data sampled indicate that 53% were taken from animals older than two years, and 47% pets under that age. Regarding the type of dog breed, we found that 50% of the serum samples corresponded to mixed breeds, followed by crosses of small breeds such as poodle and chihuahua (29%); these two groups made up 79% of the samples, the gender of the sampled animals, 55% correspond to males and 45% to females. The place of habitual residence of the dogs was also considered an important factor related to the epidemiology of the disease; 94.34% of the dogs lived within the home, while 53% of the sampled dogs had contact with the street. We observed that 10.38% of the dogs remained inside the house, 38.68% inhabited both the interior and exterior of the house, and the majority (45.28%) remained only in courtyards. Regarding the type of floor in the place inhabited by the dogs, 36% corresponded to cement and 39% to cement and dirt; the rest of the animals stayed only on dirt (25%).With

regard to the water supply in the homes, 88% have piped water, and 12% reported using drums or basins for water storage. Drainage was present in 90% of the households. Regarding the number of people living in each household, the most frequent value was five residents per household (25.47%), followed by households with four inhabitants (23.58%) as well as other with less people. Rodents were reported in 75% of the households. A serological study of 106 samples from dogs living in the city of Culiacan, Sinaloa, indicated a prevalence of *Leptospira Interrogans* of 17%; the serovars detected were: Wolffi, Bratislava, Australis, Canicola, Grippotyphosa, Pyrogenes, Hardjo, Icterohaemorragiae, Pomona, Hebdomadis y Shermani. The observed frequency for serovars, from high to low was: Wolffi (66.6%), Bratislava (22.2%), Australis (16.6%), Canicola (11.1%), Grippotyphosa (11.1%), Pyrogenes (11.1%), Hardjo (5.5%) Icterohaemorragiae (5.5%), Pomona (5.5%), Hebdomadis (5.5%), Shermani (5.5%). Epidemiological variables corresponding to neighborhoods (socioeconomic stratum), number of pets per household, dogs with or without owner age, breed, stay of pets type of floors water supply, drainage, water stored in drums and basins and presence of rodents showed no significant differences. There was a significant difference in the gender of pets ($P < 0.05$; OR= 2.9) and the dogs who stay at home and have access to the street was marginally significant (P= 0.06).

DISCUSSION

Serological positive samples were found in colonies with similar characteristics of housing, construction, access routes and similar socioeconomic status, unpaved streets with garbage presence in yards and street, with accumulation of "pots", with poor basic sanitation and poor management of stored water; all these characteristics were risk factors for the disease (Meeyam *et al.,* 2006). The eleven serovars identified include those mentioned as very pathogenic to humans of their characteristics of hemolysins production, mainly by serovars Icterohaemorrahgiae and Pomona, which are responsible for hemoglobinuria and vascular damage (Goldstein *et al*., 2006). Positive animals do not present clinical signs typical of the disease and their owners not mention seen them sick. We also observed the presence of multiple infections in a single host (up to 6 serovars). The vaccines conventionally applied in veterinary clinics of Culiacan usually include 4 serovars: Canicola, Grippotyphosa, Icterohaemorrhagiae and Pomona, but the serovars Bratislava, Pyrogenes, Shermani Ballum, Australis, Hardjo y Hebdomadis, which were found in the study and are pathogenic to humans were not included, creating the potential risk that pet owners could get infected by these serovars (Geinsen *et al.,* 2007). The prevalence observed in this study (17%) is such than that reported for Mexico by several authors in other studies conducted with animals suspected of leptospirosis or at high risk, including stray dogs, animals in veterinary clinics and rabies centers, and dogs in close coexistence with domestic animals such as cattle, pigs, goats and sheep (Cárdenas *et al*., 2003). The serovars vary from region to region. Davis *et al.* (2008) observed a predominance of the serovars Autumnalis, Icterohemorrhagiae and Canicola in healthy dogs of the state of Washington, with a seroprevalence of 17%. Sinaloa have detected 20 serovars in humans and animals. Studies in Mexico refer up to five serovars in dogs detected in the different regions studied unlike the eleven serovars found in this study (Luna *et al.,* 2008). The gender of pets was statistically significant ($P < 0.05$; OR= 2.9), with females having higher prevalence compared to males, which agrees with Hernandez *et al*. (2012). Ward *et al.* (2002) reported male dogs used for work and shepherding are in greater risk of contracting the disease. The dogs who stayed at home and had access to the street were marginally significant (P= 0.06) to have contact with other animals, urine or contaminated water, and can be inferred that there is a greater risk of being infected with bacteria (Meeyan *et al*., 2006; Alton *et al*., 2009; Kikuti *et al*., 2012). We identified seropositive dogs to *Leptospira Interrogans* in Culiacan, Sinaloa. Seropositivity was associated with risk factors, and creates the possibility of contagion to other animals and humans.

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ACTUALIZATION OF STRATEGIES FOR ONE-HEALTH IN THE CONTEXT OF ANIMAL HYGIENE EDUCATION IN WEST AFRICA

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SUMMARY. In view of a perennial problem in curricula for veterinary public health that does not connect epizootiology with animal hygiene, coupled with the increasing challenge of neglected and emergence zoonoses in West Africa, we embarked on a revision of curricula of higher education for surveillance of human-animal diseases in West Africa. The objective was to mainstream one-health model into animal hygiene training. A logical framework for one-health in the context of animal hygiene was concurrently implemented at the Centres for Control and Prevention of Zoonoses (CCPZ), Ibadan, Nigeria and Njala, Sierra Leone from January 2012 to December, 2015, based on a 5-year strategic plan for improving postgraduate training for surveillance of human-animal diseases in West Africa. The novelty of the curricula featured course modules on statistical methods for human-animal disease surveillance, spatial exposure science, time-trend modeling, indigenous knowledge, attitude and practices, molecular detection and diagnostic methods, design of logical framework for disease control and prevention, alongside monitoring and evaluation of disease control project in one-health mode. Enrolled trainees were strictly from universities, ministries of health and agriculture within partner institutions and countries in West Africa. Thus, we convened international collaboration and solidarity in one-health mode for higher education in human-animal disease surveillance. We successfully trained 5 doctoral students, 30 masters' students and 210 participants in short courses. About 50 peer-reviewed scientific publications emanated from research conducted by trainees and mentors. We concluded that CCPZ's systematic epizootiology curriculum has effectively mainstreamed one-health considerations into animal hygiene training thereby offering a more effective surveillance network for learning about logical framework for control of neglected zoonoses in West Africa.

Keywords: Curriculum, Human-Animal disease surveillance, West Africa.

INTRODUCTION

Animal Hygiene is the part of Animal Health Care that deals with the quality of the environment where animals live (adapt and maintain the environment) in such a way that animal health is safeguarded. Animal hygiene as part of Veterinary Public Health and Preventive Medicine studies the effects of animate and inanimate environmental variables on the health of animals to achieve a strategic prevention of diseases, promoting animal health and ensuring age-specific as well as species-specific welfare needs of such animals (Strauch, 1986; Tielen, 2000). There is a critical need for general public hygiene and in particular, animal hygiene in several communities in West Africa with its increasing challenge of neglected and emergence zoonoses (Esuruoso, 2013; Olugasa *et al.*, 2014). Understanding the inextricable connection between animal hygiene and veterinary public health to human-animal diseases surveillance and one-health education, especially epizootiology science and service is crucial to meeting this challenge (Olugasa *et al.*, 2003; Esuruoso, 2009). The definition of animal hygiene, though encapsulated in all subject areas taught under Veterinary Public Health and Preventive

Medicine, is not a commonly used term in West Africa. For example, the three units within a typical Department of Veterinary Public Health and Preventive Medicine in West Africa, namely; veterinary public health, veterinary preventive medicine, and wildlife and fish ecology and diseases do not have such nomenclature as animal hygiene. On the other hand, public health veterinarians so enthusiastic about one-health collaboration with physicians often show a palpable coldness towards needed reciprocal collaboration with animal husbandry professionals in efforts toward mainstreaming animal hygiene. This is often interpreted as an avenue to "promoting quackery". The objective of this case study on improving postgraduate programmes for human-animal disease surveillance was to actualize a strategy for mainstreaming one-health model into animal hygiene training through broad collaborative human-animal disease surveillance curricula in West Africa.

MATERIALS AND METHOD

A logical framework was designed to engage four of the oldest Universities in West Africa on a review of postgraduate programme and curriculum in epizootiology (Table 1). The goal was to improve human-animal disease surveillance training in West Africa. The University of Ibadan (established in 1948 and oldest University in Nigeria) in collaboration with Cuttington University, Liberia (1889 - third oldest University in West Africa), Njala University, Sierra Leone (off-shoot of University of Sierra Leone, 1827 and oldest University in West Africa), and University of Ghana (established in 1948 and oldest University in Ghana), formed a consortium for major activities, including revision of curriculum for professionalism in human-animal disease surveillance, one-health and animal hygiene as lead competence of Master of Science (M.Sc.) in Epizootiology in the sub-region. The logical framework with set purpose, indicators and means of verification (Table 1) was implemented from January 2012 to December, 2015.

The activities commenced with a revision of an existing M.Sc. Epizootiology curriculum by stakeholders, producing a Systematic Epizootiology for postgraduate training and developed a Certificate of Participation short course in human-animal disease surveillance in West Africa. The team conducted training for a critical mass of personnel, enrolled mainly from ministries of health and agriculture, and university consortium for human-animal disease surveillance in West Africa. The consortium convened a sub-regional (international) collaboration for one-health and animal hygiene in higher education in West Africa. The consortium organized conferences, workshops, seminars and training programmes for stakeholders in human-animal-environment health across West Africa. The work produced technical reports and scientific publications that captured disease surveillance and exposure science into a one-health pavilion (Olugasa, 2014).

RESULTS

The inter-connectivity between geography and human-animal disease surveillance are the core modules (courses) for effective learning of animal hygiene and exposure science. Teaching modules in zoonoses and environmental hygiene offered course work that showcased the links with animal hygiene in animal health and human health within common environment (Table 2). Enrolment for M.Sc. Epizootiology at the University of Ibadan steadily increased from an average of 1 in 2006 through 2011 to an average of 7 in 2012 through 2016. This trend was driven by scholarship opportunity for one-health and animal hygiene foci. A total of 5 Doctoral students, 30 Masters' students were mentored. In addition 210 trainees participated in short course human-animal disease surveillance in the 5 years of the project.

DISCUSSION AND CONCLUSION

Animal Hygiene programme is well over 120 years old in Europe (Stuart, 1986). However, its absence in West African education has been a major deficit that supported neglected, endemic and emerging zoonoses in the sub-region (Alexander *et al*., 2015). The surveillance of pathogens at the human-animal-environment interface in particular, is an essential baseline for animal hygiene training. As such, an effort to keep the ongoing mainstream beyond current level, the alumni of systematic epizootiology programme in human-animal disease surveillance have facilitated the creation of sub-regional geospatial data profile on priority human-animal diseases. The first output, the Liberian Lassa fever geospatial data profile, was created and used to model Lassa fever epidemics in Liberia (Olugasa *et al.*, 2014). The Lassa fever data profile thus set a stage for institutionalizing animal hygiene and one-health education, science and service in epizootiology (Olugasa, 2014). We concluded that a systematic epizootiology curriculum has effectively mainstreamed one-health considerations into animal hygiene training. It also offered the basis for a pavilion of peer-reviewed papers, featuring animal hygiene status, community-by-community in the sub-regional Journal of Epizootiology and Animal Health in West Africa.

Table 1. Logical framework for mainstreaming animal hygiene and one-health education in West Africa

	Narrative	Indicator	Means of verification	Assumption
Goal	Improving postgraduate programmes for human-animal disease surveillance in West Africa	Teaching one-health and animal hygiene courses in public health and animal science departments	One-Health and animal hygiene learning centres at Universities in Ghana, Liberia, Sierra Leone and Nigeria	Personnel and funds available for setting-up learning centres in five Universities
Purpose	Mainstreaming one-health and animal hygiene in higher education, West Africa	Topics on exposure science, environmental variables, animal and human health risks	Number of courses that feature animal hygiene, one-health and geo-spatial map evaluation	Institutional politics may derail implementation in major departments
Output	Learning materials produced; training held in Ghana, Liberia, Sierra Leone and Nigeria for agriculture, epizootiology, public health and related fields	Supply of learning materials on field work, teaching and research on disease surveillance at the human-animal-environment interface	Molecular, serologic diagnostics; spatial and spatio-temporal epizootiology laboratory; pre- and post-intervention observational data	Country and institutional ownership of revised or developed curriculum; adoption by stakeholders with diverse institutional needs addressed.
Activities	Curriculum review workshop; stakeholders meet to consider and adopt revised/developed curriculum; learning materials development; conduct on-site training	Reports on one-health, animal hygiene and epizootiology curricula, technical guidelines and quality assurance; developed materials and training attendance	Course modules on animal hygiene topics, environmental exposure data profile, one-health collaboration and training implementation	Experts available to convene workshop, develop learning materials and train-the-teachers to sustain animal hygiene education

Table 2. Human-animal disease surveillance modules cover animal hygiene and exposure education

Title of course/module	Description of course or modular content
Indigenous Knowledge	Harnessing native intelligence and knowledge about local beliefs, and health seeking preferences in relation to zoonoses relevant to each community.
Disease Mapping	Identification of environmental variables (drivers) associated with disease events, spatial analysis methods, defining risk factors, risk mapping and visualization with geographic information system (GIS) laying emphasis on priority zoonoses.
Disease Time-Trend Modelling	Development of time plot, trend equation and seasonal index of disease cases, model computation, forecasting of disease outbreaks and designing of logical framework for their control and prevention.
Molecular Diagnostic Methods	Safety measures in sample collection, storage and analysis; nucleic acid (DNA and RNA) extraction, storage and analysis, polymerase chain reaction (PCR), purification of amplicon, gene sequencing and phylogenetic analysis.

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IMPROVED PROTEIN COCKTAILS COMPLEMENT BOVINE PURIFIED PROTEIN DERIVATIVE FOR *IN VITRO* DIAGNOSIS OF SUBCLINICAL BOVINE TB

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SUMMARY. Bovine tuberculosis (bTB) in livestock is a serious problem in developing countries. The control and eradication of bTB is mainly based on a test-and-slaughter policy, therefore diagnosis is vital for success. The IFN-γ release assay (IGRA) is a blood-based assay that improves detection of infected cattle at early stages that escape skin testing. Due to its apparent lower specificity by the use of bovine purified protein derivative (PPDB), improvements to *in vitro* test with specific proteins have been performed. Transcripts of DosR (dormancy survival regulon) genes from *Mycobacterium bovis* associated to non-replicative persistence (NRP) phase of growth have been identified by our group in lymph nodes of IGRA negative bovines. This led us to hypothesize that DosR-related proteins may potentiate the IFN-γ response to PPDB in animals with a subclinical infection. We evaluated peripheral blood cellular immune responses of three hundred dairy cattle against two protein cocktails containing four *M. bovis* NRP phase proteins, as a complemented format of the IGRA PPDB based test. Bacterial infection was confirmed directly in lymph node tissues by bacteriological culture and by direct PCR. An increased blood IFN-γ production was observed in 176 (58%) animals by protein complemented IGRA, and fourty samples were positive converters according test criteria. Only 18 out of 176 IFN-γ high producing animals were also positive to *M. bovis* isolation, and fifty seven animals without evident tuberculous lesions (NVL) at slaughter contained *M. bovis* DNA in tissue samples. Thus, animal infection was evidenced by means of protein cocktail supplementation of IGRA test without bacterial culture confirmation, probably containing low bacterial loads in a dormant state of infection. In conclusion, NRP phase proteins of *M. bovis* have the potential to complement PPDB, increasing sensitivity of the IFN-γ *in vitro* test for bTB diagnosis.

Key words: *In vitro* test, subclinical infection, bovine tuberculosis

INTRODUCTION

Bovine tuberculosis (bTB) in livestock is a serious problem in developing countries where eradication programs have been insufficient to accomplish eradication. Diagnosis is performed by the tuberculin skin test (TST) worldwide however, the interferon-gamma (IFN-γ) release assay (IGRA) is used as an ancillary tests to supplement the TST. It has been recognized that some infected animals do not respond to the TST but show intact cell-mediated immunity (CMI) responses to the IGRA test (Pollock and Neill 2002). By other hand, it has been suggested that animals with positive TST and IGRA tests with no macroscopic lesions in tissue (NVL group) may have a non-replicative persistence (NRP) phase of mycobacterial infection (Alvarez *et al*. 2009). In human medicine, IGRA version of test for subclinical TB is mainly based on antigenicity to specific RD1 antigens such as ESAT6 and CFP10. Therefore, the

aim of the present study was to assess if improved protein cocktails including RD1 antigens and proteins of the so called DosR of *M. bovis* could improve sensitivity for bTB diagnosis.

MATERIAL AND METHODS

Heparinized blood samples from 300 dairy cattle were obtained from the caudal vein just before sacrifice at a local abattoir in the central region of Jalisco, Mexico, where the prevalence of bTB is above 2% according TST. Animals with visible lesions were classified as VL, otherwise, as non-visible lesions (NVL). Central parts of lymph nodes were pooled and prepared for direct tissue PCR and bacteriological analysis of each animal. Blood samples were assessed with IGRA assay (Bovigam, Prionics AG, USA) for bTB diagnosis using independently two protein cocktails of four DosR antigens or in combination with ESAT6 and CFP10 in a concentration of 4 µg/ml/peptide for blood stimulation. Total genomic DNA was isolated from lymph nodes by using Phire Animal Tissue Direct PCR kit (Thermo Scientific). We used highly specific primers JB21 to amplify a 500 bp fragment of the putative gene called RvD1-Rv2031c of *M. bovis* (Cardoso *et al*. 2009). Different lymph node tissue samples of the same animal were cultured at 37°C in Stonebrink's medium for primary isolation.

RESULTS

Production of IFN-γ in response to protein cocktails with four (DosR proteins) and six (DosR proteins, ESAT6, and CFP10) antigens in combination with PPDB was assessed stimulating animal blood samples. Although an increase of IFN-γ was observed in 176 animals when the antigenic components of the diagnostic kit were improved with both recombinant antigen cocktails (Figure 1), forty eight out of 300 animal blood samples were positive by test criteria; however, in additional 40 (negative) samples surpassed the 0.1 cut-off value, a magnitude considered for positive samples being considered as positive converters. Protein cocktail consisting of four DosR recombinant proteins in mixture with PPDB induced on average 25% more production of IFN-γ compared to PPDB stimulus alone. The protein cocktail of six recombinant antigens in combination to PPDB, induced on average 50% more IFN-γ in blood showing an additive immunological synergistic effect. In the NVL group of cattle, 68 animals were IGRA positive (including those positive converters), however mycobacterial DNA was present in 54 (30%) of these animals but with no-cultivable bacteria, together demonstrating superior sensitivity of the complemented IGRA test. Nonetheless, a larger number of NVL animals (57) with a negative IGRA test were also positive to *M. bovis* DNA, but negative to bacteriological culture (Table 1).

DISCUSSION

The *in vitro* IFN-γ assay for diagnosis of bTB (Bovigam®) is being incorporated as an ancillary test to the intradermal one to maximize the detection of *M. bovis* of infected animals. Improvements consisting in the use of cocktails of specific proteins to augment specificity of the test have been done (Denis *et al*. 2007). DosR antigens have been described as good antigen-specific T-cell inducers in individuals that lack clinical signs of active TB (Leyten *et al*. 2006). Under these considerations, we investigated the immunogenicity of antigen complemented PPDB using protein cocktails consisting of proteins belonging to both, stationary phase (DosR) and actively phase (RD1) of growth of *M. bovis*. It was decided to use protein cocktails to induce peripheral blood CMI together with PPDB as a whole. Of 176 IFN-γ overproducer animals, 45 animals that showed the same tendency to overproduce IFN-γ

remained as negative to IGRA test as assumed by the standard cut-off value, but 40 samples reached and surpassed the 0.1 cut-off value by additive stimulus of antigenic mix and were considered positive converters. Only 18 out of 176 animals (9 with VL) of IFN-γ overproducer animals in antigen supplemented IGRA test were also positive to bacteriological tissue culture, interestingly 57 and 9 animals that belonged to the NVL group with a negative IGRA test, were positive for *M. bovis* DNA and bacteriological culture tests, respectively. This preliminary data suggests that animals with uncultivable bacteria probably belong to the recognized group of non-responder/uncultivable animal samples which has been largely speculated that may contain low bacterial loads in a chronic non-replicative persistence state (Vordermeier *et al*. 2008), making probable that bTB indeed would have a NRP state of infection. Notwithstanding, these findings make it difficult to define a particular hallmark of a LTBI of *M. bovis* in cattle, and probably both active and latent infection coexist. This consideration may impose important implications on how new ancillary immunological tests like complemented IFN-γ test should be interpreted in relation to the gold standards in cattle bTB diagnosis. Special care should be taken to not consider that IFN-γ *in vitro* test only detects infection in animals where *M. bovis* is actively replicating.

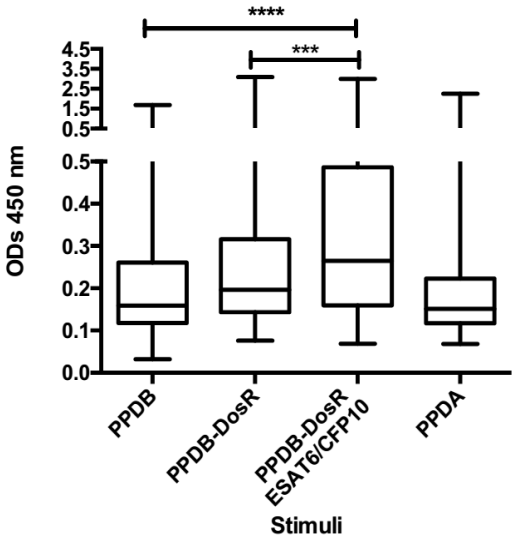

Figure 1. Amount of IFN-γ released in blood after stimulation with protein cocktails and PPDB. ODs obtained from the individual animals (n = 300) are represented inside boxes.

Table 3. Results of post-mortem examination of cattle with augmented IFN-γ production by supplemented IGRA test.

Scrutinized cattle (n = 176)			
VL[a] (n = 33)	19%	NVL[b] (n = 143)	81%
IGRA[c] +ve (n = 20)	11%	IGRA +ve (n = 68)	39%
C[d] +ve/PCR[e] +ve (n = 6)	3%	C +ve/PCR +ve (n = 8)	5%
C +ve/PCR -ve (n = 3)	2%	C +ve/PCR -ve (n = 0)	0%
C -ve/PCR +ve (n = 9)	5%	C -ve/PCR +ve (n = 54)	30%
C -ve/PCR -ve (n = 2)	1%	C -ve/PCR -ve (n = 7)	4%
IGRA -ve (n = 13)	7%	IGRA -ve (n = 75)	42%
C +ve/PCR +ve (n = 7)	4%	C +ve/PCR +ve (n = 9)	5%
C +ve/PCR -ve (n = 2)	1%	C +ve/PCR -ve (n = 0)	0%
C -ve/PCR +ve (n = 2)	1%	C -ve/PCR +ve (n = 57)	32%
C -ve/PCR -ve (n = 2)	1%	C -ve/PCR -ve (n = 9)	5%

[a]Animals with visible lesions.
[b]Animals without visible lesions.
[c]Interferon-gamma release assay.
[d]Culture for *M. bovis*.
[e]Direct PCR from lymphatic tissue.

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MARBOFLOXACIN ACTION IN AMASTIGOTES OF *LEISMANIA CHAGASI* IN MACROPHAGES OF BALB/C MICE

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SUMMARY. Canine Visceral Leishmaniasis (CVL) is a zoonosis of worldwide distribution and a serious public health problem in Brazil. It is caused by *Leishamnia chagasi (infantum*) and is a systemic disease whose control depends on the success of the treatment. Marbofloxacin is a third generation fluorquinolone with leismanicide action in macrophages amastigotes on the host. In the present study, we evaluated the marbofloxacin effect in BALB/c mouse peritoneal macrophage cultures infected with promastigotes of *L. chagasi* by quantification of inflammatory cytokines and cytotoxic metabolites. Sample of *L. chagasi* was isolated in hamster (*Mesocricetus auratus*) from a dog with CVL in an epidemic area for the disease and the inoculum for evaluation of the marbofloxacin cytoxicity was grown in medium LIT. First, we evaluated the cell viability of non-infected macrophages cultured with marbofloxacin in the concentrations of 100, 500 and 750 µg/mL through MTT assay. Next, macrophages were infected with promastigotes, in a proportion of 5:1 (promastigotes: macrophage), and the cultures were incubated with or without marbofloxacin in different concentrations for 6 and 24 hours. Phagocytic index and production of hydrogen peroxide (H_2O_2), nitric oxide (NO), IL-1β, IL-10, IL-6, and TNF-α were determined. The results were analyzed by GraphPad software and paired medians were compared by ANOVA. It was observed that with the increasing of the concentrations of marbofloxacin, cell viability decreased compared to control. The concentration of 500 µg/mL showed good cell viability ($p<0,01$). *L. chagasi*-infected macrophages treated with the higher concentration of the drug showed lower counts of amastigotes and exhibited more cellular vacuoles ($p<0,0632$). The production of H_2O_2, IL-1β, IL-6, and TNF-α decreased according to the increasing of concentrations of the drug and to the decreasing of the phagocytic index. Levels of NO increased, showing upregulation of macrophage response. The results suggests early action and leishmanicid effect of marbofloxacin.

Key words: treatment, zoonosis, leishmaniosis

INTRODUCTION

Canine Visceral Leishmaniasis (CVL) is a zoonosis of worldwide distribution and a serious public health problem in Brazil. It is considered the second most important protozoonosis and one of the six main infectious-parasitary diseases in the world. It is caused by an obligate intracellular protozoan parasite of the *Leishamnia chagasi* (*infantum*) species and the vectors are phlebotomines (*Lutzomyia longipalpis* in Americas). It affects both humans and animals, where dogs are the main domestic parasite reservoir, infected through phlebotomine females bite during its blood feeding in the peripheral blood of the reservoirs where metacyclic promastigotes are inoculated, known as the infective stage of *Leishmania*. In the vertebrate host the promastigote forms are phagocytosed by macrophages and differentiate into amastigote forms. (TRONCARELLI et al., 2012). The clinical signs in the dog are usually systemic and of chronic evolution. It is observed apathy, weight loss, abdominal distension due to hepatosplenomegaly, palpable spleen, lymphadenomegaly, skin lesions and/or alopecia, emaciation,

ocular lesions, onicogryphosis, haemorrhages, diarrhea, vomiting, pneumonia and locomotor and neurological alterations are related, the clinical presentations are dependent on the animal's immune response, being able to reach severe cases of debilitation (GREENE, 2012). It is a disease whose control depends on the vector control and success of the treatment, which aims to control clinical signs, improve the animal's immunity and reduce parasitic burden at non-transmissible levels (NOLI and SARIDOMICHELAKIS, 2014). Parasitological cure with anti-leishmania drug is extremely difficult, in this way it is of great importance the study of alternative drugs for the treatment of leishmaniasis. Marbofloxacin is a third generation fluorquinolone developed for veterinary use with leismanicide action in macrophages amastigotes on the host, its molecule shows a very high volume of distribution, which is widely diffused throughout the organism (VOULDOUKIS, 2006). In the present study, we evaluated the marbofloxacin effect in BALB/c mouse peritoneal macrophage cultures infected with promastigotes of *L. chagasi* by quantification of inflammatory cytokines and cytotoxic metabolites.

MATERIAL AND METHODS

The sample of *L. chagasi* was isolated in hamster (*Mesocricetus auratus*) from a dog with CVL in epidemic area for the disease and the inoculum for evaluation of the marbofloxacin cytoxicity was grown in medium LIT. For the macrophages culture, 45-days male BALB/c mice were kept in the Experimental Immunopathology Laboratory (LIPE) of the Faculty of Sciences, UNESP / Bauru. First, the cell viability of non-infected macrophages cultured with marbofloxacin in the concentrations of 100, 500 and 750 µg/mL, was evaluated through MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Next, macrophages were infected with promastigotes, in a proportion of 5:1 (promastigotes:macrophage), and the cultures were incubated with and without marbofloxacin in different concentrations for 6 and 24 hours. Phagocytic index and production of hydrogen peroxide (H_2O_2), nitric oxide (NO), IL-1β, IL-10, IL-6, and TNF-α were determined. The results were analyzed by GraphPad software and paired medians were compared by ANOVA.

RESULTS

It was observed that with the increasing of the concentrations of marbofloxacin, cell viability decreased compared to control. The concentration of 500 µg/mL showed good cell viability with 88% viability ($p<0,01$). It was observed that samples containing only macrophages and *L. chagasi* produced inflammatory metabolites and cytokines as a response to the inflammatory process. With the addition of marbofloxacin, there was a reduction in the production of inflammatory mediators, decreasing according to the concentration of marbofloxacin. Furthermore *L. chagasi*-infected macrophages treated with higher concentration of the drug showed lower counts of amastigotes and exhibited more cellular vacuoles ($p<0,0632$) (Figure 1). The production of H_2O_2, IL-1β, IL-6, and TNF-α decreased according to the increasing of concentrations of the drug and to the decreasing of the phagocytic index, IL-10 levels were not significant. Levels of NO increased, showing upregulation of macrophage response. These results suggest early action and leishmanicid effect of marbofloxacin.

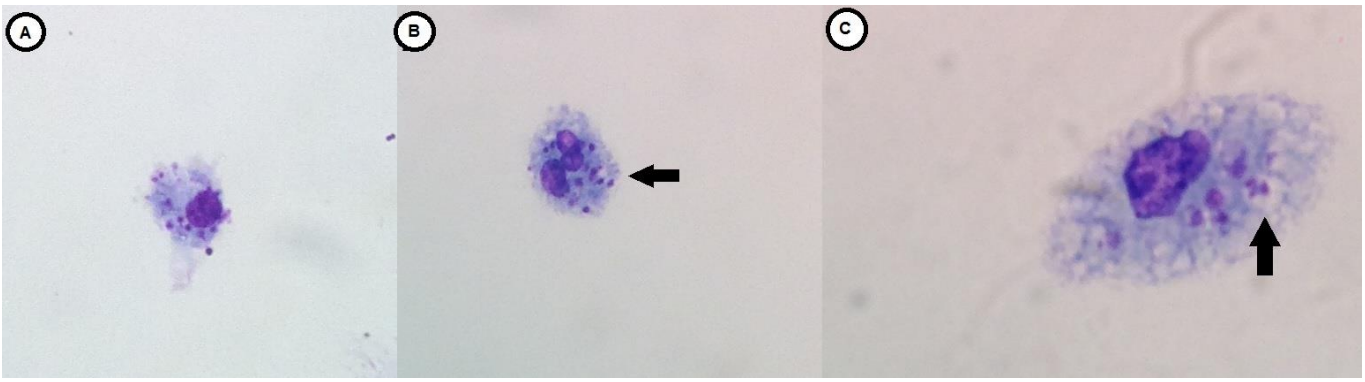

Figure 1. A: The concentration of 100 µg / ml of marbofloxacin; B: Concentration of 500 µg / ml of marbofloxacin, arrow shows formation of vacuoles; C: Concentration of 750 µg / ml of marbofloxacin. Arrow points out more vacuoles involving the amastigotes. The parasite involved by the vacuole (arrow) suffered fragmentation. Increase x40 (A and B). Increase x100 in immersion (C).

DISCUSSION

Currently, the association of meglumine antimoniate with allopurinol (Noli and Auxilia, 2005) has been used for the treatment of LVC in some countries. However, in Brazil treatment is not allowed because the complete elimination of the parasite does not occur. With the results obtained in the present study, analyzed as a whole (reduction of amastigotes associated with the presence of vacuoles and decrease of inflammatory metabolites and cytokines), it is suggested that marbofloxacin has an early action. The result of the cytotoxicity assay suggests that cell viability decreases with increasing product concentration, which corroborates the data of Vouldoukis et al. (2006), which despite the decrease in viability, is not toxic for most macrophages up to 500mg/ml. The effect of marbofloxacin at its highest concentration showed a lower number of amastigotes, probably due to its inactivation. This can be demonstrated at first by intracellular vacuoles. The results show strong evidence of the leishmanicidal action of marbofloxacin, and it may be considered as an alternative for the treatment of LVC. Further studies are suggested in this line of research, comparing the action of marbofloxacin with that of other drugs, as well as studies in experimental animals such as hamsters, for the best evaluation of the drug.

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IMMUNO-STIMULATING COMPLEX AS ADJUVANT FOR RECOMBINANT VETERINARY VACCINE AGAINST RABIES VIRUS

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SUMMARY. Rabies virus is transmitted to humans mainly by rabid dogs. Mass vaccination of dogs has been the mainstay of successful canine rabies control. The rabies virus glycoprotein is the antigen responsible for the induction of neutralizing antibodies by the host immune system, protecting it against viral infection. Immuno-stimulating complexes (ISCOMs) are cage-like adjuvant particles composed of cholesterol, phospholipid and saponins. As the immunization with a recombinant rabies virus glycoprotein (RVGP) have been shown good preliminary results and ISCOM adjuvant possesses efficient antigen delivery, their combination may be adequate. We studied formulations of ISCOM to deliver RVGP expressed on *Drosophila melanogaster* S2 cells. RVGP was expressed by S2MtRVGP-H cells after induction with $CuSO_4$ (700 µM. To produce ISCOMs in a quick manner, cholesterol and phosphatidylcholine were dissolved in ethanol and the resulting solution was rapidly injected into a stirred, aqueous solution of Quil-A, stirring for 48h. Mice were divided into 6 groups (n = 5) comprising a negative (saline) and a positive control group (commercial vaccine), RVGP in both monomeric and trimeric form (1.0 µg RVGP/dose) with and without ISCOM (15 µg/dose). The vaccination schedule comprised prime and one booster dose 30 days after the first dose. Four weeks after the booster dose, mice were challenged with the fixed rabies virus sample CVS (Challenge Virus Standard). Serum samples were collected and the immune response was evaluated by the titration of anti-RVGP antibodies by ELISA (Platelia). ISCOM samples prepared from ether injection were collected and presented homogeneous ISCOM-like particles. Particles morphology was assessed by TEM, 7 and 30 days after preparation and showed to be similar and stable. We found that mice immunized with ISCOM presented higher number of survivors than without adjuvant.

Keywords: rabies, vaccine, rVGP, orally, adjuvant, antibody, RFFIT, Platelia, booster.

INTRODUCTION

Vaccination against canine rabies is the most effective prophylactic measure to prevent cases of human rabies and has impacts on human health, public health economics, wildlife conservations and animal welfare (Cleaveland et al., 2006). In Brazil, domestic animal vaccination campaign is carried out based on annual boosters due to particular vaccine characteristics to consider an animal immunized. The vaccines used today are from inactivated virus plus adjuvant. Protection is considered by a level of high antibody, because effective immune response to rabies virus depends on their rapid neutralization before its spread. Although rabies vaccines are available and free, there is an important fault coverage due to the need of constant boosters, which leads many owners to stop vaccinating their pets. Having

this in mind, the development of new vaccines against rabies should aim increase the interval between booster doses, or even facilitating the administration process.

Rabies is a viral infectious disease that affects the central nervous system (CNS) with acute and fatal evolution, which predominantly affects mammals. It is distributed worldwide, with about 40,000 to 70,000 human deaths per year, almost all of them in developing countries, especially in Asia and Africa (Brazil, 2008; Queiroz et al., 2009). Although rabies can infect and be maintained by several different host species, domestic dogs are by far the most important source of infection to humans, with more than 95% of human cases caused by bites from rabid dogs (WHO, 1999; 2004). Globally dog rabies kills more people than yellow fever, dengue fever and Japanese encephalitis and more than 7 million people are exposed to the virus each year (Coleman et al., 2004; Knobel et al., 2005). More radical methods of controlling rabies, such as the reduction of the dog population, were not effective (WHO, 2004; Cleaveland et al., 2006). Therefore, mass vaccination is the main method of effective control of canine rabies provided that vaccine coverage reaches 70% (WHO, 1999), and if vaccination coverage is not maintained, the disease can rapidly re-establish itself (Cleaveland et al., 2006). The thing is that each human death is entirely preventable, and the economic burden of canine rabies easily reduced. Much has already been done with the advancement of molecular biology, since the discovery of Pasteur's first anti-rabies vaccine in 1885. In the intervening 130 years, improvements in vaccine immunogenicity, cell culture and inactivation techniques have led to the development of safe and highly efficacious vaccines for both humans and animals. Vaccines available for prevention or treatment after infection are proven to be effective but costly to produce (Hildegund, 2009), with difficulty in controlling production and lack of adequate chemical/molecular definition, leading to important variations between different suppliers. This reality makes it interesting, from both public and economic point of view, to develop a cheaper, more effective, and as safe as vaccines with inactivated rabies virus, especially for veterinary use. Now we have all the tools that are needed to eliminate rabies, using mass dog vaccination. The purpose of this research is to develop a prototype vaccine based on the use of a recombinant glycoprotein from rabies virus envelope. This glycoprotein expressed in S2 cells in an extensively characterized system, has demonstrated immunogenic properties in preliminary tests. We intend to use this antigen combined with different adjuvants and the use of different routes of immunization in animal model, verifying levels of protection. Obtaining a new formulation for the prevention of canine rabies may generate an innovative new vaccine, especially if it is effective is could be proven orally. The World Health Organization recommends the single dose parenteral application of the commercial vaccine for dogs and cats, with annual boosters. Much has been debated and taken into account regarding the accessibility of dogs to vaccination worldwide, especially in Africa and Asia. It is believed that from 30 to 70% of the dogs are ‘stray’ or ‘ownerless’, therefore they could not be vaccinated (Boegel and Joshi, 1990). However, recently studies have shown that a significant portion of dogs have been shown to be accessible for parenteral vaccination while the inaccessible ones make up only 15% of the population (WHO, 1988; Bogel and Joshi, 1990; Matter et al., 2000; Kayali et al., 2003). In areas where cultural factors and homeowners with breeding standards reduce accessibility to parenteral vaccination, oral vaccination can offer increased vaccine coverage without the difficulties of logistics, delays, and expensive programs involving house-to-house visits. Therefore, oral vaccination combined with parenteral vaccination can provide an effective and economical methodology to reach the goal of 70% coverage (Cleaveland et al., 2006).

MATERIAL AND METHODS

For the purpose of studying new alternatives of recombinant vaccines and products of biotechnological interest, the recombinant glycoprotein of rabies virus has been expressed in cells of *Drosophila melanogaster* Schneider 2 (S2 cells). To obtain recombinant cell lines, the cDNA containing the rabies

virus glycoprotein (RVGP) gene was cloned into a plasmid (containing resistance marker) under the control of a metallothionein (Mt) promoter that was transfected into cells S2. RVGP was expressed by S2MtRVGP-H cells after induction with $CuSO_4$ (700 µM). Cells were considered adequate for the expression of immunogenic RVGP (Astray et al., 2008; Lemos et al., 2009) which has been shown to be capable of protecting animals from experimental infection with rabies virus (Yokomizo et al., 2007). The amount of RVGP was quantified by ELISA (Pasteur Institute, Paris). Preliminary results indicate that these preparations are promising but require the introduction of adjuvants to improve their performance as an immunogen. ISCOMs have been extensively used in the experimental development of vaccines, including rabies (Fekadu et al., 1992; Koraka et al., 2014). Similar to micelles, these synthetic immunostimulants are synthesized from cholesterol, lipids and saponins derived from plants (*Quillaja*). Its lipid composition makes it a cage like shape, with about 40 nm, that encapsulates the antigen and presents it efficiently. To produce ISCOMs in a quick, simple and efficient manner, Cholesterol and phosphatidylcholine were dissolved in ethanol and the resulting solution was rapidly injected into a stirred, aqueous solution of Quil-A, stirring for 48h at room temperature. Mice were divided into 6 groups (n = 5) comprising a negative control group (saline) and a positive control group (commercial vaccine), RVGP in both monomeric and trimeric form (1.0 µg RVGP/dose) with and without ISCOM (15 µg/dose). The vaccination schedule comprised prime and one booster dose 30 days after the first dose, by subcutaneous route. Four weeks after the booster dose, mice were challenged with the fixed rabies virus sample CVS (Challenge Virus Standard) by intracranial inoculation. Serum samples were collected and the immune response was evaluated by the titration of anti-RVGP antibodies by ELISA (Platelia).

RESULTS AND DISCUSSION

Samples of ISCOM prepared from ether injection were collected and presented homogeneous ISCOM-like particles. The morphology the particles was assessed by TEM 7 and 30 days after preparation. Even after 30 days, particles showed to be very similar and stable. Results of challenge studies show that mice immunized with monomers showed lower antibody titers and protection. Although virus challenge returned different degrees of protection, we found that mice immunized with trimeric or monomeric forms combined with ISCOM presented higher number of survivors than when immunized with one of the two forms without adjuvant.

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NOVEL *Rhodococcus equi* VIRULENCE PLASMID (pVAPN) TYPE IDENTIFIED IN BOVINES AND HUMAN FROM BRAZIL

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SUMMARY. *Rhodococcus equi* is a well-recognized agent of pyogranulomatous infections in animals, and was described as an emergent pathogen among immunocompromised humans, particularly people living with HIV/Aids. Its pathogenicity has been attributed to presence of circular plasmid-encoded virulence-associated proteins (Vap), associated with survival into phagocytic cells. Three main levels of virulence are recognized among strains: virulent (VapA), major cause of suppurative pneumonia in foals; intermediately virulent (VapB), identified in immunosuppressed humans and *Suidae* lymph nodes; and avirulent, found in farm soil and human with rhodococcosis. Recently, a novel *R. equi* linear plasmid type was characterized (pVapN), identified in bovine lymph nodes ("bovine type"), humans, and in a dog. Curiously, some humans with rhodococcosis had no history of contact with livestock or farms. Similarities in VapB *R. equi* strains from humans and pigs suggested recently that pig-to-human infection might occur by ingestion of pig carcasses contaminated with VapB-strains through lymph nodes and/or feces. In this scenario, this study investigated pVapN (VapN) using PCR in 49 *R. equi* strains obtained from 24 *R. equi* isolates of human (including people living with HIV/Aids), and 25 bovine isolates recovered from lymphadenitis (n=23), pneumonia (n=1), and mastitis (n=1) cases. One *R. equi* strain isolated from a human patient and eight from bovine lymphadenitis (1 mediastinal, 2 mesenteric and 5 submandibular lymph nodes) were VapN-positive. The present study provides evidence that contamination of slaughtered bovine carcasses with *R. equi* harboring pVapN might occur by means of lymph nodes contents. This circumstantial evidence might partially explain rhodococcosis in humans without a clear history of contact with livestock or farms. VapN detection in *R. equi* strains from bovine feces and other human patients is ongoing. To our knowledge, these preliminary results show, for the first time, *R. equi* strains carrying VapN-plasmids in human and bovines in the Americas.

Key words: Rhodococcosis, virulence profile, VapN-plasmids

INTRODUCTION

Rhodococcus equi is a well-recognized opportunistic agent of pyogranulomatous infections in livestock, companion animals, and wildlife (Prescott, 1991; Takai et al., 1997). In the last decades, *R.*

equi was described as an emergent pathogen among immunocompromised humans, particularly people living with HIV/Aids (Ribeiro et al., 2011a).

Pathogenicity of microorganism has been attributed to presence of circular plasmid-encoded virulence-associated proteins (Vap) related with survival into phagocytic cells (Takai et al., 2003). Two major levels of virulence were recognized among strains: virulent (VapA) and intermediately virulent (VapB). The VapA-positive isolates are considered major causes of severe suppurative pneumonia and ulcerative enteritis in foals (Ribeiro, 2016); whereas the VapB-positive isolates have been primarily detected in the lymph nodes of pigs and wild boars, with and without lymphadenitis (Takai et al. 1996; Ribeiro et al., 2011b) and in immunosuppressed individuals, particularly people living with HIV/Aids (Takai et al. 2003).

Currently, a novel *R. equi* linear plasmid type was characterized (pVapN), identified in bovine lymph nodes ("bovine type"), humans (Ocampo-Sosa et al. 2007; Vazquez-Boland et al., 2013), and in a dog (Bryan et al., 2017). Curiously, some humans with rhodococcosis had no history of contact with livestock or farms. Similarities in types of VapB *R. equi* strains from humans and pigs suggested recently that pig-to-human infection might occur by ingestion of pig carcasses contaminated with VapB-strains through lymph nodes and/or feces (Takai et al. 2003; Lara et al., 2015). In this scenario, this study investigated pVapN (VapN) in 49 *R. equi* strains obtained from bovines and humans with rhodococcosis.

MATERIAL AND METHODS

A total of 25 samples from bovines recovered from lymphadenitis (n=23), pneumonia (n=1), and mastitis (n=1) obtained in the central region of the State of São Paulo, Brazil, were subjected to microbiological culture. In addition, 24 *R. equi* strains from human with rhodococcosis identified in Brazil (including people living with HIV/Aids) previously isolated predominantly among pneumonia and organ abscesses were included in this study. All the samples were submitted to microbiological culture on conventional sheep blood agar (5%), incubated aerobically for 3 days at 37ºC. The suspected *R. equi* colonies were classified using conventional phenotypic methods (Quinn et al., 2011). *vapN* gene was investigated through polymerase chain reaction (PCR) described previously (Ocampo-Sosa et al. 2007; Vazquez-Boland et al., 2013). VapN detection in *R. equi* strains from bovine feces and other human patients is ongoing.

RESULTS

One *R. equi* strain isolated from a human patient and eight from bovine lymphadenitis (1 mediastinal, 2 mesenteric and 5 submandibular lymph nodes) were VapN-positive.

DISCUSSION

R. equi is an opportunistic pathogen widespread in soil and farm manure. Occasionally, the microorganism been identified in the feces of livestock and rarely has it been isolated from the feces of humans (Prescott 1991).

Traditionally, inhalation, wounds contamination, contact with soil, manure of feces of livestock, and direct contact with livestock (horse and bovine) appears to be the major route of *R. equi* transmission to humans (Takai, 1997; Ribeiro, 2016). Recent evidence supports that the ingestion of pig products or undercooked pork represents a possible source of *R. equi* infection in humans (Takai et al., 2003; Ribeiro et al., 2011a,b). This hypothesis is especially valid to individuals without history of contact with pig breeding, because the high prevalence of *R. equi* VapB-positive harboring similar plasmids types identified in the lymph nodes of pigs (Takai et al., 1996; Ribeiro et al., 2011b) and in humans with pneumonia (Takai et al., 2003; Ribeiro et al., 2011a).

Here, we observed the presence of *R. equi* in the lymph nodes of slaughtered bovines harboring the VapN-plasmid. Likewise pigs, these results provide circumstantial evidence that the contamination of slaughtered bovine carcasses with pathogenic *R. equi* might occurs thorough lymph nodes contents. This finding represents a probable route of transmission of this bacterium to humans through the consumption of contaminated meat, particularly to immunosuppressed people with no history of contact with bovines or farms. To our knowledge, these preliminary results show, for the first time, *R. equi* strains carrying VapN-plasmids in human and bovines in the Americas.

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GENES ASSOCIATED TO VIRULENCE AND *IN VITRO* ANTIMICROBIAL SUSCEPTIBILITY OF *T. PYOGENES* ISOLATED FROM BOVINE MASTITIS

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SUMMARY. *Trueperella pyogenes* is opportunistic bacterium characterized by pyogranulomatous infections in livestock, commonly refractory to conventional antimicrobial therapy, although bovine mastitis appears to be the major clinical sign of this pathogen. Recently, the genes which encode exotoxin pyolysin (*plo*) and factors that promote adhesion of pathogen to host cells such as fimbriae (*fimA, fimC, fimE, fimG*), neuraminidases (*nanH, nanP*) and collagen-binding protein (*cbpA*) have been associated to virulence. The present study investigated the multidrug resistance pattern of isolates and the presence of genes *plo, fimA, fimC, fimE, fimG, nanH, nanP* and *cbpA* in 50 *T. pyogenes* strains obtained from clinical bovine mastitis. Eighteen isolates were subjected to *in vitro* modified disk diffusion method using 18 commercially antimicrobials from 10 different groups of drugs. The most effective drugs against pathogen were ampicillin, azithromicin, ceftiofur, penicillin and gentamicin with 100% of efficacy; whereas the highest resistance rates of isolates were observed to bacitracin (55.5%), neomycin (27.8%) and lincomycin (27.8%). Antimicrobial multiple resistance index – AMRI (>0.3) was found in 6 (33.3%) isolates. The most common genes detected among isolates were: *plo* (50/50=100.0%), *fimA* (49/50=98.0%), *nanP* (37/50=74.0%), *fimE* (29/50=58.0%), *fimC* (27/50=54.0%), *nanH* (22/50=44.0%), *cbpA* (4/50=8.0%) and *fimG* (3/50=6.0%). The main associations of genes among isolates were *plo, fimA, fimE, nanH* and *nanP* (7/50=14.0%); *plo, fimA, fimE, fimC, nanH* and *nanP* (6/50=12.0%); *plo, fimA, fimE, fimC* and *nanP* (6/50=12.0%); and *plo, fimA, fimC* and *nanP* (6/50=12.0%). *plo, fimA, fimC, fimE, nanH* and *nanP* are, apparently, the major genes associated to virulence of bovine mastitis by *T. pyogenes* in Brazil. The occurrence of multidrug resistance patterns among isolates reinforces that first-choice antimicrobial treatment should be based on *in vitro* resistance profile, because the responsible use of antimicrobials for livestock is an emergent One Health concern, to conserve these drugs for human therapy approaches.

Key words: *Arcanobacterium pyogenes*, virulence factors, multidrug resistance

INTRODUCTION

Trueperella pyogenes (formerly *Arcanobacterium pyogenes*), is opportunistic bacterium characterized by diverse clinical manifestations in livestock and companion animals, commonly refractory to conventional antimicrobial therapy (Ribeiro et al., 2015). Pathogenicity of organism is attributed to various mechanisms, including development of pyogranulomatous reactions. To date, pyolisin, a potent cytolisin associated to tissue damage, is considered the major virulence factor of the pathogen. Furthermore, neuraminidases (*nanH* and *nanP* genes), fimbriae (*fimA, fimC, fimE, fimG*), and collagen-binding protein (*cbpA*) associated to mucosal adherence and colonization (Jost & Billington 2005) have also been associated with virulence of *T. pyogenes,* particularly in bovine mastitis (Zastempowska and Lassa, 2012) and uterine diseases (Bicalho et al., 2012). The present study investigated the multidrug resistance pattern of isolates and the presence of genes *plo, fimA, fimC, fimE, fimG, nanH, nanP* and *cbpA* in 50 *T. pyogenes* strains obtained from clinical bovine mastitis.

MATERIAL AND METHODS

A total of 50 *T. pyogenes* strains obtained from clinical bovine mastitis in the State of São Paulo, and Rio Grande do Sul, Brazil, were used. Diagnosis of clinical mastitis was carried out using strip cup test (Radostitis et al., 2007). All the milk samples were collected aseptically and subjected to microbiological culture on defibrinated sheep blood agar (5%), incubated aerobically and also at 5% CO_2 atmosphere, at 37ºC for 96 hours. Simultaneously, the same samples were plated on MacConkey media and kept under the same aerobic conditions described above. Colonies compatible with *T. pyogenes* were submitted to conventional phenotypic tests (Quinn et al. 2011).

Eighteen isolates were submitted to the *in vitro* antimicrobial disk diffusion test according to the CLSI guidelines for aerobic bacterium (CLSI, 2014) with some modifications, due to absence of standard protocols and breakpoints to interpretative inhibition zones for some coryneform bacteria (Ribeiro et al., 2015), such as *T. pyogenes*. The isolates were cultured on sheep blood agar (5%) under aerobic conditions at 37ºC. After 48 hours, pure colonies were inoculated in tubes containing brain-heart-infusion broth (3mL) supplemented with 30µL of Tween 80. Afterwards, the tubes were vortexed to decrease clump formation, and incubated at 37 ºC for 48 hours, until the appropriate optical density (OD) of inoculum equivalent to a 0.5 in the McFarland scale (Condas et al. 2013). *Streptococcus pneumoniae* was used to define inhibition zones (Zastempowska and Lassa, 2012; Ribeiro et al., 2015).

Eighteen commercially antimicrobials from 10 different groups of drugs were used *in vitro*, as follows: (1) cefalosporins (ceftiofur, 30 µg; ceftriaxone, 30 µg; cefoperazone, 75 µg); (2) penicillin and other betalactamic derivates (penicillin, 10 IU, ampicillin, 10 µg, oxacillin, 10 µg), (3) aminoglycosides (gentamicin, 10 µg; amikacin, 30 µg; neomycin, 30 µg), (4) amphenicols (florfenicol, 30 µg), (5) fluoroquinolones (enrofloxacin, 5 µg; ciprofloxacin, 5 µg), (6) macrolides (azithromycin, 15 µg; erythromycin, 15 µg), (7) tetracyclines (oxytetracycline, 30 µg), and (8) sulfonamides (trimethoprim/ sulfamethoxazole, 25 µg), (9) lincosamides (lyncomicin, 2 µg), and (10) peptides

(bacitracin, 10 IU). Multiple antimicrobial resistance indices (AMRI) were calculated by determining the ratio of the number of antimicrobial class(es) against which each isolate was resistant in relation to the total number of tested classes (10 classes). Strains with index values > 0.3 were considered potential sources of resistance genes (Krumperman, 1983).

plo, fimA, fimC, fimE, fimG, nanH, nanP, and *cbpA* genes were investigated through polymerase chain reaction (PCR) described previously (Zastempowska and Lassa, 2012), using specific primers.

RESULTS

The most effective drugs against *T. pyogenes* were ampicillin, azithromicin, ceftiofur, penicillin and gentamicin with 100% of efficacy. In contrast, the highest resistance rates of isolates were observed to bacitracin (55.5%), neomycin (27.8%) and lincomycin (27.8%). In addition, antimicrobial multiple resistance index – AMRI (>0.3) was found in 33.3% (6/18) isolates.

The most common genes detected among isolates were: *plo* (50/50=100.0%), *fimA* (49/50=98.0%), *nanP* (37/50=74.0%), *fimE* (29/50=58.0%), *fimC* (27/50=54.0%), *nanH* (22/50=44.0%), *cbpA* (4/50=8.0%) and *fimG* (3/50=6.0%). The main associations of genes among isolates were *plo, fimA, fimE, nanH* and *nanP* (7/50=14.0%); *plo, fimA, fimE, fimC, nanH* and *nanP* (6/50=12.0%); *plo, fimA, fimE, fimC* and *nanP* (6/50=12.0%); and *plo, fimA, fimC* and *nanP* (6/50=12.0%).

DISCUSSION

Over the last decades, *T. pyogenes* has been implicated as a primary cause of different clinical manifestations in domestic animals (Radostits et al. 2007; Quinn et al. 2011), although bovine mastitis appears to be the major clinical sign of this pathogen (Zastempowska and Lassa, 2012; Ribeiro et al., 2015). Traditionally, the pathogen is refractory to conventional therapy. Development of pyogranulomatous reactions, intracellular location of pathogen, and improper use of drugs may be related to unsuccessful of therapy against *T. pyogenes* infections in livestock (Radostits et al. 2007; Quinn et al. 2011). In this context, bacterial resistance to antimicrobials and the emergence of multidrug-resistant microorganisms are emergent public health threats (Giguère et al., 2010). Indeed, 33.3% (6/18) of our isolates showed multiple antimicrobial resistance indices to three or more antimicrobials used. Based on our findings, the selection of first-line antimicrobials for therapy of *T. pyogenes* infections should be based on local *in vitro* resistance patterns (Zastempowska and Lassa, 2012; Ribeiro et al., 2015). In fact, the responsible use of antimicrobials for domestic animals is an emergent precept of the *One Health* concept, to conserve these drugs for human therapy approaches.

plo, *fimA*, *nanP*, *fimE*, *fimC*, and *nanH* are, apparently, the major genes associated to virulence of bovine mastitis by *T. pyogenes* in Brazil. Similar results were found in *T. pyogenes* isolated from bovines mastitis in Poland (Zastempowska and Lassa, 2012), besides some differences among frequency of genes. Studies focusing on the major virulence factors of animal pathogens, including *T. pyogenes*, are critical to provide data regarding the molecular epidemiology and pathogenicity of isolates causing distinct clinical manifestations, as well as hypothetical differences among the virulence of strains from different geographical areas (Ribeiro et al., 2015).

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HISTOPATHOLOGICAL AND MOLECULAR DIAGNOSIS OF *Trypanosoma cruzi* IN DOMESTIC CATS FROM BRAZIL

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SUMMARY. Chagas disease, which etiologic agent is the protozoa *Trypanosoma cruzi*, affects domestic animals such as dogs (*Canis lupus familiaris*) and cats (*Felis catus*).Once infected, they may act as source of infection and risk factor for human beings, because they are considered the major reservoir hosts of *T. cruzi* in the domestic environment by displaying high infectivity for triatomines, becoming important reservoirs for the disease. The objective of this work was to ascertain parasitological and molecular diagnoses of *T. cruzi* in pinna tissue from domestic cats (*Felis catus*). For this, pinna tissue samples from 40 cats at the Zoonosis Control Center of the municipality of Três Lagoas, Mato Grosso do Sul, Brazil, were evaluated by means of histochemistry and the Polymerase Chain Reaction (PCR). The results from the parasitological examination showed that 55% (22/40) of the cats were positive, presenting intracellular amastigotes of *T. cruzi*. Through PCR, it was observed that 45% (18/40) of the cats were positive for *T. cruzi*, and all of these findings were concordant with the histochemistry. There was no significant difference (p value < 0,05) between the two techniques by the Fisher test. Both techniques enabled identification of *T. cruzi* in these cats, thus showing the importance of these animals within the epidemiological context of the disease, as possible reservoirs and food sources for triatomines, especially in transmission cycles in domestic and peridomestic areas.

Keywords: *Trypanosoma cruzi*, cat, diagnosis

INTRODUCTION

Chagas disease, which etiologic agent is the protozoa *Trypanosoma cruzi*, affects domestic animals such as dogs (*Canis lupus familiaris*) and cats (*Felis catus*). Once infected, they may act as source of infection and risk factor for human beings, because they are considered the major reservoir host of *T. cruzi* in the domestic environment by displaying high infectivity for triatomines, becoming important reservoirs for the disease (Beard et al., 2003; Gürtler et al., 2007; Cardinal et al., 2008; Gurtler; Cardinal 2015). This situation confirms the need for greater attention and investigation to the role of cats in the urban and peri-urban cycle of Chagas disease.

Among the diagnostic techniques of Chagas disease, the serological techniques are the most common, but there is possibility of cross-reaction with other trypanosomatids, such as *Leishmania* spp. (Caballero et al., 2007; Nieto et al., 2009; Umezawa et al., 2009; Luciano et al., 2009).

The use of parasitological methods decreases the false positive diagnosis, but does not determine the species of the parasite. The problem has been solved by molecular tests, that are more specific and detect species of parasites by DNA fragments (Lucheis et al., 2005). Thus, it becomes necessary the accuracy of detection of *T. cruzi* infection in domestic animals, assess the potential risk of this zoonosis disease in these species and propose relevant measures of trypanosomatids control in the urban environment (Enriquez et al., 2013).

In this work, we report the detection of *Trypanosoma cruzi* in pinna tissues of domestic cats, by the association of parasitological technique of histochemistry and by the association with the molecular test of Polymerase Chain Reaction (PCR).

MATERIAL AND METHODS

Pinna tissues of 40 cats from the Zoonosis Control Center (ZCC) from Três Lagoas, MS, Brazil, were harvested after being euthanized, with the owner's consent, according to the routine of the institution. Some of the pinna's fragments were fixed in buffered formalin for histochemistry diagnosis and elsewhere were stored in a freezer at -20° C for molecular diagnosis.

After fabrication of microscopic slides, all tissues were hydrated in xylene/ethanol and stained with hematoxylin/eosin (HE). The tissues were evaluated under light microscopy at 1000x magnification for visualizing amastigote forms, being classified as negative tissues (without presence of amastigote forms) and positive tissues (when the parasites were visualized inside or outside of macrophages). Among the positive tissues, they were sub-classified according to Giunchetti et al. (2008).

DNA from the samples were extracted using the commercial kit Illustra Genomic Prep Cells & Tissue™ Mini Spin (GE Healthcare®). For PCR were used primers TCZ1 (5'-CGAGCTCTTGCCCACACGGGTGCT-3') and TCZ2 (CCTCCAAGCAGCGGATAGTTCAGG 5'-3'), amplifying a region of 188 base pairs (bp) of a specific tracking of nDNA (Virreira et al., 2003). The amplification conditions were one cycle to initial denaturation at 96°C for 2 minutes; 30 cycles of denaturation at 94°C, annealing at 60°C and elongation at 72°C for one minute and a cycle of 72°C for ten minutes. As a positive control of the reaction was used the strain "Y" of *Trypanosoma cruzi*, proceeding from the Institute of Tropical Medicine of São Paulo, Brazil. As negative control it was used sterile Milli-Q water.

RESULTS

Through histochemistry technique, 22 out of 40 cats (55%) were positive, while 18 (45%) were positive by PCR. Four animals (10%) were positive only by histochemistry technique. Among the positive animals to histochemistry (22), eighteen animals (81,8%) were also positive to PCR. There was no significant difference (p-value <0,05) between the two techniques by the Fisher test.

DISCUSSION

To our knowledge this work is the first report of the amastigote forms detected in pinna skins of domestic cats from Três Lagoas, Mato Grosso do Sul, Brazil, analyzed by the parasitological test of histochemistry and confirmed by PCR for *Trypanosoma cruzi*. Previous studies confirmed the

possibility of domestic cats as reservoirs and its capacity of infection in Chagas disease, by performing parasitological and molecular tests with their blood (Eloy; Lucheis, 2012; Enriquez et al., 2013, 2014).

Twenty-two out of 40 cats (55%) were positive to histochemistry technique. This parasitological technique provides a reliable result when applied to animals with high parasite load, since the visualization of amastigote forms and structures (nucleus, kinetoplast and cytoplasm) creates some difficulty for the correct identification and may produce inconclusive results (Bourdoiseau et al., 1997). To date, there are no studies in the literature involving the parasitological diagnosis for *T. cruzi* from domestic cats' tissue samples. We have few studies that report the finding of protozoans in blood by hemoculture, which is a parasitological technique considered laborious and also requires molecular confirmation to define the isolated species in culture (Alves et al., 2011; Braga et al., 2014.)

Through PCR, 18 tissues of 40 animals (45%) confirmed the positivity for *T. cruzi*. The detection of four non-positive animals for *T. cruzi* to PCR can be explained by the infection with other *Trypanosoma* species. There is a possibility of *Leishmania* infection in cats, previously reported in several studies (Chatziz et al., 2014; Braga et al., 2014). So, we have performed PCR for *Leishmania* in all cats, and the results were all negative, confirming the positive results of amastigotes for *Trypanosoma cruzi* in evaluated tissues.

The molecular technique of PCR, due to its sensitivity and specificity, is indicated for confirmatory testing in cases of inconclusive diagnosis and cross-reactivity between *Leishmania* spp. and *T. cruzi* (Troncarelli et al., 2009). Therefore, the association of histochemistry and PCR techniques contributed to confirm the diagnosis of *T. cruzi* in cats of this study. This reinforces the importance of domestic cats as natural reservoirs for this and others trypanosomatids (Cardinal et al., 2008; Eloy; Lucheis, 2012). Preventive measures for infection are needed, such as the maintenance of dogs and cats out of the internal environment of homes, mainly in areas where the presence of the vector is known, reducing therefore the risk of transmission (Gurtler; Cardinal, 2015). Moreover, the improvement of diagnostic methods for the correct and proper identification of feline infection through *T. cruzi* is critical to the recognition of this species, with the dog as a reservoir host in the transmission cycle of Chagas disease.

The association of parasitological technique of histochemistry and the PCR allowed the identification of *T. cruzi* in pinna tissues of cats, concluding that cats may act as important reservoirs in the epidemiological cycle of Chagas disease. It emphasizes the need of more studies to verify the potential risk of infection by triatomines vectors and to humans.

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Trypanosoma cruzi INFECTION IN WILDLIFE IN A HIGH-END GATED COMMUNITY IN SOUTHEASTERN BRAZIL

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SUMMARY. The discussion on the participation of wildlife in the transmission of zoonosis has become particularly important in recent years, following the CDC's "One Health" initiative and growing environmental changes. A consequence of environmental change is fauna modification, including vectors and host pathogens. In this context, one important disease is American trypanosomiasis (AT), caused by *Trypanosoma cruzi* and transmitted, among other forms, by triatomine feces. AT primarily affects rural populations, where frequent vector contact occurs. In Brazil, chronic cases predominate due to vector infection acquired in past.

In Campinas city (22°53'20" S 47°04'40" W), São Paulo State, southeastern Brazil, there is an environmentally protected area (EPA), which, despite its biological wealth, has undergone intense anthropogenic changes in recent decades. In order to investigate *T. cruzi* infections in EPA wildlife, we captured free-ranging mammals for four consecutive days per month, over 12 consecutive months (April 2014 to March 2015).

Subcutaneous microchips were applied to identify each mammal and blood samples were collected. Genomic DNA was extracted and polymerase chain reaction was performed with primers P35-P36 and TCZ1-TCZ2, which amplify 330 and 188 base pair products, respectively. Amplicon identification was performed by agarose gel electrophoresis and confirmed by Sanger genetic sequencing.

Positive results were obtained for 14/82 (17.1%; 95%CI 8.9-25.2%) mammals. Genetic sequencing allowed the confirmation of *T. cruzi* infection in three *Didelphis albiventris* (white-eared opossum) for the first time in this region, showing 97% to 100% similarity with GenBank sequences (accession numbers KY421668 to KY421671). Interestingly, one of these opossums was positive in its first capture and also in its recapture, three months after.

These results are important for public health, because in this EPA cohabitation with wildlife, humans and their pets occurs and *T. cruzi* was detected in blood in a wild region where there may be vector species.

Key words: Chagas' disease, wildlife, molecular

INTRODUCTION

American trypanosomiasis or Chagas disease is a zoonotic infection by the protozoan parasite *Trypanosoma cruzi*, discovered and described by Carlos Chagas (1909). It is an important public health problem, especially due to its life-threatening potential and substantial costs with treatment and vector control (WHO, 2016).

T. cruzi parasites are mainly transmitted by contact with faeces and or urine of infected triatomine bugs, which typically live in the wall or roof cracks of poorly-constructed homes in rural or suburban areas. They usually become active at night and bite an exposed area of skin, defecating close to the bite, which permits that parasites enter the body. Other forms of transmission are: consumption of contaminated food, blood transfusion, congenital transmission, organ transplants and laboratory accidents (WHO, 2016).

Estimates indicate that about six to seven million people worldwide are infected with *T. cruzi* and 21 Latin American countries have endemic areas of transmission, including Brazil (WHO, 2016). In this country there is a predominance of chronic cases resulting from vector transmission in past decades.

The latest survey conducted in all the rural territory of Brazil from 2001 to 2008, except in Rio de Janeiro State, points that 0,03% of children under five years old living in rural areas were infected by *T. cruzi*, but 0,02% have concomitant maternal positivity, indicating congenital transmission and demonstrating success in controlling vector transmission (Ostermayer et al., 2011). However, control measures related to the sylvatic cycle of Chagas disease are actually in evidence in Brazil, due to the increase in this type of transmission in recent years (Brasil, 2015).

It is evident from the history of Chagas disease that the primary causes for the transmission of the infection to people are the anthropogenic environmental changes, especially deforestation, which brings people into closer contact with disease-carrying vectors (Steverding, 2014). These environmental changes are increasingly frequent and provide close contact between man and pets with wildlife.

In Campinas city (22°53'20" S 47°04'40" W), São Paulo State, southeastern Brazil, there is an environmentally protected area (EPA), which, despite its biological wealth, has undergone intense anthropogenic changes in recent decades due to urbanization. We performed the polymerase chain reaction (PCR) in blood samples of wild mammals from EPA-Campinas, in order to investigate the occurrence of *T. cruzi* infections and to verify the risk of transmission of Chagas disease to humans and pets inhabiting this area.

MATERIAL AND METHODS

We captured free-ranging mammals for four consecutive days per month, over 12 consecutive months (April 2014 to March 2015) in 18 different points of the territory of EPA-Campinas. Subcutaneous microchips were applied to identify each mammal and blood samples were collected. The capture, containment, handling and sampling were carried out according to guidelines for the use of wild mammals in research, as recommended by the American Society of Mammologists (Sikes & Gannon 2011).

DNA extraction of total blood samples was performed within 48 hours of the conclusion of each capture, using a QIAamp DNA mini kit in a QIAcube® DNA extractor (Qiagen®, Netherlands). PCR was performed with two primers pairs, P35 (5'AAATAATGTACGGGGGAGATGCATGA3') and P36 (5'GGGTTCGATTGGGGTTGGTGT3') (Sturm et al., 1989) and TCZ1 5'CGAGCTCTTGCCCACACGGGTGCT3') and TCZ2 (5'CCTCCAAGCAGCGGATAGTTCAGG3') (Moser et al., 1989), which amplify specific 330 and 188 base pair products, respectively for each pair.

The reaction mixture contained 1.3 µL of buffer (50mM KCl, 20mM Tris-HCl pH 8.4), 0.4 µL MgCl2 (1.6 µM), 0.25 µL of each oligonucleotide (0.2 µM), 0.25 µL dNTP (0.2 mM), 0.25 µL of Platinum Taq DNA polymerase (Invitrogen, Brazil) and 8.3 µL of ultrapure water, with 1 µL of extracted DNA with a minimal concentration of 10 ng/µL. As reaction controls we used extracted DNA from *in vitro* cultures of *T. cruzi* and ultrapure water.

Amplicon identification was performed by 1.5% agarose gel electrophoresis containing 1.0 μL/10 mL of SYBR® safe DNA gel stain (Invitrogen®, Life Technologies, USA) and they are confirmed by Sanger genetic sequencing.

The prevalence of *T. cruzi* infection in the study area and its respective 95% confidence interval (95%CI) were calculated using Stata software, v. 11.0 (StataCorp LP, USA).

This research was approved by the Ethics Committee on Animal Use of Campinas State University (protocol no. 3296-1) and by the Brazilian Institute of Environment and Renewable Natural Resources, through the Biodiversity Authorization and Information System (IBAMA, SISBIO, no. 42926-1/2).

RESULTS

Positive PCR results were obtained for 14/82 (17.1%; 95%CI 8.9-25.2%) mammals with at least one of the primer pairs. Genetic sequencing allowed the confirmation of *T. cruzi* infection in three *Didelphis albiventris* (white-eared opossum) for the first time in this region, showing 97% to 100% similarity with GenBank sequences (accession numbers KY421668 to KY421671). Interestingly, one of these opossums was positive in its first capture, in June 2014, and also in its recapture, three months after.

DISCUSSION

In recent times, urbanization and other human activities linked to deforestation led to an increase in the spread of Chagas disease. Besides that, triatomine vectors have a remarkable ability to quickly adapt to newly created environments and to new hosts, which led to the establishment of domestic transmission cycles between pets and humans (Steverding, 2014).

Opossums (*Didelphis* spp.) are considered natural hosts of *T. cruzi* and the parasite is able to maintain extracellular multiplicative stages in the lumen of the scent anal glands of these animals (Deane et al., 1984). Infection or evidence of infection by *T. cruzi* in opossums are reported in other studies (Deane et al., 1984; Schweigmann et al., 1999; Rabinovich et al., 2001; Gurgel-gonçalves et al., 2004; Herrera et al., 2005; Tenório et al., 2014; Bhattacharyya et al., 2015; Costa et al., 2015).

Besides that, the genus *Didelphis* is able to maintain high and long-lasting parasitemias (Jansen et al., 2015). In our study, the finding of one *D. albiventris* with at least four months lasting parasitemia is important when considering the possibility of vector transmission after blood meal and the synanthropic habit of opossums.

The results reported here are also important in a scenario of interruption of vector transmission, which occurs in Brazil. In this situation, one of the great difficulties is the awareness of local health institutions regarding the need to maintain vigilance and control actions, especially when there are the occurrence of other diseases of greater repercussion, such as dengue (OPAS, 2011).

Besides that, the results have implications for the disease control. It is known that human cases of Chagas disease can accidentally occur when man enters natural ecotopes of the infection (Coura & Borges-Pereira 2010). Thus, the results point to the need of constant epidemiological surveillance activities to verify the circulation of *T. cruzi* and its vectors, which is of extreme importance to avoid the establishment of reemergence of acute cases of Chagas disease by vector transmission.

It is not possible to rule out the possibility of enzootic *T. cruzi* transmission cycles or re-infestation of houses by infected sylvatic triatomine bugs (Bhattacharyya et al., 2015) in areas like EPA-Campinas, where there are wild animals with parasitemia.

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MOLECULAR EXPLORATION OF GENETIC RESISTANCE AGAINST BOVINE TUBERCULOSIS IN RIVERINE BUFFALO

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Abstract

Introduction: Being an agrarian country, Pakistan is primarily dependent upon livestock for its economy. Role of animal sector is not only crucial for determining national GDP but also pivotal as provider of necessary food items and their byproducts. It has been estimated that species of livestock can give milk about 29.472 million tones and beef about 1.115 million tons annually. Among different species of livestock, buffalo stands out as an efficient converter of poor quality roughages into highly valuable products as milk and meat. Buffalo contributes about 68% of total milk produced in Pakistan. Production potential of these animals has been admired internationally. But production capacity has been found reversely related with fertility and health. More would be the production poorer would be the fertility. This opens the opportunity to genetically study the significant loci for production and reproduction traits. To test this hypothesis, present research was planned to study CYP11b1 and CYP19A1 genes as candidate for resistance against bovine tuberculosis in river buffaloes.

Animals, Materials and Methods: Selected animal species was Riverine Buffalo. Blood samples were collected from true representative of river buffalo. Primers were designed from coding regions of the two genes and them these were amplified and sequenced. Comparison of sequences provided genetic variations and then statistically analyzed.

Results: In CYP11b1, seven variations were identified and in CYP19A1, four novel variations were identified. After statistical analysis, one of CYP11b1 and two of CYP19A1 were found significantly associated with natural resistance against bovine tuberculosis.

Conclusions: It was concluded that animals with high natural genetic resistance are pretentious in future selection programs.

Key words: Polymorphism, Immunity, CYP11b1, Buffalo, Association

Introduction

In many parts of the world, improvements have been made in many of the breeds on the basis of novel selection signatures in significant parts of the genes and still our quest for new markers is in the run. In present study, CYP11b1 and CYP19A1 were studied at genomic level for identification of novel polymorphisms in the coding region of the gene (Khatib et al. 2006). These genes have been found associated with genetic resistance against infectious agents (Kataoka et al. 2000; Heyen et al. 1999; De Koning et al. 2001; Rodriguez-Zas et al. 2002; Olsen et al. 2002; Awad et al. 2010; Schopen et al. 2011). The present study explored the association of a single nucleotide polymorphism (SNP) in the both genes with resistance against bovine tuberculosis in river buffaloes of Nili-Ravi breed. Polymerase chain reaction technique was performed for genotyping the animals. A total of fifteen SNPs were identified. Identified selection signature can serve as genetic marker for section of superior buffaloes to enhance the reproduction potential of our animals.

Materials and Methods

Sampling strategy: A total of 50 animals of Nili-Ravi buffalo breed were selected from government and private livestock farms (Buffalo Research Institute, Pattoki; Livestock experimental Station,

Okara). Animals were categorized into two groups. Group-1 included animals in first month of their second lactation with milk fat content more than 8% (n=50). In group-2, animals were selected with same cyclic stage (first month of second lactation) but with milk fat content less than 8% (n=50). Then selected animals were subjected to blood sampling. 10mL blood was collected from each animal in EDTA added vaccutainer. Blood was immediately transferred to the ice cooler and was shifted to Molecular biology and Genomics lab. In Institute of Biochemistry and Biotechnology, University of Veterinary and Anima Sciences, Lahore for further processing.

Genomic DNA extraction, PCR amplification and sequencing: DNA was extracted by using organic DNA extraction protocol reported by Maryam et al. (2012) with some modifications. Specific sets of primers (table-1) were used to amplify the all exons of OLR1 gene. Then PCR amplicons were purified and sent for DNA sequencing.

Bioinformatics analysis: Identified sequences were aligned with CYP11b1 and CYP19A1 genes sequence reported in cattle (AC_000162.1) and total of fifteen SNPs were identified (table-2). These variations were tested for Hardy Weinberg Equilibrium and only one (P17H) was found obeying HWE and was selected for association analysis.

Results

CYP11b1 and CYP19A1 genes were studied for the identification of biomarkers. Khatib et al. (2006) identified the genetic variation in this gene that was associated with milk fat content. In present study, total fifteen polymorphisms were identified (table-2). Out of these 15, four were intronic and remaining eleven were exonic. From these eleven, five were synonymous and were not changing any amino acid. Remaining six were non-synonymous. Ratio of transition and transversion is 1.15:1.

Results of single marker analysis depict the distribution of alleles indicated that CYPb-5[P= 0.1306 >0.05] , CYPb-6 [P= 0.0913>0.05], CYPa-10 [P= 0.1014>0.05], CYPa-11 [P= 0.2403>0.05] and CYPa-13 [P= 0.1410>0.05] were non-significant and following Hardy-Weinberg equilibrium indicating that the alleles were randomly distributed throughout the population, no migration had occurred, no bottlenecks happened. While for loci CYPb-1 [P= 0.0001<0.05], CYPb-2 [P= 0.0010<0.05], CYPb-3 [P= 0.0005<0.05], CYPb-4 [P= 0.0013 <0.05], CYPa- 7[P= 0.0011<0.05], CYPa-8 [P= 0.0010<0.05], CYPa-9 [P= 0.0015<0.05], CYPa-14 [P= 0.0021 <0.05], and CYPa-15 [P= 0.0002 <0.05] as probability value of Chi-square test was below 0.05, suggesting that population at these polymorphic sites was indicating significant deviations from Hardy-Weinberg equilibrium. These results have been given in table-2. All of mutations were novel and were not reported before. Most of variations were identified in exon-4. Khatib et al. (2006) also reported associated mutations in exon-4.

Discussion

Out of total fifteen variants, one was found obeying Hardy Weinberg Equilibrium (HWE). Description of identified variations has been mentioned in table-2. Chi square testing was performed on these variations and P-value (>0.05) was calculated. Results of genotypic and allelic frequencies are given in table-3 and 4. The allele frequencies identified at this position were not consistent with those of Khatib et al. (2006), Komisarek & Dorynek (2009) and Wang et al. (2012) who reported 0.46, 0.43 and 0.42 for allele A and 0.54, 0.57 and 0.58 for allele C in US, Polish and the Israeli Holstein cattle populations, respectively. However, they are consistent with the frequencies reported by Schennink et al. (2009) with 0.71 and 0.29 for alleles A and C in an experiment with a Dutch Holstein population. Allele C was found associated with high immunity levels. HWE analysis revealed significance of identified loci in local buffalo population. Genotypic and allele frequency were also calculated. Kataoka et al. (2000) studied CYP11b1 and found similar genotypic frequency. The present study is an example of candidate gene approach to find some novel variations at population level. This study is first step in finding some probable markers for genetic resistance in Nili-Ravi buffalo that can be used in future selection and breeding program.

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Table-1: List of Primers

Sr #	Primer Name	Primer Length	Product Size	Primer Sequence (5'-3')
1	*CYP11b1* F1	24	385	CACAGATTCACCACTTTCCCTTCC
2	*CYP11b1* R1	21		CCACACCCAGGCATTGTAGTT
3	*CYP11b1* F2	20	465	GATATTGAATCCCAGCTCCT
4	*CYP11b1* R2	20		CATCTTCCCATTCACTCCTA
5	*CYP11b1* F3	19	508	GTTGGGTTGATTTGTTGTC
6	*CYP11b1* R3	21		GGACCTCTAATGTAGAACCTG
7	*CYP11b1* F4	20	490	AGTCTGGGTGTAATTCTGAC
8	*CYP11b1* R4	20		CTTTACAGCGATGTCTAGTG
9	*CYP19a1* F1	19	390	GCTCCACTAGACATCGCTG
10	*CYP19a1* R1	20		CAGTGAGAAGCCCACACATC
11	*CYP19a1* F2	21	512	CCAACTCTCTCACACAAGGAC
12	*CYP19a1* R2	20		GGACTTGGAACAAGTTAGGG
13	*CYP19a1* F3	20	497	GGATCTGGAGGAAAAGAAGG
14	*CYP19a1* R3	18		GCAAAGGCAATGTAGTGA
15	*CYP19a1*F4	19	453	CCTAACTCAAGGTCACAGC
16	*CYP19a1* R4	19		GGACAAGCCAATTTAAGAC
17	*CYP19a1* F5	19	574	CTTGGAATCACATGGTAGT
18	*CYP19a1* R5	21		GAGATTCTAGTCCATGAAATC

Table-2: Polymorphic sites detected in the *CYP11b1 (CYPb) and CYP19a1* (CYPa) region

Genetic variants	Transition/ Transversion	Chi test (P>0.05)
CYPb-1	Transversion	1.2051 **
CYPb-2	Transition	0.0010 *
CYPb-3	Transversion	0.0005 *
CYPb-4	Transversion	0.0013 *
CYPb-5	Transversion	0.1306 **
CYPa-1	Transversion	0.0913**
CYPa-2	Transition	0.0011*
CYPa-3	Transversion	0.0010*
CYPa-4	Transition	0.0015*
CYPa-5	Transition	0.1014**
CYPa-6	Transition	0.2403**
CYPa-7	Transition	0.0000
CYPa-8	Transition	0.1410 **
CYPa-9	Transition	0.0021 *
CYPa-10	Transversion	0.0002 *

**Significant*
***Non-significant*

Table-3: Allele Frequency for all Loci of *CYP11b1 and CYP11a1*

SNP ID	Allele Frequency		Minor Allele Frequency
CYPb-1	**C**	**A**	0.2771
	0.7229	0.2771	
CYPb-5	**A**	**C**	0.0120
	0.9880	0.0120	
CYPa-1	**A**	**C**	0.0723
	0.9277	0.0723	
CYPa-5	**T**	**C**	0.2530
	0.7470	0.2530	
CYPa-6	**T**	**C**	0.0241
	0.9759	0.0241	
CYPa-8	**G**	**A**	0.2289
	0.7711	0.2289	

Table-4: Genotypic Frequency for all Loci of *CYP11b1 and CYP11a1*

0.2683	0.1951	0.5366
0.5484	0.0645	0.3871
0.4516	0.0968	0.4516
0.2439	0.5854	0.1707
0.2195	0.5122	0.2683
0.5366	0.1951	0.2683

ANTIGENIC AND GENOTYPIC CHARACTERIZATION OF RABIES VIRUS ISOLATED FROM BATS (MAMMALIA: CHIROPTERA) FROM MUNICIPALITIES IN SÃO PAULO STATE, SOUTHEASTERN BRAZIL

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SUMMARY. Bats have aroused growing attention in the public health sphere because they are considered the main reservoir of the rabies virus (RABV) in the Americas, in places where canine rabies is under control. Antigenic and genetic studies of RABV isolates have been used to describe the epidemiological profile of rabies and to detect possible hosts/reservoirs for different epidemiological cycles. Thus, this study describes the antigenic and genotypic characterization of 19 RABV isolates from central nervous system samples of non-hematophagous bats (Mammalian: Chiroptera). These bats were diagnosed RABV- positive by direct fluorescent antibody and the mouse inoculation test. Antigenic characterization using a panel of eight monoclonal antibodies revealed that 7/19 RABV isolates from these bats belonged to variant 3, for which the hematophagous bat species *Desmodus rotundus* is the main reservoir, and 1/19 RABV isolate from an insectivorous bat belonged to variant 4, which is characteristic of these bats. The remaining 11/19 RABV samples were divided into six non-compatible profiles. The isolates were subjected to reverse transcriptase polymerase chain reaction for the N gene and partially sequenced. Genetic characterization of these isolates was performed by grouping the sequences obtained with known RABV lineages. The sequences were grouped in clusters by the phylogenetic inference neighbor-joining method, together with another 89 homologous sequences obtained from GenBank. This analysis grouped the isolates into four known lineages: *Nyctinomops* Brazil, *Myotis* Brazil, *Eptesicus* Brazil and *D. rotundus* Brazil, as well as another cluster that may define a RABV lineage not yet characterized, here named *Myotis* Brazil II, for which bats of the genus *Myotis* apparently act as reservoirs. This assumption of a new lineage is also based on the observation of amino acid substitutions, with an average intraspecific identity of 99.8%, varying from 99.6 to 100.0% for nucleotides and 100.0% for amino acids.

Key words: rabies virus; bats; antigenic characterization; genotypic characterization.

INTRODUCTION

Rabies is a viral infectious disease that affects the central nervous system (CNS) with acute and fatal evolution, which predominantly affects mammals. It is distributed worldwide, with about 40,000 to 70,000 human deaths per year, almost all of them in developing countries, especially in Asia and Africa (Brasil, 2008).

The disease is caused by Rabies virus (RABV), a RNA virus that belongs to Order *Mononegavirales*, family *Rhabdoviridae* and genus *Lyssavirus*. The virus presents a negative sense (3'- 5'), single-strand and non-segmented RNA genome. It is 100 to 250 nm long, 75 nm in diameter and has 11932

nucleotides that codify the five structural proteins: N (nucleoprotein), P (phosphoprotein), M (matrix protein), G (glycoprotein) and L (RNA polymerase) (Wunner, 2007).

In terms of amino acid sequence similarity, protein N is the most conserved among the genus Lyssavirus, although a relatively high degree of genetic diversity is found in some small regions of the gene N in the different genotypes. For this reason, in order to detect RABV by reverse transcriptase polymerase chain reaction (RT-PCR), the gene of protein N is the most commonly used (Wunner, 2007). Molecular characterization of this gene identifies 14 species (ICTV IC, 2016).

Molecular studies of the RABV have shown that there are many reservoirs for this genotype. These different reservoirs play a fundamental role in the specific maintenance of each viral variant in the environment (Velasco-Vila, 2002; Favoretto, 2013).

Sylvatic rabies has grown in importance due to the synanthropic habits of bats and other wild mammals, which began to inhabit urban and transitional areas as a consequence of the greater availability of food and the environmental impact provoked by anthropogenic changes in their habitat (Kotait, 2007).

Antigenic and genetic studies of RABV isolates in Latin America have been used to describe the epidemiological profile of rabies and to detect possible hosts and reservoirs involved in different epidemiological cycles of the disease. Understanding the evolution of RABV is fundamental to determining the phenotypic and genotypic variability of the viral population. The appearance of viral variants in new hosts and their ability to change are important in the development of new vaccines, for example. Moreover, it is known that minor mutations in certain areas, sometimes of only one amino acid, can modify the pathogenicity and virulence of RABV (Ito, 2001).

Thus, we conducted a study to genetically and antigenically characterize 19 samples of RABV isolated from non-hematophagous bats from five cities of São Paulo State, Brazil, in order to determine the molecular epidemiology of the virus in these animals and in the region studied.

MATERIAL AND METHODS

We conducted a study of 19 RABV isolates from the CNS of non-hematophagous bats. The isolates were obtained by first and second passages in mice inoculated with CNS material of the following genus/species of bats: four *Artibeus lituratus*, four *Artibeus* spp.; two *Myotis nigricans*, four *Myotis* spp.; four *Molossus* spp. and one *Nyctinomops* spp. The identification and classification (genus/species) of the bats studied was performed using the taxonomic keys of (Vizotto, 1973) and (Gregorin, 2002).

The antigenic analysis was performed per the protocol describe by (Diaz, 1994) by the indirect immunofluorescence technique, using panel of eight monoclonal antibodies (MAbs) against the *N* protein of RABV (Mattos, 1998).

Total RNA of the central nervous system (CNS) of these animals was performed using the TRIzol method (Invitrogen, Carlsbad, CA, USA). RT-PCR was performed per the protocol standardized at the Molecular Biology Laboratory of the Pasteur Institute in São Paulo, SP. The PCR for partial amplification of gene *N* was conducted per the protocol described by (Orciari, 2001) and (Oliveira, 2010). Purification of PCR products was performed using QIAquick® Gel Extraction kit (QIAGEN, Netherlands). Purification of sequencing reaction was performed using Sephadex™ G50 fine (GE Healthcare Life Sciences, Uppsala, Sweden). After purification, the sequences were obtained using the ABI-3130 genetic analyser (Applied Biosystems, Carlsbad, CA, USA).

For the editions of sequences and the phylogenetic analyzes we use the program BioEdit v. 5.0.9 (Hall, 1999), and software MEGA7 (Kumar, 2016).

RESULTS

Antigenic characterization by the MAbs panel revealed eight different antigenic profiles of the RABV isolates, according to the municipality the bats came from. The RABV isolates were grouped as follows: 36.8% (7/19) in variant AgV-3, characteristic of the hematophagous bat *Desmodus rotundus*, six of which were isolated from frugivorous bats of the genus *Artibeus* – two *A. lituratus* – and one isolated from an insectivorous bat of the genus *Molossus*; 5.3% (1/19) bat samples in variant AgV-4, characteristic of the insectivorous bat *Tadarida brasiliensis*, which was identified in one insectivorous bat *Myotis* spp.

However, six patterns of non-compatible (NC) results were identified when compared with profiles established by the MAbs panel: NC1, which was characterized in 21.0% (4/19) of RABV isolates and involved two bats of the genus *Molossus*, one *Myotis* and one *A. lituratus*; profile NC2, which was identified in 15.8% (3/19) of the samples, with isolates from three bats, *A. lituratus*, *Myotis* spp. and *Myotis nigricans*; and finally other four distinct profiles, NC3, NC4, NC5 and NC6, which were each identified in one of the samples 5.3% (1/19), respectively, in the genera of insectivorous bats *Molossus* (one), *Nyctinomops* (one) and *Myotis* (two; one of which was the species *Myotis nigricans*).

Of the 19 samples, 16 presented positive sequences, which in the genetic characterization allowed the grouping of 12 isolates in four previously known strains:

lineage *D. rotundus* Brazil in six frugivorous bats of the genus A*rtibeus* and one insectivorous bat of the genus *Molossus*; lineage *Nyctinomops* Brazil in one *Molossus* and one *A. lituratus*; lineage *Myotis* Brazil in two insectivorous bats of the genus *Myotis*; and lineage *Eptesicus* Brazil in one *Molossus.* However, four RABV isolates formed a distinct cluster, two of them isolated from bats of the genus *Myotis*, one from the genus *Molossus* and one from the species *A. lituratus*, with bootstrap value of 100%.

DISCUSSION

In Brazil, bats were responsible for the transmission of 14% of human rabies cases from 1990 to 1995 and 5.0% from 1996 to 2001, but their contribution has grown to 63.8% from 2002 to 2009, such that they are now the main agent of rabies transmission to humans in Brazil (Brasil, 2011).

Studies that provide genetic information on RABV are necessary, among other aspects, to trace the origins of infections and to develop effective control programs (Benjathummarak, 2016). In this study, we analyzed 19 RABV isolates from four different genera/species of non-hematophagous bats from five municipalities in mid-western São Paulo State. Their antigenic and genetic characterization enabled us to elucidate and evaluate the molecular epidemiology of the RABV and the role of bats in virus distribution, which is a pioneering study in the area investigated.

Antigenic characterization showed interesting results, eight different antigenic profiles were identified in the RABV isolates studied. Profile AgV-3, characteristic of the hematophagous bat *D. rotundus*, represented the largest group, which was identified in seven non-hematophagous bats (six *Artibeus* and one *Molossus*), a result which corroborates with the findings of studies conducted in Brazil and Argentina (Kotait, 2007; Delpietro, 1997; Queiroz, 2012). Likewise, a RABV isolate from an insectivorous bat of the genus *Molossus* was antigenically characterized as AgV-4, which is a characteristic variant of insectivorous bat *T. brasiliensis*. Six antigenic profiles were classified as non-compatible when analyzed with MAbs panel, totaling 11 isolates, a result also verified in other studies (Oliveira, 2010; Queiroz, 2012; Almeida, 2011).

In relation to genotypic characterization, the RABV isolates were grouped in four different previously described lineages (Oliveira, 2010): *Nyctinomops* Brazil, *Myotis* Brazil, *Eptesicus* Brazil and *D. rotundus* Brazil. It is worth highlighting that groups were demonstrated based only on the phylogenetic tree topography, due to the size of the sequenced DNA fragment, which prevented us from determining the presence of specific molecular markers for each lineage.

The results obtained here lead us to conclude that agreement between the antigenic characterization, based on MAbs directed to the viral nucleoprotein, and the genotype classification, based on the partial sequencing of the N gene, was achieved in 37% (7/19) of the RABV isolates. Thus, we suggest a review of the MAbs panel in order to update it and include new antigenic profiles that have been repeatedly reported in several Brazilian studies.

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AFRICAN SWINE FEVER IN POLAND – EPIDEMIOLOGICAL REPORT

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SUMMARY. The study presents an epidemiological report and cartographic analysis of the African Swine Fever (ASF) spread in Europe (2007 – 2016) with a particular impact on current situation in Poland. The ASF virus is a highly virulent microorganism from Asfarviridae family, genus Asfivirus, affecting domestic pigs and wild boars by the oral or nasal contact with infected material. The disease presents different clinical signs depending on the virulence of the virus, but mortality of infected animals usually approaching 100% in 5-20 days after infection.

The introduction of ASF disease was noticed in Georgia in 2007 and the virus has spread unexpectedly to Russia, Belarus and Lithuania, finally reaching Poland. On 17 February 2014 the first ASF outbreak was reported in Grzybowszczyzna (Podlaskie province) and 119 cases of the disease in wild boars was confirmed. There were no scientifically proven reports of direct contacts between wild boar and domestic pigs and no correlation between wild boar density and infected areas was observed. However, the disease was also found in domestic pigs in a backyard farms and large pig holdings and since the first notified case on 19 July 2014 only 23 confirmed outbreaks of ASF in pig farms were reported.

The ASF spread was successfully stopped in Polish buffer area and the eradication programme in affected pig holdings was implemented simultaneously with depopulation of the wild boar in eastern provinces. Nevertheless, there are widespread possibilities of the ASF virus transmission to other countries and present epidemiological situation in Poland has to be considered as a priority animal health problem in Europe.

Disease prevention and new anti-infective approaches

CURRENT LEVEL OF GHG EMISSION REDUCTIONS IN POLISH AGRICULTURE

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SUMMARY

Climate change and combating it are one of the priorities of the Common Agricultural Policy (CAP), which are aimed to reach the reduction targets set by the European Union. Previous national Rural Development Programmes (RDP) indirectly contributed to the GHG reduction effect mainly through the measures of the Agri-Environmental Programme. Therefore, an attempt was made to estimate the effects of this reduction. The current NVZ status (7.53% UAA) allows for an annual reduction of 494.2 million tons of CO_{2eq}, which corresponds to 1.36% of total emissions from agriculture. Thanks to the RDP, a total of 62 thous. ha were converted into forest. This means that the RDP resulted in the sequestration of 2.193 million tons of CO_{2eq}, and this figure (0.017%) should be deducted from the total emissions from UAA. Another activity that indirectly reduces GHG emissions is organic farming (0.61% of total GHG). The support for permanent grassland areas is estimated to reduce 81 million tons of CO_{2eq}, (0.22%). The measures for the conservation of endangered bird species and natural habitats covered a 0.31% of total GHG emissions. Sustainable agriculture is indirectly responsible for a reduction of 1.27% CO_{2eq} from total agricultural emissions, which is 478.12 tons of CO_{2eq}. The soil and water protection package was implemented in the area of 854.96 thous ha.The evaluation presented above shows that it is impossible to reduce GHG emissions in Polish agriculture by more than 10%, based solely on the RDP measures implemented to date. Therefore, it is advisable to undertake new, strictly dedicated reduction measures in the next programming perspective.

Key words: GHG, agriculture, reduction, RDP

INTRODUCTION

Climate change and combating it are one of the priorities of the Common Agricultural Policy (CAP) for the years 2014-2020, which are aimed to reach the reduction targets set by the European Union. At the level of the Member States, implementation of the CAP priorities and objectives depends on internal decisions and the subsequently adopted Rural Development Programme (RDP). In previous programming periods, the GHG reduction effect resulted indirectly from the activities connected with environmental protection and good agricultural and environmental conditions. To this must be added effects from the activities in Nitrate Vulnerable Zones (NVZ) and support for investment in agricultural holdings. In both of these cases, the emission reductions were influenced by measures related to the storage of natural fertilizers and methods of fertilization. In addition, account must be taken of the sequestration of CO_2 by building up soil organic matter. It should be remembered, however, that the direct and indirect actions must be stable over time for the reduction effect to occur.

MATERIALS AND METHODS

The effects of mitigation activities were analysed based on the number and size of accomplished activities contained in:

- the evaluation data and the executive reports for consecutive RDPs (Ministry of Agriculture and Rural Development),
- the databases of the Agency for Restructuring and Modernization of Agriculture,
- the prognosis on the extent of access to the RDP 2014-20 (Ministry of Agriculture and Rural Development),
- the interim reports and analyses of the National Centre for Emissions Management,
- the reports of the Main Inspectorate of Commercial Quality of Agri-Food Products.

The calculations were made using the official IPCC data found in the "2006 IPCC Guidelines for National Greenhouse Gas Inventories" and in the "Catalogue of GHG reduction methods for national agriculture" (2015).

RESULTS

GHG reduction percentage was related to the estimates from the basic scenario for 2005, in accordance with the IPCC. A cumulative list of the reduction effect of RDP activities to date is given in Table 1. NVZs in Poland currently amount to 1.227 million ha (KZGW, 2016). The amount of reduction for the previous programming period is regrettably small due to the limited scope of NVZs (0.5-1.5% of Poland's area). Assuming that the current NVZ status (7.53% UAA) is retained, the annual reduction of CO_{2eq} will be 494.2 million tons, which corresponds to 2.39% of GHG emissions from domestic UAA (1.36% of total emissions from agriculture). As regards the modernization of agricultural holdings in terms of animal housing systems and fertilizer storage, indirect GHG reduction in 7,000 farms is estimated at 7.3 million tons of CO_{2eq}, which is only 0.02% of total emissions. The total reduction effect due to support of organic farming is estimated to be 0.61% of total GHG emissions from agriculture. As regards renewable energy sources in the previous programming period, it seems that the 80 agricultural biogas plants do not qualify only as the effects of the RDP, because they benefited from support of the National Fund for Environmental Protection, and their reduction effect is recognized in the ETS sector as part of the energy industry. However, if allowing for the estimation of abandoned CH_4 and N_2O emissions from natural fertilizers as a fermentation substrate, the effect of these facilities could be established as 0.031% of total emissions from agriculture. Another activity which indirectly reduced GHG, was the support provided by the RDP to 279,226 ha of permanent grasslands. This effect, which nominally reaches 20% of GHG emission in relation to the intensively used area, is estimated at 81 million tons of CO_{2eq}. The measures for the conservation of endangered bird species and natural habitats, in and outside Natura 2000 sites, covered a total of 373 thous. ha and 0.31% of total GHG emissions from agriculture. In the subsequent programmes, sustainable (integrated) farming was constantly supported. The indirect reduction effect is mainly related to soil fertilization and cultivation, but also includes animal husbandry. This measure encompassed 1.1 million ha of UAA, which in terms of the size of the emission reduction amounts to 1.27% of CO_{2eq} from total emissions from agriculture. The soil and water protection package was implemented in the area of 854.96 thous. ha. The indirect effect of GHG reduction is related here to the use and ploughing in of catch crops, periodic prohibition of crop production practices and fertilization, and at least partial preservation of surface vegetation in the winter period. The total reduction effect of these measures is estimated at 15%, which corresponds to 221 million tons of CO_{2eq} and 0.59% of CO_{2eq} from total agricultural emissions.

Outside the scope of the completed RDPs are the reducing effects of set-aside and ploughing crop residue. The latter are estimated for N_2O emissions by the National Centre for Emissions Management, but without CO_2 sequestration. Thus, the total emission of 3 Gg N_2O from crop residue should be decreased by this disregarded effect. A change in estimation methodology which accounts for this effect could reduce the emissions by as much as 5.2% of CO_{2eq}. However, this effect must be reduced

by 0.2% of increased emission due to N_2O, which is formed from nitrogen compounds found in the residue. When converted, this sequestration corresponds to 1033.9 million tons of CO_{2eq}, and thus a reduction of 2.85% of CO_{2eq} from the domestic GHG emissions from UAA. Set-aside is foreseen as a part of Ecological Focus Areas (EFA) in 2014-2020, but the present data (GUS, 2016) already show that 475 thous. ha of such UAA exist in Poland. The effect of this measure may reach 97% of standard GHG emission per unit area and depends on the type of crop as well as the soil. Assuming that the average reduction is just 47% per ha, we can account for an emission reduction of 225.9 million tons of CO_{2eq} at national and annual level, which corresponds to 0.62% of total emissions from agriculture. In the current RDP for 2014-2020, climate change is recognized as the thematic objective. It is estimated that 30% of all domestic holdings will be covered by greening. This practice has not been mentioned in the directory of practices and so a proper valuation of the effect is difficult to make. According to various sources, it can be assumed that this measure will allow for a 2-5% reduction of GHG emission, which would correspond to 1814.7 million tons of CO_{2eq} per year, introduced gradually until 2030. Payment for legume crops is considered as specific support. It was included in the RDP as part of Ecological Focus Areas, with an estimated share of 3-7% of the greening area. The effect of legume crop support is currently estimated at 368,9 thous. ha, which is equivalent to 2.5% UAA. Translation of this utilized agricultural area into GHG emission reduction is not conclusive. First, the National Centre for Emissions Management attributes the nitrogen-fixing effect of these crops to N_2O emission. Second, the directory of practices estimates this reduction at just 10%, but the latest research reports a 50% reduction accounting for carbon footprint. Therefore, the reduction is estimated between 1.1 and 238 million tons of CO_{2eq} per year. Even with the maximum reduction, it will form 0.66% of total emissions from agriculture. Uncertain reduction potential is apparently offered by the investment in physical assets of agricultural holdings. Considering the proposals submitted to date and the maximum possible reduction effect of this measure, one may speculate that by 2020, the support will be used by 3167 holdings with a total of 158 350 livestock units. It can be assumed with a high degree of uncertainty that this may constitute up to 902 million tons of CO_{2eq} reduction per year (up until 2020), which means reducing total agricultural emissions by 2.49% in relation to 2005. In the immediate term, it is possible to adopt an action programme aimed at preventing dispersal of nitrogen compounds in the environment, or other equivalent solutions dictated by the verdict of the EU Court of Justice on implementation of the Nitrate Directive. Its impact in terms of indirect reduction of GHG is estimated at 1 800 million tons of CO_{2eq} by the year 2030, which corresponds to 4.95% of annual domestic emissions from agriculture.

Table 1. The impact of the current implementation of CAP on the size of GHG emission reduction from the domestic agriculture.

No.	**Name of action/package**	**Size of emission reduction (million tons of CO_{2eq})**	**Size of emission reduction up to 2005 (%)**
RDP, 2007-2013			
1.	Modernization of agricultural holdings	7.3	0.02
2.	Afforestation of agricultural land	2.193	0.017
3.	Organic farming	224	0.61
4.	Renewable energy sources	11.3	0.031
5.	Permanent grasslands	81	0.22

6.	Conservation of habitats	117.3	0.31
7.	Sustainable agriculture	478.12	1.27
8.	Soil and water protection	221	0.59
9.	Ploughing of crop residue	1033.9	2.85
10.	Set-aside	225.9	0.62
Others, 2007-2013			
11.	Nitrate Vulnerable Zones	494.2	1.36
12.	Liming	486	1.34
Total 2007-2013		**3382.21**	**9.24**
RDP, 2014-2020			
13.	Greening	1814	4.9
14.	Leguminous plants	238	0.66
15.	Investments in physical assets	902	2.49
Others, 2014-2020			
16.	Action programme (Nitrate Directive)	1800	4,95
Total 2014-2020		**4754**	**13.0**
Total 2007-2020		**8136.21**	**22.24**

DISCUSSION

The assessment made above shows that it is possible to obtain an almost 10% minimum GHG reduction threshold in domestic agriculture, exclusively based on RDP activities that have already been implemented and replicated onto the immediate perspective, as well as on other activities supported by the Ministry. The new measures and packages of the current programme have a much higher additional potential (8.05%), but their implementation is subject to uncertainty in relation to the commitments actually made. Furthermore, the need to adopt the action programme aimed to limit dispersal of nitrogen compounds in the environment will increase the total reduction effect up to 13%. The magnitude of this potential is directly associated with the coverage, from which small and part of medium-sized holdings will be excluded. Unfortunately, it is currently difficult to say how far it will be possible to fully document these calculations, such that they can be approved by the European Commission. It seems particularly difficult to incorporate soil carbon sequestration into emission balancing, which is a significant novelty in this area. Of great importance will be inclusion or exclusion of the carbon footprint, which may replace well-established methods with completely new ones. Because of the political will, stated in different EU documents, to identify the significance of climate change, it seems necessary to adopt new, dedicated reduction activities in the current or the next programming perspective, especially with regard to livestock husbandry. In the current perspective, it is still possible to more precisely word several legal acts on agricultural production, rules of support, and documenting of reduction technique implementation. Without this type of monitoring, it will be difficult to gain acceptance of the final estimate of the GHG reduction effect.

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IMMUNE MODULATING ACTIVITIES OF SULFATED POLYSACCHARIDES OF GREEN ALGAE (*ULVA ARMORICANA*) EXTRACT

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SUMMARY. Antibiotics have been used for a long time in animal production to protect animals against pathogens. However, facing the increasing resistance of bacteria to antibiotics, alternative to antibiotics are being looked at. Marine algae contain in their cell wall water soluble sulphated polysaccharides with potential biological activities such as anticoagulant, antiviral, antibacterial and immune-modulating activities that are being explored to be used as an effective alternative to antibiotics. A crude extract (MSP) containing sulphated polysaccharides was prepared from the green algae *Ulva armoricana*. The ability of this MSP to stimulate the expression of the immune response mediators was evaluated using an *in vitro* system of porcine differentiated intestinal epithelial cells IPEC-1. Three doses (1.0, 0.1 and 0.01 mg/ml) were tested in comparison with E. coli O111:B4 LPS as positive control and with cells incubated alone as negative control. The MSP was also tested on a human embryonic renal cell line, HEK293 which expresses TLR4/MD2/CD14, TLR2, TLR5, TLR9, NOD1 and NOD2, in order to identify which TLR or NLR receptor was involved. Expression of IL-8 with ELISA was used as a marker of stimulation. Analysis by RT-qPCR showed increased expression of several cytokines including TNFα (83-fold), IL-1α (23-fold), IL-6 (31-fold), IL-8 (314-fold) and CCL20 (159-fold). When tested with HEK293 cells on several membrane receptors, the MSP stimulated the expression of immune factors via the activation of TLR4 membrane receptor. These results showed that this MSP has the capacity to stimulate, *in vitro*, the expression of cytokines involved in the immune response. This suggests that this extract could be used as a new prophylactic strategy to stimulate the immune response of animals and reduce the use of antibiotics in farms.

Key words: algal polysaccharide, immunity

INTRODUCTION

Antibiotics have been used for a long time in animal production to protect animals against pathogens. However, facing the increasing resistance of bacteria to antibiotics, alternative to antibiotics are being looked at. The use of marine algae arouses an increasing interest, thanks to the nutritional quality of seaweeds, as well as their high content in biologically active molecules (Wijesekara *et al.*, 2010; Evans and Critchley, 2014). Marine algae contain in their cell wall water soluble sulphated polysaccharides with specific physicochemical properties and biological activities that could be used in the pharmaceutical industry, in agriculture or as additives for humans and animals (Barcelo *et al.*, 2000; O'Sullivan *et al.*, 2010; Chojnacka *et al.*, 2012). Anticoagulant, antiviral, antibacterial and immune-modulating activities of marine algae are thus being explored to be used as an effective alternative to antibiotics. In the present study, an extract-rich in marine algae sulphated polysaccharides (MSP) was prepared from the green seaweed *Ulva armoricana*, harvested in Brittany. This extract was tested *in*

vitro for its capacity to stimulate the expression of immune response mediators via the activation of Toll Like Receptor (TLR).

MATERIAL AND METHODS

A crude extract (MSP) containing algal sulphated polysaccharides was prepared from the green algae *Ulva armoricana*. MSP contains 11.6% of neutral sugars, 7.3% of proteins, 12.2% of uronic acids and 26.4% of sulphated polysaccharides. The ability of MSP to stimulate the expression of the immune response mediators was evaluated using an *in vitro* system of porcine differentiated intestinal epithelial cells IPEC- 1. Three doses (1, 0.1 and 0.01 mg/ml) were tested in comparison with *E. coli* O111:B4 LPS as positive control and untreated cells as a negative control. MSP was also tested on a human embryonic kidney cell line, HEK293, transfected either to express none (null) or one specific immune receptor (TLR4/MD2/CD14, TLR2, TLR5, TLR9, NOD1 and NOD2), in order to identify which TLR or NLR receptor was involved. Expression of IL-8 with ELISA was used as a marker of stimulation.
Data are expressed as the mean value ± S.E.M. of triplicate assays and were analysed using the Kruskal-Wallis test followed by Bonferroni-Dunn post-test group comparison tests of means using the GraphPad (GraphPad Prism version 4.00 for Windows; GraphPad Software, USA). Statistical differences between the various treatments were considered significant when P values were <0.01.

RESULTS

RT- qPCR assay showed that MSP, at the dose of 1 mg/ml, induces an increased expression of several cytokines by IPEC-1 cells in comparison with untreated cells (Table 1). The relative expression of IL-8 (Fc = 313.53 ± 47.5), CCL20 (Fc = 159.44 ± 42.5), TNFα (Fc= 82.63 ± 15.8), IL-1α (Fc= 22.96 ± 3.2) was significantly (P<0.01) increased compared to the control (Figure 1). Furthermore, MSP increased significantly (P<0.01) the relative expression of IL-6 (Fc = 30.58 ± 7), IL-1β (Fc = 4.92 ± 1.6), and TGFβ (Fc = 4.83 ± 0.7 Fc) mRNA (Table 1). However, MSP did not produce any significant change on the relative gene expression of IL-10 and IL-12 cytokines, and CCL25 and CCL28 chemokines (Table 1). When tested with HEK293 cells on several membrane receptors, the MSP stimulated the expression of immune factors via the activation of TLR4 membrane receptor.

DISCUSSION

These results show that MSP has the capacity to stimulate, *in vitro*, the expression of cytokines involved in the activation, recruitment and migration of lymphocytes, as well as dendritic cells, to modulate the immune response. This stimulation seems to come from the activation of TLR4 receptor. However, this route of activation of immune mediators may not be exclusive. These results suggest that MSP could be used as a new prophylactic strategy to stimulate the immune response of animals and reduce the use of antibiotics in farms. Its use as an adjuvant in the frame of mucosal vaccination may also improve the immune response of the host organism.

Table 1. Main functions of target mediators and influence of 1 mg/ml of immune-modulating MSP on their gene expression (** $P<0.01$)

Mediator	Main functions	Degree of stimulation of expression compared to control
TNFα	Phagocytosis and chemo-attraction of neutrophils.	82.63 ± 15.79**
IL-1α	Proliferation of CD4+ T-cells and fibroblasts, proliferation and maturation of B-cells.	22.96 ± 3.16**
IL-8	Recruitment of neutrophils, phagocytosis.	313.53 ± 47.54**
CCL20	Recruitment of lymphocytes and dendritic cells, antimicrobial activity.	159.44 ± 42.52 **
IL-6	Differentiation of cytotoxic T-cells, proliferation and maturation of B-cells and excretion of IgA.	30.58 ± 7.03 **
IL-1β	Proliferation, differentiation and apoptosis, expression of adhesion molecules and chemokines.	4.92 ± 1.63**
IL-12p40	Production of IFN-γ, differentiation of Th1 cells, activation of NK cells and development of cytotoxic T-cells.	3.88 ± 0.66**
TGFβ	Differentiation of B-cells, switch IgM/IgA, induction of regulatory T-cells.	4.83 ± 0.66**
PPARγ	Transcription factor with anti-inflammatory function, inhibition of TNFα and IL1β production.	3.71 ± 0.78**
IL-12p35	Production of IFN-γ, differentiation of Th1 cells, activation of NK cells and development of cytotoxic T-cells.	4.19 ± 0.63
IL-10	Differentiation and production of IgA+ plasma cells, control of inflammatory response, stimulation of regulatory T-cells.	2.54 ± 0.61
CCL25	Migration et homing of T-cells and IgA+ plasma cells.	6.20 ± 2.47
CCL28	Migration et homing of T-cells and IgA+ plasma cells.	3.24 ± 1.11

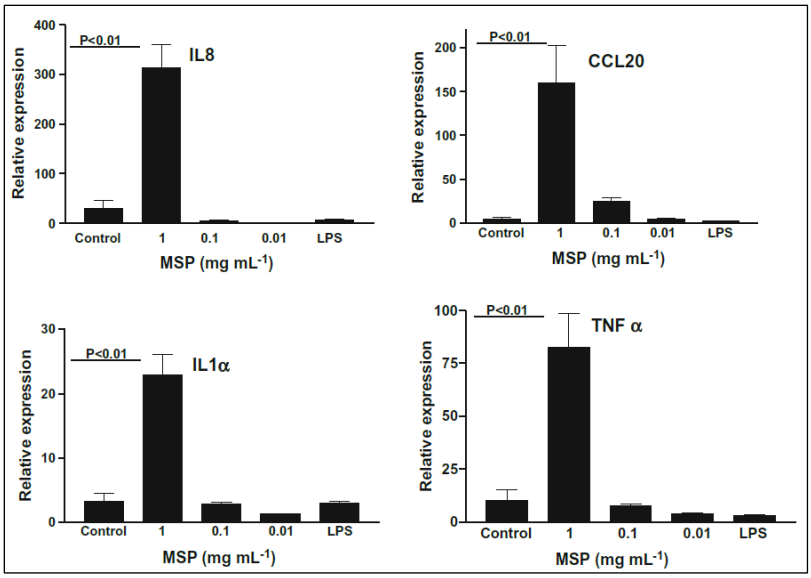

Figure 1. Relative expression of IL-8, CCL20, IL-1α and TNFα by intestinal epithelial cells

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Antibiotics use in animal production: resistance and consequences

DIFFERENCES OF PHENOTYPIC RESISTANCES BETWEEN *E. COLI* ISOLATES FROM "OLD" ANIMAL HOUSE DUST SAMPLES

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SUMMARY. A retrospective study recently showed that dust from farm animal houses can be a reservoir for antimicrobial-resistant *Escherichia coli* for at least 20 years. Depending on the origin of the dust, isolates differed in the number of antimicrobial resistances. However, the resistance rates of single antimicrobials were not compared yet between isolates from different groups of animals. This study compares the antimicrobial drug resistance in *E. coli* isolates originating from up to 20 years old sedimentation dust samples from three pig barns, five fattening poultry barns and one laying hen house. Overall 79 isolates from 54 different dust samples were tested for antimicrobial susceptibility to 10 antibiotics using a microdilution test. An exact conditional logistic regression was used to test significant differences ($p < 0.05$) between antimicrobial resistances of isolates from the different animal groups. Isolates from fattening poultry barns were significantly more often resistant to amoxicillin/clavulanic acid, ampicillin and trimethoprim/sulfamethoxazole than isolates from laying hen houses. They were also significantly less often susceptible to ampicillin and tetracycline when compared to isolates from pig barns. In contrast, resistance to gentamycin was higher in isolates from pig barns. *Escherichia coli* from pig barn dust were also significantly more often resistant to trimethoprim/sulfamethoxazole than isolates from laying hen house dust. Although the random collection of samples does not represent a systematic approach for comparing antibiotic resistance profiles, clear differences are observed indicating different antibiotic regimes applied in the sampled husbandries. Investigating dust samples can be a useful method to give proof of the antibiotics used in an animal house. However, some results like the relatively high occurrence of chloramphenicol-resistant isolates in all kinds of dust samples cannot simply be explained by the use of this antibiotic. Multiple resistances or cross resistance must be taken into account and should be further investigated.

Key words: *Eschrichia coli,* dust, antimicrobial resistances

INTRODUCTION

Dust from livestock buildings contains high amounts of microorganisms which mainly originate from the animals, their faeces, bedding material and feed (Carpenter, 1986). Among these microorganisms are potential pathogens and antimicrobial-resistant bacteria. For instance, pathogenic and antibiotic-resistant *Escherichia coli* were found in airborne dust of pig and poultry barns (Letourneau et al., 2010; Laube et al., 2014). Furthermore, it is known that dust from poultry houses can be a reservoir of *E. coli* (Harry, 1964). However, the long-term survival of *E. coli* in animal house dust was unclear and a recently published publication reported about the cultivability of *E. coli* from more than 20 years old stored dust samples from pig and poultry barns (Schulz et al., 2016). Fifty-three percent of the isolates were multidrug resistant and associations between the number of antibiotic resistances and the origin of the isolates were found. In this recent study, the rates of resistances to the tested antibiotics were not

compared between the isolates from different animal groups. Therefore, this paper aims to investigate the differences between resistance rates of *E. coli* isolates from stored dust samples of laying hen houses, fattening poultry barns and pig barns.

MATERIAL AND METHODS

Isolation, identification and antimicrobial susceptibility testing of *E. coli* from dust samples was described by Schulz et al. (2016). However, the present study includes only isolates from the non-selective McConkey agar (without supplemented ciprofloxacin). The age of isolates varied approximately between six and 20 years. Overall 79 *E. coli* isolates and their antimicrobial profiles, 18 out of nine dust samples from laying hen houses, 42 out of 31 dust samples from fattening poultry barns and 19 out of 14 dust samples from pig barns in Germany, were analysed in the present study. The resistant phenotype of each isolate was analyzed by a microdilution test with the VIZION® system (TREK Diagnostik Systems Ltd., West Sussex, England). Plates (plate codeCMV3AGNF) were inoculated with *E. coli* suspensions as proposed by the manufacturer's protocol. Antibiotic resistance to amoxicillin/clavulanic acid (AUG2), ampicillin (AMP), cefoxitin (FOX), ceftriaxone (AXO), chloramphenicol (CHL),ciprofloxacin(CIP), gentamicin (GEN), sulfisoxazole (FIS), tetracycline(TET), and Trimethoprim/sulfamethoxazole (SXT) were analyzed by means of the Sensititre™ SWIN™ Sofware (p/N SW514-2, 27-Feb-14) adjusted to use CLSI (Clinical and Laboratory Standards Institute) breakpoints (CLSI, 2014). For each antimicrobial the association between occurrence of resistance and different animal groups was analysed separately using logistic regression. As some groups occurred for which no resistances were found (Figure 1), we conducted exact conditional logistic regression using SAS, version 9.3, procedure GENMOD (SAS Institute Inc., 2012), performing exact Score tests and exact Odds Ratios estimates (Stokes et al., 2012). Antimicrobials for which the three animal groups showed no variation in resistances no regression model was fitted, which was the case for AXO and FIS. In this study p-values ≤ 0.05 were interpreted as statistically significant.

RESULTS

Escherichia coli isolates from dust of laying hen houses showed resistances to five antibiotics, isolates from broiler barns to seven antibiotics and isolates from pig barns dust to nine antibiotics (Figure 1). Except for AXO and FIS the proportions of resistance isolates differed between the origins. Isolates from dust of broiler barns indicate higher resistant rates to AUG2, AMP, CHL, CIP, TET and SXT compared to the rates of isolates from pig barns and isolates from laying hen houses. Resistances to GEN and FOX were only detected in isolates from pig barns. The exact conditional logistic regression revealed significant differences between the rates of antibiotic resistant isolates from different animal groups in cases of AUG2, AMP, GEN, TET and SXT (Table 1). Isolates from fattening poultry barns were significantly more often resistant to AUG2, AMP and SXT than isolates from laying hen houses and also more resistant to ampicillin and tetracycline than isolates from pig barns. In contrast, resistance to gentamycin was significantly higher in isolates from pig barns when compared to isolates from fattening poultry barns. *Escherichia coli* from pig barn dust showed also significantly higher resistance rates to SXT as compared to isolates from laying hen house dust.

DISCUSSION

Although the random collection of dust samples does not represent a systematic approach for comparing antibiotic resistance profiles (Schulz et al., 2016), clear differences are observed between the resistance rates of *E. coli* isolates from dust of fattening poultry barns, pig barns and laying hen houses. Higher resistance rates of isolates from fattening poultry compared to isolates from laying hens seems to be typical and might be explained by differences in the antimicrobial treatment regimes (Kaesbohrer et al., 2012). In the present study differences were significant for three potentially used antibiotics (AUG2, AMP, SXT). Furthermore, the proportion of resistances to AMP, TET and CHL of *E. coli* from stored dust of fattening poultry were clearly higher as compared to data published for Germany in 2014 (EFSA, 2016). This might reflect a change in antimicrobial usage in fattening poultry barns over the past 20 years. However, in the case of CHL the situation seems to be more complex. The use of CHL is prohibited since 1994 in the European Union. Therefore, persisting resistance might be explained by the transfer of co-resistance (Szmolka and Nagy, 2013). A significant higher resistance to gentamycin was observed in isolates from pig barns. Apramycin is widely used to treat diseases in pigs and enzymatic cross-resistance could be a reason for the higher resistance against gentamycin in isolates from pig barns (Herrero-Fresno et al., 2016). In conclusion, dust in animal houses is a reservoir of resistant and multidrug resistant *E. coli*. The analysis of isolates from stored dust samples showed that *E. coli* surviving more than 20 years allowed a look into the past concerning differences in resistance profiles associated to the origin of the dust samples.

Table 1. Results of the exact conditional logistic regression analysing the association between the resistant rates and animal groups for each antibiotic. Differences are significant when $p < 0.05$.

Antibiotic	AUG2	AMP	FOX	CHL	CIP	GEN	TET	SXT
P-value	0.0321	0.0001	0.4684	0.1698	0.2334	0.0043	0.0277	0.0001

Table 2. Significant pairwise differences ($p < 0.05$) of proportions of resistances between different animal groups.

Animal group	Animal group	Antibiotic	Odds ratio	95% confidence limits	P-Value
Fattening poultry	Laying hens	AUG2	0.094	0 – 0.480	0.0099
Fattening poultry	Laying hens	AMP	0.082	0. 012 – 0.408	0.0009
Fattening poultry	Pigs	AMP	0.053	0.008 – 0.263	< 0.0001
Pigs	Fattening poultry	GEN	0.069	0 – 0.426	0.0126
Fattening poultry	Pigs	TET	0.235	0.057 – 0.910	0.0341
Fattening poultry	Laying hens	SXT	0.022	0 – 0.111	< 0.0001
Pigs	Laying hens	SXT	0.033	0 – 0.186	0.0002

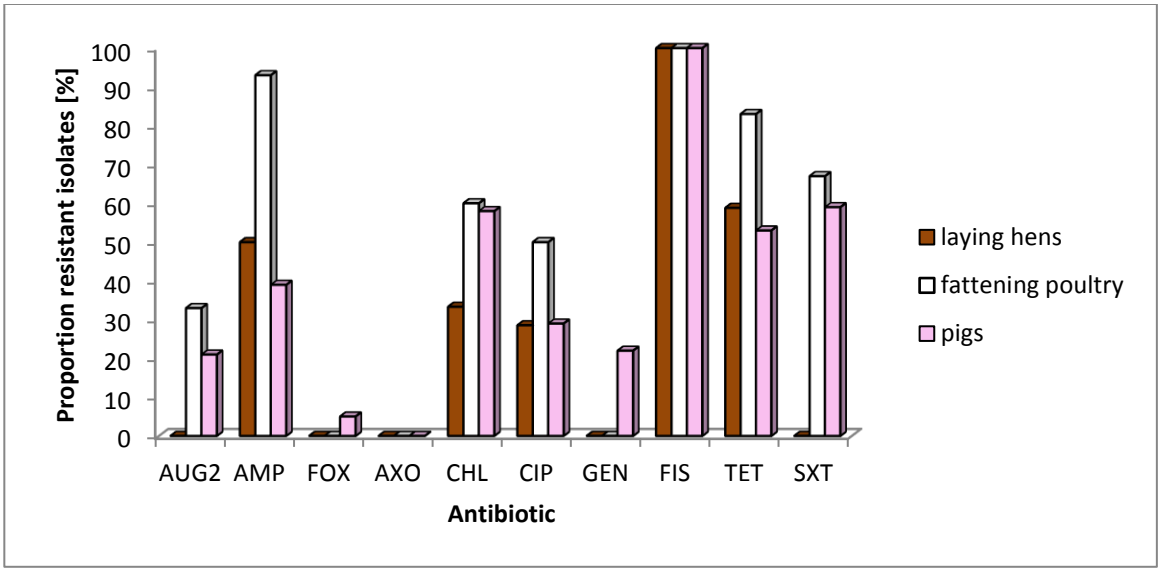

Figure 1. Proportion of resistant *E. coli* isolates from dust of laying hen houses, fattening poultry barns and pig barns.

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PHENOTIPIC CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DAIRY COWS WITH SUBCLINICAL MASTITIS IN SMALL DAIRY HERDS

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SUMMARY

The mastitis by *Sataphylococcus aureus* affects the production and milk quality. The objective was to determine the frequency of subclinical mastitis associated to *S. aureus* phenotypes in dairy herds. The Wisconsin test was performed in 527 milk samples from 19 herds at Toluca valley. Bacterial isolation was performed in blood agar McCokey, Vogel Jhonson with potassium tellurite at 37 ° C for 18 to 24 h. The hemolysin phenotypes was carried out on blood agar plates with erythrocytes of different species, in reduced atmosphere of CO_2 at 37ºC,as control *S. aureus* strain Wood 42. Antibiotypes were identified with the *in vitro* susceptibility test using the Kirby Bauer method, against *S. aureus* ATCC 25923 and disc units: penicillin 10 IU, ampicillin 10 µg, dicloxacillin 1 µg, gentamicin 10 µg, lincomycin 20µg. erythromycin 30 µg, kanamycin 30 µg, neomycin, 30 µg, cefotaxime 30µg, tetracycline 30 µg, novobiocin 30µg, spiramycin 100µg, sulfamethoxasol / trimethoprim 30µg.Capsular phenotypes type 5 and 8 were detected by serum agglutination test. Results were evaluated using the Chi-squarer test ($p < 0.05$). The overall frequency of *S. aureus* was 22.4% and coagulase negative *Staphylococcus* 8.5%. Other agents observed in minor proportion were *Streptococcus agalactiae* 6.2%, *Bacillus spp*., 5.2% ($p > 0.05$). *Micrococcus spp*., 3.8% ($p < 0.05$). The proportion of *S. aureus* isolates was similar in dairy herds. Wisconsin test reactions related to the isolation of *S. aureus* in the estimated somatic cells level $<100 \times 10^3$ / mL, were 8.5% and $> 2500 \times 10^3$ / mL were 45% ($p < 0.05$).The *S.aureus* phenotypes were higher for α and β toxins, lower proportion for αβ, αδ; the capsular serotype 8 was predominat in the herds ($p < 0.05$). Resistant antibiotypes often observed were ampicillin and penicillin 92.8% ($p < 0.05$). The S.aureus phenotypes identified in small dairy herds are compromising the udder and public health.

INTRODUCCION

Small holders family dairy herds is a predominant production system in the Toluca Valley *S. aureus* mastitis is considered a limiting factor in the production (Antunes, 2000;Manjarrez,2010). The transmission agent is increased by the conditions of handling and hygiene during milking (Baselga et al., 1994b).The detection of subclinical mastitis by *S. aureus*, allows establishing a causal association with the production environment and herd management (De Oliveira et al., 2000). The virulence factors has been considered the importance of hemolysins by its leucocidal and cytotoxic effect produced by α and β toxins (Velazquez,2010). The capsular exopolysaccharide of *S. aureus* present in the isolates of cases of mastitis are frequently related to the *S.aureus* capsular serotypes 5 and 8 (Sutra y Poutrel, 1990). Resistance and multiresistance of *S. aureus* strains to antibiotics in dairy herds constitutes an important public health alert (Lee et al., 2004). The study was conducted to identify *S. aureus*

phenotypes related to the somatic cell level estimated in the Wisconsin test in dairy cows in family-type production units.

MATERIAL AND METHODS

A cross - sectional study was carried out by means randomized sampling in 19 small family dairy herds in the mexican highlands, in the Toluca Valley in the municipalities of Toluca, Lerma, Tenango and Almoloya de Juárez. 366 milk samples of dairy cows were obtained in various stages of production, taking aseptically a sample of milk composed of the glandular quarters in a volume of 25 mL., deposited in a glass tube preserving at 4 ° C. La prueba de Wisconsin se realizó para obtener el número estimado de células somáticas presentes en la leche x 10^3 / ML (National Mastitis Council,2005). Isolation and identification of agents was carry out by spread 0.01 ml of milk on blood agar plates, Maconckey and Vogel Jhonson agar (potassium tellurium / 0.001 g / L), incubated at 37 ° C for 18 A 24 h. Colony forming units (CFUs) were described and identified by gram staining, coagulase, catalase *Staphylococcus* assays and Voges Proskauer. Mannitol in anaerobiosis, fermentation of maltose and trehalose. Coagulase-positive and coagulase-negative Staphylococcus isolates were identified with the Api-Staph system and gram-negative bacteria by Api-20E. The phenotypic characterization of hemolysin types was carried out on blood agar plates with erythrocytes of different species, incubated at 37 ° C in a reduced atmosphere of CO^2. As a control strains S. aureus wood 42 and Newbold 604. Identification of antimicrobial resistance antibiotics was carried out by the modified Kirby Bauer method using as a control the *S. aureus* strain ATCC 25923. *S. aureus* isolates were inoculated into the Muller-Hinton broth tubes incubated at 37 °C for 4 hours, a 0.5 turbidity of Mac Farland, on the Muller Hinton agar were transferred 0.01 mL., Uniformly distributed on agar plates with swabs to place unidisks 10 μg of penicillin, ampicillin 10 μg, dicloxacillin 1 μg, gentamicin 10 μg, kanamycin 30 μg, neomycin 30 μg, streptomycin 10 μg, cefotaxime 30 μg, lincomycin 2 μg, erythromycin 15 μg., Tetracycline 30 μg, novobiocin 30 μg, 100 μg spiramycin, 30 μg sulfamethoxasol / trimethoprim. The plates were incubated at 37 ° C for 18 to 24 hours. The bacterial growth inhibition was expressed in mm in wich was compared with the reference tables in order to consider the isolates as resistant, sensitive and intermediate. The phenotypes of capsular serotypes 5 and 8were identified according to the plaque agglutination test.The results were evaluated considering the observed frecuencies in the Wisconsin reactions and the in vitro sensitivity assessed using the Ji-squared test ($p < 0.05$) using the Microsoft Sytem 2010 Megastat complement software.

RESULTS

The frequency of isolation of *S. aureus* was 22.4%, in a lower proportion were coagulase negative *Staphylococcus* 8.5%. Other agents observed were *Streptococcus agalactiae* 6.2%, *Bacillus spp.,* 5.2%, *Micrococcus spp.,* 3.8%. The distribution of *S. aureus* isolates considering the estimated somatic cell level in the Wisconsin test. At the level of <100 x 103 / ml., It was 8.5%. The highest frequency of isolation of *S. aureus* was 45%, obtained at a level> 2500 x 103 / mL ($p < 0.05$). The distribution of isolates of *S. aureus* among the dairy family units of the technified type was not performed in the different municipal regions evaluated ($p > 0.05$). The toxin phenotypes α and β toxins were shows the larger proportion and a lower proportion αβ,αδ associations. Phenotypes of capsular serotypes 5 and 8 were widely distributed in dairy herds, predominating among them the phenotype of the capsular serotype 8 ($p < 0.05$). *S. aureus* antimicrobial resistant phenotypes for to ampicillin and penicillin were common 92.8% ($p < 0.05$), in contrast to the higher in vitro sensitivity of the bacterium for cefatoxin, sulfamethoxasol / trimethoprim, dicloxacillin, tetracycline, erythromycin, spiramycin neomycin and gentamicin.

DISCUSSION

In the dairy herds studied, was observed a high frequency of *S. aureus* infection in wich as the main causative agent of subclinical mastitis associated with *S.aureus* phenotypes with resistance to β-lactam antibiotics related to ORSA / MRSA strains (Gentillini *et al.*,2000). The presence of capsular exoplisaccharide affects phagocytosis and complement activity during intraglandular mammary infection,the exopolysaccharide capsular *S. aureus*, serotypes 5, 8 and NT in combination with the production of α, β, γ hemolysins (Baselga et al., 1994). Virulence factors Significantly increase the pathogenicity of *S.aureus* strains and the persistence of the infection. The infection of the mammary gland is favored by the conditions of handling and hygiene of the milking in the herds, due to the absence of measures of prevention and control of mastitis. In other hand the increase in the resistance of *S. aureus* to the antimicrobial drugs and the presence of the virulence factors phenotypes in the isolates obtained from the dairy herds studied, it is proposed the search of alternatives oriented to an improvement of the systems by means of the prevention, control and monitoring of *S. aureus* mastitis for to improve the quality and safety of milk and the economy production , the somatic cell level in milk estimated in the Wisconsin test in dairy herds showed a significant proportion of elevated reactions> 2500x103 cells / mL., by the exposure to the pathogens the production environment of dairy cows and the absence of strategies for prevention and control of mastitis (Makovec y Ruegg 2006). The virulence factors were related to phenotypes of *S. aureus* in association to α and β toxin increased the cytotoxic and leucocidal ability on neutrophils of the mammary gland they are favoring the development of the glandular infection (Velázquez, 2010). The high resistance expressed in the antibiotic implies a significant health risk of dissemination of MRSA.

CONCLUSSION

Subclinical mastitis in family-type dairy farms shows a high frequency related *S. aureus* infection associated with phenotypes that express the agent's virulence factors imply a risk to animal and human health.

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ESBL-PLASMIDS INTERFERE WITH BIOFILM FORMATION,

COMPETITIVE ADHESION AND SERUM RESISTANCE

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INTRODUCTION: ESBL-producing *E. coli* have become abundant all over the world and especially clonal lineages like ST131 and ST648 are of utmost importance. This study investigated the influence of ESBL-plasmids on non-resistance factors like biofilm formation, competition with commensals, competitive adhesion, serum resistance and motility of the host bacteria.

MATERIAL AND METHODS: Seven triplets of ESBL-carrying wild-type (WT) strains, their corresponding ESBL-plasmid-"cured" variant (PCV) and a complementary ESBLcarrying transformant (T) where analyzed in long-term colony, competition assays with commensals in co-cultures, competitive adhesion on IPEC-J2 cell lines, serum resistance, swimming motility, Biolog phenotypic microarrays, whole-genome sequence and RNA-sequence analysis.

RESULTS:
For some of the triplets we detected enhanced curli and/or cellulose production and a reduced swimming capacity of the WT and T strain compared to their PCV. RNA sequencing revealed the chromosomally-encoded *csgD*-pathway as a key factor involved. Biolog results pointed towards a similar metabolic behavior of WT, PCV and T. In addition, ESBL plasmids played protective role against serum bactericidal activity and did not no generally present a burden to the host in competition assays against commensal *E. coli* in co-cultures and adhesion assays on epithelial cell lines. For some of the strains carriage of ESBL-plasmids was even advantageous for competition.

CONCLUSIONS:
Our phenotypic and RNA sequencing results clearly indicate an influence of ESBLplasmids on non-resistance factors important for virulence and survival of the pathogenic strains, presumably contributing to their pandemic success.

DYNAMICS OF *STAPHYLOCOCCUS AUREUS* AND COAGULASE NEGATIVE *STAPHYLOCOCCUS* INFECTION IN DAIRY COWS DURING THE SUMMER-AUTUMN PERIOD

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SUMMARY. *Staphylococcus aureus* (*S aureus*) is the most important agent of mastitis in family dairy production units, affecting milk production and food safety of milk and dairy products. Coagulase-negative *Staphylococcus* (CNS) are currently considered of importance in the cronic infection of the mammary gland, emphasizing the persistence of infection associated with *S aureus*. The aim of the study was to evaluate the dynamics of *S aureus* and CNS infection in dairy cows with subclinical mastitis during summer to autumn period in a small scale dairy herd at Toluca valley, México. Thirty cows on the milking production line were studied. The California mastitis test was performed, milk individual samples were taken from the mammary gland quarters. 10 µl of milk were inoculated in blood-agar and Mannitol salt plates. The isolated were described and identified by hemolysis and coagulase, catalase, Voges Proskauer test and maltose, trehalose acid reactions and mannitol fermentation in anaerobiosis. The confirmation of *S. aureus* isolates and CNS identification were performed using the API Staph system. The results were evaluated using contingency tables and the Chi-square test ($p < 0.05$). The average infection rate for *S aureus* was 60.05% in contrast with 39.55% isolated CNS. During the summer to autumn period.The CNS identified in the herd were species such as *Staphylococcus epidermidis*, *S.chromogenes*, and *S. simulans* 31.6%, 47.4% and 17.5% respectively. The 3.75% of CNS species not were identified. During the study period, there was a variable tendency to increase CNS and the reduction of *S. aureus* infection in studied dairy cows.

Environmental pollution by animal production

REDUCTION OF GAS EMISSIONS FROM POULTRY PRODUCTION USING BIOFILTER WITH WATER CURTAIN

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SUMMARY

The aim of the study was to determine the reduction potential of gas emissions by filtration of air from indoor poultry housing. Determinations were made of the groups of gas compounds in unfiltered air from poultry housing and from using an air biofilter and water curtain with different mixtures that formed biofilter bed. The experiment used mixtures of 3 biofilter beds and air forced into these beds from climatic-respiration chambers, in which 600 Isa Brown laying hens were kept. The layers were kept on litter in groups, 50 birds per chamber in 3 replicates. The following mixtures were used as beds for biofiltration: peat (highly absorbent), chopped straw (relatively high absorptive capacity) and chopped tree bark (highly porous). The highest emission reduction (92%) was obtained for NH_3 (bed no. 2 contained 50% straw). NO_x and CO_2 reduction was highest for bed no. 1, which contained 50% peat. VOC, NO_2 and CH_4 reduction was not confirmed by statistically significant differences. The best filtration properties in the described biofilter were characteristic of the mixtures whose beds contained more peat, followed by those rich in straw, and the worst for those with sawdust. The range of biofiltration showed selective reduction associated with the chemical nature of the emitted compounds, which may suggest that not only the physical properties but also the chemical properties of the bed mixtures have a considerable effect on the efficiency of the ongoing pollution reduction processes.

Key words: GHG emissions, biofilter, poultry.

INTRODUCTION

Late 20th-century global climate change, which is characterized by an increase in the mean surface temperature of Earth, has drawn close attention of scientists. A special role in this process is played by livestock production, notably poultry farming (Atkinson et al., 1996). This is all the more important as farms are governed by a number of directives that not only regulate the quality of products but also determine and define the environmental impact of production. In addition to large quantities of meat and eggs, farms with a high concentration of birds produce excreta as well as emitting large amounts of ammonia, carbon dioxide, nitric oxides, and methane (Rotz, 2004; Kim and Patterson, 2003; Kristensen et al., 2000; Wiliams et al., 1999; Wathes, 1998). Therefore, all attempts to minimize the impact of poultry farms are considered a major area of research in poultry production technology.

MATERIAL AND METHODS

The experiment used mixtures of 3 biofilter beds based on peat (1), straw (2) and sawdust (3), as well as air forced into these beds from climatic-respiration chambers, in which 600 Isa Brown layers were kept. Birds were fed according to Polish poultry feeding standards (2005) and the farm feeding scheme, with constant access to water. The experiment used the litter management system, in which each group of layers occupied a separate climatic-respiration chamber with optimum microclimate based on zootechnical standards. The study was performed in three replications from May to August, from September to December, and from January to April. Exhaust gases were biofiltered with the following mixtures: (1) 50% peat, 25% chopped straw and 25% sawdust; (2) 50% chopped straw, 25%

peat and 25% sawdust; (3) 50% sawdust, 25% peat and 25% chopped straw, which were placed in the biofilter to which mechanically exhausted air from the building was supplied. In the biofilter, water from the curtain circulation system was flowing through the beds, thus eliminating the molecules of dissolved chemical compounds. Prior to use in the biofilter, the bed (0.9m × 0.9m × 0.9m) with experimentally determined composition was experimentally conditioned and stabilized for microflora. During the replications, the following measurement data were collected: air temperature in climatic-respiration chambers, supply ducts and exhaust ducts; relative humidity in climatic-respiration chambers, supply ducts and exhaust ducts; air speed in climatic-respiration chambers, supply ducts and exhaust ducts; concentrations of VOC, ammonia, nitric oxides, methane and carbon dioxide in climatic-respiration chambers, supply ducts and exhaust ducts, and at the exit of biofilter with water curtain; and composition and conditioning of biofilter beds.

RESULTS

Based on the results presented in Table 1, it was concluded that the filter bed mixtures used in the water curtain biofilter showed the largest, highly significant difference in reducing NH_3 and NO. NH_3 emission was reduced the most (92%) by mixture 2, which contained 50% straw, 25% peat and 25% sawdust, followed by mixture 3, composed of 50% sawdust (86% reduction). The most efficient in reducing NO emission were mixtures 3 (50% sawdust) and 1 (50% peat). These mixtures reduced NO emission by almost 87 and 88%, respectively. The highly significant reducing properties of all the three mixtures for NH_3 and NO were not observed for NO_2, because none of them reduced the emission of this gas. The reducing efficiency of mixtures 1 (50% peat) and 3 (50% sawdust) was reconfirmed for the emission of NO_x, again with a highly significant difference in limiting the emission of this oxide in relation to the control group (77 and 76%, respectively). In the case of CO_2, only mixture 1 slightly reduced the emission of this gas. For VOC and CH_4, no emission reduction was observed in relation to the control group.

Tab. 1. Emission rate of chemical compound groups from layer management following the use of biofilter with water curtain (kg/bird/year)

Group of compounds	**Group/Biofilter with water curtain**			
	No filtration	**(1) biofilter bed mixture**	**(2) biofilter bed mixture**	**(3) biofilter bed mixture**
VOC	0.0032a	0.0032a	0.0031a	0.0032a
NH_3	0.037A	0.012B	0.003C	0.005C
NO	0.0086A	0.0011B	0.0041C	0.0010B

NO_2	0.0010a	0.0011a	0.0015a	0.0013a
NO_x	0.0096A	0.0022B	0.0056C	0.0023B
CH_4	0.042a	0.041a	0.042a	0.042a
CO_2	14.2a	13.5b	15.3a	15.5a

DISCUSSION

In terms of the reduction of gas emissions through biofiltration in layer management, this method proved highly efficient when biofilter with water curtain was used for three gases: NH_3, NO and NO_x, but the efficiency was dependent on the type of filter bed (Tymczyna et al., 2007 a, b, 2004; Hartung et al., 2001; Atkinson et al., 1996). In the case of the other groups of compounds, it is worth noting the lack of reduction of nitric oxides with an oxidation state of +4 as well as CH_4 and VOC, and also the slight reduction of CO_2 by the filter bed mixtures. This problem requires further study, although the literature suggests that complete elimination of, for example, VOC molecules would be inadvisable because they often serve an informative function for a species (e.g. pheromones) (Mayrhofer et al., 2006; Stuetz and Nicolas, 2001). In the case of the biofilter with water curtain, which was used in laying hens and, in an earlier study in fattening pigs, it is worthwhile noting selectivity of the filtering, which contributes to reducing different groups of compounds, as confirmed for poultry emission research conducted by Cooperband and Middleton (1996) and Goyal et al. (2005). Mixture 1 (50% peat) caused the greatest reduction of NO_x (0.0022 kg/bird/year) and CO_2 (13.5 kg/bird/year). Mixture 2 (50% straw) reduced NH_3 emission the most, down to 0.003 kg/bird/year. Mixture 3 minimized NO emission to 0.0010 kg/bird/year. Thus, the efficiency of reducing different groups of chemical compounds present in poultry management, is determined by different mixtures of the biofilter bed with water curtain. Variation in the composition of the mixtures, which was considered when they were formulated, accounts for a considerable importance of its absorptive capacity, i.e. physical properties, as is the case for peat, which is able to absorb nitrogen (McCrory and Hobbs, 2001), and, indirectly, the possibility of using the mixture components by microflora as a nutrient (Tymczyna et al., 2007). The literature reports the need to maintain a high C/N ratio, similarly to the material intended for composting (Goyal et al., 2005; Tiquia, 2005; Bicudo et al., 2002; Barrington et al., 2002; McCrory and Hobbs, 2001). Although straw has a relatively good absorptive capacity, it is not porous enough, and the carbon it contains is not as easy to use as a substrate by the microorganisms. Hence the use of mixed substrate as a biofilter bed, which allows its natural characteristics to be used (Cloirec et al., 2001). Apart from the physical properties of the filter mixture components, an essential role in selectivity is played by their chemical properties, which influence the reduction rate of selected chemical compounds from poultry operations (Cooperband and Middleton, 1996). Based on the present findings, the following generalizations concerning the reduction of gas emissions from layer production through the use of water curtain for air biofiltration can be made: the use of the experimental biofilter proved effective in reducing the emission of three gaseous compounds: NH_3, NO and NO_x. The best filtration properties were shown by the mixture high in peat, which was most effective in reducing the emission of two gases (NO_x and CO_2). Less efficient filtration was ensured by the mixtures with a high straw (NH_3) and sawdust (NO) content.

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REDUCTION OF BIOAEROSOLS IN EXHAUST AIR OF A BIOFILTER BY REGULATION OF THE MOISTURE CONTENT

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SUMMARY: The air of pig barns contains large amounts of bioaerosols. Harmful bioaerosols may be transmitted via the exhaust air to neighbouring animal houses and residential dwellings. Exhaust air washers combined with a biological cleaning stage are used to prevent harmful bioaerosol emissions. However, the reduction efficiency of the biological stage is probably depending on the moisture content of the material. In a current study the effect of controlled moisture content in the biological stage on the reduction of bioaerosols is investigated. This paper reports on preliminary results.
The study is conducted in a pig barn with a stock of 840 fattening pigs. The exhaust air is cleaned by a three stage system consisting of a physical, a chemical and a biofilter. The biofilter is divided in two parts. One contains wood chips, the other part contains paper pads. The moisture content of the biological cleaning stage is controlled by TimeDomainReflectrometry. Air samples were taken before the first (physical filter), behind the second (chemical filter), and behind the third (biofilter) filter by using emission-impingers according to a Verein Deutscher Ingeneure (VDI) guideline (4257 part 2). Impinger solutions were analyzed to determine the total plate count (TPC) of mesophilic bacteria, the number of fungi and the concentrations of endotoxins, so far.
Preliminary results showed that moistured filters with a water content of approximately 10% to 25% by weight reduced the TPC from $1.3x10^5$ cfu/m³ to $2.4x10^4$ cfu/m³ in the treated air behind both filter materials. Furthermore, a reduction of fungi, streptococci, MRSA and endotoxins was detected. However, the optimized moisture content of filter materials to reduce bioaerosols is not known yet and it seems that outdoor temperature and solar radiation influences the moisture content of both filter materials significantly

Key words: Bioaerosols, Impingement, Moisture

INDRODUCTION

To prevent harmful bioaerosols from getting into the environment by the exhaust air of pig barns, implementation of air washing technology is legally required in some regions. One solution for air cleaning is a three staged air washer with a biological cleaning stage. It is known, that the biofilter is able to prevent harmful bioaersol emission. A former study showed that the reduction efficiency depends on the moisture content in the biological stage in composting facilities with different materials (Mäule and Fischer, 2004). However, it is not known which optimal moisture content is needed to keep the bioaerosols most efficiently out of the exhaust air from pig barns. At least one reason for this is that monitoring the moisture content in inhomogeneous material is difficult. It is suspected, that there is a correlation between the moisture content in the biological stage and the reduction of bioaerosols, too. Therefore, in this current study the concentrations of bioaerosols in untreated air and exhaust air of one pig barn are analyzed at different conditions. Simultaneously, the moisture content is detected permanently with a special TimeDomainReflectrometry-system in two different materials. An additional idea is to detect the external influences on the moisture content.

MATERIAL AND METHODS

The investigation was conducted in a forced ventilated pig barn with a three staged system for air cleaning (MagixX-P, Big Dutchman, Germany). The stock of maximum 840 fattening pigs (Danzucht x Duroc) in two compartments varies considerably during the sampling period, which was considered in the analysis. Samplings were carried out on nine days from June till December 2016, yet. The whole biofilter gets moistured by sprinkling water in different intervals of sprinkling and recess. The moistening varies over the seasons and during the day. Overnight with a basic setting of ten seconds sparkling and 3,600 seconds recess alternating. From April till June (section A) in the morning the interval of sparkling is ten second longer and in the afternoon 15 seconds longer than the basal settings. From July till September (section B) and from October till December (section C) in the morning the interval of sparkling is extended for five seconds. In the afternoon in sections B the interval of sparkling is extended for ten seconds and in section C for five seconds. In section A two samplings were taken, in section B three and in section C there were four sampling days.

Air samples were taken from three locations of the air cleaning system: in the untreated air before the physical filter, behind the chemical filter and behind the biofilter. The biofilter is divided into two parts: one containing wood chips, roughly torn root wood from conifers (Hempelmann gmbH; Germany), and the second containing paper pads (CELdec® 7060-15, Munters, Sweden). The samples were taken on special points in compliance with the VDI guideline (4257 part 2). According to these directions, emission-impingers (Laborglas Keiner, Mühlheim an der Ruhr, Germany) were used to sample bioaerosols. The samples were brought to the laboratory within 3 hours at a temperature of 4-6°C and then stored overnight in a fridge at the same conditions.

Temperature and humidity were measured with thermo-hygrometers (Date logger Hydrolog-D HygroClioS Temperatur /RH; Rotronic GmbH, Germany) and Infralog Rugged Visual loggers (Driesen und Kern, Germany) with external sensors). Weather data was documented by a metrological station (Wetterstation Uniklima 7, TOSS, Germany.

The moisture content of wood chips and paper pads was gathered by a special TimeDomainReflectometry (TDR) measuring system called TAUPE, which is an electromagnetic determination system for continuous monitoring of moisture even in non-homogenous material (M. Stacheder et al, 2009). Four sensors were installed parallel in the biofilter over the entire area, two 60 cm and 200 cm about the floor, each one half in paper pads and the other in wood chips. These sensors are called p 1-4 and w 5-8.

In the lab the impinger's inlet tubes were rinsed with 10 ml of sterile phosphate buffer Sorensen into the sampling vessel, filled with 30 ml of the same buffer before sampling. The samples were edited and analyzed according to the VDI guideline 4253 part 3, without accumulation of fungi by filtration. Measurement parameters were TPC of mesophilic bacteria (tryptic soy agar; 36° C, 48 h, aerobically) and contents of endotoxins. For analysis of bacterial endotoxins the limulous amoebocyte lysate test (Lonza, USA) was used after storing an aliquot of the samples at – 18° C overnight.

Statistical analyses with SAS software version 9.3. (SAS Institute Inc., Cary, NC, USA) were used to look for significances in the data about the data of bacteria, fungi and contents of endotoxins, up to now. Because of small sample numbers the data was first tested for normal distribution by Univariate Shapiro-Wilk-test and proved to be not normally distributed. Therefore, the Wilcoxon Signed Rank-test was used for non-parametric statistic in the data analysis. A P-value of less than 0.05 was considered to be significant.

RESULTS

The moisture content in the biofilter is given in uncalibrated water content in % by weight. On the nine sampling days the moisture content was between 9 and 22% by weight. There were differences in the moisture content between paper-pads and wood-chips. Nevertheless in both parts of the biofilter it was shown_ that the material was moister inside at the button than outside at the top. This effect was higher in the paper pads. The moisture content in paper pads increased faster than in wood chips. In section A of moistening both materials were dampest. In section B the whole biofilter was moister than in section C. The

differences in the moisture content between the separate positions in the biofilter were larger in section B and C than in section A.

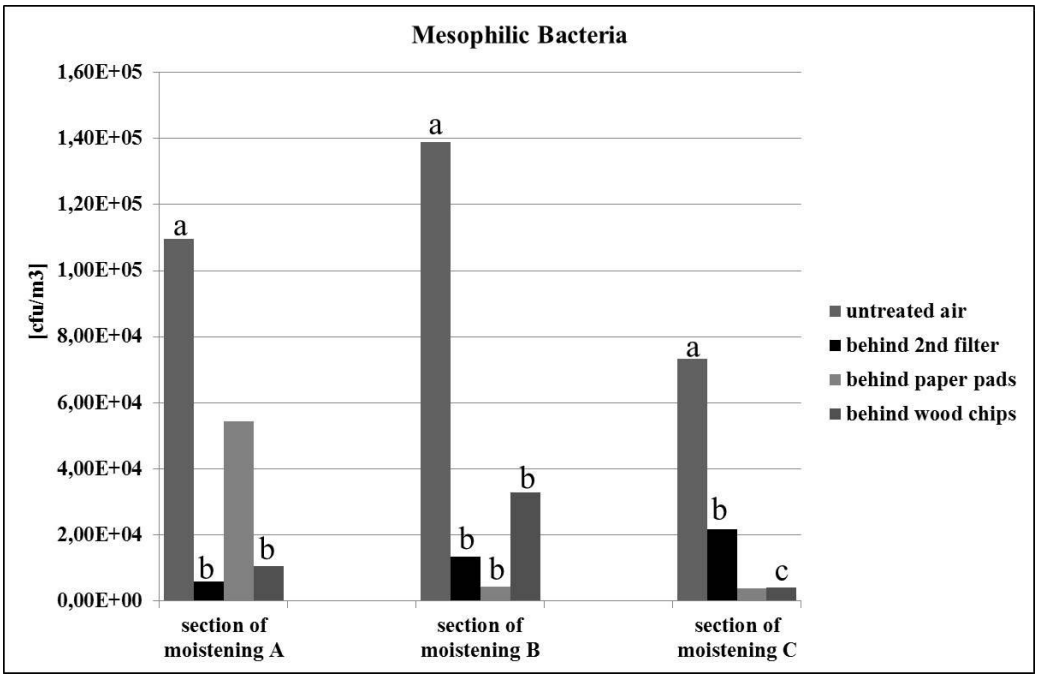

Figure 1: Averaged contents of the TPC of mesophilic bacteria on different samplings points in the three sections of moistening; Significances are marked with a, b and c

The results of all nine samplings in 2016 showed that the TPC of mesophilic bacteria was in range, with the TPC of the first five preliminary samplings, mentioned in the summary.
The content of mesophilic bacteria in the untreated air was $1.03x10^5$ cfu/m³ in the arithmetic mean. Samples from behind the second filter showed an averaged TPC from $1.55x10^4$ cfu/m³, behind the paper pads 1.53 $x10^4$ cfu/m³ and behind the wood $1.52x10^4$ cfu/m³.
The content of endotoxins was in the range of 50 EU/ml in untreated air in section A of moistening, less than 20 EU/ml in section B and less than 5 EU/ml in section C.
In section B of moistening there was a significant reduction of mesophilic bacteria when untreated air had passed the first and second filter. The reduction of the contents of endotoxins was significant in section A and B of moistening. The reduction of the mesophilic bacteria passed after the air had and the paper pads section wood chips, for In cases B and C of moistening, was the air significant, too. biofilter of the contents of endotoxins the lower reduction after three had passed the was also significant but than in both materials in all sections of

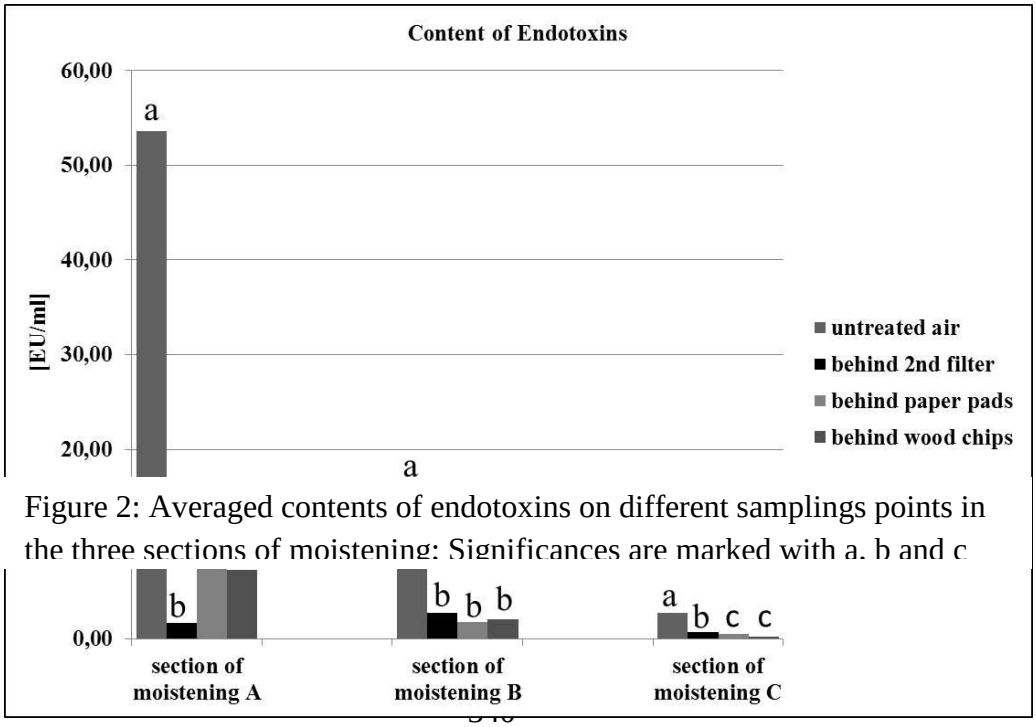

Figure 2: Averaged contents of endotoxins on different samplings points in the three sections of moistening; Significances are marked with a, b and c

moistening. The number of fungi increased behind the paper pads. The number of fungi in the air behind the second filter increased as well. In section A of moistening there was a reduction in the number of fungi behind the wood chips, but it was not significant. The number of fungi increased in the sections B and C behind wood chips, too. In Section C of moistening the reduction of mesophilic bacteria and the content of endotoxins were significant between the air from behind the second filter and those behind the biofilter in both materials.

DISCUSSION

The results of the TDR-measurements regarding to the moisture content showed that paper pads possess a higher water absorption capacity in less time than the wood chips. However, the water storage capacity of paper pads is less than in wood chips, so that the moisture content drops faster again. The differences in the moisture content from the inner and outer part of the biofilter can be attributed to the influences of the climate and gravitation. While inside the temperature and humidity of air is very similar all the time, the climate outside follows the climatic conditions: temperature and humidity of air, evaporation, sunshine hours and rainfall. Gravitation seems to be the reason for the lower parts of the whole biofilter being moister. All these influences lead to a decoupling effect from the inner and outer part of the biofilter in both materials, which is larger in the paper pad. But these effects are just analyzed by descriptive statistic and further analyses will follow.

The reduction of mesophilic bacteria and the contents of endotoxins in the air before the air washer, behind the second filter and explicitly behind the biofilter varied widely and seemed to depend on the different sections of moistening. In case of the both analyzed parameters in section C of moistening, there is a significant reduction between the air from behind the chemical filter and the cleaned air behind paper pads and wood chips. This indicated that the reduction might depend on the moisture content of the biofilter. The results showed that the biofilter has no capacity of significantly reducing fungi under the present circumstances. This was also described in a former study of Mäule und Fischer (2009). There seemed to be even some differences in the reduction efficiency between paper pads and wood chips by filtrating fungi. These differences seemed to depend on the different moisture content, but to proof this there have to be some more investigations at different conditions. All in all the reduction efficiency for fungi is not regarded as probable as for bacteria and endotoxins. Therefore there have to be more analyzes under specific conditions of moistening.

It is also important to determine the actual values of moisture contents, which are still uncalibrated up to now, for an assessment for optimized settings in three staged air washers.

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NATURAL FERTILIZERS AS A SUBSTITUTE FOR MAIZE SILAGE IN AGRICULTURAL BIOGAS PRODUCTION

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SUMMARY

Agricultural biogas production is one of the energy uses of biomass, providing an opportunity to reduce the harmful effects of natural fertilizers on the soil, water and air. In the European Union, maize silage and slurry is the most common feedstock for biogas production. The aim of the study was to formulate fermentation beds in which part of maize was replaced with natural fertilizers derived from different livestock species. The experiment used 8 mixtures of substrates containing variable proportions of solid natural fertilizers collected from dairy cows, laying hens, mink, and foxes; maize silage; and cattle slurry, to obtain appropriate dry matter content ensuring the flow of substrate. The control group was a substrate composed of maize silage and cattle slurry alone.

Chemical analysis of the individual components was followed by formulation of substrate mixtures to ensure C/N ratio of 26:1 and dry matter content of 13%. The mixtures were fermented using microfermenters equipped with substrate dispensers and mixers, corresponding to wet fermentation. Fermentation was held under anaerobic mesophilic conditions (37°C) with complete control over the direction and parameters of the ongoing processes (pH, amount and composition of biogas, etc).

The results obtained showed that natural fertilizers could be a partial substitute (up to 21%) for maize silage in methane fermentation. The best results in terms of methane yield per unit of substrate organic dry matter (ODM), no different from the utilization of maize silage (330.3 m^3 CH_4/ton of ODM), were obtained for fox manure (304.1 m^3 CH_4/ton of ODM) and chicken manure (298.4 m^3 CH_4/ton of ODM). Except for the mixtures higher in chicken manure, all the other mixtures had similar methane production rates (MPR) as the control group.

Key words: GHG, agriculture, reduction, RDP

INTRODUCTION

The energy value of biomass available in Poland is estimated at 800 PJ. Biogas production is one of the elements of the energy use of biomass. This term is defined as the biodegradable fraction of products, waste and residues from agriculture (including vegetal and animal substances), forestry and related industries, as well as the biodegradable fraction of industrial and municipal waste (Directive 2001/77/EC). Research on the kinetics of biogas production have led to a conclusion that carbohydrate substrates are used more rapidly than fats. It was hypothesized that natural fertilizers used as a substrate for methanogenesis, can replace maize silage without considerably reducing process efficiency.

MATERIAL AND METHODS

The experiment used 8 mixtures of substrates (3 kg each) containing variable proportions of solid natural fertilizers collected from dairy cows, laying hens, mink, and foxes; maize silage; and cattle slurry, to obtain appropriate dry matter content ensuring the flow of substrate. The control group was a substrate composed of maize silage and cattle slurry alone.

Chemical analysis of the individual components was followed by formulation of substrate mixtures to ensure C/N ratio of 26:1 and dry matter content of 13%. Composition of the substrate mixtures is shown in Table 1. The mixtures were fermented using microfermenters equipped with substrate

dispensers and mixers, corresponding to wet fermentation. Microfermenters were fitted with flow meters for gases such as methane, oxygen, carbon dioxide, and hydrogen sulfide. Fermentation was held under anaerobic mesophilic conditions (37°C) with complete control over the direction and parameters of the ongoing processes (pH, amount and composition of biogas).

Each substrate was fermented for 36 days at a constant temperature of 37°C, with continuous collection of biogas from the microfermenters. Single-stage fermentation (without hydrolysis and additional fermentation) was used. The experiment was conducted in 3 replications.

RESULTS

As regards the pH of substrate mixtures, significant differences were found between two groups of substrates (Tab. 1). A markedly more acidic pH was characteristic of the cow manure mixtures and the control mixture (from 5.24 to 5.48). The other groups of mixtures had a pH in excess of 6.0, while the pH of poultry manure substrates can be considered as neutral (6.35 and 6.34). However, the differences were not sufficiently high to modify the methane fermentation process, as evidenced, among others, by the final digestate pH, which fell within the neutral range (Tab. 3). All of the substrates had a dry matter content of 13.3 – 14.07%. Significant differences did appear for the content of organic dry matter (ODM) (Tab. 1). Total nitrogen content in the substrate mixtures was strongly influenced by supplemental manure, especially that from fur animals (Tab. 1). These groups showed a significantly higher level of total nitrogen (e.g. 4.32%, mink manure group 2) in relation to all the other groups. Total nitrogen content may be the limiting factor of methane fermentation as one of two components of the C/N ratio, for it accounts for the microflora nutritional requirements and may range between 80 and 7 (Schröder and Uenk, 2006). However, the optimum figure is 20-30, which results in an almost 80% methane biogas content. Chemical oxygen demand (COD) is a parameter used to assess water quality, or the pollutant load of wastewater or liquid natural fertilizers. In the case of the substrate mixtures for methane fermentation, the studied groups did not differ significantly in this respect. COD value closest to unity was obtained for the control mixture from maize and slurry (1.0).

As regards the course of fermentation (Tab. 2), the highest methane concentration in biogas was determined for mink manure (65.1 and 63.6%) and fox manure groups (63.5%). In production practice this is a very high value that determines the possibility of further treatment of biogas for use in the gas network or in the means of transport (Abatzoglou and Boivin, 2009). The second most abundant gas in biogas is carbon dioxide. In the control groups, this concentration showed significant differences and assumed the highest values in the control group (41.4%) and in the groups high in poultry manure and cow manure (40.4 and 40.3%, respectively). In the case of hydrogen sulfide, all such groups differed significantly between one another, and highly significantly only within the mink manure mixtures. A similar situation occurred for the concentration of ammonia (266 ppm), but the differences were lowly significant. Most of this gas was found during fermentation of mink manure (843 ppm) and fox manure (825 ppm). The highest productivity of biogas per ton of dry matter was obtained from the control substrate (511.4 m^3). This value was highly significantly higher compared to the other substrate groups. The least biogas was obtained per DM unit for the mink manure mixtures (301.4 and 298.0 m^3) and the high poultry manure mixture (307.9 m^3). A better description of the methane-producing properties of substrate is offered by methane yield per ton of organic dry matter. In this regard, again the highest value was obtained for the control group (330 m^3 CH_4). A reduction of substrate dry matter during fermentation is the result of their "degassing". Indirectly it may reflect the amount of ethane produced, more specifically the biogas itself. The highest DM reduction was characteristic of the fox manure mixture (69.10%), the control mixture (66.84%) and the mixture with a higher content of mink manure (67.88%). Of similarly limited use are the last two indicators of the fermentation process, namely reduction in chemical oxygen demand and methane yield in terms of reduced COD (Tab. 2). They are

more useful with regard to environmental protection when the substrates contain effluents or other waste unsuitable for use in agricultural biogas plants. The highest reduction concerned the fox manure substrate (72.08%) and the low poultry manure substrate (66.03%).

Table 1. Average physicochemical parameters and composition of substrates

Component	Type of substrate							
	Poultry manure 1	**Poultry manure 2**	**Cow manure 1**	**Cow manure 2**	**Mink manure 1**	**Mink manure 2**	**Fox manure**	**Maize**
pH	6.40a	6.35a	5.15b	5.45b	6.18a	6.03a	6.02a	5.48b
Dry matter [%]	13.77	13.57	13.23	13.03	13.23	13.70	14.47	13.27
Organic DM [% DM]	81.63a	81.17a	87.33b	86.83b	79.10c	78.67c	76.43d	88.87b
Ash [% DM]	18.83aA	18.37aA	12.67bB	13.17bB	21.33cC	20.90dC	23.57eD	11.13fE
Total nitrogen [% DM]	3.44aA	3.73aA	2.45bA	2.55bA	4.13acA	4.32cA	3.78acA	1.79dB
Ammonium nitrogen [% DM]	1.50aA	1.8aA	0.87bB	1.04abAB	1.69aA	1.9aA	1.02abAB	0.64bB
COD [g O_2/g DM]	0.93	0.84	0.87	0.95	0.82	0.92	0.81	1.00
Total phosphorus [mg/kg DM]	4630aA	6912.3bB	5932abAB	6562bB	7912.3cC	8112.3C	7512.3cC	1748eD

ab – significant differences at P≤0.05; AB – significant differences at P≤0.01

Table 2. Basic average parameters of substrate methane fermentation

Component	Type of substrate							
	Poultry manure 1	**Poultry manure 2**	**Cow manure 1**	**Cow manure 2**	**Mink manure 1**	**Mink manure 2**	**Fox manure**	**Maize**
CH_4 [%]	60.2a	59.7a	58.5 ab	58.3ab	65.1c	63.6d	63.5d	57.4b
CO_2 [%]	38.3 a	40.4b	39.5ab	40.3b	32.3 c	35.7d	36d	41.4b
O_2 [%]	0.3aA	0.2bA	0.3 aA	0.2bA	0.6 cB	0.3aA	0.2bA	0.2bA
H_2S [ppm]	279aA	324bA	330bA	389cB	532dC	654eD	743fE	155gF
NH_3 [ppm]	403a	564b	554b	579b	760b	843c	825d	266e
Average MPR [m^3 CH_4/m^3d]	0.87a	0.67b	0.78ab	0.74ab	0.7ab	0.7ab	0.88a	0.92ac
Biogas yield per ton of DM [m^3]	404.6aA	307.9bB	408.5aA	369.4cC	301.4bB	298dB	366.1cC	511.4eD
Methane yield per ton of ODM [m^3]	298.4a	226.4b	275.2b	246.8b	248.0b	240.9b	304.1a	330.3a
Reduction of DM [%]	49.89aA	57.25bA	53.14cA	60.09bcA	62.21cAB	67.88dB	69.10dB	66.84dB
Reduction of COD [%]	66.03aA	58.06bB	57.37bB	38.59cC	57.0b8B	57.54bB	72.08dD	58.57bB
Yield [dm^3 CH_4/kg reduced COD]	0.441aA	0.341bB	0.437aA	0.727cC	0.420aA	0.360bB	0.397abAB	0.499aA

ab – significant differences at P≤0.05; AB – significant differences at P≤0.01

DISCUSSION

It is not easy and conclusive to assess the usefulness of substrate mixtures (with natural fertilizers, with a higher content of natural fertilizers, and with a lower content of maize silage) for methanogenesis. One should consider, on the one hand, the volume of methane obtained as an energy carrier, and, on the other, the possible environmental effect of using fur animal manure, because the latter has no legal status as a natural fertilizer and should be treated as waste. Finally, one should consider the competitiveness of maize as energy source compared to its use in ruminant feeding, as well as the economic aspect, namely the cost of methane production (Kowalczyk-Juśko et al., 2009; Laaber, 2011). In terms of methanogenesis efficiency, equally good results as for maize were obtained by using fox manure as well as a low proportion of poultry manure in the substrates. In this way, silage proportion was reduced from 40 to 21 and 17%, respectively. However, can the other mixtures, the use of which reduced the process yield by about 30% in relation to ODM, find practical application? Although the yield of methane is lower in them, its concentration in biogas exceeds 58% for such substrates. To reduce the costs, many agricultural biogas plants accept methane concentrations of 45%, which is the minimum needed for co-generators to work (Resch et al., 2006). In addition, apart from the substrate high in poultry manure, all the others did not differ in average MPR, which is a very important parameter for biogas plant efficiency. As indicated by market analyses, maize silage prices gradually increase, which locally may discredit its use for energy co-generation. Therefore, the European Union shows second and third generation of energy sources that are not in competition with their use in feeding and as a feed (Sunada et al., 2012). Based on the present study, the results obtained and their analysis, several conclusions must be made concerning the possibility of using natural fertilizers in the substrate mixtures for methane fermentation.

1. Natural fertilizers may act as an effective substitute for maize silage in methane fermentation conducted in agricultural biogas plants while retaining the proper C/N ratio of 26.
2. Best results in terms of methane production per ODM unit of the substrate, which matched those of maize silage, were obtained for fox manure and poultry manure.
3. Except for the mixture with a higher content of poultry manure, all the other mixtures, in terms of MPR, had a comparable methane yield per unit of substrate.
4. The product obtained from methane fermentation of the mixtures with natural fertilizers is characterized by high fertilizer value, in terms of both nitrogen and phosphorus content.

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INFLUENCE OF TANNINS EXTRACT ON PRESENCE OF *Escherichia coli* IN FAECES OF FEEDLOT CATTLE

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SUMMARY. High concentration of bovines confined in large feedlots and dairy farms implies risk of spread to environment large amount of bacteria as *E. coli*. Several studies *in vitro* suggest that tannins can inhibit *Escherichia coli* growth. This research was performed to determine the influence of hydrolysable and condensed tannins on presence of *Escherichia coli* in faeces of feedlot cattle. Two experiments were conducted. Experiment 1, faecal samples from 20 finishing bullocks were pooled, divided in three sub-samples and randomly assigned to treatments as follows: 1) No addition (CTRL); 2) Mixed with 0.6 % of condensed tannins (CT); or 3) Mixed with 0.6 % of hydrolysable tannins (HT). Faeces were exposed to environment during 0, 24, 48 and 72 h, incubated in a selective medium for *E. coli* and after 24 h, CFU/g was accounted; results were analysed by ANOVA for a completely randomized design (CRD). Experiment 2, thirty bull calves were randomly assigned to treatments as follows: 1) fed a growing diet (CTRL); 2) CTRL supplemented with 0.6% of CT; or 3) CTRL supplemented with 0.6 % of HT. After 28 d, faecal samples were incubated in a selective medium for *E. coli*, and CFU/g was accounted and analysed by ANOVA for a CRD. Experiment 1, tannins x exposition time interaction was significant (P = 0.08); *E. coli* CFU/g were similar (P > 0.20) across treatments within the first 24 h, however, at 48 and 72 h of environment exposition, CT decreased (P = 0.08) *E. coli* account in relationship to CTRL. Experiment 2, tannins supplementation had not effect on *E. coli* (P = 0.20). These results suggests that condensed tannins are able to decrease *E. coli* account when added directly to bovine faeces, however, when CT are offered the presence of *E. coli* in faeces of bovines is not affected.

Key words: Bovines, *Escherichia coli*, faeces, Tannins.

INTRODUCTION

Plant tannins are natural polyphenolic compound produced by secondary metabolism of a wide variety of plants (Frutos *et al*., 2004). The biological role of tannins in plants is related to protect against infection, insects or herbivores (Khanbabace and Van Ree, 2001; Duval and Averous, 2016). Their antimicrobial activity has been the focus of research in several fields, as pharmacology and animal nutrition (Smith *et al*., 2003). In some *in vitro* studies (Chung *et al.*, 1998; Hoshino et al., 2000), both condensed and hydrolysable tannins inhibit *Escherichia coli (E. coli)* growth. Some researchers considered exploring the possibility of a reduction of *E. coli* presence in cattle faeces using tannins (Wells *et al.*, 2005; Min *et al*., 2007; Gutierrez-Bañuelos *et al*., 2011), which has been due to that *E. coli* is regular inhabitant of the digestive tract in bovines (Callaway *et al*., 2009), and high concentration of bovines confined in intensive facilities, as large feedlot and dairy farms, mean high amount of manure produced (Dungan, 2010). The objective of this study was to evaluate the influence of hydrolysable tannins and condensed tannins extract on the presence of *E. coli* in faeces of feedlot cattle.

MATERIAL AND METHODS

Two experiments were conducted at the Experimental Station of the feedlot yard "Ganadera Los Migueles"; and in the laboratories from Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, Mexico (24° 51' N, 107° 26' W, 57 m a.s.l.).

Experiment 1. Faecal samples were taken from rectum of 20 finishing bullocks (415.6 ± 53.8 kg) housed in five corrals (n= 5/corral). Faeces obtained from the five bullocks in a same pen were blended to obtain one composite sample per pen. Then, the pen-composite samples were split into three sub-samples of 80 g and randomly assigned to 3 treatments as follows: 1) Faecal sample without tannins extract addition (Control); 2) Control plus 0.6 % of hydrolysable tannins extract (HT); and 3) Control plus 0.6 % of condensed tannins extract (CT). Hydrolysable tannins extract from chestnut (*Castanea sativa*) was delivery as NutriP (Silvateam; San Michele Mondavi, Italy); and condensed tannins extract from quebracho tree (*Schinopsis balansae*) was provided as Bypro (Indunor, S. A.; Buenos Aires, Argentina). Faeces and tannins in each treatment were thoroughly mixed and then were placed directly on the soil of the cattle management aisle sited, in the back side of its respective pens. Aliquots of 1 g de faeces of each repetition (3 treatments by 4 pens) were taken at 0, 24, 48 and 72 h of exposure to the environment. Faeces collected were placed in a culture tube and diluted in 9 ml of buffered peptone water (DPW; Becton, Dickinson and Company, Sparks, MD). Then, 1 ml of primary dilution was taken and dissolved again in 9 ml of buffered peptone water (Wells *et al.,* 2005; Varel *et al*., 2008). Finally, 100 µl of the decimally diluted sample were spread by the extension method in Petri dishes with a selective culture medium for faecal coliforms (CHROMagar™ ECC; Dr. A. Rambach; Paris, France); this procedure was performed by duplicate for each treatment. The planted Petri dishes were incubated at 44 °C during 48 h. Once complete the incubation time, the blue stained colonies, indicative of *E. coli* presence, were accounted, the mean of duplicate determination for each sample was recorded as *E. coli* colony forming units per g of faeces (CFU/g faeces).

Experiment 2. Thirty *Bos indicus* bull-calves (230.8 ± 12.7 kg) were placed in six corrals (n=5/corral) as described in Experiment 1. Bull-calves were fed *ad libitum* a growing diet (71:29 of roughage: concentrate) that contained 46 % of corn silage. After a 21-d adaptation period, faecal samples were taken directly from rectum of each bull-calve. Additionally, a 2-g faecal sample was taken individually for pH determination after being dissolved in 2 g of distillate water into a 100 ml beaker (Wheller and Noller, 1977). Faecal samples were placed in sterile plastic bags and transported to laboratory, where *E. coli* CFU/g was determined as described in Experiment 1. Once obtained faecal samples, bull-calves were randomly assigned to three treatments as follows: 1) Growing corn silage diet without tannins extract addition (Control); 2) Control plus 0.6 % of hydrolysable tannins extract (HT); and 3) Control plus 0.6 % of condensed tannins extract (CT). Bull-calves were fed during 28 d with its respective treatments, and faecal samples were taken at day 28 again from each bull-calve, and *E. coli* CFU/g of faeces was measured as described previously.

Statistical analyses. All incubations were performed in duplicate for each faecal collection or treatment. Prior of statistical analysis in both experiments, the results of CFU/g faeces were transformed to $\log_{10}$ of CFU/g faeces. Results of the Experiment 1 were analysed by ANOVA for a completely randomized design (Hicks, 1973) with a 3 x 4 factorial arrangement of treatments (3 tannins schedule x 4 times of exposure to environment). Pen was considered the experimental unit, and separation of means was performed using LSD test (Berry *et al*., 2006). Results of Experiment 2 were analysed by ANOVA for a completely randomized design (Hicks, 1973). Each bull-calve was the experimental unit, and separation of means was performed using LSD test (Berry *et al*., 2006). The statistical analysis was performed with the Statistix program, version 9.0 (2007).

RESULTS

Experiment 1. Results of the influence of tannins extract addition to feedlot cattle faeces and exposition time to the environment on presence of *E. coli* are shown in Table 1. An interaction between tannins extract addition and exposition time was observed ($P = 0.08$), where during the first 24 h of exposition, the *E. coli* $\log_{10}$ CFU/g account was similar ($P > 0.20$) among treatments; however, the response to CT extract addition changed at 24 h exposure, and at 48 and 72 h, the amount of *E. coli* $\log_{10}$ CFU/g was lower ($P = 0.08$) in faeces with 0.6 % of CT compared with Control. The addition of hydrolysable tannins to bovine faeces did not induce ($P > 0.10$) change on the account of *E. coli* growth.
Experiment 2. Dietary supplementation of both hydrolysable and condensed tannins did not affect ($P>0.20$) *E. coli* population in faeces. The faecal pH was 7.03±0.33 with no effect of treatments.

Table 1. *Influence of tannins extract addition to feedlot cattle faeces and exposition time to the environment on presence of Escherichia coli expressed as* $\log_{10}$ CFU/g *of faeces*

Exposition	Tannins extract			S.E.M.* (n = 4)	*P* value		
	Control No tannin	Hydrolysable tannin	Condensed tannin		Control v. HT	Control v. CT	HT v. CT
0 h	4.2	4.3	4.1	0.218	0.91	0.90	0.85
24 h	3.6	4.2	4.1	0.152	0.27	0.34	0.84
48 h	4.1	2.3	1.1	0.556	0.22	0.06	0.39
72 h	4.1	3.2	1.8	0.621	0.28	0.08	0.48

*Standard error of the mean

DISCUSSION

The reduction of *E. coli* in faeces by condensed tannins corroborates its antimicrobial effect; however the mechanism remains unclear (Wells *et al.*, 2005). A possible pathway is the ability of CT to form complex with proteins under pH conditions in ending portion of bovines small intestine (Frutos *et al*., 2004; Wheller and Noller, 1977); when CT form stable complexes with cell proteins induces hydrophobic effects, modifying the Na^+ / H^+ ion exchange that can results in death of the bacteria (Haslam, 1996). The faecal pH 6.31 of the actual experiment could provide condition for it. Deprivation of bacteria from some key minerals is another option (Dungan, 2010), polyphenols forming strong complexes with several mineral ions (Haslam, 1996); catechine-Cu (II) complex exposed *in vitro* bactericidal activity against *E. coli* (Hoshino *et al*., 2000). Chung *et al*. (1998) observed a reduction of *E. coli* growth after that added tannic acid in culture media, removed tannic acid-iron precipitate complex and added iron again *E. coli* growth. Smith *et al.* (2003) suggest that CT is toxic for *E. coli* under aerobic culture media because they generate H_2O_2. Min *et al*. (2007) observed *in vitro* an effect against *E. coli* by effect of HT and CT. There are few experiments where tannin was added directly to faeces; Wells *et al.* (2005) observed that death rate of *E. coli* O157:H7 inoculated to faeces increases when *trans*-cinnamic acid and *p*-cumaric acid were added to faeces of cattle. The lack impact of HT on *E. coli* growth in faeces is opposite to other report done by Min *et al.* (2007). Several researches documented activity of HT against *E. coli* (Akhtar *et al.*, 2015; Chung *et al*., 1998; Hoshino *et al.*, 1999). Results suggest that tannins extract from quebracho looks as candidate as alternative to decreases participation of *E. coli* from bovine manure in contamination of food and water (Dungan, 2010); some possible application in pre-harvest feedlot cattle area (Gutierrez-Bañuelos *et al.*, 2011), and manure utilized for land fertilization (Oliveira *et al*., 2012). In the experiment 2, the lack effect of tannins on *E. coli* shedding, could be partially explained with base in pH dependence tannins to expresses or not its ability to link proteins; when tannins are ingested, insoluble tannin-protein complexes are formed; but are dissociate in the abomasum and anterior duodenum (Frutos *et al*., 2004),

where pH is < 3.0 (Wheller and Noller, 1977); however in the last part of small intestine pH values turns slightly alkaline (Christiansen *et al*., 1990). The faecal pH = 7.03 found in the faeces of bull-calves indicate that the large intestine pH of these animal was close to neutrality. Under intestinal conditions of neutral pH, it is feasible that tannins formed newly stable complexes with undigested component from the diet, and with endogenous proteins from digestive secretions and tissue abrasion (Frutos *et al*., 2004), in this way when tannins flow to large intestine, they were already linked and unable to forms new bonds with proteins from bacteria cell membrane. Wells *et al*. (2005) observed that lower faecal pH was associated with higher death rates of *E. coli*. Results suggest that condensed tannins from quebracho is able to decrease *E. coli* CFU account when is added directly in bovine faeces, but spend more than 24 h to be evident its effect; however, when condensed tannins is fed to bull-calves, the presence of *E. coli* in faeces is not modified.

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WATER QUALITY AT DIFFERENT SITES OF RAINBOW TROUT BREEDING FARM

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SUMMARY. Rainbow trout is very sensitive to water quality. Intensive farming raises some problems which may be reflected in some physico-chemical and microbiological quality at different sites of the farm. The quality may also change with respect to season. The highest level of microbial contamination was detected in the effluent from the raceway, respectively, in the breeding raceway. Higher number of bacteria in these sampling sites is probably related to rainbow trout feeding and production of excrement. The highest value of nitrate was found in winter and the highest level of COD was measured in the spring. This was probably related related to the production of excreta and subsequent organic pollution of water in the breeding raceway. The level of suspended solids were the highest in the raceway and in the effluent from raceway, particularly in the spring. Nowadays fish breeding farms are affected by decreasing volume of available water and its worsened quality. This involves reduction of oxygen in water, accumulation of metabolites of fishes, increased levels of ammonia, CO_2, NO_2, NO_3, total suspended solids and bacterial load.

Key words: water quality, rainbow trout

INTRODUCTION

Reduced quality of water results in stress and increased susceptibility to diseases, affects uptake of food and growth of fish and leads to overall decrease in the quality of life (Cooke 2000; Ellis 2002). In any case, the quality of water is the key factor for determination of carrying-capacity of the cultivation system. The toxicity thresholds depend strongly on the species and size of fish. The influence on aquaculture environment changes and involves conflicts between demands of various users of aquaculture products, changes in hydrological regimens, introduction of exotic species into free nature and pollution of water resources (Read, 2001). According to Boyd (2003), pollution of water sources by wastes has been the most frequent reason for complaints and the related concerns attracted attention of official bodies in the majority of developed countries. The investigated fish farm Diviaky is located in Slovakian region of upper Turiec, close to spa town Turčianske Teplice, near to natural reserve Veľká Fatra. Water supplied to this breeding farm is taken from the river Turiec, the only Danube Salmon (*Hucho Hucho*) reserve in the central Europe. The river flows out of the reservoir Turček which supplies with drinking water the area of upper Nitra river. The quality of water, natural conditions and top-quality feed obtained from Denmark guarantee excellent quality of rainbow trout produced by this farm. The aim of the study was to investigate the physico-chemical and microbiological quality of water at four sites on the farm used for breeding of rainbow trout and compare changes in relevant parameters between different sampling sites in individual seasons.

MATERIAL AND METHODS

Samples were collected at selected sites of the fish farm Diviaky in individual seasons (spring, summer, autumn, winter) as follows: six samples were obtained from each of the 4 sampling sites (site 1 – Rakšiansky brook; site 2 − accumulation reservoir; site 3 – breeding raceway; site 4 – water discharged from breeding raceways). Physico-chemical evaluation included determination of pH, total suspended solids (TSS), ammonium (NH_3), nitrates (NO_3) and chemical oxygen demand (COD_{Mn}). Microbiological examination focused on plate counts of mesophilic bacteria and total coliforms, *E. coli* and faecal streptococci. Examinations were carried out according to relevant regulations set by legislative requirements on determination of physico-chemical and microbiological parameters of surface water.

RESULTS

Microbiological examination and comparison of the results of samples taken in individual seasons showed that the highest plate counts of mesophilic bacteria were detected at sites 4 (raceway effluent) and 3 (raceway) (Fig. 1, 2). The higher bacterial loads at these sampling sites is probably related to rainbow trout feeding and production of excrements. Plate counts of coliform bacteria were the highest in spring at sampling site 1 (water source – Rakšiansky brook) and in summer at sites 1 (influent brook) and 3 (breeding raceway). *E. coli* bacteria were present in higher numbers in individual seasons at all sampling sites. Faecal streptococci were detected in water samples taken at sampling sites 1, 2 and 4 only in summer. The highest microbial contamination was recorded in the summer and autumn and the lowest in spring and winter which can be ascribed partially to lower water temperature in these seasons. Comparison of results of physico-chemical determination (pH, NO_3, COD_{Mn}, total suspended solids TSS) at all sampling sites in individual seasons showed that the highest level of nitrate was reached in winter and the highest level of COD_{Mn} was measured in spring. The biggest differences in physico-chemical parameters between sampling sites were recorded in the spring in chemical oxygen demand (COD_{Mn}) in the effluent from the raceway. This was probably caused by increased production of excreta and subsequent organic pollution of water at this sampling site. The level of total suspended solids was the highest in the raceway and in the effluent from raceway, particularly in spring.

The increased levels of COD_{Mn} are also related to the way of management of rainbow trout breeding as during winter intensity of feeding is decreased but regular removal of mud and excrements from breeding raceways is not carried out so this material collects on the bottom and results in higher organic pollution. Also the self-cleaning capacity of water in the source (brook) and accumulation reservoir is reduced due to unfavourable climatic conditions (low temperature, freezing of surface) as compared to spring and autumn. This was confirmed also by the level of suspended solids which was higher in spring. Cleaning of raceways or fishing are activities that are associated with introduction of pollutants which increase the load on water supplied to fish (Dumas and Bregheim, 2001). Saturation of water with oxygen was similar throughout the year.

The level of pH was the highest in summer and autumn when it reached 8.04 and the lowest in winter when it ranged between 6.09 and 6.43. The pH is an important parameter as the ammonium is present in the ionic form at pH above 8 which is toxic to fish brood. The level of ammonium during our investigations did not exceed 0.2 $mg.l^{-1}$. Characterisation of locations, monitoring of water quality and characterisation of solid wastes introduced to water are important tools used in the control of quality of water. Special attention is paid to the character of suspended and deposited solid substances produced within the farm. Suspended solids can have negative effect on aquaculture systems (Chen, 1993) and when released into the environment can negatively influence water biotops (Stephens and Farris, 2004). It was also proved that various pollutants (nutrients) are related to increased concentration of total suspended solids (Teichert - Coddington, 1999). Flow-through aquaculture systems produce and discharge into the environment effluents which contain increased levels of nutrients and solid substances. Such wastewaters can affect significantly recipient water if not treated properly (Forenshel, 2001; Miller and Semmens, 2002; Schulz, 2003). Owing to increased interest in implementation of environmentally friendly activities and sustainable fish breeding, the aquaculture branch oriented recently on practices which allow one to reduce the quantity of discharged waste through effective management of feeding and treatment of effluent. Individual governments are also involved in taking measures focused on environmental protection, including regulation, control and monitoring procedures for minimisation of potentially harmful effects of aquaculture (Read, 2001; Bergheim and Brinker, 2003). Breeding of rainbow trout and other fish with the use of energy rich extruded feed affect substantially the quality of effluent (Viadero, 2005; Maillard, 2005). On the other hand, the water supplied to fish farms also affects most of the parameters of wastewater. Feeding of fish is the only factor which impacts all monitored parameters. Increase in production/intensity of feeding increases levels of waste nutrients. Fish feed is the only source of nutrients added to aquaculture system (Bergheim and Asgard, 1996). A portion of supplied feed is undigested and becomes a waste. Some of the undigested feed is eliminated as faeces (Green, 2002) which are composed mostly of organic carbon and phosphorus (Cripps and Bergheim, 2000). The remaining portion is excreted as dissolved nutrients through gills, particularly as ammonium, and in urine in the form of phosphates and ammonium (Green, 2002).

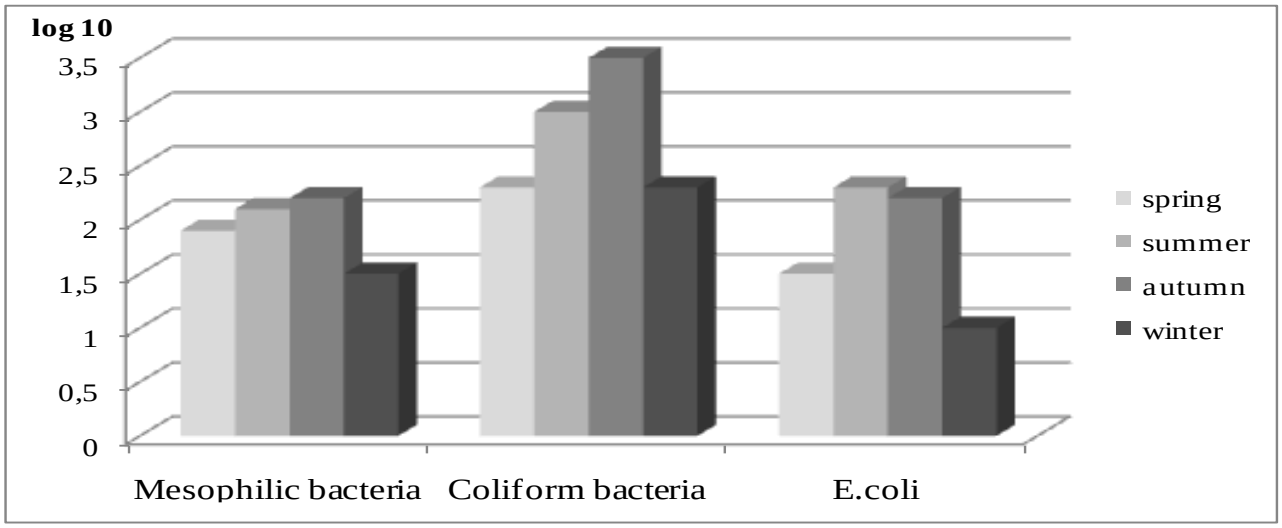

Figure 1. Comparison of the results of samples taken in individual seasons in site 4 – water discharged from breeding raceways.

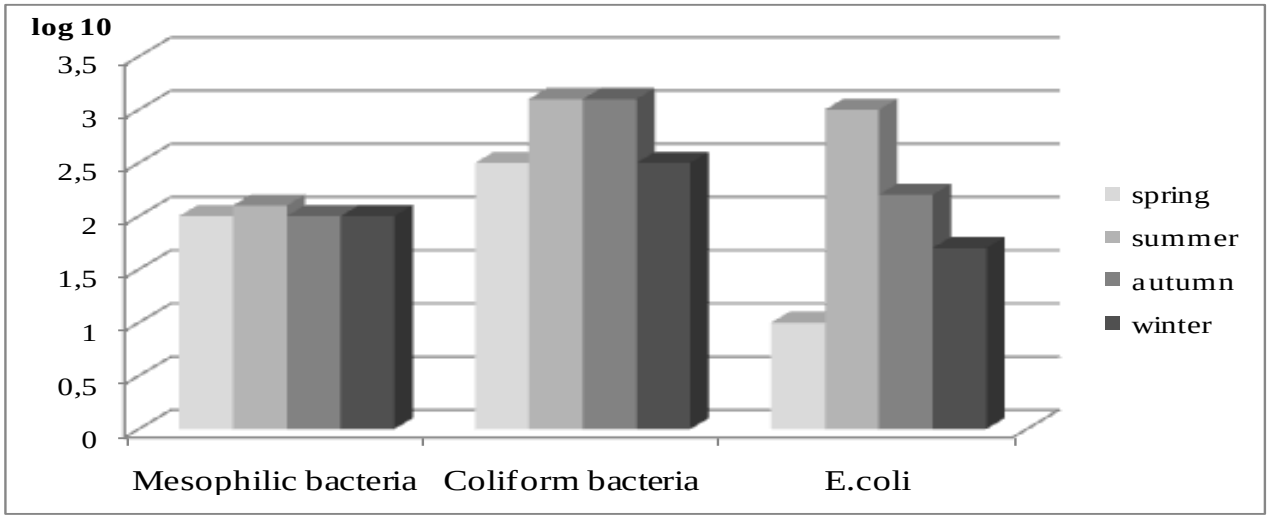

Figure 2. Comparison of the results of samples taken in individual seasons in site 3 – breeding raceway.

DISCUSSION

Quality of water is very important for fish, especially for rainbow trout which is more sensitive than other less valuable fish species. Decreased quality at some sampling sites or in different seasons may be related to the intensive rearing method of rainbow trout as in winter the intensity of feeding is reduced, but at the same time periodic cleaning of breeding raceways is not performed and faecal matter accumulates on the bottom resulting in increased total organic pollution of discharged water. The management of feeding and treatment of effluents affect the most the potential pollution of water bodies by aquaculture units. Involvement of legislation bodies in taking protection measures is important for minimisation of potentially harmful effects of aquaculture.

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MICROBIOLOGICAL AND CHEMICAL CONTROL IN THE ENVIRONMENT OF THE WATER TREATMENT FACILITY

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SUMMARY. The aim of the study was microbial and chemical evaluation of wastewater samples and a detection of antibiotic resistance in *E.coli* isolated from environment of wastewater treatment plant. Organic and inorganic chemicals as a nitrogen or phosphorus in treated water released into the recipient leads to pollution and finally eutrophication. The microbiological content of the wastewater is variable. High levels of antibiotic resistance bacteria are found in river sediments downstream from a wastewater treatment plant. The cleaning processes lead to the removal of organic matter (nitrogen, phosphorus, BOD5, COD, etc.), which could significantly affect the quality of water in rivers. Each determined chemical parameters in treated water were in required level during the year. In the investigated samples were found out enterobacteria *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*. In E. coli strains were detected an antibiotic resistance and the highest levels were detected in ampicilin and cephalosporins. ESBL phenotype and ESBL genes resistance (CTX-M genes) were revealed in E. coli strains.

Key words: wastewater, chemical parameters, microbiological control

INTRODUCTION

Thousands of tons of antibiotics have been administered to humans and animals since the discovery of penicillin. Antibiotics are never fully metabolized and are excreted as the parent compound or as its metabolites with urine and feces (Harnisz et al. 2015).

Water treatment facilities are one of the main sources of releasing of antibiotic resistant micro-organisms into various compartments of the environment worldwide and present health risks to humans and animals (Colomer-Lluch et al., 2014).

Also small streams and rivers, local inputs of wastewater treatment facilities may also become an important sources of nutrients, emerging pollutants, organic mater and also the above mentioned antibiotic resistance bacteria (Proia et al., 2016).

Extended-spectrum beta-lactamase-producing (ESBL-producing) *Enterobacteriaceae – E. coli, Klebsiella pneumoniae, Enterobacter cloacae* are widely distributed and can be found in humans as well as in various animal species, food and even in environmental samples (Laube, 2014).

ESBL-producing bacteria are resistant to most beta-lactam antibiotics, including 3rd and 4th generation cephalosporins, and are often additionally resistant to multiple other classes of antibiotics. ESBL-production was typically associated with infections caused by *Klebsiella pneumoniae* and *Escherichia coli* (Blaak et al., 2014).

In the samples with ESBL phenotype is very important to find a presence an ESBL genes in enterobacteria. The incidence of ESBLs of the CTX-M type have increased dramatically. Nowadays ESBL genes are much more frequently found in *E. coli* from waste water treatment plant and sludge. The majority of these ESBL genes belongs to the CTX-M- group (Blaak et al., 2014; Reinthaler, 2010).

MATERIAL AND METHODS

Our study was based on a detection of microbial and chemical evaluation of wastewater samples. The investigated samples were collected from the influent and the effluent of the wastewater facility. An individual species of enterobacteria from the water environment were identified by Maldi Tof biotyper. From samples of water were isolated E. *coli* strains and minimal inhibitory concentrations (MIC) of 20 antibiotics were detected according to CLSI:VET01-S2 (CLSI, 2013).
For detection of ESBL genes (CTX-M group genes) in investigated *E. coli* strains was used PCR analysis.
Subsequently, we also determined chemical parameters of the wastewater - pH, chemical oxygen demand, total phosphorus, total nitrogen and ammonia nitrogen.

RESULTS

The cleaning processes lead to the removal of organic matter - nitrogen, phosphorus, COD, etc.), which could significantly affect the quality of water in rivers.
The microbiological and chemical content of the wastewater is variable. The efficiency of cleaning process in investigated wastewater treatment plant is very high (Tab. 1). Each determined chemical parameters in treated water were in required level only chemical oxygen demand (COD) was higher than limit (125 $mg \cdot l^{-1}$).

Table 1. Chemical and microbial evaluation of wastewater samples

Parameters	**Influent**	**Effluent**	**% of efficiency**
Chemical parameters (limit)			
pH (6,0 – 9,0)	7.8	7.2	-
N /sum (40 $mg \cdot l^{-1}$)	47.06 $mg \cdot l^{-1}$	4.06 $mg \cdot l^{-1}$	95.25 %
N-NH4 (30 $mg \cdot l^{-1}$)	39.22 $mg \cdot l^{-1}$	2.8 $mg \cdot l^{-1}$	92.86 %
COD (125 $mg \cdot l^{-1}$)	821.6 $mg \cdot l^{-1}$	328.3 $mg \cdot l^{-1}$	60.4%
P/sum (4 $mg \cdot l^{-1}$)	283 $mg \cdot l^{-1}$	1.2 $mg \cdot l^{-1}$	99.5 %
Microbiological parameters			
Mesophilic bacteria	$9{,}1 \cdot 10^5$ $CFU \cdot ml^{-1}$	$2{,}5 \cdot 10^4$ $CFU \cdot ml^{-1}$	97,1%
Fecal coliform bacteria	$6{,}4 \cdot 10^4$ $CFU \cdot ml^{-1}$	$1 \cdot 10^3$ $CFU \cdot ml^{-1}$	98,4%
Coliform bacteria	$2{,}8 \cdot 10^5$ $CFU \cdot ml^{-1}$	$1{,}4 \cdot 10^4$ $CFU \cdot ml^{-1}$	91,4%
Fecal enterococci	$1{,}8 \cdot 10^5$ $CFU \cdot ml^{-1}$	$1 \cdot 10^3$ $CFU \cdot ml^{-1}$	99%

In the investigated samples of waste water were found out also individual species of enterobacteria - *E. coli, Klebsiella pneumoniae, Proteus mirabilis*. Their identification was corfirmed by Maldi Tof biotyper.
The highest level of antibiotic resistance in investigated *E. coli* strains was detected to ampicilin 88%. We found out a frequent occurrence of *E.coli* resistant to cephalosporins - ceftiofur (52%) and ceftriaxone (34%). ESBL phenotype was confirmed in 17% of *E. coli* strains and the occurrence of ESBL genes (CTX-M group genes) was detected in 47 % examined samples. These findings showed increased prevalence of enterobacteria with ESBL genes in the wastewater treatment plant.

DISCUSSION

The emergence and diffusion of antibiotic-resistant bacteria cause major public health problem for many years. Many autors deal with the study of antibiotic-resistance of microorganisms originated from municipal wastewater. *E. coli* strain collected from different wastewater samples showed high resistance to ampicillin (AMP) and chloramphenicol (CAF), used as representative antibiotics for the

efficacy against Gram-positive and Gram-negative bacteria (Zanotto, C. et al., 2016). High level of antibiotic resistance were found out also to ampicilin in our study. In addition, there was detected resistance to cephalosporins, while resistance to chloramphenicol were not demonstrated.

Extended-spectrum β-lactamase (ESBL) and quinolone-resistant *Enterobacteriaceae* were revealed in hospital effluent, sanitary effluent, inflow sewage and wastewater treatment plant. Resistance to cephalosporins and quinolone was observed in 34.4% of *E. coli* (Conte et al., 2017).

Biological oxygen demand (BOD_5) or chemical oxygen demand (COD) analysis is widely used to evaluate organic pollutants in water systems as well as the efficiency of wastewater treatment plants. The level of the COD in the influent of the wastewater treatment plant reached 694 $mg \cdot l^{-1}$ in the influent and around 300 $mg \cdot l^{-1}$ in the effluent of the wastewater treatment plant (Hussain et al., 2013). In our study, were detected similarly values for chemical oxygen demand (COD) 821$mg \cdot l^{-1}$ in the influent and 328 $mg \cdot l^{-1}$ in the effluent.

Wastewater treatment processes are not specifically designed to degrade all dangerous organic contaminants. These are consumed by aquatic organisms, which can present a hazard to the whole food chain (Rajasulochana et Preethy, 2016).

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ENVIRONMENTAL POLLUTION IN RENDERING PLANT AND PROCESSESING OF WASTEWATER

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SUMMARY. Animal carcasses and secondary raw materials of animal origin in rendering plants are important source of dust, gases and microorganisms which contributes to the risk of pollution of the environment. Processed material may contain also antibiotic-resistant micro-organisms which can find their way into the processed product and the atmosphere. The aim of the study was to determine the levels of gases, dust particles, airborne micro-organisms and resistant isolates of *E. coli* in the rendering plant and its surroundings.
From the environmental samples were the minimal inhibitory concentrations of *E. coli* strains detected by colorimetric broth microdilution method according CLSI guidelines.
In *E.coli* isolates obtained from the samples were detected ESBL, fluorochinolone resistant isolates and CTX-M type.
Antimicrobials used in animal production have the potential to bioaccumulate in the animal carcasses and processing materials in rendering plant, which can be used as fertilizer and animal feed.
Key words: carcases, antibiotic resistant bacteria, bioaerosol

INTRODUCTION

Processing of animal carcasses and secondary raw materials of animal origin in rendering plants is an important source of gases, dust and biological pollutants which contributes to the risk of pollution of the environment.
Inappropriate use of antibiotics in intensive animal production but also because of practices in the agricultural industry, the processed material may contain also antibiotic-resistant micro-organisms which can find their way into the processed product and the atmosphere.
Handling with materials of animal origin in rendering plant include many processes - unloading of raw material brought for processing, its sorting, primary processing and sampling, sterilisation, separation of fat and feed meals of animal origin, pressing, processing of feed meals, expedition and processing of animal fat. The operation premises are divided to a section used for common processing of material of categories I and II and a separate section for processing of material of category III.
Destruction (crushing of material to 50 mm particles) and sterilisation of animal by-products using the temperature 133°C, pressure 3 bars during 20 minutes ensure hygienisation and limit risk of the transmission of microorganisms to the environment. These parameters are critical control points in the process of processing of raw material in the rendering plant (Regulation EC 1774/2002). In the dryer the solid part is separated from the liquid one (water) and the dried meat-bone meal is pressed during which process the fat is separated from meat-bone mash.
The meat-bone meal obtained from the material of category III is then ground and sifted to the required size of particles and is used for production of pet granules. The meat- bone meal obtained from the material of categories I and II is transported to a cement factory for burning. The refined fat is used as an alternative fuel or is burned. The technological procedure in the rendering plant includes processing

of wastewater and biological air washer which also considerably decrease the hygiene-epidemiological risks.

MATERIAL AND METHODS

Bioaerosols were collected by means of a sampler MAS-100Eco. The MAS-100 Eco air monitoring system is a compact sampler intended to use of standard Petri dishes with respective nutrient agars.
Susceptibility (MIC - minimal inhibitory concentration) of *E.coli* isolates from bioaerosol was determined by colorimetric broth microdilution method according to CLSI guidelines (2008).
CTX-M betalactamases were investigated in positive *E.coli* strains by means of PCR (Carattoli et al. 2008).

RESULTS

Only low levels of airborne micro-organisms were detected (Figure 1) which is mostly related to high automatisation and close system of the entire procedure of processing of the raw material of animal origin and the fact that the majority of processes takes place in a closed system.

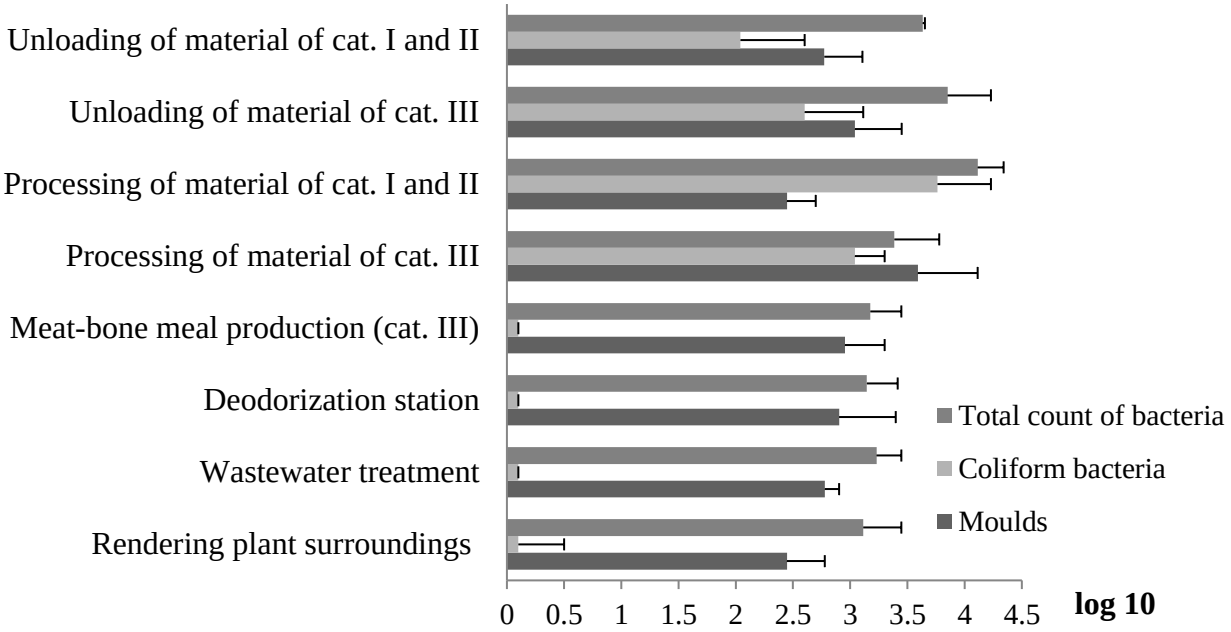

Figure 1 Mean and maximal concentration of airborne micro-organism in sampling places of the rendering plant

Unloading of the raw material and wastewater treatment are critical points with regard to potential inhalation of pathogenic micro-organisms
Critical sites with regard to are unloading of the raw material and wastewater treatment. In this part of rendering plan the raw material is dumped from collecting containers of the transport vehicles and it is associated with potential aerosolization of liquids, such as blood, intestinal contents and similar.

Table 1 MIC levels and percentage of resistance in *E. coli* from environmental samples of rendering plant

ATB	Resistance %	Mic XG	ATB	Resistance %	Mic XG
AMP	100	115,9	**NAL**	42,86	13,1
A+IB	28,57	10,8	**CIP**	0	0,1
CFT	0	0	**ENR**	0	0,3
CTR	28,57	3,3	**TTC**	42,86	7,2
CFF	28,57	1,5	**CMP**	28,57	4,9
CFQ	28,57	2,0	**FLO**	0	4,9
GEN	0	0,3	**COT**	42,86	29,0
STM	42,86	9,8	**CLO**	0	0,3
NEO	14,29	3,6			

The results of microbial inhibition concentration (Table1) showed that the percentage of resistance in *E.coli* isolates was the highest in ampicilin, streptomycin, nalidoxic acid, cotrimoxazol and tetracycline.

In *E.coli* isolates we were determined TEM-1,2/SHV-1:low (57,14%), ESBL TEM (14,29%), fluorochinolone resistant isolates (42,86%) and CTX-M type (14,3%).

Hofacre et al. (2001) inspected rendered animal protein products from poultry feed mills and other meat and bone meal samples. They found that a high percentage of feed sample (85%) contained bacteria resistant to ampicillin, amoxicillin, clavulanic acid or cephalothin, whereas few samples contained bacteria resistant to ciprofloxacin, kanamycin or trimethoprim/sulfamethoxazole. Many of the isolated bacteria contained integrons, genetic elements that mediate multiple drug resistance.

DISCUSSION

Processing of raw materials in rendering plants may be associated with production of bioaerosols containing considerable numbers of pathogenic micro-organisms.

In the past, many cases of diseases, such as psittacosis, Q-fever, anthrax, leptospirosis, brucellosis, tularemia, equine viral encephalitis and other, were recorded in the rendering plant personnel (Fleeger and Lillquist, 2006). In workers in rendering plants and abattoirs there was also increased incidence of liver tumours due to increased concentration of aflatoxins and ochratoxins in their working environment (Douwes et al. 2003).

Rendered animal protein products are often included in poultry feeds and could potentially serve as a source of antibiotic resistant bacteria.

Many of the drugs used in animal agriculture and human medicine are the same or very similar including, but not limited to, betalactams (penicillin, ampicillin, cloxacillin), tetracyclines, sulfonamides and potentiated sulfonamides, cephalosporins, and fluoroquinolones (McEwen and Fadorka-Cray, 2002). Exposure to zoonotic pathogens harbouring resistance to antimicrobials of clinical importance may lead to diseases with few or no treatment options in humans.

Antimicrobials used in poultry production have the potential to bioaccumulate in poultry feathers but available data are scarce. Following poultry slaughter, feathers are converted by rendering into feather meal and sold as fertilizer and animal feed, thereby providing a potential pathway for re-entry of drugs into the human food supply (Love et al., 2012).

According to our results the highest concentration of airborne micro-organisms occurred in the areas of unloading and processing of the raw material. Therefore it is very important to ensure that the

unloading ramp area is completely enclosed and thus prevent escape of the produced bioaerosol to the surrounding areas.

Adequate protection of the personnel working in the rendering plant and strict hygiene regulations decrease considerably the risk of transfer of dangerous diseases. The transmission of airborne particles can secure also the minimal protection zone (3000 m) between rendering and animal farms, human dwelling, slaughter houses and other facilities.

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Regulation EC 1774/2002 laying down health rules concerning animal by-products not intended for human consumption

WHOLE GENOME SEQUENCE ANALYSIS OF ESBL- E. COLI OF ST 131 AND ST648 FROM VARIOUS HABITATS

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ESBL-producing *E. coli* of ST 131 are paradigmatic for the success of MDR-pathogens combining virulence with antimicrobial resistance. While the reasons for the success of ST131 *E. coli* are yet not fully understood other pandemic lineages of ESBL-producing *E. coli* have been emerging recently, like ST648 or ST410. To gain more insights into their interspecies transmission that contributes to the success of those pandemic lineages of *E. coli* we used whole genome sequencing (Illumina HiSeq2000) to comparatively analyze 196 isolates of ST131 and ST648 originating from human and veterinary clinical samples as well as wildlife and the environment worldwide. The phylogenetic analysis was based on the core genome and the accessory genome. In addition we screened for resistance and extra-intestinal virulence genes (VAGs). Although high rates of resistance and virulence genes were found in both STs, isolates of ST131 significantly harbored more VAGS (median 57) than ST648 isolates (median 41). On the contrary ST648 isolates harbored significantly more resistance genes (median 11 vs 7). When comparing the content of antimicrobial resistance genes and VAGs no clear positive or negative correlation between resistance and virulence associated genes could be detected for both STs. A separate core genome alignment and maximum likelihood phylogeny was obtained for all 88 ST648 and 108 ST131 genomes. Isolates from humans, domesticated animals wildlife and the environment are distributed throughout both of the phylogenetic trees suggesting an equal cross species movement of strains of both STs. In addition neither the type of the disease nor the country of isolation clustered in both trees suggesting a generalistic lifestyle of both ESBL lineages. This suggests that *E. coli* ST648 is a pandemic host generalist pathogen capable of frequent inter-species movement like it has been reported for ST131 and our data is underlining the urgency of the one health approach.

Animal welfare in an environmental perspective; a case study of life cycle analysis of pig production

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Sustainability within animal production is a highly complex issue with numerous interconnections within the production system. Besides the sustainability aspects generally discussed, agriculture also involves issues related to animal husbandry and animal welfare. In order to evaluate sustainability in farm animal production, life cycle analysis can be used for quantifying the environmental impact. In this paper aspects of pig welfare were investigated in relation to the sustainability of the integrated pork production chain.
An interdisciplinary project developed descriptions of supply chains of pork produced based on empirical data from a Swedish region in 2012. The set-up of the project was that experts on production along the supply chain designed environmentally improved systems. The next step was to challenge the improvements considering their possible consequences on products and systems from different perspectives: e.g. food safety, animal welfare, consumer appreciation and costs. Three future scenarios were created and compared to the current situation (reference); 1. Reduced impact on local ecosystems; 2. Optimized plant nutrient use; and 3. Reduced climate impact. The finalized supply chains for pork were analyzed regarding environmental impact with LCA, as well as from the other perspectives.
All scenarios decreased the environmental impact of the pork production compared to the current system. Results for the animal husbandry part of the pork chain revealed that there were many similarities between the three solution scenarios. Furthermore, taken the whole production chain into account, the differences in environmental impact were found to be marginal. However, morbidity and mortality had a substantial impact on the amount of pork produced. The environmental impact of the housing systems has to be considered in relation to systems' effect on the health and welfare to get an accurate estimation of the full productions chain. Thus, improved animal welfare is essential for shaping a more sustainable pig production.

Animal production in developing countries

INVOLVEMENT OF ENVIROMENTAL CONDITIONS IN DAIRY PERFORMANCES OF TUNISIAN HERDS

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SUMMARY. Besides the genotype and the physiological stage of the cows, phenotypic values in dairy cattle depend on the contribution of multiplicity of factors which are related to its vital environment and husbandry conditions that vary considerably in accordance to environmental disparity. This study aimed to quantify the contribution of these fixed factors in the expression of dairy potentialities of Holstein cows raised under the Tunisian conditions and to genetically assess herds for 305-days milk yield. The study has concerned 23 280 Holstein cows which belong to 307 herds and were calved between 2006 and 2011. A total of 32 688 lactation performances were computed according to 8 monthly records and standardized to 305 days. Cows were divided into 8 herd size groups according to which the variance analysis of the 305-days milk yield was performed by a fixed linear model and the genetic evaluation was carried by a uni-trait animal model with permanent effect. Raw average milk yield has reached 4975 kg (± 2708 kg) while the average standardized performance was about 5770.67 kg (± 2396 kg), with respective maximums of about 16 064 kg and 14 838 kg. Heritabilities and repeatabilities have ranged respectively between 0.02 and 0.07 and 0.20 to 0.38 by group of herds. High permanent environment variance components were observed compared to the additive genetic merit especially in small herds. The 305-days yields were found to be deficient in the Tunisian Holstein herds highlighting a limited genetic potential expression that reflects unstable environment conditions and unsuitable husbandry of the Tunisian dairy cows, especially in small herds.

Key words: Interaction, dairy genotype, Tunisian environment

INTRODUCTION

Profitability of dairy cattle system essentially rests on the herd productivity level, so it is important to take into account the herd performance influencing factors which are related to the natural environment, the management circumstances and the genetic potential of the used animals (Gantner *et al*., 2010). In fact, the herd performance is expressed by the genotypes interaction with the different components of their breeding environment and genotype is a fixed and specific component that determines the genetic variability between animals while breeding environment considerably vary, due to the existence of a large environmental disparity. Therefore, a good genetic potential cow reared under poor conditions, her productivity remains low. Then choosing a high-performance genetic material is absolutely important in order to ensure sustainable production whether it has to be associated with suitable environment conditions in order to maintain high genetic potential expression (Agabriel *et al.*, 1990). In this context, the present study aimed to quantify the environment

contribution in the expression of dairy performances in Holstein cows raised under the Tunisian conditions and to genetically assess herds for 305-days milk yield (MY305).

MATERIAL AND METHODS

Data used is from the official milk recording conducted by the Agency of Livestock and Pasture (O.E.P) and represented 32 688 305-days milk yield recorded on 23 280 Holstein cows in their first, second and third parity, calved between 2006 and 2011 and belonging to 307 herds. Herd sizes (HS) vary between 20 and 1712 cows and were classified into 8 balanced herd size groups according to which analysis were performed. MY305 variance was analyzed by a fixed linear model:

$$Yijklmnop = \mu + Pi + CLYj + CLMk + MFl + CFCm + Hn(HCo) + HCo*CLYj + eijklmnop$$

Where Yijklmnop is the observed MY305 on the cow in parity i (i = 1, 2, 3), calved in month k (k = 1,..., 12) during the year j (j = 2006,..., 2011), milked with l frequency (l = 1, 2, 3), for the m interval between calving and first control (m = 1,..., 67), in herd n within the herd class o (o= 1, 2, 3, 4) and in the herd class o within the calving year j. μ = overall mean; P = the parity effect; CLY = the calving year effect; CLM = the calving month effect; MF = the milking frequency effect; CFC = the interval between calving and first control effect and e = the residual errors. Estimation of the least squares was carried out by the General Linear Model procedure (GLM) of SAS program (Statistic Analysis System, 2003).

Genetic evaluation was carried by a uni-trait animal model with permanent effect based on the method of the restricted maximum likelihood where contemporary groups were defined as herd*calving year:

$$Y = Xb + Za + Wep + e$$

Where y is a vector which contained MY305, b is the vector of the fixed effect solutions, a is the vector of the genetic random effect solution, ep is the vector of the permanent environment random effect solutions, X, Z and W are matrixes relating phenotype observations to the different effects and e is the vector of residual. The estimation of genetic parameters, the prediction of breeding values as well as the estimation of the permanent environment components were made simultaneously for each herd class using the VCE 5.1 programme (Variance component estimator).

RESULTS

Raw average milk yield reached 4975 ± 2708 kg and the average standardized yield was 5770.67 ± 2396 kg with respective maximums of about 16 064 kg and 14 838 kg. Variance analysis has showed that MY305 was specifically explained at the level of each herd size category. The coefficients of determination and variation for each class have represented distinct degrees of significance for each common variation factor (Table 1), where highest coefficients were found within small herds.

Table 1. Determination and variation Coefficients of MY305 by herd size category.

Herd size category	R^2	CV
HS = 50	64.82	27.29
50 < HS< 100	39.57	25.71
100 ≤ HS < 150	28.88	28.02
150 ≤ HS < 220	42.38	27.35
220 ≤ HS < 300	16.51	27.21
300 ≤ HS< 400	41.73	23.85
400 ≤ HS ≤ 600	8.10	24.06
T > 600	3.87	27.22

Variance components estimates, heritabilities, repeatabilties and the predicted values of additive genetic and permanent environment component were calculated (Table 2). Heritabilities and repeatabilities have ranged respectively between 0.02 and 0.07 and 0.20 to 0.38 and permanent environment variance components were found to be higher than the additive genetic merit especially in small herds.

Table 2. Determination and variation Coefficients of MY305 by herd size category.

Herd size category	σ^2_p	σ^2_a	σ^2_{ep}	σ^2_e	h^2	r
HS = 50	1207783,77	29949,178	297485,921	880348,670	0,02	0,27
50 < HS< 100	1728120,57	4160,748	595182,412	1128777,408	0,002	0,34
100 ≤ HS < 150	1975268,44	142919,607	403467,340	1428881,492	0,07	0,27
150 ≤ HS < 220	1702383,45	110150,499	397237,845	1194995,109	0,06	0,29
220 ≤ HS < 300	2256372,28	75947,639	682594,690	1497829,952	0,03	0,33
300 ≤ HS< 400	1819710,27	88240,383	439716,929	1291752,962	0,04	0,29
400 ≤ HS ≤ 600	2776239,06	178068,235	387194,097	2210976,730	0,06	0,20
T > 600	3511324,79	93889,249	1242777,243	2174658,293	0,02	0,38

σ^2_p**:** phenotypic variation; σ^2_a**:** genetic additive variance; σ^2_{ep}: permanent environment variance; h^2: heritability; r: repeatability; HS: herd size.

DISCUSSION

Holstein cows produce about 9411 kg per lactation and compared to the average found in this study, the productivity level is considered quite low in Tunisia, which can be explained either by climatic changes that have occurred during the studied years, or by changes in the management of herds that reflect inadequate husbandry conditions according to the reported explanations of Rekik *et al.* (2003). The variance analysis results explain distinct degrees of significance for the common variation factor within each herd size category to demonstrate a specific variability of the performances due to the various climatic conditions and the adapted husbandry modes that implies the satisfactory dairy performances expression. The highest coefficients were perceived within small herds to interpret an aggravated environmental sensitivity. By considering the total genotypic and environmental variance, genetic parameters were relatively too low, compared to those reported in the literature (Hammami *et al.*, 2009; Ben Gara *et al.*, 2006), as well as strong variance components of the permanent environment were observed compared to those additive genetic which reflects a signification interaction between genotypes and the environment, as Minveille (1990) has explained. In the same context, Hammami *et al.* (2009) found the same trend within Holstein cows in Tunisia. This explains that the genetic potential expression of Holstein cows in Tunisia is limited and that the observed phenotypic variability

is due essentially to non-genetic factors attesting a major contribution of relatively difficult environment, especially for small herds. Moreover, the distribution of the standard genetic deviations according to the herd size categories shows a low genetic diversification within and between herds explaining a low genetic gain and an ultimate effect of divergence between environments (Figure 1). In the same context, Hamrouni et *al.* (2010) found a significant effect of the interaction between genotype and region in dairy cattle of Tunisia proving that genotypes respond differently to situations presented by a given environment. Milk yields the Tunisian Holstein herds highlight a limited genetic potential expression due to unstable environment conditions and unsuitable husbandry of dairy cows, especially in small herds.

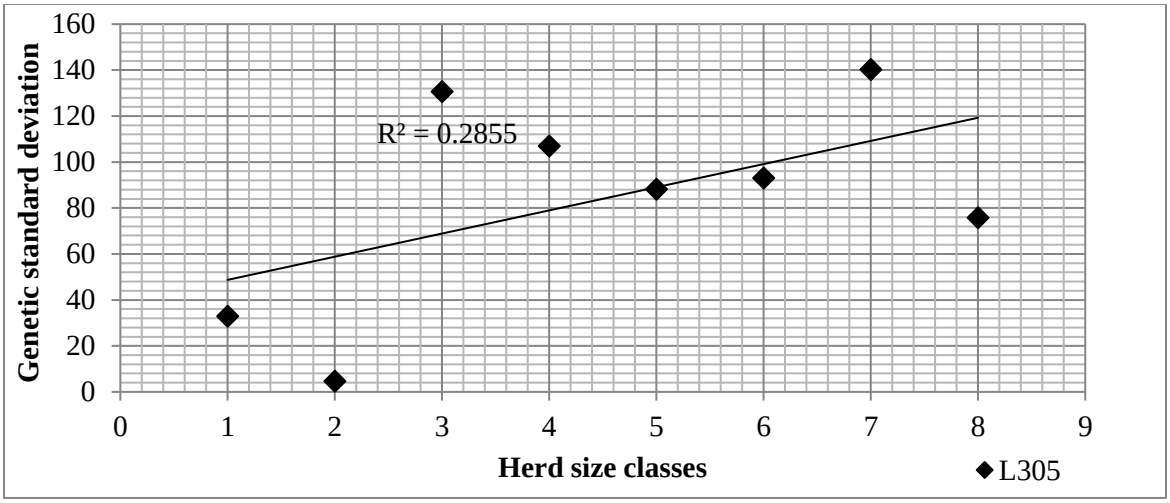

Figure 1. Genetic standard deviations distribution according to herd size categories

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EFFECT OF TECHNOLOGY TRANSFER ON SUSTAINABLE LIVESTOCK PRODUCTION OF DEVELOPING COUNTRIES

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SUMMARY. To link research to technology, transfer is an indispensable factor to achieve competitiveness, economic growth and development. Nevertheless, there has been a marked lack of success in transfer of new technologies in developing countries (UNCTAD, 2014). This paper considers issues related to the role of technology transfer to help achieve sustainable livestock production. The area of study is located in the counties of Iguala, Coyuca de Benitez and Ometepec, in the state of Guerrero, Mexico. Twenty seventh Professional Service Provider (PSP) were trained for knowing how to transfer the model of GGAVATT (Farm Group for Validation and technology Transfer) and assigned to 570 small production units (PU) of dual-purpose livestock with an average of 31 heads each one; therefore, 18 038 dual-purpose livestock were utilized to evaluate adoption (technology transfer) of innovating farming practices such as: management, nutrition, forages, genetics and reproduction, animal health and, administration and organization in the circumstances of individual producers. The 570 farmers were organized in 27 GGAVATT´s and assisted, through 264 educational events offered by PSP, to improve livestock farming methods and techniques to increase production efficiency. The main livestock innovating production practices adopted by farmers were: genetics and reproduction, administration and organization, and animal health with 70, 65 and 61% of the 570 production units, respectively. On the other hand, nutrition and management practices were adopted by 51 and 48% of the total units. Adoption of specific technologies on these areas is described. Age, schooling and extra income determine the adoption of livestock systems, as well as other factors including the geographical location, the level of development of human capacities and skills, and infrastructure. Technology transfer often requires an adaptation of the technology to the conditions in the transfer destination to improve its effectiveness and impact.

Key words: Livestock, technology transfer, GGAVATT

INTRODUCTION

In developing countries, two thirds of the world's livestock are raised; however, only one fourth of meat and one fifth of milk is produced in these countries. This data suggests that there is a high potential to increase the sustainable livestock production and productivity through the transfer of new technologies (Ehui and Shapiro, 1998). Therefore, the objective of this study was to evaluate the type and grade of adopted technology of 570 dual purpose livestock producers with the model of GGAVATT (Farm Group for Validation and Technology Transfer), as well as their social, technical and productive aspects.

MATERIALS AND METHODS

Characteristics of the producers and study area. Five hundred and seventy producers grouped in 19 GGAVTTs were allocated to 27 PSP who were hired by the State Government of Guerrero for a period of 8 months (June 2013 to January 2014). The PSP that were aimed at training and transferring technology, as well as evaluating technology adoption by the producers. The cattlemen are located in the counties of Iguala, Coyuca de Benitez and Ometepec, all from the state of Guerrero, Mexico, and correspond to those grouped in the stratum with more than 20 livestock head that represent about 45% of the producers of the State (Cervantes et al., 2005).

PSP training. The methodological training of the PSP consisted in the attendance of 5 courses proposed by the National Institute for Forestry, Agriculture and Livestock Research (INIFAP): 1) Induction to livestock strategy, 2) Methodology for diagnostic evaluation, 3) Implementation of the GGAVATT, 4) Administration of farms, and 5) Management of the SI-UTEP (Information system of the specialized livestock technical unit).

Producers training. Two hundred and sixty fourth events of interactive training were offered to groups of no more than 30 producers each one (Table 1). The technical subjects were selected according to the main interest of the cattlemen.

Design of data collection. The design of data collection considered three phases: 1) Initial, 2) Tracing and, 3) Final.

Table 1. Zootechnical discipline and number of events of group training.

Zootechnical discipline	Talks	Courses	Modules	Total	%
Livestock management	37	9	15	61	23
Genetics and reproduction	29	16	11	56	21
Animal health	30	6	9	45	17
Forages	13	12	14	39	15
Animal nutrition	16	12	3	31	12
Administration and organization	22	1	9	32	12
TOTAL	**147**	**56**	**61**	**264**	**100**

RESULTS AND DISCUSSION

General profile of the producers. Average age 53.5 years; illiterate is high with an average of 12% of farmers that do not know how to read and write; the activity is performed mainly by men (89 %) and about 11% for women. The producers have an average of 35 years of experience. This data is very similar to that found by Cervantes et al. (2005) and Ponce-Mendez et al. (2016). On the other hand, about 18 000 dual purpose cattle head on a semi-intensive system were attended; therefore, the average herd per production unit (PU) was 31 heads; whereas, the average production unit for grazing and forage production was 25.5 and 6.2 hectares, respectively.

Adoption of technology. Genetics and reproduction was the technical area with the highest adoption. Initially there were 287 PU (50.4%) while at the final phase this number increased up to 400, perhaps because cattlemen are very aware of reducing the open days of calving and to improve genetics (Table 2), similar results were found by Ponce-Mendez et al. (2011). Administration and organization, and animal health, were technical areas where there was an important increase, mainly in “using log” and “deworming and vaccination”. Bustos et al. (2008) reported higher values of adoption; however, they

compared six years of intervention. On the other hand, nutrition and livestock management had little increment perhaps due to the high costs of inputs; nevertheless; in PU for milk production, Guevara (2010) found values of adoption of technology up to 100% after 5 years of training and transferring technology. Industrialization of the dairy products is one of the main problems for farmers, although just a few farmers elaborated cheese and yogurt, the adoption of this technology was significant.

Table 2. Adoption of technology by technical areas, number and percentage of PU at initial and final phase.

Technical areas	PU's Initial phase (June 2013)		PU's Final phase (January 2014)	
	Number	%	Number	%
Genetics and reproduction:				
Pregnancy diagnosis	106	18.6	172	30.2
Artificial insemination	65	11.4	98	17.2
Puerperium management	44	7.7	45	7.8
Replacement of cows	39	6.8	39	6.8
Estrus synchronization	33	5.8	46	8.1
TOTAL	**287**	**50.4**	**400**	**70.2**
Administration and organization:				
Using log	195	34.2	306	53.7
Model of GGVATT	24	4.2	29	5.1
Purchase and sale of inputs	15	2.6	12	2.1
ID SINIIGA	0	0	13	2.3
Entry to PGN	0	0	12	2.1
TOTAL	**234**	**41.1**	**372**	**65.3**
Animal health:				
Deworming and vaccination	188	33.0	235	41.2
Diagnosis of subclinical mastitis	44	7.7	83	14.6
Sanitary measures, breeding	42	7.4	35	6.1
TOTAL	**227**	**39.8**	**353**	**61.9**
Nutrition:				
Mineral supplementation	138	24.2	172	30.2
Feeding strategies	57	10.0	24	4.2
Elaboration of diets and portion	49	8.6	58	10.2
Nutritional blocks	31	5.4	28	4.9
Molasses-urea supplementation	14	2.5	9	1.2
TOTAL	**289**	**50.7**	**291**	**51.5**
Livestock management:				
Management of calves	102	17.9	96	16.8
Hormonal implants	30	5.3	41	7.2
Good milking practices	40	7.0	41	7.2
Early weaning	30	5.3	29	5.1
Management replacements	24	4.2	27	4.7
Dehorning calves	24	4.2	24	4.2
TOTAL	**250**	**43.9**	**258**	**45.3**
Forages:				
Forages conservation practices	66	11.2	71	12.5
Establishment of improved grassland	43	7.5	56	9.7

Rotational grazing	3	0.5	20	3.5
TOTAL	**112**	**19.6**	**147**	**25.8**
Industrialization:				
Elaboration of cheese and yogurt	**0**	0.0	**7**	**1.2**

CONCLUSIONS

The socioeconomic and cultural level of producers is low; in addition, their age reflects the abandonment of farmland by young people, who migrate to other states or countries. The adoption of technology, despite the short term of technical advice and training activities, was relatively high in some of the technical areas, although this technology is considered basic. Nevertheless, PSP´s must remain for a longer time training and transferring technology to the farmers. The GGAVATT model induces the farmer, to some extent, to adopt innovation as a result of the interactive training and transferring technology; therefore, the PU coverage in the state should be wider.

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GASTROINTESTINAL PARASITES IN SHEEP IN XOCHIMILCO, MEXICO CITY

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SUMMARY. Parasitic gastroenteritis caused by gastrointestinal nematodes is considered the most important disease of sheep grazing around the world, as it can cause weight loss in animals, diarrhoea and even death; in order to maintain control of these internal parasites, there have been used anti-parasitic, however, irrational and continued use has caused to appear to know more resistant gastrointestinal parasite to the action of chemicals. Then, becomes of interest know the actual geographical distribution of the nematodes ´genus that parasite to sheep around the word. The Xochimilco area in Mexico City forms a special ecosystem inner the urban surrounding; it is formed by a network of water channels that includes infinity of small islets and have template jungle vegetation type. The aim of this work is to determine the prevalence of gastrointestinal disturbances nematodes in sheep of Xochimilco area, Mexico City. From May to September of 2013, in a complete randomly sampling procedure, faecal samples from 250 sheep were taken directly from the rectum. Sheep involved in actual study were male and female, from 6 months to three years old, and from different breed and its crosses that represented the actual sheep population of Xochimilco area. In faecal samples using the McMaster technique eggs of nematodes by gram of faeces were accounted. Only two genuses of nematodes were found: *Nematodirus* sp. and *Chabertia* sp. However, these nematodes were present in the 54% (134/250) of sampled sheep. It is concluded that *Nematodirus* sp. and *Chabertia* sp. Are the nematodes genus's present in Xochimilco, Mexico. And its high prevalence indicates that represent a severe health challenge for the Xochimilco sheep herd.
Key words: Parasites, sheep, gastrointestinal.

INTRODUCTION

The gastrointestinal nematode is one of the natural mechanisms that regulated the animal populations in the different ecosystems in the eagerness to maintain this ecosystem. However, the animal protein requirements bordered the man to surround spaces where the largest number of animals are kept. With this, parasitic irrigation has increased and the negative effects of NGI on the herds are in sight (Aguilar *et al.*, 2009).

Parasitic gastroenteritis caused by gastrointestinal nematodes (NGI) is considered the most important disease of grazing sheep worldwide, causing weight loss, diarrhea and even death (Sutherland and Scott, 2010).

The distribution and abundance of gastrointestinal nematodes vary according to climatic conditions, particularly rainfall and temperature, as well as within and among ruminant species (Amarante *et al.*, 2013).

The most used strategy to control NGI is to break the life cycles of these organisms through the application of antiparasites at intervals determined by the ecological region, species to be controlled, residual efficacy (persistence of antiparasitic) and the customs of the producer.
Some of the main genera of NGI that can be found in sheep can be observed in Table 1, however, it is reported in the literature that Haemonchus contortus is the most important NGI in the tropics. However, infections under natural conditions are always mixed. As a result, the different strategies recommended for the control of this nematode species do not always have the expected effects, since in their absence the other parasitic populations occupy the place of the eliminated parasite and its pathogenicity is increased causing similar effects in the animals (Martínez *et al.*, 2007)
Antiparasitics have been successfully used in parasite control. However, its continuous and irrational use has led to the generation of NGI strains resistant to the action of these chemicals (McMahon *et al.*, 2013).
Due to the problem of resistance to the parasites it is necessary that the producers design and implement programs of integral control of parasites. Among the main problems that directly affect the health of ruminants and consequently are reflected in their productivity, are those caused by gastrointestinal nematodes; (Sutherland and Scott, 2010). The aim of this work is to determine the prevalence of gastrointestinal disturbances nematodes in sheep of Xochimilco area, Mexico City**.**

MATERIAL AND METHODS

The work was carried out in the Xochimilco Delegation, which has a tropical mountain climate. Its average annual temperature ranges between 12 ° C and 18 ° C, with an average annual rainfall of 700mm (Galicia, 2006).

Table 1. Genus and species of gastrointestinal nematodes affecting sheep.

Digestive system	Gender	Species
	Haemonchus	*contortus*
Abomasum	*Teladorsagia (Ostertagia)*	*circumcincta*
	Trichostrongylus	*axei*
	Cooperia	*curticei*
	Trichostrongylus	*colubriformis, vitrinus*
Small intestine	*Nematodirus*	*filicollis, spathiger*
	Bunostomum	*trigoncephalum,*
	Strongyloides	*papillosus*
Large intestine	*Oesophagostomum,*	*columbianum, globulosa*
	Trichuris	*ovis*

(Aguilar *et al*., 2008).

Sampling was done directly from the rectum with a plastic glove, which was used both hygienically to not dirty the hands as well as container of the same samples. Once obtained, they were identified and taken to the laboratory. The parasite identification was done by the flotation method.

RESULTS

Of the 250 individuals sampled, only 134 (54%) samples were detected positive. The eggs that were found were Nematodirus, Marshallagia marshalli and Chabertia.

DISCUSSION

As mentioned by Amarante *et al.,* 2013 gastrointestinal nematodes can be found in regions where there is rain and there are good temperatures for the rearing of ruminants as it was in the sampled region.

Coinciding with Aguilar et al., 2008, which mentions that one of the genera that affect sheep is Nematodirus, in the present study was found as Marshallagia marshalli and Chabertia. Therefore, it is possible to conclude that *Nematodirus* sp. and *Chabertia* sp. Are the nematodes genus's present in Xochimilco, Mexico. And its high prevalence indicates that represent a severe health challenge for the Xochimilco sheep herd.

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DECREASED PROLIFICACY IN RABBITS INDUCED BY *Taenia pisiformis* CYSTICERCOSIS

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SUMMARY. Some parasitic infections can affect host's reproduction. This fact has been widely documented in several vertebrate species, involving detrimental effects on their reproductive capacity reflected in a decrease in the number of offspring and the lack of care to the offspring. Regarding T. pisiformis cysticercosis, little is known about the consequences on prolificacy in infected rabbits. In this study, 10 week-old New Zealand does were either infected with 1,000 Taenia pisiformis eggs (n=5) or uninfected (n=5). Seven weeks after infection, each doe was mated ensuring two ejaculations. The total number of kits born alive and dead were visually counted at birth and litter weight was determined the day after delivery, and at weaning. At day 30 postpartum, does were humanely sacrificed and the number and location of cysticerci and granulomas was registered. Serum progesterone levels were measured before mating, the day of mating, 10, 20 and 25 days after mating, and after delivery, using the coated tube radioimmunoassay kit (Immunotech S.R.O.). Body weight, weaned kits and BMI were analyzed with an ANOVA test; the weighs and number of kits born dead or alive with a Mann-Whitney test; the weight of litter, voluntary food intake, and P with an ANOVA test and Tukey post-test. T. pisiformis infection induced a decrease in prolificacy of does, which was not related to increased mortality of kits during lactation, nor with loss of body condition of the mother. It seems likely that the infection-induced decrease in prolificacy is related to changes in the endocrine system of the host during pregnancy, as circulating P4 levels were found to be dramatically altered in infected animals.

Key words: Rabbit, Cysticercosis, Prolificacy

INTRODUCTION

Prolificacy alterations in hosts infected by parasites have been recognized in several phyla, especially in arthropods and mollusks, but it has been few studies in vertebrates, mostly in mammals (Sarasa et al., 2011). Understanding the mechanisms involved in the host's driven reproduction modulated by parasites is essential for the comprehension of disease mechanisms induced by parasites and its consequences to the host. In rabbits, the most frequent cestodiasis is caused by *Taenia pisiformis* (*T. pisiformis*) (Szkucik et al., 2014), whom become infected when they are fed water or food contaminated with eggs of *T. pisiformis*, developing metacestodes mainly in the peritoneal cavity and mesentery. In the liver, the infection originates granulomatous lesions, causing economic losses due to confiscation in slaughterhouses (Flatt and Campbell, 1974). A study in wild hares showed that deworming with ivermectin increases fecundity (Newey and Thirgood, 2004). Additionally, it was reported that female rabbits infected by *Trichostrongylus retortaeformis* produced smaller litter size

compared to healthy does (Dunsmorje 1981), and some authors found a relationship between overall body condition and fecundity of rabbits (Martínez-Paredes et al., 2012). Little has been studied about the consequences of *T. pisiformis* cysticercosis in infected rabbits, particularly in does, where there is a gap of information about the consequences of this parasite on host prolificacy. The aim of this study is to address the effects of infection with *T. pisiformis* cysticercosis in rabbit farms, and correlate parasite burdens to body condition of pregnant rabbits, P_4 levels, and prolificacy, evaluated as the number and weight of does offspring and litter size at weaning.

MATERIAL AND METHODS

Animal care and experimentation practices were performed with adherence to official Mexican regulations (NOM-062-ZOO-1999). Ten 8 week-old nulliparous New Zealand does (*Oryctolagus cuniculus*) were uninfected or orally infected with 1,000 eggs of *T. pisiformis* by oesophageal administration using a sterile plastic tube. Animals were kept individually in 90x50x40 cm wire cages equipped with a gate that allowed free transit to a 20 cm depth nest, fed *ad libitum* with Conejina Purina® (15.5% protein and 15.0% fiber) and water. At 7 weeks of infection, does were crossed with sexually experienced New Zealand bucks (Jiménez et al., 2012). Total number of kits born dead or alive were visually counted at birth and litter weight was determined using an electronic scale the day after delivery. Blood samples were obtained from the marginal ear vein using a syringe, blood serum was kept at -20° C until analysis of Progesterone (P_4) concentration, using the coated tube radioimmunoassay kit (Immunotech S.R.O.) (Hallal-Calleros et al., 2013). The body mass index (BMI) was obtained as described by Sweet et al. (2013). After weaning (30 days postpartum), rabbits were humanely sacrificed and the number and location of cysticerci and granulomas were registered.

RESULTS

The infection of does with eggs of *T. pisiformis* induced a decrease of 62% in the number of kits born alive. The average number of kits born alive in the infected group was 3.2±1.6, where the lowest number of kits was 0, while in the controlled group the average was 8.6±1.1, with the lowest number of kits being 4 ($P=0.046$) (Fig. 1A). Mortality at birth in the infected group was 0.4±0.2, representing two stillbirths from 18 kits, while in the control group was zero in a total of 43 kits born, however, there were similar ($P=0.51$) (Fig. 1B). After 30 days of nursing, 16 rabbits (3.2±3.7) were weaned in the infected group and 40 rabbits were weaned in the uninfected group (8 ± 2.3) (Fig. 1C), representing a decrease of 60% in the number of weaned rabbits induced by the infection. The weight of the litters was evaluated at 24 hrs, 7, 14, 21 and 28 days after birth, observing, a trend of 48% of decrease in litter weight induced by the infection with no significant difference ($P>0.05$) (Fig. 1D). The infection induced an increase of 64% in uterus weight of infected rabbits, while weight of the liver and spleen were similar ($P>0.05$) between control and infected groups (Not shown). No differences were found in food intake of pregnant rabbits, nor in the average body weight or BMI. No obvious signs of disease such as abnormal prostration or shaggy hair in infected animals were observed. Serum P_4 levels during pregnancy were higher ($P<0.05$) in infected females compared to controls during days 10 and 25 of gestation. No difference was found ($P>0.05$) in P_4 levels before pregnancy and at delivery, between infected and control rabbits (Fig. 2). An average of 24.5±7.17 hepatic granulomas, ranging 1 to 46, and

3.3±0.91 metacestodes ranging 1 to 5, were found in infected rabbits. Hepatic granulomas represent 37.5% of infection, 25% cysticerci were found free in peritoneum, 31.25 % was attached to uterine tubes, and 6.25% in heart. No cysticerci or granulomas were found in control group.

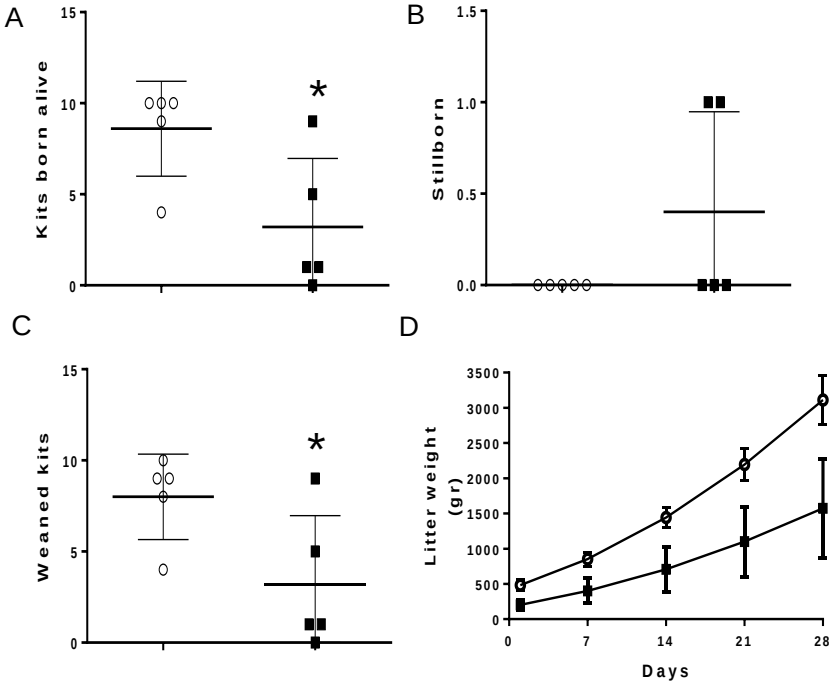

Figure 1. *T. pisiformis* cysticercosis decreases the offspring in does. (A) Number of kits born alive, (B) stillbirths, (C) weaned after 30 days, (C) and litter weight at weaning, from infected (■) or healthy does (○). Mean±SD. Kruskall-Wallis Test *P<0.05.

DISCUSSION

Our results confirm and extend the notion that a parasite infection, particularly with an intestinal helminth, reduces the reproductive outcome in the infected host, however, the post-reproductive parameters of an infection has been less studied. We demonstrated that cysticercosis by *T. pisiformis* decreases prolificacy of does. Cysticercosis by *T. crassiceps* alters the endocrine environment and the behavior of its host, in male mice chronically infected testosterone and estradiol levels are comparable to those of a female (Larralde al., 1995), while females infected chronically lose the continuity of the estrous cycle (Arteaga-Silva et al. 2009). It has been reported a sophisticated communication between the host and the parasite during *T. crassiceps* cysticercosis, which also results in loss of male prolificacy. It is unclear whether cysticerci castrates their mammalian hosts or if the loss of prolificacy is a host strategy in fighting infection. Therefore, *T. pisiformis* infection causes loss of prolificacy probably by alterations that involve changes in P_4 levels during pregnancy. Our results are of interest for the management and production of rabbits and suggest that loss of prolificacy caused by cysticerci of the genus *Taenia* are broader than previously considered.

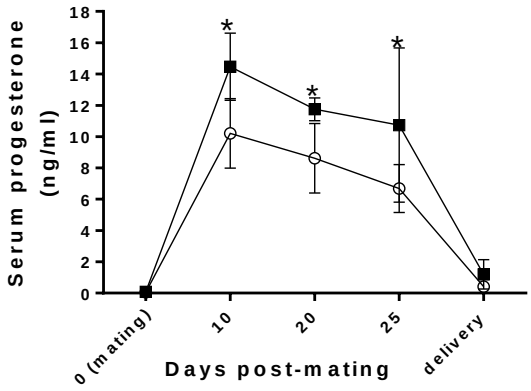

Figure 2. T. pisiformis cysticercosis modifies serum P_4 levels during pregnancy. Mean±SD, Mann-whitney *P<0.05.

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INFLUENCE OF NON-ENZYMATIC ANTIOXIDANTS ON QUALITY OF BEETAL BUCK SEMEN AT 4°C"

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INTRODUCTION: An experiment was designed to evaluate the role of vitamin E and glutathione in improving the seminal parameters during hypothermic storage of liquid semen at 4°C for 72 hours.

ANIMALS, MATERIALS AND METHODS: The semen ejaculates were collected by artificial vagina from 6 bucks (Beetal) during the normal reproduction season. The samples were centrifuged and the seminal plasma was removed. The sperm pellet was diluted with Tris based extender and divided into 3 groups. Group T1: control samples without antioxidants, group T2: samples supplemented with tocopherol @ 3mM and group T3: samples supplemented with glutathione @ 1mM. The samples were evaluated for progressive motility, percent liveability, percent abnormal spermatozoa and acrosome integrity after liquid preservation for 0, 24, 48 and 72 hours. The level of lipid peroxidation and antioxidant enzymes, viz., glutathione peroxidase (GPx) and superoxide dismutase (SOD) were estimated after liquid preservation for 0 and 72 hours.

RESULTS: It was observed that after storage of semen at 4°C upto 72 hours, the progressive sperm motility, percent liveability, percent abnormal spermatozoa and percent intact acrosomes were significantly ($P<0.05$) higher in group T2 and T3 samples as compared to control. However, level of lipid peroxidation in T2 and T3 group was significantly ($P<0.05$) lower after 72 hours of incubation at 4°C. Similarly, GPx and SOD values were significantly ($P<0.05$) increased in T2 and T3 group after 72 hours of storage at 4°C.

CONCLUSSIONS: Thus it can be concluded that vitamin E and glutathione supplementation @ 3mM and 1mM respectively while preserving the semen samples at 4°C helped in maintaining the seminal parameters up to 72 hours and protected the spermatozoa from oxidative damage.

IMPACT OF HEAT STRESS ON REPRODUCTIVE INDICES OF RABBIT BUCKS AT IBADAN, NIGERIA

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INTRODUCTION: Heat stress often blamed for suboptimal reproductive efficiency inflicts heavy economic losses reflected in limiting the breeding season of rabbits. Many factors affect seminal traits and thus it is crucial to define suitable protocols to improve spermatozoa characteristics during peak periods of heat stress. Hence, it is possible to produce more doses of semen with higher "expected" fertility and with less variability all year round. This study aimed to investigate semen quality and oxidative status of four exotic breeds of rabbit buck at peak temperature humidity index (THI) of Ibadan, Nigeria.

MATERIALS AND METHODS: Four Exotic breeds of rabbit consist of Fauve de Bourgogne, Chinchilla, British spot and New Zealand White (10 rabbits per breed) allotted to experimental cages in a completely randomized design. Semen samples were collected from the animals at 7th week of exposure to peak THI in Ibadan, Nigeria. The samples were analysed for semen quality and seminal fluid were assayed for Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and total antioxidant activity (TAA).

RESULTS: Results obtained showed that animals were exposed to very severe heat stress during the experimental period, Mass activity and motility of spermatozoa in New Zealand White bucks was significantly ($p<0.05$) higher than Fauve de Bourgogne bucks. Functional sperm membrane integrity of 54.35% (Fauve de Bourgogne), 48.43% (chinchilla), 49.56% (British Spot) and 58.94 % for New Zealand White bucks were obtained. Seminal total antioxidant activity was significantly ($p<0.05$) highest in New Zealand White bucks and significantly ($p<0.05$) least values was obtained in British Spot. Seminal lipid peroxidation was significantly ($p<0.05$) least in British Spot.

CONCLUSION: It could be concluded New Zealand white bucks had best semen quality, this could be due to its high oxidative status, though British spot bucks had least seminal lipid peroxidation.

DETERMINATION OF UDDER HEALTH STATUS AND THE QUALITY OF THE MILK IN DAIRY COWS OF TÉJARO, MICHOACÁN, MEXICO.

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SUMMARY. The objective was to determine udder health status and milk quality in dairy cows from Téjaro, Michoacán through somatic cell counts (SCC), by the California test (CMT) and DE Laval Cell Counter (CCD). A total of 20 samples of milk obtained from the production units of Téjaro, Michoacán, were analyzed. Sampling was performed at random, considering the owners' disposition regarding the collaboration of the sampling. To determine the quality of the milk was based in the Mexican normatively NMX-F-700-COFOCALEC-2004, as well as the California Test. A mean of 660,000 ± 17,000 SC/mL was obtained from the milk samples. Regarding the California Test, 85% of the samples turned out to be within the range established by Saran and Chaffer. (2000), as good quality and fit for human consumption. The amount of SC found in milk samples from cows in Tejaro, is in the range suggested as the US standard, which mark > 400,000 to 750,000 SC / mL respectively and according the NMX -F-700-COFOCALEC-2004, which establishes a limit of 1,000,000 SC / mL. It is concluded that the state of health of the udder is good and the milk produced in the municipality of Téjaro Michoacán is of good quality since it complies with the parameters established at national and international level, through the California and CCD Test, for which is suitable for public consumption, without risk. The SCC and the CMT have shown good correlation and have been useful in the diagnosis of the health status of the udder and milk health, since the CMT could give negative results to those herds that presented low CCS indicating that can provide a similar and reliable result

Biosecurity

THE IMPORTANCE OF MICROBIOLOGICAL ANALYSIS OF DRINKING WATER INTENDED FOR ANIMAL CONSUMPTION

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SUMMARY. The waterborne pathogens potentially causing illness include: bacteria, viruses, parasites (protozoa and helminthes). Most waterborne pathogens are introduced into drinking water by its contamination with human and animal faeces, they do not grow in water and they induce infection in the gastrointestinal tract following the ingestion. Besides ingestion, other routes of transmission can include inhalation of aerosols, leading to infections of the respiratory tract (e.g. *Legionella*) and cutaneous contact by bathing, leading to infections of the skin and brain (e.g. *Naegleria fowleri*). In Romania, it is considered that the water intended for animal consumption must have the same quality as the drinking water, because there isn't a distinct quality standard for water intended for animal consumption. Therefore, in this study/paper, the indicator parameters for the microbiological contamination of the drinking water, such as: coliform bacteria, *Escherichia coli*, enterococci, colony formatting micro-organisms at 22 °C and 37 °C, *Pseudomonas aeruginosa* and *Clostridium perfringens*, are defined and described. The importance of their analysis in the context of the appreciation of the quality of drinking water is marked, the standardized methods of analysis and the current European and Romanian legislation for the interpretation of the results of the analysis are also presented. A comparative study on the microbiological quality of the water intended for animal consumption, at the national level, performed in the period 2007-2015, is also presented. A marked decrease in the number of water samples was observed, as well as the necessity of monitoring the water quality as a major objective, in order to maintain the state of health and the level of animal production, to prevent outbreaks of transmissible diseases among animals and from animal to human, to protect the environment and, last but not at least, to protect the human health.

Key-words: drinking water, indicator parameter, microbiological analysis.

INTRODUCTION

The waterborne pathogens potentially causing illness include: bacteria, viruses, parasites (protozoa and helminthes). Most waterborne pathogens are introduced into the drinking water by its contamination with human and animal faeces; they do not grow in water and they induce an infection in the gastrointestinal tract following the ingestion. Besides this route, other routes of transmission can include inhalation of aerosols, leading to infections of respiratory tract (e.g. *Legionella*) and cutaneous contact by bathing, leading to infections of the skin and brain (e.g. *Naegleria fowleri*). In Romania, it is considered that the water intended for animal consumption must have the same quality as drinking water, because there isn't a distinct quality standard for the water intended for animal consumption (Decun, 2007). Therefore, the scope of this paper is to define and present the main indicator-parameters of the microbiological contamination of the drinking water and their importance for the assessment of the drinking water quality. ***Coliform bacteria*** are Gram-negative, aerobic and facultatively anaerobic, non-spore-forming bacilli, capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose. This group includes the genera with faecal origin as: *Escherichia, Citrobacter, Klebsiella, Enterobacter, Serratia, Hafnia,* and environmental origin (capable of

multiplication in water and soil). Their presence indicates an inadequate treatment, biofilm formation through entry of foreign material (soil, plants) in the distribution systems and water tanks. ***Escherichia coli*** is a member of the thermo-tolerant coliforms group, capable of lactases fermentation at higher temperature, but it is different from the coliforms by its ability to produce indole from triptophan and β-glucuronidase enzyme. *Escherichia coli* is considered the **most suitable indicator for faecal contamination** because it is present in an important quantity in human and animal faeces. ***Enterococci*** are a subgroup of faecal streptococci represented by the following species: *Enterococcus faecalis, Enterococcus faecium, Enterococcus durans, Enterococcus hirae*. They are Gram-positive bacteria, relatively tolerant of sodium chloride solution (6,5%) and alkaline pH levels, facultatively anaerobic. Enterococci are relatively specific for faecal pollution because most species don't multiply in a water environment, but some can originate from other habitats, e.g. soil, in the absence of faecal contamination. They are considered the **indicators of recent faecal pollution** like *Escherichia coli*, but they tend to survive longer in a water environment, and are more resistant to drying and chlorination. ***Micro-organisms formatting colonies*** are represented by bacteria and fungi, capable of growing on rich growth media, without inhibitory or selective agents, over a specified incubation period and at a defined temperature (22 °C and 37 °C). Here are included: micro-organisms sensitive to the disinfection processes (coliform bacteria), micro-organisms resistant to disinfection (sporulated micro-organisms), microorganisms that rapidly proliferate in treated water in absence of residual disinfectants and opportunist pathogens such as: *Acinetobacter, Aeromonas, Flavobacterium, Klebsiella, Moraxella, Serratia, Pseudomonas, Xanthomonas*. There is no evidence of an association of any of these organisms with a gastrointestinal infection through the ingestion of drinking-water in the general population. The microorganisms that grow at 37 °C originate from human and animals with warm blood and those growing at 22 °C are of aquatic origin. Their number should be as low as possible, a big number providing an inadequate treatment, presence of biofilm and lack of integrity of distribution systems. ***Pseudomonas aeruginosa*** is a member of *Enterobacteriaceae* Family, unsporulated, with a weak mobility. It synthesizes pigments like: pyocianin, pyoverdin and pyorubin, and is considered a hygiene indicator. ***Clostridium perfringens*** is a species of *Clostridium* Genus, represented by a group of Gram-positive, anaerobic, sporulated (spores are resistant in unfavourable conditions of aquatic habitat: UV irradiation temperature, pH extremes and chlorination), sulfite-reducing bacilli. It is considered an **indicator** with high specificity for **intermittent faecal pollution**, an indicator of protozoa and enteric viruses and a useful indicator for the effectiveness of the filtration, because the *Clostridium perfringens* spores are smaller than oocysts or cysts of protozoa.

MATERIAL AND METHODS

A comparative study concerning microbiological quality of the water intended for animal consumption, at the national level, was performed in the period 2007-2015, by collecting data from the County Sanitary Veterinary and Food Safety Laboratories and the Institute for Diagnosis and Animal Health Bucharest. In Romania, the main microbiological parameters for the quality assessment of the drinking water intended for animal consumption are: coliform bacteria, *Escherichia coli*, enterococci and micro-organisms formatting colonies at 22 °C and 37 °C. The specialists used the methods of analysis of microbiological parameters mentioned in the EU Directive 2015/1787 for the modification of EU Directive 98/83/EC on the quality of water intended for human consumption. The evaluation of the number of colony formatting micro-organisms at 22°C and 37°C /ml is made by the inoculation of 1 ml of sample, and another 1 ml of sample decimal dilution in Petri dishes, an operation performed two times, followed by the counting of colonies grown on culture medium – yeast extract agar – after aerobic incubation at 22±2 and 36±2 °C and the calculation of weighted average. These are the steps for the detection and enumeration of *Escherichia coli* and coliform bacteria, by the membrane filtration

method: filtration of 100 ml of samples; transfer of the membrane on selective medium – chromogenic coliform agar (CCA), with substrates for enzymes as: β-galactozidase (synthesized by coliforms) and β-glucuronidase (synthesized by *Escherichia coli*); incubation of the plate at 36 ± 2 °C, 21 ± 3 h; examination of the colonies grown on the plate; the dark blue-violet - β-galactozidase-positive and β-glucuronidase-positive colonies are considered *Escherichia coli* confirmed/plate and pink-red colonies -β-galactozidase-positive-coliform bacteria that are not *Escherichia coli* presumptive and their confirmation of the latter by oxidase test (negative). The principle of the method for the detection and enumeration of intestinal enterococci is based on the filtration of 100 ml of sample, the incubation of the membrane on Slanetz-Bartley medium, that contains sodium aside (for inhibition of Gram-negative bacteria) and 2,3,5-triphenyltetrazolium chloride (colourless substance), that is reduced formazan (red) by enterococci. By the transfer of membrane with typical colonies on bile-aesculin-azide agar, preheated at 44°C, the hydrolysis occurs in 2 h (the final product, 6,7-dihidroxicumarin, combines with iron (III) ions in order to give a tan-coloured to black compound that diffused into the medium).

RESULTS

The number of colony formatting micro-organisms at 22°C and 37°C /ml represents the weighted average of numbers of colonies/plates inoculated as mentioned above. The number of coliform bacteria /100 ml represents the number of coliform bacteria /plate, expressed as sum between the number of *Escherichia coli* confirmed/plate (dark blue-violet colonies - β-galactozidase-positive and β-glucuronidase-positive) and the number of coliform bacteria that are not *Escherichia coli*, both presumptive (pink-red colonies - β-galactozidase-positive) and confirmed (oxidase-negative)/plate. The number of intestinal enterococci /100 ml is the number of presumtive colonies that grow on Slanetz-Bartley medium (pink, red or maroon colonies) and confirm on bile-aesculin-azide agar (black colonies, with blackening of medium under the colonies). The data presented in the Table 1 on the quality of the drinking water intended for animal consumption in the period 2007-2015 were collected from the County Sanitary Veterinary and Food Safety Laboratories and the Institute of Diagnosis and Animal Health Bucharest.

Table 1: A comparative study on the microbiological quality of the water intended for animal consumption, at the national level, performed in the period 2007-2015

Year	Total number of analyzed samples	Total number of adequate samples	Percentage of adequate samples	Total number of inadequate samples	Percentage of inadequate samples
2007	15523	10682	68,81 %	4841	31,19 %
2008	5851	4060	69,38 %	1791	30,62 %
2014	951	890	93,58 %	61	6,42 %
2015	843	801	95,01 %	42	4,99 %

DISCUSSION

The interpretation of these microbiological parameters was performed according to the Law 311/2004 for the modification and completion of Law 458/2002 on the quality of potable water, transposed form the EU Directive 98/83/EC on the quality of water intended for human consumption. In the period 2007-2015, a marked decrease of number of water samples from 15523 to 843 (94,95 %) was observed: a small difference between 2014 and 2015 (11,35 %), high difference between 2007 and 2008 (62,31%) and between 2008 and 2014 (83,74 %), which requires the necessity of monitoring the water quality by lab analysis (Figure 1). It was observed also an increase in the percentage of adequate samples: 68,81%

(2007), 69,38% (2008), 93,58% (2014) and 94,95% (2015), that is correlated with an improvement in the quality of the drinking water intended for animal consumption (Figure 2).
It is necessary to monitor the water quality in order to observe the animal welfare principles, to preserve the health status and the level of animal production, to prevent outbreaks of transmissible diseases among animals and from animal to human, to protect the environment and, most importantly, to protect human health.

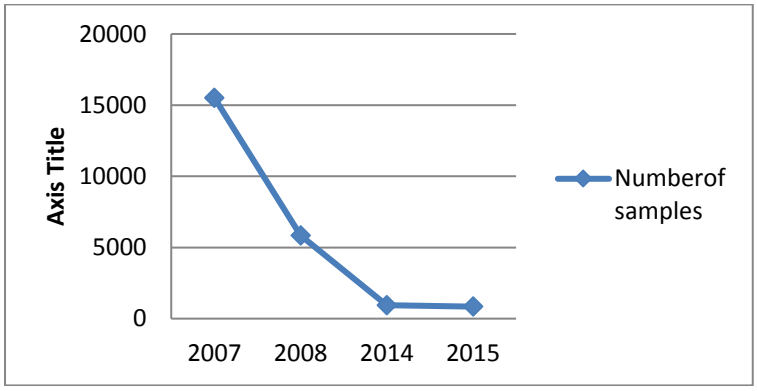

Figure 1. Number of samples analysed in the period 2007-2015

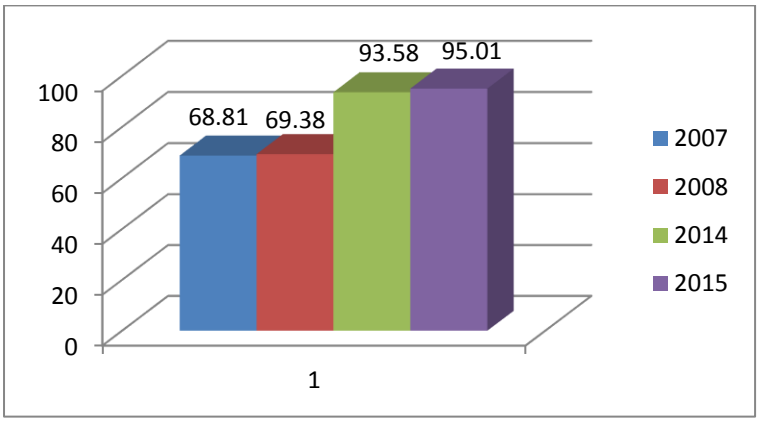

Figure 2. Percentage of adequate samples

ACKNOWLEDGMENTS

I want to thank the specialists from the County Sanitary Veterinary and Food Safety Laboratories for providing the data on the microbiological quality of the water intended for animal consumption**.**

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SR EN ISO 7899-2:2002 - Water quality – Detection and anumeration of intestinal enterococci. Part 2: Membrane filtration method.

SR EN ISO 9308-1:2015 - Water quality – Detection and anumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method for waters with low bacterial background flora.

ARE THE "TOP" 25% IRISH PIG FARMS DOING SOMETHING DIFFERENT IN TERMS OF BIOSECURITY PRACTICES RELATED TO STAFF AND VISITORS?

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SUMMARY: The objective of this study was to identify differences in biosecurity practices related to staff and visitors between the top (T25) and bottom 25% (B25) of surveyed Irish pig farms in terms of herd size and production parameters. Forty-eight Irish farms were surveyed using the BIOCHECK.UGENT® protocol, a biosecurity benchmarking system comprising 108 questions developed by Ghent University, Belgium, currently used in several EU countries. Questions regarding external and internal biosecurity and related to human circulation and hygiene were selected for analysis. The farms were ranked into four different groups for herd size, piglet mortality %, number of pigs produced per sow per year and feed conversion ratio. The bottom and top 25% of farms for each production criterion were selected. Data was analysed using Chi-square tests. Regarding herd size, 100% of T25 farms obliged visitors to check-in before entering the farm versus 75% of B25 (P = 0.064). A hygiene lock was available and always used by 91.7% of T25 versus 58.3% of B25 farms (P = 0.059). Farm buildings were only accessible to visitors from the hygiene lock in 58.3% of T25 versus 16.7% B25 (P = 0.035). There was no difference in the percentage of T25 and B25 herd size rankings that had a strict separation between the clean and the dirty area of the hygiene lock; farms where visitors had to wear specific clothing/shoes; farms where hands had to be washed before entering the buildings and between different sections within the farm. Additionally, there was no difference between farm ranking for performance traits and the biosecurity practices. Results show that the percentage of farms engaging in some of these practices is low irrespective of their ranking. However, larger herds tended to implement stricter external but not internal biosecurity practices.

Key words: Biosecurity, pigs, production parameters

INTRODUCTION

Good biosecurity is crucial to prevent the introduction and spread of diseases in pig herds. Farms with higher biosecurity status have lower antimicrobial usage and higher daily weight gains (Laanen et al., 2013); indicating that higher biosecurity levels may have a positive impact on pig health and performance. Thus, it is important to identify the specific biosecurity practices that might lead to better performance. We hypothesised that better producing farms would adhere to stricter biosecurity practices compared with the less producing farms. Therefore, the objective of this study was to identify differences in biosecurity practices related to staff and visitors between the top (T25) and bottom 25% (B25) of surveyed Irish pig farms in terms of herd size and production parameters.

MATERIAL AND METHODS

Forty-eight farrow-to-finish Irish pig farms were surveyed using the BIOCHECK.UGENT® protocol (available at www.biocheck.be), a biosecurity benchmarking system comprising 108 questions developed by Ghent University, Belgium, currently used in several EU countries. The BIOCHECK.UGENT® protocol is subdivided into external (i.e. purchase of animals and semen; transport of animals, remove manure and dead animals; feed, water and equipment supply; entrance of personnel and visitors; vermin and bird control; environment) and internal (i.e. disease management; farrowing and suckling period; nursery unit; fattening unit; measures between compartments and use of equipment; cleaning and disinfection) biosecurity. Farmers were initially contacted by phone and a visit to the farm was scheduled upon the farmer's agreement to participate in the study. Further, for each of the surveyed farm, production parameters (i.e. herd size, piglet mortality %, number of pigs produced per sow per year and feed conversion ratio) were retrieved from the Irish national database (Teagasc e-Profit Monitor System) for the year 2015.

Statistical analysis. Questions related to human circulation and hygiene from both the external and internal biosecurity subdivisions were selected for analysis. Each farm was considered the experimental unit. Farms were ranked into four different groups for herd size, piglet mortality %, number of pigs produced per sow per year and feed conversion ratio using PROC RANK of SAS (SAS Inst. Inc., Cary, NC). The T25 and B25 farms for each production criterion were selected for analysis. A Chi-Square test (PROC FREQ, SAS) was performed to investigate the relationship between biosecurity and production parameters. Alpha level for determination of significance and trends were 0.05 and 0.10, respectively.

RESULTS

Descriptive statistics for the four production criteria for the B25 and T25 farms are presented in Table 1. The percentage of T25 and B25 farms that answered "**yes**" to the biosecurity questions by each ranking criterion is presented in Table 2. Regarding herd size, 100% of T25 farms obliged visitors to check-in before entering the farm versus 75% of B25 (P = 0.064). A hygiene lock was available and always used by 91.7% of T25 versus 58.3% of B25 farms (P = 0.059). Farm buildings were only accessible to visitors from the hygiene lock in 58.3% of T25 versus 16.7% B25 (P = 0.035). There was no difference in the percentage of T25 and B25 herd size rankings that had a strict separation between the clean and the dirty area of the hygiene lock; farms where visitors had to wear specific clothing/shoes; farms where hands had to be washed before entering the buildings and between different sections within the farm. There was no difference between farm ranking for performance traits and the biosecurity practices except for 45.8% of T25 farms ranked for number of pigs produced/sow./year where hands are washed and/or disinfected between different compartments compared with 25% of B25 farms that did the same. Additionally, in none of the surveyed farms clothing is changed between compartments

Table 1. Descriptive statistics for the top (T25) and bottom 25% (B25) of 48 surveyed Irish pig farms[1] ranked into four different groups for herd size, piglet mortality %, number of pigs produced per sow per year and feed conversion ratio

	B25% (n =12)				T25% (n = 12)			
Production criterion	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Herd size	281	70.78	161	381	1163	513.93	763	2525
Piglet mortality, %	13.18	0.79	12.20	14.30	6.53	1.76	5.80	7.90

Pig produced/ sow/year	23.13	1.10	20.90	24.10	28.67	1.41	27.30	31.80
Feed conversion ratio	2.54	0.06	2.47	2.68	2.18	0.30	2.10	2.33

[1]Farms were ranked using PROC RANK of SAS (SAS Inst. Inc., Cary, NC)

Table 2. Percentage of top (T25) and bottom 25% (B25) of 48 surveyed Irish pig farms[1] ranked according to four different production criteria that answered "YES" to questions related to human circulation and hygiene from both the external and internal biosecurity subdivisions of the BIOCHECK.UGENT® protocol.

	Herd size		**Piglet mortality**		**Pig produced/ sow/year**		**FCR[2]**	
	B25%	**T25%**	**B25%**	**T25%**	**B25%**	**T25%**	**B25%**	**T25%**
EXTERNAL BIOSECURITY								
Visitors check in before entering to the farm	75.0[b]	100.0[a]	83.3	83.3	91.7	100.0	91.7	91.7
Pig contact free period of 12h required to enter the farm	91.7	91.7	100.0	100.0	100.0	100.0	100.0	91.7
Hygiene lock available and always used by visitors before entering to the farm	58.3[(b)]	91.7[(a)]	75.0	75.0	58.3	83.3	83.2	75.0
Farm is only accessible for visitors from the hygiene lock	16.7[b]	58.3[a]	33.3	33.3	33.3	16.7	50.0	33.3
Strict separation between the clean and the dirty area of the hygiene lock	26.7	25.0	16.7	16.7	16.7	16.7	33.3	16.7
INTERNAL BIOSECURITY								
Hands are washed and/or disinfected between different compartments	83.3	91.7	16.7	16.7	50.0[a]	91.7[b]	16.7	25.0
Disinfection baths are located between different compartments	33.3	33.3	25.0	25.0	50.0	33.3	41.7	33.3

[1]Farms were ranked into four different groups for herd size, piglet mortality %, number of pigs produced per sow per year and feed conversion ratio using PROC RANK of SAS (SAS Inst. Inc., Cary, NC).
[2]Feed Conversion Ratio
[(a), (b)] Denote tendencies; $P > 0.05$ and ≤ 0.10

DISCUSSION

Biosecurity has an impact in production parameter and usually, farms with higher biosecurity levels also had better performance; thus having a good understanding about the biosecurity practices implemented in such farms could help to provide advice to less producing farms that could lead to significant improvements in pig health and performance. Questions related to human circulation and hygiene from both the external and internal biosecurity subdivisions were selected for this study. This was done because it seems that greater important is giving to other biosecurity aspects such as purchase and transport of animals and the removal of dead animals from the premises (biosecurity aspects over which farmers do not have 100% control) and less attention is paid to the protocols follow to allow visitors inside the farms or practices follow inside the farm by both farm staff and visitors which could lead to the introduction of pathogens to the herd. For example, measurements between compartments received the lower internal score in previous studies using the BIOCHECK.UGENT® protocol (Laanen et al., 2011; Postma et al., 2015). We expected that top ranked farms followed stricter biosecurity practices than bottom ranked farms. A higher percentage of farms practiced more external than internal biosecurity measurements related to human circulation. It seems that farmers see more threatening outside factors and disregard the potential benefit of also having good internal biosecurity practices. It could also be possible that most of the farms would follow biosecurity practices such as having a visitors' registry or a hygiene lock available because it might be required by quality certification schemes. Furthermore, results suggest that farmers are not aware of the importance to carry out the biosecurity practices themselves. A similar result was observed by Backhans et al. (2015) where only 32% of farmers carry out hygienic measurements themselves. From the four production criterion chosen for analysis, only herd size was associated with following extricter biosecurity practices. This was also observed by Laanen et al. (2013) and Backhans et al. (2015) in Belgian and Swedish pig herds, respectively. According to Laanen et al. (2013) management in larger herds must be more professional and more organised.

CONCLUSIONS

In conclusion, considerable improvement can be achieved in terms of biosecurity practices related to human circulation and in educating farmers about the important of training themselves and their personnel to follow the biosecurity practices imposed to visitors regardless farm of ranking.

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Nutrition, feed and additives

INFLUENCE OF BIFIDOBACTERIUM SPECIES ON FUNCTIONAL STATUS OF RUMEN

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SUMMARY. The aim of this study was to determine how the administration of probiotic feed supplements affects microflora and microfauna of the rumen of cattle, how it impresses the basic chemical and biological processes in the rumen, and also to check their influence on the total digestibility of feed in the cannulated cattle. For the experiment we used two adult cows of Aberdeen Angus breed with implanted permanent cannula, whom probiotics *Bifidobacterium sp.* were administered daily. From the samples of rumen fluid we analyzed the amount of ammonia, volatile fatty acids, protozoa and pH. The impact of probiotics has not been demonstrated in testing the influence of probiotics on the different variables with fixed effect of an individual. When testing the influence of probiotics without the effect of an individual, obtained data of acetic and butyric acid came out in the linear model the best. In their dependence numbers of protozoa were increasinig. Since we tested only two experiment individuals, there is a strong effect of the individual here. These results indicate that the effect of probiotics *Bifidobacterium sp.* on the functional state of the rumen is low. These results could be affected by the low number of probiotic application replications, and also by the low number of animals.
Key words: cannula; cattle; protozoa

INTRODUCTION

The rumen hosts a large number of microorganisms, including bacteria, protozoa and fungi, that function on the base of strict anaerobic ambience. These microbes degrade plant fiber to non-fibrous carbohydrates, proteins, volatile fatty acids and ammonia. Ammonia is used by microbes as energy and own source of nitrogen needed for their growth (Fraga et al., 2013; Gillespie and Flanders, 2014). This implies that these microorganisms have an important role in maintaining the stability of rumen ambience and health of the host (Castillo-Gonzalez et al., 2014).

MATERIAL AND METHODS

For the experiment was used two adult cows of breed Aberdeen Angus with implanted permanent rumen cannula (ø 13 cm) to evaluate the impact of administration of probiotics genus *Bifidobacteriunm sp.* (10^7 . g^{-1}). Experimental animals were housed loosely in box loges with ad libitum access to the drinking bowls with water and lick. The average body weight during the experiment was in the first animal 799 ± 7.1 kg, in the second animal 594 ± 9 kg. Probiotics *Bifidobacterium sp.* were administered in a lyophilized form of 2 g each, stirred in 100 ml of drinking water and applied through the cannula into the rumen, each day at 9:00 PM during the whole habituating and experimental period. The experiment was performed in two reruns, which had a consistent pattern of activities. In each of them both cows were included gradually. In the third controlled period animals received the basic

feeding ration (BFR). During the whole experiment stable microclimate was monitored using datalogger.

RESULTS

At first the influence of variables on the amount of ciliates in rumen without the effect of an individual was tested. We used LM (Table 1) from which the values of pH ($F = 4.1674$; $p = 0.04$) and the amount of acetic acid ($F = 6.6834$; $p = 0.011$) were significant. Then LM was simplified into the resulting linear model that best describes the obtained data, where the variable acetic acid was significant ($p = 0.001$) and the butyric acid was inconclusive ($p = 0.08$). Subsequently data were tested towards the influence of a group (experiment, control) with the fixed effect of an individual. The overview of the results of each test, where all variables were inconclusive. The resulting linear model best describes influencing on the amount of ciliates by monitored variables, quantity [mM^{-1}] of butyric, propionic and acetic acid, the pH level and amount of ammonia in the rumen fluid in both animals. The overview of the results of each model, that tested each variable towards the group (experiment, control) with the fixed effect of an individual. The p value was inconclusive in all variables. Simultaneously, the effect of the individual was always significant. Summary results of testing of individual variables towards a group (experiment, control) with a fixed effect of the individual. P value for all variables was inconclusive. The effect of the individual was significant in the share of ash and NDF.

DISCUSSION

It was demonstrated that probiotics have an effect on the stabilization of pH, on the improvement of nutrient intake from the rumen microbiota to the host and on the improvement of ruminal ambience (Chiquette et al. 2012). The influence of probiotics on the pH was not proved in this study. It was proved only the influence of an individual that notes also Ritz et al. (2014). Probiotics did not have any effect on the amount of VFA in rumen fluid, the same as in other studies (Qadis et al., 2014). Wang et al. (2016) report that probiotics reduced the concentration of propionic and acetic acid, which is the opposite of the Beauchemin et al. (2003) study. In our study only the influence of the individual on all type of VFA was demonstrated. Inconclusiveness of our experiment could also be caused by a low number of animals. It was given mainly due to spatial and financial limitations. It is also very complicated to obtain permission for cannulation of animals and the manipulation itself. In studies Qadis et al. (2014) and Lee et al. (2004) twelve cannulated individuals were used for the experiment and Guedes et al. (2008) used only three individuals, that is also a low number for the statistical treatment. In conclusion, the data collected in our experiment and subsequent analyzes having used linear models proved neither conclusive results, nor the affect of probiotics *Bifidobacterium sp.* Results of the experiment could be influenced by a low number of probiotic replications, and also by a low number of tested animals. The low number of animals is caused mainly as a result of high cost of their operation, by the demandingness of the cannula application itself and by obtaining the permission for the animal manipulation, too. Moreover, the results could have been influenced by the short period of the cannula application, or by the fast alternation of individual groups (experiment and control). Eventually, inconclusiveness of our results could have been affected by the kind of administered probiotics itself (*Bifidobacterium sp.*), and by the amount of the dose. In further research there would

be useful to monitor also the influence of amino acids in the rumen, because their increase would positively affect the digestibility of feed. It would also be appropriate to try other types of probiotics, or to test their various dosages.

Table 1. Basic linear model before simplification without the effect of an individual.

	Df	Sum Sq	Mean Sq	F value	P
pH	1	1.0332	1.03317	4.1674	0.04354*
acetic acid	1	1.6569	1.65693	6.6834	0.011**
butyric acid	1	0.6646	0.66457	2.6806	0.10436
propion acid	1	0.1101	0.11014	0.4442	0.50644
ammonia	1	0.0937	0.09372	0.378	0.5399

Signif. codes: ** 0.01 ; * 0.05

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POTENTIAL USE OF OLIVE CAKE BY PRODUCTS IN OSSIMI SHEEP IN EGYPT

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SUMMARY. The use of low-cost of by product such as olive cake (OC), has the potential to reduce the production costs. The current research was designed to study the effect of using different levels of olive cake as a partial replacer of barseem hay on sheep performance. A total of 30 ewes and 21 lambs of local Ossimi were involved in this study which carried out at Sids Experimental Farm, belonging to Animal Production Research Institute , Ministry of Agriculture, Cairo, Egypt. The Study was carried out in two phases, In phase I, thirty ewes were divided into three equal groups of 10 ewe each, G1 used as control (no replace), OC levels 10% and 20% were used replacing barseem hay as G2 and G3, respectively. In phase II, twenty one weaned lambs assigned to three equal groups received the same pervious treatments and they used in feeding trial. The experiment was designed according to the complete randomized design (CRD). No significant effect for OC treatments was observed on gestation length, no of service per conception, litter size and daily & total milk yield. On the other hand, the treatments of OC had a significant effect ($P < 0.05$ or $P < 0.01$) on litter weight at birth , body weight at third and fourth month, and lambs total gain, both of OC treatments were better than control. Regarding to the results of current research, it can be conclude that olive cake can replace to sheep diets to levels up to 20% without any negative effect on growth performance and milk yield.

Key words: Olive cake, barseem hay, productive performance, sheep.

INTRODUCTION

Insufficiency and high cost of traditional feed ingredients have driven the attention of survey in the direction of beneficial of non- traditional feedstuffs in livestock ration. The use of this feedstuffs reduce the competition between humans and livestock for traditional food grains and reduces the cost of animal rations which represented about 50-70% from total cost in projects of animal production. One from these traditional feed is called olive cake, the solid residue generated after extraction of oil from olive crop. The nutritive value of this by-product varies greatly with the processing system. Sadeghi *et al.* (2009) used olive cake in growing Zel sheep. They analysed olive cake and found out that 87.6 dry matter, 7.6% crude protein, 38.7% crude fiber, ether extract 5.7%, NFE 40.6%, NDF 68.9%, ADF 51.2% and lignin 31.3%. As well as high in residual water of 24% and fat content of 9% which cause rapid fermentation. Therefore, the period of utilization of crude olive cake as fresh material is short and (Ishfaq *et al*., 2015). However, they probably utilized in animal ration reducing their pollution possibly and providing non- traditional feed- stuff. The complementarily of this olive cake in ruminant ration is limited. The aim of present study to investigate the impacts of partial replacer of barseem hay by olive cake on sheep performance.

MATERIAL AND METHODS

The experiment took place at Sids Experimental Farm, belonging to Animal Production Research Institute, Ministry of Agriculture, to investigate the effects of feeding different levels of olive cake as partial replacer of barseem hay. G1 was used as control (no replace), Olive cake (OC) levels 10% and 20% were used replacing barseem hay (40% roughage in ration) as G2 and G3, respectively.

Responses were reproductive traits, daily and total milk yield of ewes, and growth performance of lambs. Concentrate Feed Mixture (CFM) ingredients content as follow decorticated cotton seed meal (22%), yellow corn (35%), wheat bran (20%), soybean meal (15%), molasses (5%), ground lime stone (2%) and common salt (1%). The study was done in two phases: In phase *I*, 30 ewes weighing an average of 42.50±1.85 kg were chosen randomly and divided into three equal groups of 10 ewe each to investigate the effects of OC levels on reproductive efficiency, milk production traits and growth performance on their lambs. In phase *II*, 21 weaned Ossimi lambs weighing average of 23.66±1.03 kg were randomly assigned to three equal groups received the same pervious treatments and they used in feeding trial to study the growth performance of lambs fed tested rations. In both phases animals were fed on DM basis to cover their nutrient requirements according to NRC (1985). Milk yield was estimated by the lamb suckling weight differential technique (Economides,1987); after weaning (8 wks), ewes were milked twice daily and were considered dried off when the amount of milk reached less than 200 ml/d. Only ewes having single lambs were included in the study and milk samples were taken biweekly during lactation period. Three digestibility trials were conducted to evaluate the digestibility and nutritive value of the experimental rations. Feed and fecal samples were analyzed according to AOAC (2003). Data were analyzed statistically by GLM of SAS (2006). The model used was: $Y_{ij} = \mu + T_i + E_{ij}$, where, Y_{ij} is the observation of traits; μ is the overall mean; T_i is the effect of i (treatments) and E_{ij} is the experimental random error. Duncan's test was used to detect differences among means.

RESULTS

Proximate analysis of the tested rations are illustrated in Table 1. The crude OC had low CP, high crude fiber, EE and a relatively low NFE. Digestion coefficients and nutritive values for different levels of OC fed by sheep are presented in Table 2. Effects of tested rations on some reproductive traits, daily and total milk yield are shown in Table 3. No significant effect was observed for tested rations on previous traits except on litter weight at birth ($P<0.05$). Growth performance of lambs fed their dams tested rations are shown in Table 4. There was a significant effect for tested ration fed to ewes on daily gain, final BW and total gain ($P< 0.05$) for their lambs, while no significant effect was observed on initial body weight. Body weight (BW), average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) are presented in Table 5. There was no effect for tested ration on initial BW, but there was a significant effect ($P< 0.05$ or $P< 0.01$) of rations on BW at third and fourth month of age and total gain, as well as ADFI in different periods are observed. Values of BW, total gain and ADFI were significantly greater in OC10 and OC 20 compared to control (OC0).

DISCUSSION

The result of analysis tested rations in current study are in agreement with that reported by Martin Garcia *et al*. (2003) who found that OC was higher in NDF (62.4%), gross energy (18.7MJ kg-1), tannin content (1.38%) and a high part of the N attached to the ADF fraction (70.6 % of CP), while poor in CP (7.9%) on DM bases. Similar trend was observed by Filya *et al*. (2006) and Aboul-Fotouh and Rady (2015). Different results for digestion coefficients and nutritive values in many works which used OC in fed sheep ration may be due to the olive by-products varies greatly influenced by factors like geographical origin, procedure of production, processing system and percentage of replacing OC in the ration. Generally, in this study the levels of OC in rations had greater values compared with those from control (Table 2). This mean that is possible to combine OC in sheep diets without negative effect on fertility performance and milk yield (Table 3). Filya *et al*. (2006) concluded that raw OC can be used successfully maximumally up to 150 g kg-1 in lamb fattening diets. This result is supported by Aboul-Fotouh and Rady (2015), who suggested that the use of 25% raw OC ration had no adverse

effect on lamb's performance. Nefzaoui (1999) reported that the negative effects of OC, low energy and digestible protein contents and its richness in lignin. Depending upon the processing, ADF bound nitrogen is 80 to 90% of total nitrogen is fixed on lignocellulose, thus not digestible. Also, ruminants are sensitive to fat intake above 5% of DM in the ration. Therefore, we suggest that the level of OC in the sheep diet should not exceed 30-40%, otherwise animal performance will be drastically affected. Thus, using different levels of OC as a partial replacer of barseem hay in the current study in the range of limit levels of previous studies and the negatively effect on fertility, milk production and growth performance, was minimized.

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Table 1. Chemical analysis of feed ingredients and tested rations.

Items	DM	DM composition (%), basis					
		OM	CP	EE	CF	NFE	ASH
CFM	90.85	91.34	14.65	2.45	18.66	55.59	8.66
Olive Cake (OC)	88.67	92.65	8.80	5.80	36.75	41.30	7.35
Berseem Hay	91.94	87.42	12.52	1.93	29.42	43.55	12.58
OC10	90.52	91.86	14.28	2.84	19.39	55.37	8.14
OC20	90.19	92.38	13.91	3.23	20.12	55.14	7.62

DM: dry matter, OM:organic matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract

Table 2.Digestion coefficients and nutritive values for different levels of olive cake fed by sheep.

Items	DM	OM	CP	EE	CF	NFE
Digestion coefficients						
Level of Sig.	*	**	**	NS	NS	*
CFM (OC0)	68.38b	71.54b	76.21b	69.44	42.86	77.56b
OC10%	71.18ab	75.59a	79.96a	69.97	46.97	80.75a
OC20%	73.17a	77.69a	82.02a	71.60	45.40	82.49a
± SE	0.85	0.91	0.87	1.24	1.20	0.86
Nutritive values						
	TDN	SV	DCP	DE		
Level of Sig.	*	*	NS	**		
CFM (OC0)	66.59b	64.33b	10.89	2.86b		

OC10%	69.71a	67.41a	11.42	3.00a	
OC20%	70.72a	68.40a	11.72	3.04a	
± SE	0.69	0.70	0.16	0.03	

TDN: Total digestibility nutrients, SV: Starch value, DE: Digestible energy, DCP: Digestible crude protein. OC: Olive cake and SE: Standard error

Table 3. Means ± SE and level of significance for effect of tested rations on reproductive, daily and total milk yield traits.

Item	GL	NSC	LS	LWT	DMY	TMY
General Mean	150.46±0.24	1.23±0.08	1.17±0.07	4.78±0.23	772.81±70.3	48.69 ±4.4
Level Of sig.	NS	NS	NS	*	NS	NS
CFM (OC0)	±0.59150.86	±0.201.43	±0.0000.1	±0.12b4.00	575.10±83.3	36.23±5.3
OC10	±0.29150.20	±0.101.10	±0.131.20	±0.39a5.31	±87.4854.67	53.85±4.5
OC20	±0.41150.44	±0.151.22	±0.151.30	±0.41ab4.76	±90.4888.67	55.98±6.3

GL: gestation length, NSC: no of services per conception, LS: litter size at birth, LWT: litter weight at birth, DMY: daily milk yield, TMY: total milk yield.

Table 4. Means ± SE and level of significance for growth performance of lambs fed their dams tested rations.

Item	Level of olive cake				
	General mean	OC (0)	OC10	OC20	Level of sig.
Initial BW, kg	4.01±0.11	4.05±0.10	3.90±0.18	4.08±0.25	NS
Daily gain, g	170.51± 0.01	±0.01145.77	169.05± 0.01	185.07± 0.01	*
Final BW, kg	±0.48 b16.82	±0.90b14.93	±0.69ab16.68	±0.74a17.96	*
Total gain. Kg	±0.4212.79.	±0.90b10.93.	±0.61ab12.68	±0.57a13.88	*

Table 5. Means ± SE and level of significance for growth performance of lambs fed tested rations.

Item	Level of olive cake				
	General mean	CFM (OC0)	OC10	OC20	Level of sig.
1) Body Weight (BW)					
Initial BW., kg	23.44±0.25	23.17±0.48	23.50±0.43	23.67±0.42	NS
First Mo., kg	±0.35 25.83	±0.5825.00	±0.4826.17	±0.7426.33	NS
Sec. Mo., kg	±0.6331.44	±1.0929.50	±1.1232.33	±0.6732.50	NS
Third Mo., kg	36.67±0.59	±0.70b35.17	36.33 1.26ab	38.50±0.62a	*
Fourth Mo., kg	41.33±0.81	38.67±1.20b	41.16±1.17ab	44.17±0.95a	**
Total gain, kg	17.89±0.72	15.50±1.12b	17.67±0.88ab	20.50±0.85a	**
2)Average Daily Feed Intake (ADFI)					
First Mo., Kg	1.08±0.08	0.942±0.03b	1.07±0.02a	.22±0.03a	*
Sec. Mo., Kg	±0.03 1.21.	±0.58b1.26	±0.04a1.32	±0.03a1.48	*
Third Mo., kg	±0.041.40	±1.06b1.04	±0.05a1.19	±0.02a1.53	**
Fourth Mo., kg	1.61±0.05	±0.07b1.00	±0.05a1.10	0.04±1.22a	*
3)Average Daily Gain (ADG)					
IBW-30d, g	0.168±0.01	0.145±0.03	0.172±0.02	0.188±0.04	NS
30-60d, g	±0.01 0.194	±0.020.189	±0.020.188	±0.030.205	NS
60-90d, g	±0.020.165	± 0.020.151	±0.020.163	0.183±0.03	NS

90-120d, g	0.149±0.03	±0.040.140	0.02±0.148	0.160 ±0.04	NS
4) Feed Conversion Ratio (FCR)					
First Mo., Kg	6.41±0.58	6.48.±1.37	6.26 ±0.98	6.50±0.48	NS
Sec. Mo., Kg	6.98±0.58	±0.586.66	±0.47 7.04	±0.447.24	NS
Third Mo., kg	7.16±0.58	6.80 ±1.37	7.28 ±1.37	7.40 ±1.37	NS
Fourth Mo.,kg	7.40±0.58	25.7 ±1.37	7.35 ±1.37	7.60 ±1.37	NS

[a,b,c] means within the same row having different superscripts significantly different (* P<0.05 and ** P<0.01). NS = Not significant

PRODUCTIVE RESPONSE OF GROWING PIGS TO ORGANIC ZINC SUPPLEMENTATION

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SUMMARY. The objective was to evaluate the influence of organic zinc supplementation on performance of growing pigs under hot weather conditions, 96 pigs 84 d of age (33.8 ± SE 0.96 kg BW) were used in a complete randomized block design experiment (DRCB). The experiment was conducted from August to October of 2016, and treatments were: 1) Diet based on corn and soybean meal, with nutritional supply according to the production stage with no supplemental Zn (Control); 2) Control plus 120 ppm of organic Zn (120ZnM); 3) Control plus 240 ppm of organic Zn (240ZnM), and 4) Control plus 360 ppm of organic Zn 360ZnM). Organic zinc was provided as zinc methionine (ZnMet) from the premix Zinpro 120. In groups of eight (4 males and 4 females), pigs were placed in 12 pens (3 pens per treatment), and pen was the experimental unit. Pigs were weighed at days 1 and 42, feed intake, air temperature and relative humidity was recorded daily. Results were analysed by ANOVA and orthogonal contrasts were performed for linear quadratic and cubic effects of Zn supplemental level. Across the experiment the average temperature was 30.4 °C; relative humidity 73%, and THI 82. Pigs final weight, daily weight gains and daily feed intake decreased quadratically ($P \leq 0.05$) with increasing Zn supplementation level and responses were similar between Control and 360ZnM. Feed conversion tended ($P = 0.08$) to be improved linearly as organic Zn level increased, with mean values of 2.97, 2.83, 2.90, and 2,70 ± 0.080 kg feed/kg gain, for Control, 120ZnM, 240ZnM, and 360ZnM, respectively. The results indicate that consumption of diets supplemented with 360 ppm Zn from ZnMet improve the productive performance of growing pigs under heat weather conditions.

Key words: Zinc methionine, pigs, productive performance

INTRODUCTION

Heat stress induces alterations in the metabolic system (Baumgard and Rhoads, 2013); these alterations include the decrease in the release of thyroid and growth hormones, which decreases the basal metabolic rate (Aggarwal and Upadhyay, 2013), affecting the expression of genes and proteins involved in metabolism of energy and nutrients (Sanz *et al.*, 2015). Zinc is a trace mineral with proven importance for the function of more than 300 enzymes (Chasapis *et al.*, 2012). The metabolic action of Zn includes energy metabolism, protein synthesis, nucleic acid metabolism, integrity of epithelial tissue, repair and cell division, transport and utilization of vitamin A, and absorption of vitamin E (Borah *et al.*, 2014). Dietary Zn has been shown to improve and prevent the reduction of intestinal integrity during heat stress (Sanz *et al.*, 2014), decreases intestinal permeability of piglets during weaning (Zhang and Guo, 2009), promotes the restoration of the intestinal epithelium (Song *et al.*, 2015) and improves protein metabolism in pig (Pearce *et al*., 2015). Because the requirements of Zn increase during the heat stress (Lagana et al., 2007), it has been suggested that Zn supplementation could be used to attenuate the decrease in serum Zn during periods of high ambient temperatures (Li *et al.*, 2015). The diets for pigs are generally supplemented with inorganic Zn ($ZnSO_4$ or ZnO) to ensure the required input; the $ZnSO_4$ being the inorganic source with the highest bioavailability (NRC, 2012); however, under normal physiological conditions and with adequate intake, only 5 to 15% of diet Zn is apparently absorbed (McDowell, 2003). In recent years, the use of organic sources of Zn has been

explored because of its greater bioavailability (Sahin *et al*., 2005). The objective of the present study was to evaluate the influence of organic zinc supplementation on performance of growing pigs under hot weather conditions.

MATERIAL Y METHODS

The study was performed from August to October of 2016 in the Experimental Unit for Fattening Pigs of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autonoma de Sinaloa, in the pig farm "La Huerta", located in the municipality of Culiacán, Sinaloa, Mexico (24° 49' 38" N and 107° 22' 47" W, and 60 m o s l); annual mean air temperature of 24.9 °C, (range 45 °C to7 ºC), and 671.4 mm of rainfall. Ninety-six 84-d-old breed pigs (42 males and 42 females; 33.8 ± SE 0.96 kg BW) were used in a complete randomized block design experiment (CRBD). Pigs were individually weighed, were grouped in three blocks by initial weight and sex, and inner each block in groups of eight (4 males and 4 females), pigs were placed in 4 pens by block, and pen was the experimental unit. From each block, pens were randomly assigned to treatments as follows: 1) Diet based on corn and soybean meal, with nutritional supply according to the production stage with no supplemental Zn (Control); 2) Control plus 120 ppm of organic Zn (120ZnM); 3) Control plus 240 ppm of organic Zn (240ZnM), and 4) Control plus 360 ppm of organic Zn (360ZnM). Organic Zn was provided as zinc methionine complex (ZnMet) from Zinpro®120 (Zinpro Corporation, Eden Prairie, MN, USA). In groups of eight (2 males and 2 females), pigs were placed in 12 pens (3 by treatment), and pen was the experimental unit. It seems that you already included a sentence to explain the allocation of pigs to treatments, pens, blocks etc. This sentence might be removed. The pigs were weighted, identified and housed in groups of eight in 7 x 1.5 m pens (10.5 m^2). Pens includes 1.5 m^2 of pond, with concrete floor and fully roofed, equipped with hopper plastic feeder and integrated metallic pacifier. Pigs had permanent access to drinking water and free access to feed. Besides been weighed at the beginning (day 0), the pigs were also weighed at day 42 of the experiment to determine the daily weight gain (DWG). The feed offered to each pen was recorded daily to determine daily feed intake (DFI). Based on the DWG and DFI, the feed conversion (FC = DFI/DWG) was calculated. The temperature (t ° C) and relative humidity (HR, %) data were taken with a thermo-hygrometer located inside the experimental unit and recorded daily during the experimental period. The temperature and humidity index (THI) was calculated using the formula THI = [0.8 x ambient temperature] + [(% HR / 100) x (room temperature - 14.4)] + 46.4 (Mader *et al*., 2006). Data were analysed by ANOVA (Steel and Torrie, 1985) for a CRBD. Orthogonal contrast for linear, quadratic and cubic effects of Zn level. Effects were considered significant when $P \leq 0.05$, and a trend was considered when $P = 0.05$ to 0.10. Pen was considered as the experimental unit. All calculations were performed using the version 8 of Statistix® Statistical Package.

RESULTS

Across the experiment the average temperature was 30.4 °C; relative humidity 73%, and THI 82. The influence of Zn supplementation from ZnMet on the productive response of growing pigs are shown in Table 1. Pigs final weight, DWG and DFI decreased quadratically ($P \leq 0.05$) with increasing Zn supplementation level and responses were similar between Control and 360ZnM. Feed conversion tended ($P = 0.08$) to be improved linearly as organic Zn level was increased.

DISCUSSION

Heat environmental conditions can adversely affect to animals (Chauhan *et al*., 2014). High temperatures alone can be deadly, but in many areas, high humidity also contributes significantly to elevated heat indices which incorporates both ambient air temperature and relative humidity in order to

produce an index of how hot it feels (Parsons, 1995). The warm and humid conditions during the summers, imply some risks for pigs to be involved under heat stress. The high average temperature and relative humidity (30.4 ° C and 73% HR) during 42 days of the present study were severed enough to have the pigs under risk of heat stress and subjected to endanger and emergency range (THI between 80 and 84; Mader *et al*., 2006). Hot environment decreases FI and growth rates of livestock. Both FI and body weight (BW) losses can be quite dramatic in pigs. In growing pigs, 24 h of heat stress caused a 50 % reduction in FI and BW loss of almost 3 kg (Pearce *et al.*, 2013). Pigs reared at 33°C lost almost 1 kg BW over a 6-day period and FI was reduced by 300 g (Collin, 2001). In a longer-term study, pigs heat-stressed at 32°C for three weeks had a reduction in FI of 771 g which equated to a 32 % decrease (Renaudeau *et al*., 2013). In a diurnal pattern of heat stress (27-37°C) finishing pigs had a reduction in DGW (0.87 kg to 0.58 kg) and a 26% reduction in FI over a 28-d period (Song *et al*., 2011). Plasma concentrations of Ca, K, Na and Zn in animals subjected to heat stress decrease with high environmental temperatures (Pearce *et al*., 2013). Because the requirements of Zn increase during heat stress (Lagana *et al*., 2007), it has been suggested that Zn supplementation could be used to attenuate the decrease in serum Zn during periods of high ambient temperatures (Li *et al*., 2015). During heat stress in laying Japanese quail, supplementation of $ZnSO_4$ improved FI, egg production, egg quality, feed efficiency and nutrient digestibility (Sahin and Kucuk, 2003). In broiler chickens, supplementing $ZnSO_4$ improved weight gain, and feed efficiency while also reducing oxidative stress (Kucuk *et al*., 2003). It also improves intestinal barrier function during heat stress in a swine model (Sanz *et al*., 2014). Although, FI, DWG and FW in the present study decreased quadratically with increasing Zn supplementation level, responses of 360ZnM were similar than Control. However, FC improved linearly with increasing Zn supplementation level. Although the statistical response was linear for FC, biologically only the 360ZnM level was superior to Control (See Table 1). The best feed conversion was obtained in pigs fed with 360 ppm of additional Zn, with a 9% improvement in feed efficiency, respect to control group. These results were similar to those observed by Li *et al*. (2015) in pigs fed with diet supplemented with 1,500 mg of Zn ($ZnSO_4$), under heat stress (40 °C for 5 h daily for 8 consecutive days); others studies have also suggested that the consumption of diets added with pharmacological inorganic Zn improves the productive response of the pigs (Carlson *et al*., 1999; Mavromichalis *et al*., 2001).The results indicate that diets intake added with 360 ppm Zn from ZnMet improve the productive performance of growing pigs under environment heat stress.

Table 1. Influence of the additional level of Zn from zinc methionine on the productivity of growing pigs.

Variable	Additional Zn level, mg/kg				SEM	*P*-value	Polynomials		
	0	120	240	360			Linear	Cuadratic	Cubic
Pigs	24	24	24	24					
Pens, n	3	3	3	3					
Days in test	42	42	42	42					
Starting weight, kg	33.700	34.033	33.700	33.700	0.144	0.35	0.62	0.29	0.17
Final weight, kg	59.233^{ab}	56.733^{b}	57.933^{b}	61.767^{a}	1.291	0.09	0.18	0.05	0.86
Daily weight gain, kg	0.608^{ab}	0.541^{b}	0.577^{ab}	0.669^{a}	0.029	0.05	0.13	0.03	0.73
Daily feed intake, kg	1.814^{a}	1.536^{b}	1.673^{ab}	1.802^{ab}	0.087	0.06	0.81	0.05	0.31
Feed conversion	2.967^{a}	2.833^{ab}	2.900^{ab}	2.700^{b}	0.080	0.05	0.08	0.69	0.23

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USE OF SODIUM ACETATE AQUEOUS SOLUTION IN REARING OF NEWBORN LAMBS

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Summary. It's known that in the first days of newborn animals' life are the most critical to the health and future growth and development. That's why in the neonatal period animals are most susceptible to all stresses (primarily technological ones). Since the alarm phase sympatho-adrenal system of organism is excited resulting in inhibition of parietal cells function, i.e. the phenomenon of abomasal achlorhydria is observed. As a consequence, the lamb loses appetite, resulting in a relatively low gain weight. We have attempted to eliminate the negative effects of stresses on appetite using 3% aqueous solution of sodium acetate (SAAS). Experiments were carried out in three flocks. Animals in each flock were divided into two groups of lambs with their ewes: 10 experienced and 10 control animals. Every day before the morning feeding lambs of experimental groups (EG) were given 5 ml of 3% (SAAS). This procedure was performed from the cannula of plastic syringe on the root of tongue. Animals of control groups (CG) instead of SAAS received 5 ml of tap water in the similar way. The procedure was repeated for 7 days. All lambs were weighed prior to and after the experiments. Live weight gain of lambs was estimated.
In all flocks traced one pattern. EG were significantly ahead of the CG on live weight gain. So, in the flock I. EG lambs gained 3,09±0,42 kg, in CG - 2,09±0,49 kg, i.e. 32,4%. II. EG lambs gained 2,83±0,40 kg, CG - 1,95±0,35 kg, i.e. 31,1%. III. EG lambs gained 2,22±0,35 kg, CG - 1,58±0,02 kg, i.e. 28,8%. Thus, the results of our experiments shows that only a week-long introduction to the diet 5 ml of 3% SAAS gives lambs live weight gain on average by 30%. The mechanism of action of SAAS is associated with increased appetite in young animals.

Key words: lambs, rearing, weight gain.

INTRODUCTION

It is known that in the first days of life of newborn farm animals are the most critical to the health and future growth and development. At this time, the basic foundation is laid for the productive qualities of the animal. That is why in the neonatal period animals are most susceptible to all sorts of stresses (primarily technological ones) (Shlygin, 1997). Young small ruminants are the most resistant of all farm animals to various kinds of stressors. Apparently, therefore, there are very seldom cases of various diseases, such as the digestive or respiratory system disorders of noncontagious etiology appear among the lambs. Nevertheless, all stress factors influence on the development of a young organism. Since the alarm phase sympatho-adrenal system of the animal organism is excited resulting in inhibition of parietal cells (of the abomasum) function, i.e. the phenomenon of achlorhydria is observed. As a consequence, the lamb loses appetite, resulting in a relatively low gain of live weight (Zhirkov, 2013). We have attempted to eliminate stresses if not themselves, but their negative effects on appetite using 3% aqueous solution of sodium acetate.

MATERIALS AND METHODS

Field trials were carried out under flocks belonged to breeding farm “Plemzavod Romashkovsky" which situated in Volgograd region in the period of lambing (late March to early April). Experiments were carried out in flocks of shepherd Mamahaev, shepherd Arystangaliev, and shepherd Bakhtiyarov. All animals in each flock were divided into two groups of lambs with their ewes. Each flock had 10 experienced and 10 control animals. Every day before the morning feeding lambs of experimental groups were given 5 ml of 3% sodium acetate ("Khimprom" JS, Volgograd) aqueous solution. This procedure was performed from the cannula of plastic syringe on the root of the tongue. Animals of control groups instead of sodium acetate solution received 5 ml of tap water with the similar way. Then lambs were released to their ewes. The procedure was repeated for 7 days. All lambs were weighed prior to and after the experiments. Live weight gain of lambs was estimated.

RESULTS

In all flocks traced one pattern. Experimental groups were significantly ahead of the control on live weight gain. It can be seen below. So, there were three flocks: I. of Mamakhaev, in experimental group the lambs gained 3,09±0,42 kg, in control - 2,09±0,49 kg, i.e. 32,4%. II. of Arystangaliev, in experimental group the lambs gained 2,83±0,40 kg, in control - 1,95±0,35 kg, i.e. 31,1%. III. of Bakhtiyarov, in experimental group the lambs gained 2,22±0,35 kg, in control - 1,58±0,02 kg, i.e. 28,8%. Thus, the results of our experiments shows that only a week-long introduction to the diet 5 ml of 3% aqueous solution of sodium acetate gives lambs live weight gain on average by 30%. The mechanism of action of aqueous solutions of sodium acetate is associated with increased appetite in young animals. Effect of sodium acetate on the secretory function of the stomach has been repeatedly demonstrated and patented earlier. However, this effect only applies if one ewe has a single lamb, if she has twice - first it's necessary to increase the milking ewes.
So, we showed the way of dramatic weight gain increase in rearing lambs. Moreover the drag is very cheap and ecologically pure. But for the introduction of this technology of growing lambs Russian farmers are not yet ready. For this it is necessary to completely change traditional patterns of growing lambs. The procedure for giving the drag into the every lamb’s mouth so laborious, that in the traditional sheep breeding is not economically profitable. More research is necessary.

DISCUSSION

Appetite in the newborn lambs depends primarily on the hormonal status of the young organism and GI hormones play the leading role. Moreover, before birth, leptin may inhibit endogenous glucose production by the fetal liver when adipose energy stores and transplacental nutrient delivery are sufficient for the metabolic needs of the fetus (Forhead et al., 2008). Adam et al. (2013) showed that intra-uterine growth restriction (IUGR) is involved in developmental metabolic programming in newborn lambs. Neither IUGR nor gender affected suckling activity (proxy for appetite) assessed at 3 weeks, but final NPY gene expression correlated with suckling weight gain in males. This study has revealed no effect of IUGR on early postnatal hypothalamic energy balance gene expression but a major effect of gender associated with major sex differences in adiposity and leptinemia. In the present research we did not divide lambs according to gender differences. In these fields the data of recent investigations of Clarke & Arbabi (2016) sound very interesting. They suppose that there is a complex interaction between appetite-regulating peptide neurons and kisspeptin neurons that enables the former to regulate the latter both positively and negatively. In terms of how GnRH secretion is reduced during stress, recent data indicate that GnIH cells are integrally involved, with increased input to the GnRH

cells. The secretion of GnIH into the portal blood is not increased during stress, so the negative effect is most likely effected at the level of GnRH neuronal cell bodies. A neonatal peak in rodent plasma leptin plays a central role in regulating development of the hypothalamic appetite control centres. Maternal obesity lengthens and amplifies the peak in altricial rodent species. The precise timing and characteristics of the neonatal leptin peak have not been established in offspring of either normal or obese mothers in any precocial species (Long et al., 2011) In any case further research is necessary.

CONCLUSIONS

So, it was shown the way of dramatic weight gain increase in rearing lambs. Moreover the drag is very cheap and ecologically pure. But for the introduction of this technology of growing lambs Russian farmers are not yet ready. The next step is to develop the new technological way of rearing the newborn lambs.

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INFLUENCE OF PROTEIN SOURCE ON APPARENT DIGESTIBILITY OF GROWING PIGS

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SUMMARY

With the aim of determining the influence of different protein source on apparent digestibility of nutrients in growing pigs; six crossbred pigs (BW = 42.1 ± 1.8) were used in a replicated Latin Square Design. Pigs were assigned to consume one of three diets: 1) Diet with 17.8 % CP and 3.27 Mcal ME/kg, containing sorghum 69.5 %, soybean meal 28 %, and premix 2.5 % (CONT); 2) Diet with 17.7 % CP and 3.28 Mcal ME/kg with sorghum 42.5 %, cull chickpeas 40%, soybean meal 12.0%, vegetable oil 3 %, and premix 2.5 % (CHP), and 3) Diet with 17.9 % CP and 3.26 Mcal ME/kg with sorghum 51.4 %, cull chickpeas 30%, peanut meal 14 %, vegetable oil 2%, and premix 2.5% (CHPN). Pigs were individually placed in metabolic crates (0.6 × 1.2 m). The adaptation period was 6 days and sample collection period was 4 days. From each diet and period, one kg of diet was taken as a sample and the total fecal production was collected. Apparent digestibility of DM with values of 82.04, 82.89 and 83.36 %, for CONT, CHP, and CHPN, was affected among treatments ($P < 0.05$). Apparent digestibility of crude protein was not altered ($P = 0.77$) by CHP and CHPN inclusion (78.35, 78.47 and 79.24%), and apparent digestibility of OM was not altered ($P = 0.35$) by CHP and CHPN inclusion (84.89, 84.45 and 85.36%). It's concluded that cull chickpeas and cull chickpeas-peanut meal can be used in growing pig improving nutrient digestibility.

Key words: Pigs, apparent digestibility.

INTRODUCTION

Feeding costs represent the largest proportion of investment required in the pig fattening process (García *et al*., 2012). Most of the diet of intensive-production pigs consists of cereal grains and oilseed pulps are the basis of the supplementary protein in them (García *et al*., 2010). With the increase in ingredient prices and diversification in by-products derived from the food industry that can be incorporated into animal feed, it is of interest to conduct comparative studies of the nutritional value of emerging ingredients in relation to soybean meal, which is the protein source with the most available information (Ilori *et al*., 1984); such as the peanut meal (Ranjhan *et al*., 1964; Shelton *et al*., 2001), and the cull chickpeas, available in Northwestern Mexico, which has been used in behavioral tests in different species (Obregón *et al*., 2002). However, the experimental results of the use of cull chickpeas are scarce in fattening pigs, where it has partially replaced maize and soybean paste in the diet, at levels up to 40% in growth stage and 60% in finishing, unmodified the productive performance (Obregón *et al*., 2002 Güémez *et al*., 2004,). However, there is no information in pigs of the total or apparent digestibility of the cull chickpeas in combination with peanut meal, which would allow a more efficient use of this by-product in the feeding of growing pigs, making it necessary to carry out experiments on the digestibility of cull chickpeas. For this reason the objective of the present investigation is to determine the influence of different protein sources on apparent nutrient digestibility in growing pigs.

MATERIAL AND METHODS

The experiment was carried out in the experimental unit of small ruminants and pigs, and in the food and Nutrition Research Laboratories of the Faculty of Veterinary Medicine and Animal Science of the Autonomous University of Sinaloa, in the municipality of Culiacán, Sinaloa ; located at boulevard San

Ángel s / n, colonia San Benito, at latitude 24 ° 49 'N and longitude 107 ° 32' W, at a height of 40 meters above sea level, with an average annual temperature of 25 ° C, and an annual average rainfall of 675 mm (INEGI, 1997), the climate is classified as semi-arid (INIFAP, 2002). The treatments consisted of diets that cover the nutritional requirements of the growing and finishing pigs (NRC, 1998), for the growth stage, three diets with similar amounts of protein and energy were elaborated, each of which were provided to free access to the pigs during the entire test. The animals were weighed at the beginning and at the end of the experiment, the feed served daily by the cages, with the remaining residue at the end of the experiment, to determine the daily feed consumption per animal.

To determine the apparent intestinal digestibility of the protein source, the animals to be used were housed individually in 1.2 x 0.6 m metabolic cages, with plastic grid floor, aluminium impeller, equipped with manual feeders and suction cups. Six crossbred pigs (BW = 39.1 ± 1.7) were used in a replicated Latin Square Design. Pigs were assigned to consume one of three diets: 1) Diet with 17.78 % CP and 3.27 Mcal ME/kg, containing sorghum 69.5 %, soybean meal 28 %, and premix 2.5 % (CONT); 2) Diet with 17.73% CP and 3.28 Mcal ME/kg with sorghum 42.5 %, cull chickpeas 40%, soybean meal 12.0%, vegetable oil 3 %, and premix 2.5 % (CHP), and 3) Diet with 17.9 % CP and 3.26 Mcal ME/kg with sorghum 51.4 %, cull chickpeas 30%, peanut meal 14 %, vegetable oil 2%, and premix 2.5% (CHPN). The adaptation period was 6 days and sample collection period was 4 days. The experiment consisted of three treatments (diets) with 6 replicates. The animals were fed at 8:00 am and 1:30 p.m., during three 10-day periods. From each diet and period, one kg of diet was taken as a sample and the total fecal production was collected. The fecal contents were collected once a day before serving the food, for 4 days. The total feces collections and 1 kg samples of the diets, per pork and period, were dried in a forced ventilation oven at 105 ° C for 24 hours to obtain dry matter in grams (AOAC, 1975) and mixed to obtain (CP = N x 6.25; Kjeldahl), ash by calcination at 550 ° C for three hours (AOAC, 1975).

With the results obtained the apparent digestibility of dry matter (DM), organic matter (OM), and crude protein (CP) was determined according to the formula presented by Church *et al*. (2000):

CDn = (matter consumed g - matter in faeces g / matter consumed g) 100.

The results of DM, MO, CP in feces and apparent digestibility of diets (% of consumption), were analyzed by a replicated latin square design, according to the model: $Yijk = \mu + Hi + Cj + Tk(ij) + Eijk$; where Yijk is the response variable, μ the general average of the experiment, Hi the pig effect, Cj the period effect, Tk (ij) the treatment effect (diet) and Eijk the experimental error (Steel and Torrie 1988). Using alpha of = 0.05 to accept difference between the evaluated treatments and using the variance / covariance analysis module of the procedure for General Linear Models of Version 9, of the Statistix® computational package (2007).

RESULTS

Feed Intake (2.19, 2.24 and 2.26 kg/day) was not affected by treatments ($P>0.05$) for CONT, CHP and CHPN, respectively. Apparent digestibility of DM (82.04, 82.89 and 83.36 %) was affected among treatments ($P <0.05$). Apparent digestibility of crude protein was not altered ($P=0.77$) by CHP and CHPN inclusion. (78.35, 78.47 and 79.24%). Apparent digestibility of organic matter was not altered ($P=0.35$) by CHP and CHPN inclusion. (84.89, 84.45 and 85.36%).

DISCUSSION

The results of the determination of the influence of different protein sources on the apparent digestibility of nutrients in growing pigs, indicate that the substitution of sorghum and soybean meal by cull chickpeas does not affect the digestibility of crude protein and matter organic, being able to substitute up to 42% of the soybean paste; similarly, by replacing cull chickpeas and peanut meal, the

totality of the soybean paste can be supplied, similar results were previously found by Shelton *et al.* (2001), and by Salgado *et.al.* (2001), reporting an apparent digestibility of 79% of the crude protein when using chickpeas in weaned pigs. No references were found where the apparent digestibility of the dry matter in sorghum-soybean meal diets is lower, although slightly, to that obtained for cull chickpeas with soybeans, or for cull chickpeas with peanut meal as obtained in this test. It's concluded that cull chickpeas and cull chickpeas-peanut meal can be used in growing pig improving nutrient digestibility. In addition, a decrease in the cost of production is presumed, since peanut meal is generally less expensive than soybean meal, and inclusion in the chickpea sorghum-soybean meal mix is more economical.

Table 1. Influence of protein source on apparent digestibility of growing pigs

Item	Treatments (protein source)			SEM	*P*-value
	Soybean meal (CONT)	Cull Chickpeas (CHP)	Peanut meal (CHPN)		
Pigs, n	6	6	6		
BW, kg	39.10	39.10	39.10		
Apparent digestibility, %					
Dry matter	82.04[b]	82.89[a]	83.36[a]	0.330	0.03
Organic matter	84.89	84.45	85.36	0.424	0.35
Crude protein	78.35	78.47	79.24	0.944	0.77

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EFFECT OF ORGANIC SELENIUM AND ZINC METHIONINE ON FEEDLOT AND CARCASS TRAITS OF HAIR SHEEP

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SUMMARY. In order to evaluate the effect of the supplementation of zinc methionine (ZM) and organic selenium (Se) in the finishing diet of male lambs intended for meat production, 24 lambs (average initial weight of 25±3.4 kg) of Pelibuey and Pelibuey x Dorper breeds were housed in individual cages under a completely randomized design and divided in 3 groups of treatments (8 lambs per treatment); the three treatments were: (T0) control group, no supplementation at all; (T1) group supplemented with 0.5 mg/kg DM of Se; and (T2) group supplemented with 40 mg/kg DM of Zn. The experimental period was 55 d, and the response variables recorded were daily weight gain (DWG), dry matter intake (DMI), and feed conversion (FC). The carcass traits measured were: back fat (BF) and rib-eye area (REA). Average daily gain and the Final weight were not affected both for Selenium as for Zinc supplementation ($P > 0.05$). Dry matter intake was decreased ($P < 0.05$) by Zinc-methionine supplementation. Feed conversion was better in lambs that were supplemented with Zn-methionine in relationship to unsupplemented lambs ($P < 0.01$). Se-methionine supplementation had no effect on DMI or feed conversion ($p > 0.05$). Both Se-methionine and Zn-methionine supplementation increased hot carcass weight and cold carcass weight ($P < 0.05$); however no effect of mineral supplementation was observed on carcass dressing as percentage of final weight, rib eye area or back fat thickness ($P > 0.05$). Results suggest that the supplementation to feedlot lambs with organic minerals as Se-methionine and Zn-methionine contributes to increase tissue deposition in the carcass

Key words: Sheep, selenium, zinc.

INTRODUCTION

In Mexico, the use of mineral supplements for sheep is scarce. By the other hand, sheep are known to be particularly susceptible to mineral deficiencies such as Selenium (Se), which can lead to some diseases (McDowwell, 2001). It is also known that zinc-methionine and Se-methionine are considered as metabolism modifiers, which improve the quality of the carcass when they are supplemented in organic forms by means of yeast (Domínguez et al, 2007). Selenium is an essential trace mineral found in all cells and tissues, requiring small amounts and is necessary for fertility and growth, since it forms part of multiple enzymes including glutathione peroxidase (McDowell, 1997). Likewise, Zinc is also an essential element that has importance in the metabolism of lipids, proteins and nucleic acids. Some studies have shown that the use of zinc methionine in the form of yeast (organic) improves weight gain (McDowell, 1997). For the specific case of sheep, the National Research Council (NRC, 2001) recommends 0.1 to 0.2 ppm of Se, whereas more recent reports estimate requirement in 1.0 mg / d,

with values that would have dietary concentrations for sheep in finishing between 0.30 and 0.55 ppm (NRC, 2001). For this reason, the objective of the present study was to evaluate the use of minerals, Se-methionine and Zinc-methionine, on the productive behaviour in hair sheep in the finishing stage.

MATERIALS AND METHODS

Twenty-four Pelibuey x Dorper male lambs with an initial weight of 25.66 ± 3.65 kg were housed in individual cages. They were dewormed (Ivermectin) and injected with vitamins A, D and E prior to the start of the trial. The experimental period had a total duration of 55 days and lambs were weighed in days 1 and 55. The animals were distributed according to a completely randomized design with three treatments as follows: TO) control group fed basal diet without additional supplementation of Se or Zn as methionine complexes; T1) Basal diet plus supplementation of 0.5 mg / kg of Se-methionine; T3) basal diet supplemented with 40 mg / kg Zn-methionine. The composition of basal diet is presented in Table 1. Diet samples were taken weekly (500 g) and in the laboratory determinations were performed for dry matter (DM), crude protein, ether extract, and organic matter (AOAC, 1997), neutral detergent fibre (Van Soest, et al., 1991), and rumen degradable protein ("in situ" at 24 hours incubation; Nocek, 1988). Diets were offered ad libitum twice a day (09:00 and 17:00 h, and refusals were removed daily at 08:00; the dry matter intake (DMI) was computed as DM offered, minus daily refusals. Daily gain was calculated subtracting the initial weight from the final weight of period and divided by period length days. Once completed feeding period, lambs were randomly sacrificed, and carcass characteristics were measured (Silva et al, 2005). The normality of the data was tested with the PROC UNIVARIATE and the results were analysed according to a completely randomized design with Proc GLM of the SAS, 2002.

RESULTS

Results of the influence of organic mineral supplementation are shown in Table 2. Average daily gain and the Final weight were not affected both for Selenium as for Zinc supplementation ($P > 0.05$). Dry matter intake was decreased ($P < 0.05$) by Zinc-methionine supplementation. Feed conversion was better in lambs that were supplemented with Zn-methionine in relationship to unsupplemented lambs ($P < 0.01$). Se-methionine supplementation had no effect on DMI or feed conversion ($p > 0.05$).

The effects of Se-methionine and Zn-methionine supplementation on carcass characteristics of labs are presented in Table 3. Both Se-methionine and Zn-methionine supplementation increased hot carcass weight and cold carcass weight ($P < 0.05$); however no effect of mineral supplementation was observed on carcass dressing as percentage of final weight, rib eye area or back fat thickness ($P > 0.05$).

DISCUSSION

This results is agree with obtained by Reséndiz–Hernández et al. (2012), who fed yeast Se-enriched to lambs and did no observed changes in body weight gain, but found heavier carcass as consequence of organic selenium supplementation. Similar results were obtained by Dominguez et al. 2007 supplementing Se-enriched yeast in the diet of finishing lambs. Despite the lack effect of organic Zn and se supplementation on ADG, the fact that both increases carcass weight implies that the effect of the mineral organic supplementation contributes to improve accretion of body mass, that is the best indicator of a differential bioavailability and that when availability of those minerals exists, lamb performance is beneficed when response is measured as carcass tissues that are ultimate used as food.

Mallaki et al. (2015) found an increment in daily gain and improvement on feed conversion when supplemented organic zinc to lambs as compared with observed with sulfate as Zn source.

Table 1. Composition of experimental diets

Ingredients	T0	T 1	T 2
Corn	60 %	60%	60%
Sorghum	13.5%	13.5%	13.5%
Soybean meal	10%	10%	10%
Corn straw	10%	10%	10%
Molasses,	5%	5%	5%
Buffer	0.5%	0.5%	0.5%
Minerals	1.0%	1.0%	1.0%
Selenium Methionine	0	0.5mg/kg	0
Zinc Methionine	0	0	40mg/kg
Total	100%	100%	100%
	Composition (Dry mater basis)		
Crude protein,%	13.58	13.58	13.58
NEm, Mcal/kg	2.006	2.006	2.006
Ca, %	0.58	0.58	0.58
P, %	0.35	0.35	0.35

Table 2. Effect of supplementation of selenium and zinc methionine in productive behaviour in sheep

Item	Treatments			SEM
	T0	T 1	T 2	
Starting Weight	26.30	26.84	26.54	1.05
Final Weight	39.70	41.77	40.50	1.17
ADG, kg/d	0.243	0.257	0.254	0.022
DMI, kg/d	1,037[b]	1,115[b]	998[a]	0.98
Feed Conversion	3.38[a]	3.47[ab]	3.54[b]	0.73

[ab]Different letter in row means statistical difference (P <0.05)

CONCLUSIONS

Results suggest that the supplementation to feedlot lambs with organic minerals as Se-methionine and Zn-methionine contributes to increase tissue deposition in the carcass.

Table 3. Effect of supplementation of selenium and zinc methionine on carcass characteristics of finishing sheep

Item	Treatments		
	T0	T 1	T 2
Hot Carcass Weight, kg	19.30[b]	22.34[a]	21.40[a]
Cold Carcass Weight, kg	18.09[b]	20.29[a]	20.07[a]
Dressing, %	47.60	48.57	49.55
Rib Eye Area, cm^2	12.25	12.43	12.35
Back fat thickness, mm	0.9	1.3	1.1

[ab]Different letter in row means statistical difference (P <0.05)

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EFFECTS OF HIGH FIBRE DIETS ON BEHAVIOUR AND PERFORMANCE OF PREGNANT GILTS AND THEIR PIGLETS

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SUMMARY. Pregnant sows are often subjected to food restriction, which can compromise their welfare. High fibre diets can mitigate the feeling of hunger and, consequently, improving welfare and productivity. The aims of this study were: 1) to measure the impact of feeding pregnant gilts with high fibre diet (HFD) on welfare and performance indicators and 2) to assess agonistic behaviours of their piglets. Sixteen pregnant gilts were fed a HFD (12.86% crude fibre) and 12 gilts received a low fibre diet (LFD, 2.53% crude fibre), twice daily. The behaviour of the gilts was assessed by direct observation for 4.8 hours at 4 different days throughout pregnancy (day 30, 60, 75, 90). The performance of the piglets of 22 gilts (14 HFD, 8 LFD) was assessed and the aggressive behaviour was measured by skin lesions were counted before and after weaning. The weaning was conducted at 28 days of age. The skin lesions in the piglets were counted at the day of weaning (before mixing), 24 and 48 hours after. For statistical analyses the mixed models procedure of SAS was used. LFD gilts showed sham chewing more often (frequency) and longer (duration) before feeding than after feeding ($P<0.05$). HFD gilts were heavier in the medium third of gestation (average 7,4 kg more) and at 107th day (7,5 kg) of gestation (when moved to farrowing pens) compared to LFD gilts (216 kg and 208 kg respectively). No differences were observed in other performance parameters like number of piglets born alive, number of piglets weaned, average weight at birth. HFD and LFD had no influence on the piglet's performance (average weight daily gain, weight at weaning). Piglets from HFD gilts had less skin lesions before weaning than the offspring of LFD gilts ($P<0.01$). These results show that HFD can reduce sham chewing of gilts. Piglets of HFD sows seem to be less aggressive than piglets from LFD sows – possibly because of less hunger stressed mothers.

ANTIMICROBIAL ACTIVITY OF SEVERAL ILEX SP.

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SUMMARY. Natural compounds are an important source of desired biological activity which help to improve nutritional status, enhance productivity and bring many health benefits. The aim of the study was to determine the antimicrobial activity of aqueous (H_2O), methanolic (MeOH) and choroformic ($CHCl_3$) *Ilex sp.* extracts against selected bacteria. In present work we focused on the antimicrobial activity of air-dried leaves of *Ilex aquifolium* L. (IX1), *Ilex aquifolium 'Argentea Mariginata'*(Ix2), *Ilex meserveae* '*Blue Angel*'(Ix3), and a commercially available mate (Ix4) as the reference product. Extracts were prepared as described in previous papers (Zwyrzykowska et al 2015, Erdemoglu et al 2009). The tests have been made on certain bacteria strains: *Escherichia coli* ATCC10536, *Staphylococcus aureus* DSM799. The assessment of the effects of the extracts on the growth of microorganisms were performed in the automated Bioscreen C system (Automated Growth Curve Analysis System, Lab systems, Finland). Each extracts was used at a final concentration of 0.1% (w/v). The resulting microbial growth curves were compared to control cultures in medium supplemented with MeOH, $CHCl_3$ or water (without extract). During the culture the optical density of the cell suspensions was measured automatically at 560 nm. MIC was determined for each extract and strain combination.The data were analyzed using software (Statistica ver. 10.0) and were used to generate the growth curves for each strain studied, constructed as a function of the incubation time and the absorbancy of the culture.

The obtained data showed broad antimicrobial activity against bacteria, yeasts and filamentous fungi. The methanolic and chloroformic extracts of *Ilex sp.* most inhibited all evaluated microorganisms. *Ilex paraguariensis* chloroformic and *Ilex aquifolium* L. methanolic extracts resulted in total growth inhibition of *Escherichia coli, Staphylococcus aureus.*

EFFECT OF FEEDING *Trigonella foenum-graecum* ON GROWTH PERFORMANCE OF BROILER CHICKS

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Introduction: The present work aims to investigate the effect of using the whole seeds of fenugreek and their extracts in poultry ration formulation based on Soybean and Sorghum. **Material and Methods**: For this purpose, 195 chicks (Cobb 500) were divided into 13 groups and received the tested formulations for 4 Weeks. The fenugreek was offered in a ground /milled form (FM), fenugreek residue after extraction with the solvents (FWE), light extract (ME) and dense extract (ED). Fenugreek extraction process was carried out by a mixture of hexane - alcohol - ether in the ratio of 64:32:4, respectively. The diets were adjusted to be isoproteic and isocaloric. The tested groups received 1) FM 5%, 2) FM 10%, 3) FM 15%, 4) FWE 5%, 5) FWE 10%, 6) FWE 15%, 7) SE 5%, 8) SE 10,%, 9) SE 15%, 10) ED 5%, 11) ED 10%, 12) ED 15% and 13) Control. Complete random design was applied, where three repetitions (n=5 birds/repetition) per tested formulation were used (i.e. 15 birds/formulation). The tested parameters included weight gain (g), feed intake and the feed conversion rate (FCR). **Results**: The group fed the highly extracted fenugreek gained more weight ($P < 0.05$) than the chicks in the control group (94 g), they achieved the highest ($P < 0.05$) body weight gain among all investigated groups (1422 g in average). Whereas, the lowest ($P < 0.05$) feed intake and the feed conversion rate value was reported in the group fed 10% light extract fenugreek seeds and finally the group with the highest ($P < 0.05$) feed intake was that kept in 15% of light extract fenugreek seeds . **Conclusion:** The fenugreek can be safely used to partly replace Soybean and Sorghum in broilers starter and finisher rations without any adverse effects on the bird performance.

Sustainable animal production and agro-biodiversity conservation:

efficient and alternative farming

THE USE OF PHOTOVOLTAIC CELLS IN A DAIRY CATTLE FARM

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SUMMARY

The aim of the study was to identify potential areas of energy recovery and alternative energy sources, and to determine their efficiency in dairy farming. To achieve the set goal, an energy audit of a dairy farm was performed and the use of photovoltaic cells to power building lights, water heaters, milk feeders, milking machines, as well as hot water equipment was determined. During the study, measurements were made of electricity consumption and production, generated power of the devices and quality parameters of their performance, outdoor microclimate, and incurred costs. The study was conducted in southern Poland on a farm of 350 dairy cows kept in a free-stall system in identical, heated and mechanically ventilated buildings. The experiment used 2.2 kW photovoltaic cells with a voltage of 16 V. The installation was equipped with an electricity meter, and a converter and inverter turning direct current into alternating current with a rated voltage of 230 V and frequency of 50 Hz. The system was fitted with VRLA batteries to balance the surplus and shortfall of power resulting from sunlight exposure. These sources of energy were used to power the following receivers: 3 hot water heaters with a total capacity of 300 litres, an automated milk feeder, a bucket milker, lighting of barn, calf house, heifer house and calving pen premises, which were equipped with 35 indoor luminescent receivers with a total capacity of 2.8 kW and 8 outdoor halogen receivers with a total capacity of 2.1 kW. The balance of energy necessary to power these receivers showed that the photovoltaic cells cover 1038.4% of the requirement on average (from 280% in December to 1670% in July). In conclusion, it is stated that the possibility of using photovoltaic cells in dairy cattle farming is a viable alternative for previous solutions.

Key words: renewable energy sources, cattle

INTRODUCTION

With few exceptions, photovoltaic cells are not used in livestock production despite the fact that intensive livestock production, which is considerable in scale and concentration, should develop energy-efficient methods as any other industrial activity (Dincer, 1998). The technical potential of renewable energy sources (RES) in Poland is estimated at 3850 PJ per year. This accounts for more than 90% of the energy requirement (4292 PJ in 1997) (Kamrat, 2003, Rogulska et al. 2003). The highest potential has been ascribed to geothermal energy, solar energy, and biomass. Currently 1.5% of renewable sources are used to cover the energy requirement. A wide range of livestock production technologies has been developed; in practice, however, there are also deficiencies in cattle farms with regard to the construction and microclimate of buildings, types of housing systems, and the devices and techniques used. Therefore, it is scientifically and economically important to continue optimizing the housing conditions and the energy efficiency of cattle farms.

MATERIAL AND METHODS

The experiment used 350 dairy cows (heifers and calves). Animals were kept in a free-stall system in identical, heated and mechanically ventilated buildings. Lights and items of building equipment (water heaters, milk feeders, milking machines) were additionally powered by wind generators and

photovoltaic cells depending on the experimental group task. The control groups were fitted with standard equipment. Animals were fed and kept in accordance with current standards, with constant access to water. The following renewable energy sources were used in the study: a 2.5 kW and 24 V wind generator situated 15 m above the ground level, in the prevailing wind direction. The operating range of the generator was between 2.5 and 25 m/s. The generator was electronically controlled through a computer controller, which allowed the turbine to be optimally set relative to the direction of the wind and to be stopped in very strong wind. The installation was equipped with an electricity meter, and a converter and inverter turning direct current into alternating current with a rated voltage of 230 V and frequency of 50 Hz. The system was fitted with VRLA batteries to balance the surplus and shortfall of power resulting from the speed of the wind driving the generator. The experiment also used 2.2 kW photovoltaic cells with a voltage of 16 V. The installation was equipped with an electricity meter, and a converter and inverter turning direct current into alternating current with a rated voltage of 230 V and frequency of 50 Hz. The system was fitted with VRLA batteries to balance the surplus and shortfall of power resulting from sunlight exposure. These sources of energy were used to power the following receivers: 3 domestic hot water heaters with a total capacity of 300 litres, an automated milk feeder, a bucket milker, lighting of barn, calf house, heifer house and calving pen premises, which were equipped with 35 indoor luminescent receivers (fluorescent lamps) with a total capacity of 2.8 kW and 8 outdoor halogen receivers with a total capacity of 2.1 kW.

RESULTS

The results of the energy audit of the cattle farm are presented per stall and year in Table 1. This method is generally accepted in the literature on the subject and in the farm design guidelines (Hörndahl, 2008). It allows facilities with different technologies to be compared for inputs. The highest energy consumption was used to light the facilities and to power the water heaters. This concerned the farrowing section among the facilities (consumption of 59.8 kWh/stall/year), and water heaters among the technological equipment used (50.1 kWh/stall/year). The least energy consumption per stall/year was characteristic of the lighting of calf and heifer houses (30.2 kWh/stall/year), and of the milking machine among the equipment (12.6 kWh/stall/year). The high energy consumption for lighting the farrowing house results from the need to supervise the animals and to link supervision quality directly with the production results obtained. The energy needed to light the facilities is among the largest items on the farm's energy audit, but it is not much higher than for the technological equipment. The balance of energy needed to power both of these systems shows that wind generator energy could power a maximum of 109, and photovoltaic cell energy a maximum of 10 such installations on average (Table 2). For photovoltaic cells, an average of 1038.4% energy was covered, ranging from 280.9% in December to 1699.9% in July. Solar radiation in December and January is the lowest while the buildings require longest lighting time. For the wind generator, an average of 10917.4% energy was covered, ranging from 8925% in February to 12140% in August.

Table 1. Distribution of energy consumption in a dairy farming operation

Distribution	Average energy consumption
Lighting, dairy cows	38.7
Lighting, farrowing sector	59.8
Lighting, calf and heifer houses	30.2
Water heater, barn	50.1
Milk feeding station	25.7
Milking machine	12.6

Table 2. Balance of energy needed to power the farm's lighting system, water heaters, milking machine and milk feeding station

Month	Requirement (kWh)/stall	Effective energy efficiency		Proportion covered		Maximum number of stalls powered	
		Photovoltaic cell (kWh)	Generator (kWh)	Photovoltaic cell (%)	Generator (%)	Photovoltaic cell (no.)	Generator (no.)
I	21.1	67.89	2052.2	321.8	9726.1	3	97
II	20.8	95.2	1856.4	457.7	8925.0	5	89
III	19.2	202.43	1965.4	1054.3	10236.5	11	102
IV	17.7	251.14	1935	1418.9	10932.2	14	109
V	16.6	253.89	1962.3	1529.5	11821.1	15	118
VI	14.5	240.3	1665	1657.2	11482.8	17	115
VII	15.1	256.68	1773.2	1699.9	11743.0	17	117
VIII	15.5	250.79	1881.7	1618.0	12140.0	16	121
IX	16.8	199.8	2019	1189.3	12017.9	12	120
X	18.1	151.9	2148.3	839.2	11869.1	8	119
XI	20.4	80.4	2049	394.1	10044.1	4	100
XII	21.3	59.83	2145.2	280.9	10071.4	3	101
Average	18.09	175.9	1954.4	1038.4	10917.4	10	109

DISCUSSION

Analysis of the results shows that the possibility of using renewable energy sources in dairy cattle farming is a viable alternative for current practice. Considering the total cost of unit power installed, the economic profitability of such investments must be underlined. After a minimum 6-year period of depreciation, the energy needed for the next 15 to 20 years of operation will be practically free. The repair and maintenance costs can be disregarded here because they do not differ from those required for standard powering methods. Unfortunately, the total costs of RES installations are higher than those declared by the manufacturers. The relatively high cost of a wind generator, the use of which is most efficient, results from the need to purchase a certified tower and construct expensive foundations. This cost quickly falls with increasing generator power, which is dependent on the wind potential in a given area (Clarke, 2003). Considering these costs, the present experiment showed that the wind generator is the most effective and universal to produce energy for dairy cattle farms. However, its use will be strongly dependent on the local topographical and weather conditions. While further technological

advances will bring no breakthrough for solar energy collectors (Latała, 2010; Abu-Zour, 2006), there is much room to improve the efficiency of photovoltaic cells in the near future (Esen, 2004). This will be possible after replacing expensive silicon with semiconductor paints. Once these solutions are implemented, the installation costs will decrease by several dozen percent. These changes should also lead to a revision of the national solar energy programme, especially since the results obtained indicate that the most promising prospects lie in the use of photovoltaic cells. In the knowledge that at the present stage they can efficiently support conventional energy sources on a cattle farm by covering even several dozen percent of the devices' active power requirement, RES can be considered as an important step of agriculture in combating climate change (Wang, 2005; Bos et al., 2003). This approach conforms with the objectives of national energy policy, including the diversification of energy sources. Finally for farmers themselves, it seems important that they will be able to cut down on beef production costs by reducing the energy inputs (Hörndahl, 2008).

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EFFECT OF PHOTOPERIOD IN ANESTRIC EWES SYNCHRONIZED WITH INTRAVAGINAL SPONGES

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SUMMARY
Reproductive physiology of sheep is determined by both exogenous and endogenous factors. These factors stimulate or inhibit the ability of control of the endocrine system on the development of functional gametes and childbearing potential. Seasonal anestrus in sheep caused by the photoperiod is a feature that directly affects ewe´s fertility, representing major economic losses. The aim of this study was to evaluate the effect of photoperiod with male presence on the induction of estrus using intravaginal sponges in anestrus ewes. Forty six anestrus ewes were divided into two treatments: (T1) without photoperiod (n=23 ewes with 12 h light and 12 h darkness); and (T2) with photoperiod (n=23 ewes). The photoperiod treatment consisted of 8 h light and 16 of darkness during 30 d. Both treatments had male presence. The ewes body condition score (BCS) was between 3 to 3.5 on the scale of 1 to 5. Results of T1 revealed that 16 ewes were in estrus, representing (76.19%) within 48 to 72 h, and 5 ewes did not show estrus (23.81%). In T2, 14 ewes were detected in estrus (60.86%) within in the same time period than T1, and 9 ewes (39.14%) did not show estrus. The percentages of ewes in estrus was statistically similar (P>0.05) in both treatments. In conclusion, the effect of photoperiod with the presence of male was not effective in inducing estrus in anestrus ewes.

Key words: Photoperiod, sheep, synchronized.

INTRODUCTION

Sheep are classified as seasonal poles, indicating that their breeding season is limited to a certain time of year; where it has been regulated by the amount of light hours (photoperiod). Sexual activity begins when the number of hours decreases (autumn and winter). Estrus cycles occur in intervals of 21 + - 3 days, which are interrupted by gestation or the arrival of anestrus (Balcázar, 2009). This occurs with an appearance of milk in the marking, causing variations in prices and quality of these products (Chemineau, 2014).

The absence of seasonal anestrus is a productive advantage reproducing reproductive programs throughout the year, without using hormonal drugs or other strategies, such as sexual biostimulation (Arroyo, 2011).

The reproductive physiology of sheep is determined both by exogenous factors (food, climate, and photoperiod) and endogenous (gestation, lactation, body condition). Factors stimulate or inhibit the

ability of the endocrine system to control the development of functional gametes and gestational capacity (López *et al.,* 1993). Some unique characteristics of the effect of photoperiod on sexual activity, such as stimulant effects and inhibitors of short days and long days, respectively, the presence of a phase of sensitivity 16 hours after dawn and property Of the long days of the elimination the refractoriness To the shorts of the days, they prepare the treatments in females and the males for the control of the reproduction counter-station (Chemineau, 2014). The aim of this study was to evaluate the effect of photoperiod with male presence on the induction of estrus using intravaginal sponges in anestrus ewes.

MATERIAL AND METHODS

For this study we used 50 most sexually mature criolla ewes with average age of one year, the diet is based on broken maize, wheat bran, whole sorghum and maize stubble, have free access water and the diet is supplemented With mineral blocks. The females were selected completely randomly and divided into three treatments: T1, T2 and T3 (control).

The selection criteria that were taken into account for T1 and T2 were that the ewes were sexually mature, a body condition of 3-3.5, that they had no mouth or udder problems and that they did not have a history of obstetric problems (Beltman, 2013).

The ewes were housed, and the beginning of the photoperiod for ewe lambs of T2. At the same time the corral of the ewe lambs of the T1 was modified to avoid that they entered the zone of the T2. The ewe lambs found inside the T2 were induced a period of 8 hours of light and 16 hours of darkness for 30 days. The ewes of T1 had no alterations in their photoperiod and remained separated from T2. Over time, 1 and 2 sponges impregnated with 20 mg of fluorogestone acetate were intravaginally introduced into the ewes of treatments 1 and 2 and remained in the ewes for a period of 9 days. At the time of removal of the sponges, equine chorionic gonadotrophin (eCG) 500 IU i.m. To favor more oocytes. Heat detection was performed by means of direct observation and by means of males equipped with paint on the abdomen, determining in estro those females that have marks. Stallions at the rate of 1 male per 10 females were used and were introduced at 24 h after eCG application. The males stayed away from the females to take advantage of the male effect, to intensify the effect of ovulation. Most were expected to enter estrus within 72 hrs after removal of the sponges. The effects of treatments on the presentation of jealousy and the effect of photoperiod were determined by the analysis of means using the Excel program. The means were compared by the F test, to know if there were differences between the treatments used.

RESULTS

Counting the two treatments the total lambs that presented estrus was 30 (68.18%). In both treatments no sheep went into heat the first 24 hrs. To take advantage of the male effect, the cheeks were introduced 36 hours after the removal of the sponges, with paint on the chest to mark the females that fence riding. 48 hours after the removal of the sponges, 6 ewes (26.08%) of the treatment 1 presented signs of estrus, 72 hours later 8 more ewes began to present heat, to give us a total of 14 ewes (60.86%) of treatment one. At T2 48 hours later, 4 ewes (19.04) presented signs of estrus, 72 hours later 12 (57.14%), to give us a total of 76.19% (16) ewes in estrus. At the moment of the removal of the sponges all the lambs had a non-fetid white discharge. In treatment 2 only one lamb presented problems at the time of removal of the sponges, as this was impregnated with blood and fetid pus. None of the control ewes presented estrus.

DISCUSSION

No significant differences were found between treatments 1 and 2 P> 0.05. This indicates that the effect of the photoperiod induced in treatment 2 did not help that a greater number of lambs presented heat.

In the present study, 68.18% of synchronized ewes presented estrus, a lower percentage compared to what was reported by Córdova *et al.*, (1999), where they obtained 95.8% estrus. This could be due to the fact that no antibiotic was administered at the time of sponge introduction. Uribe *et al.*, (2008), obtained 100% in terms of estrus presentation in lambs of the Belgamacia breed, where they used intravaginal devices impregnated with 0.3 g of progesterone for 14 days and an application of 500 U.I. Of PMSG. Sponges impregnated with 0.2 mg progesterone appear to be less effective. Cedillo (2008) performed a work impregnating the sponges with FGA with doses of 45 mg, 22.5 mg and 11.25 mg. Resulting in 100% jealousy in all treatments, showing that no matter the amount of FGA, as long as it falls within this range and the season in terms of photoperiod is appropriate. In comparison with our work the low percentage of ewes in estrus was due to that it was not in season as far as photoperiod is concerned.

A study carried out by Arroyo (2011) on lambs pelibuey aimed at monthly estrous activity indicates that these ewes present only a decrease of the estrous activity (57.7%) in December, all of which is not synchronized and mentions that it can be increased Up to 30% using hormones, but this only in sheep of this breed. The wool sheep in the month of December present a estrous activity of 21.5% in the same month, but it is more difficult to induce the estrus, due to the race mainly. This helps us to understand in part the reason for the low estro presentation, since the ewes used in this work are wool and do not have any crosses with ewe pelibuey that helps or favors him in the presentation of zeal throughout year.

Adib *et al.*, (2014) reports that before the introduction of the male plasma levels of FSH, LH and estrogen are at baseline levels. Immediately after introduction of the male plasma levels of estrogens increased in all lambs. This effect known as a male effect stimulates both visually and olfactorily the ewes that come into contact with it, triggering clear effects on antheric sheep (Forcada *et al.*, 2010).

In conclusion, the effect of photoperiod with the presence of male was not effective in inducing estrus in anestrus ewes.

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THE USE OF TANNINS AS GROWTH PROMOTERS IN POULTRY CHICKENS

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Plant tissues are rich in a wide variety of secondary metabolites with antimicrobial properties. Tannins polyphenols are widely distributed among several plant species where they play a protective role (i.e. pathogen attack). Tannins of different plant species have specific physical and chemical properties which enclose very different biological activities. After several in vitro and in vivo research studies in our laboratory, performed to characterize the valuable properties of different natural polyphenols, a blend of some of them were chosen to design a growth promoter in poultry. These selected tannins showed not only bacteriostatic and bactericidal activities against different pathogens, including *Clostridium perfringens*, but also antitoxin properties, without evident induction of bacterial resistance against themselves. Also, this combination of selected natural compounds was able to regulate the intestinal microbiome, the physiologic host function, the morphology of the gastrointestinal tract and the development of different infectious diseases including necrotic enteritis. The weight gain was improved when compared to non-growth promoter control under all the different conditions tested: experimental farm (1.8%), experimental pathogen challenge (5-22%) and commercial farm (4.8%). A two years evaluation in large commercial settings of different countries corroborated our laboratory findings. In all of the farms analyzed, comparing to controls using or not antibiotic growth promoters, an improving of intestinal health, podal lesions, weigh gain, reduction of mortality was measured, producing an improvement of productive parameters with a cost reduction. The available information supports the use of specific mixture of polyphenols as alternative to antibiotic growth promoters.

Precision livestock farming: techniques, risks and benefits.

THE CALF COUGH MONITOR: SOUND ANALYSIS FOR EARLY DETECTION OF BOVINE RESPIRATORY DISEASE

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In today's calf rearing systems, bovine respiratory disease (BRD) has a large economical and social cost for the farmer. Detecting and subsequently treating the disease at an early stage could reduce the impact of the disease, hence reduce the associated costs, such as reduction in production performance and veterinary costs. As coughing is a clinical manifestation of BRD it can be used as biomarker for disease detection. The objective of this study is to compare the results of an automatic cough detection tool based on sound analysis to clinical assessments taken from the calves.

Sound was recorded continuously for two rearing cycles in four adjacent calf sheds on Teagasc, Grange Beef Research Centre. The sound acquisition equipment comprised a sound card (ESI MAYA 44) and a condenser microphone (Behringer C4). Sampling frequency was set to 22.05kHz which was considered high enough to measure all sounds of interest (i.e. cough) and a 16 bit precision was used.

At least twice a week a human observer performed clinical assessments using the Wisconsin calf health scoring criteria. The criteria consisted out of the nasal discharge, eye or ear scoring (depending on which was most visible), cough, and rectal temperature. Each criteria was scored from 0 to 4 and for the final decision the cumulative sum was used to make a decision. A scoring of 5 or higher was considered as a calf affected with BRD.

By visually inspecting the amount of calf coughing some periods of increased coughing could be observed. Six out of seven from these increased periods of coughing corresponds to BRD using the clinical assessment as reference. These results shows us that the automatic detection of calf cough has the potential to be used as an early warning tool for the detection of BRD.

Husbandry of farmed animals, fishing, apiculture and aquaculture

THE EFFECT OF TWO TECHNOLOGICAL SYSTEMS FOR CALF HOUSING TO THE FUTURE PERFORMANCE OF DAIRY COW

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SUMMARY. Technology in calf housing during the milk period is one of the most important factors affecting the health and growth of calves. The aim of our study was assessment of the impact of different types of calf housing on the future performance of dairy cow. The effect of different ways of calf housing during the milk period (from birth to 60 days) was observed in Holstein cattle during 5 years period. The individual calf hutches and individual pens were located on one farm. Calf hutches were placed outside and individual pens were situated under a shelter. All individual pens had runs. The obtained values were analysed by general linear model (Statistica software package, StatSoft). The qualitative parameters were evaluated by non-parametric Kruskal-Wallis ANOVA. Calves housed in the individual pens under the shelter reached insignificantly lower live weight at the weaning in compared with the calves housed in calf the hutches. The higher prevalence of diarrheal disease and respiratory tract was detected at the calves housed in the shelter, as well as a higher mortality of calves in comparison with outdoor hutches. Heifers which were housed as calves in the hutches have had also higher life weight during the next rearing and were previously inseminated. The first-calf heifers housed in the hutches had significantly higher milk yield ($P<0.05$) and milk protein yield ($P<0.01$) for first lactation compared with the cows housed in individual pens under the shelter. The calf hutches are one of the most effective management practices for improving the health and growth of calves prior to weaning. The calf housing in the hutches provides better conditions for subsequent production and reproduction of cows.

Key words: calf, housing, performance

INTRODUCTION

Technology of calf housing during the milk period is one of the most important factors affecting the health and growth of calves. Most mortality of the calves occurs in during the first and second week of life (Broadwater and Chester-Jones, 2009). Mortality of pre-weaned calves varies from 0.3 to 14.5 %, average 4.6 % in conditions of Czech farms (Syrůček and Burdych, 2015). When choosing a technological system of housing it is necessary to consider not only the possibility of fulfilling the basic needs of animals, but also the opportunity to create suitable working conditions for a calf care taker.

In the Czech Republic are housed 74 % of all born calves in outdoor individual hutches and only 3 % in group hutches (Doležal and Staněk, 2015). Hutches are made of different materials such as wood, polyethylene, fiberglass, tarpaulin. Recently there has been an expansion of calf housing in hutches or pens under the shelter (35 % of calves), either individually or in groups. Housing in the calf house is less frequent in the conditions of Czech farms and represents 11 % of all born calves (Doležal and Staněk, 2015).

The calfhood disease effects on daily live weight gain, age at first calving and milk yield in the first and second lactation (Morrison et al., 2013; Magnier, 2014). The housing of calves and heifers affected performance and health of the adult animal (Waltner -Toews et al., 1986a,b).

The aim of our study was assessment of the impact of different types of housing of calves on the future performance of a dairy cow.

MATERIALS AND METHODS

The effect of different ways of calf housing during the milk period (from birth to 60 days) was observed in Holstein cattle during 5 years period. Individual calf hutches and individual pens were located on one farm. The calf hutches were placed outside and the individual pens were situated under a shelter. Both type of calf housing had runs. The hutches and the individual pens had all-in all-out system. The stables for heifers and first-calf heifer had continuous-flow system. The quantity and quality of bedding, feed and water were similar for all calves. The heifers had a common housing, feeding and watering and like first-calf heifers.

The year and season of birth, health status and live weight were monitored in 252 Holstein heifer calves. At heifers was determined live weight at the age of 6, 12 and 18 months and the age of the first insemination and number of inseminations per conception. At first calf-heifers were monitored milk yield, fat and protein production for 305 days of lactation, including fat and protein content.

The obtained values were analysed by general linear model (Statistica software package, StatSoft 7). The qualitative parameters were evaluated by non-parametric Kruskal-Wallis ANOVA.

RESULTS

Birth weight of heifer calves was ranged from 30.4 to 50.6 kg during monitoring. The heifer calves reached of live weight from 49.0 to 103.0 kg at weaning. The heifer calves reared in the individual pens under the shelter reached insignificantly lower live weight at the end of weaning in compared with calves reared in calf hutches (Table 1).

Table 1. Live weight depending on type of housing during rearing of heifer calves.

System of housing during rearing	Live weight [kg]														
	At the birth			At the weaning			6 months			12 months			18 months		
	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
Hutches	94	42.5	4.5	90	79.7	7.6	77	196.2	21.3	77	364.1	32.9	51	514.2	38.2
Individual pens	158	41.5	3.7	148	77.4	10.1	141	192.9	22.9	129	363.9	32.0	125	505.0	42.2
P value	0.089			0.098			0.307			0.962			0.180		

Heifers which were housed during the milk nutrition in the individual pens under the shelter, reached insignificantly lower live weight in the 6th (of -3.3 kg), in the 12th (-0.2 kg) and in the 18 months (-9.2 kg) than heifers reared in hutches (Table 1).

On the occurrence of diarrhoea and respiratory disease of calves had statistically significant effect birth year ($P < 0.001$) and season of birth ($P < 0.01$). The incidence of diarrhoea was the least affected calves born in winter. By contrast, most calves born in the summer and autumn months are diarrhoea affected. The effect of calf housing on the incidence of diarrhoea and respiratory disease has not been shown. Nevertheless the higher prevalence of diarrheal disease and respiratory tract problems was detected with calves housed in the shelter (32 %), as well as higher mortality of calves (6 %) in comparison with the outdoor hutches (diseased 25 %, mortality 4 %).

Heifers which were housed as calves in the hutches, have had live weight during the next rearing and were inseminated four days earlier (425±31 days) than heifers reared in individual pens under the shelter (429±41 days; $P=0.526$).

The number of inseminations on pregnancy rate was almost identical in both groups of heifers (2.5±1.7 - heifers housed in the individual pens under the shelter, 2.3±1.7 - heifers bred in the hutches, $P=0.608$).

Milk yield of first-calf heifers ranged from 5187 to 11873 kilograms of milk per 305 days of lactation. Fat yield reached 215 to 452 kg and protein yield was 170 to 374 kg for the monitoring period. Fat and protein content in milk varied from 2.36 to 4.84 % respectively 2.90 to 3.61%.

Table 2. Milk performance indicators depending on type of housing during rearing of heifer calves.

System of housing during rearing	N	Milk performance per 305 day of lactation									
		Milk yield [kg]		Fat [%]		Fat [kg]		Protein [%]		Protein [kg]	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Hutches	22	9267*	1401	3.57	0,45	327	43	3.27	0,17	302**	41
Individual pens	48	8454*	1475	3.67	0,50	306	49	3.21	0,15	271**	46
P value		*0.033		0.408		0.086		0.178		**0.010	

The first-calf heifers reared in the calf hutches had significantly higher milk yield by +813 kg ($P<0.05$) and milk protein yield by +31 kg ($P<0.01$) for 305 days of lactation compared with cows housed in individual pens under the shelter (Table 2).

These first-calf heifers housed in the hutches had also insignificantly higher production of milk fat (by +21 kg). A statistically significant difference in fat and protein content in milk of heifers reared in a different type of housing during calfhood has not been proved.

DISCUSSION

Many studies have shown that an optimal housing of the young calf is of great importance to bring up a profitable dairy cow (Lundell, 2015). The housing along with other management factors affected the calf body condition and its future performance (Heinrichs et al., 2005). Individual housing of dairy calves, either indoors or outside, is generally linked with improved calf health.

Most diseases of pre-weaned dairy calves are enteric or respiratory (Heinrichs et al., 1994; Wells et al., 1996), and most of these organisms become infective through inhalation or fecal-oral contact. Isolating calves from direct contact and providing adequate ventilation can markedly reduce the transmission of pathogens (Lorenz et al., 2011). Hutches have been associated with lower morbidity and mortality in dairy calves (Waltner-Toews et al., 1986a,b; Hill et al., 2011).

Many studies (Poos et al., 1982; Waltner-Toews et al., 1986a,b; Jorgensen et al., 1984; Quigley et al., 1995) have shown clearly improved growth and performance and reduced mortality when calves were housed in hutches compared to other methods. In contrast, it has been proved that the body weights were highest (by +6%) at calves kept in indoor pens and lowest for calves in the hutches (Hill et al. 2011). The calves with lower life weight at the weaning needs a longer time to reach desired weight for mating or artificial insemination i.e. a higher age at first calving. (Waltner-Toews et al., 1986b; Magnier, 2014).

A healthy calf will have a higher average daily gain, and it grows to a higher weight and stature. The heifer is more likely to have a higher milk yield at first and future lactations if she was not ill as a calf (Lundell, 2015). A significant difference in cases of diarrhoea was found between calves housed outside and calves housed inside. Earley et al. (2004) and Hill et al. (2011) found out that the incidence of respiratory disease was higher and diarrhoea incidence was lower at calves reared indoors in comparison with calves housed outdoors.

Health status of dairy heifers has been shown to have a significant impact on growth rate of calves especially during the first 6 months of life (Donovan et al., 1998).

Diarrhoea increased the mortality rate of pre-weaned calves by 3% (AFBI, 2011). Diarrhoea and pneumonia impacts not only on husbandry demands and veterinary costs, but also on future animal performance i.e. growth rates and milk yield (AFBI, 2011; Morrison et al., 2013; Magnier, 2014).

The relative decline in growth rates for pre-weaned heifer calves has been reported as 8% for calves with pneumonia alone, 18% for calves with diarrhoea alone, and 29% for calves with both pneumonia and diarrhoea (AFBI, 2011). The one treatment for pneumonia as heifer calves had approximately a 5% reduction in milk yield in the first lactation and a 10% reduction in the second lactation (Morrison, et al., 2013).

Milk production in first lactation is affected by the growth rate of heifers (AFBI, 2012; Lundell, 2015). With age at puberty in heifers is linked to physical development, reduction in growth rates can lead to a delayed age at first insemination and subsequent first calving, thus impacting on animal productivity and profitability (Morrison et al., 2013).

Calf hutches are one of the most effective management practices for improving health and growth of calves prior to weaning. Suitable material and design of hutches to maintain a dry, comfortable environment are important for the health and growth of calves. Calf housing in hutches provides better conditions for subsequent production and reproduction in this way reared cows.

ACKNOWLEDGMENTS

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NEW TOOL FOR AVERAGE DAILY GAINS MONITORING ON PIG FARMS

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SUMMARY. Around the globe, livestock producers face similar difficulties in relation to increasing worldwide competition resulting in reduced profitability of traditional livestock production. The industry is in need of new management enhancing tools in order to remain profitable. One such production enhancing technology developed by PLF Agritech, namely the Weight-Detect™ (which was designed to measure growth rate or Average Daily Gain, ADG) was tested in commercial piggery buildings in Australia over a 28 day period. In this article the performance of this technology will be discussed, particularly in relation to the benefits it can offer in supporting decision making processes on farms. The pen monitored in Australia was located in straw-based shelters using natural ventilation. Results from this study are used in this article to highlight the beneficial effects of routine monitoring of this important production related parameter in commercial livestock buildings. For example, on this farm and in the same building a growth rate variation between 972 g/day and less than 200 g/day was observed. Such large variation in growth efficiency within the same group of pigs highlighted the need to identify events such diet changes and potential health issues that can have a significant effect on production efficiency. The authors will demonstrate that such information might be used eventually to evaluate the 'quality' of management procedures used on specific farms.

Keywords: pig houses, farm building, risk factors, growth rate.

INTRODUCTION

Around the globe, livestock producers face similar difficulties affecting animal production that are beyond their control (Banhazi and Black, 2009; Banhazi *et al.*, 2012; Berckmans, 2008). Increasing consumer demand for better animal welfare and lower environmental impact are new challenges that can significantly reduce the profitability of traditional livestock production (Black and Banhazi, 2013). The industry is in need of new management enhancing tools in order to remain profitable (Wathes *et al.*, 2008). In the past two decades several new technologies were developed by different research groups that function automatically and optimise the production environment with the aim of providing real time feedback to farmers about the condition of their animals (Schulze *et al.*, 2007; Aerts *et al.*, 2008; Aydin *et al.*, 2015). Weight-Detect™, a contactless, image analysis based weighing system was developed by PLF Agritech with the aim to provide real time information about the average daily gain of a pen of pigs which was tested on one Australian farm over a 28 day period (Banhazi and Dunn, 2016). In this article the performance of this technology will be discussed with particular attention to the benefits it might offer in relation to supporting the decision making processes of farm managers on a daily basis.

MATERIALS AND METHODS

The long term weight monitoring was carried out on a farm in Australia. The pen monitored in Australia was located in a straw-based shelters using natural ventilation. In the pen, a Weight-Detect™ equipment was installed above the feeder at 2 m height. Weight-Detect™ incorporates an off-the-self depth sensor (Microsoft Kinect, Microsoft, USA) with a 6 meter maximum depth, and 0.5m minimum depth, and 640 by 480 image resolution at 30 frames per second. The depth images were captured at exactly one frame per second, and stored in a temporary file system on a Raspberry Pi 3 (Raspberry Pi

Foundation, UK). A second process running on this embedded computer then processes the images as they are captured. With a projected Infrared (IR) light pattern from the Kinect, one of the main advantages of this system is that it does not require external lighting, thus does not affect the normal wake/sleep activities of the animals. Weight prediction in real time was achieved by acquiring, analysing and extracting features and measurements from images captured by Weight-Detect^{TM} and producing the corresponding weight estimate to the image. As pigs were completely within the field of view for various length of time, only those images with a perfect contour and height model were analysed. Weight-Detect^{TM} used 3 dimensional (3D) models with automatic segmentation of individual animals from the floor (background) and each other (Figure 1).

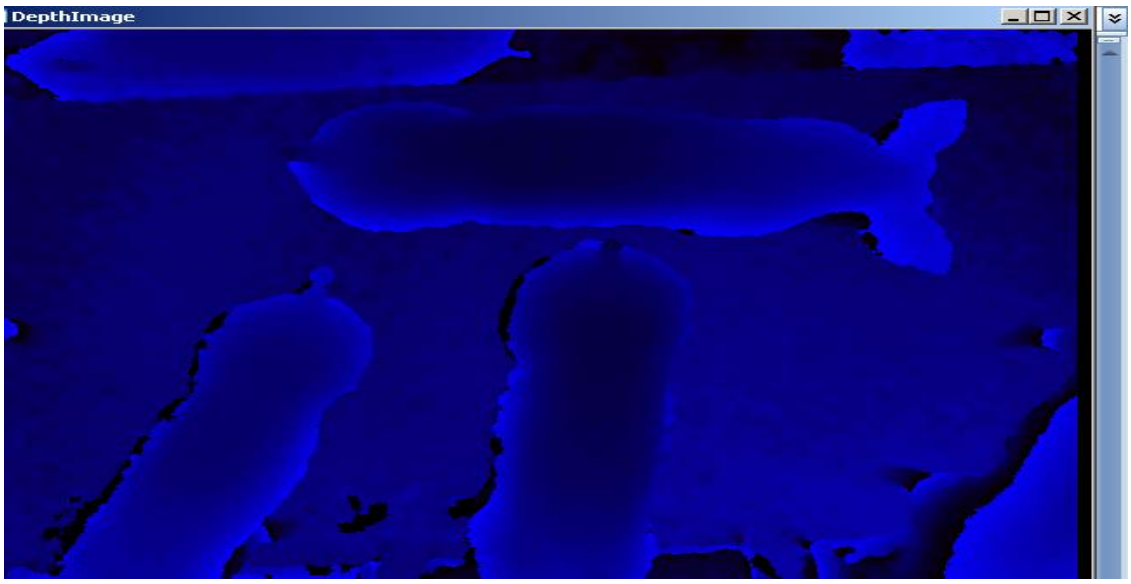

Figure 1. Segmentation of individual animals from the floor (background).

Two separate processes were used to analyse each depth image. Initially, the depth image was treated as a normal camera image, with depth corresponding to pixel intensity. The technique can then use standard image processing algorithms to segment the image into a binary image based on a minimum intensity (correlated to height) with the threshold set at a height just above the learned location of the floor plane. In this way, each distinct non-floor area will be located. Obviously, when two or more animals are close, or overlapping, the outline of the objects detected in this step will not be representative of a single animal. To confirm that a single animal exists, the object contour is scaled, rotated and matched against a library of representative templates. Once an individual animal object is confirmed, the representative point cloud is extracted from the original by selection only of those points above the floor plane, and inside the identified contour. This point cloud is then an exact representation of the maximal points of the animal as visible from the camera. Even though this novel 3D measurement is much more accurate than image analysis from visual cameras, it was still found that the head should be removed for the best correlation with the ground truth data (Brandl and Jorgensen, 1996). The measurement calculated is then a volume, which is much more statistically relevant to the weight of an animal than a simple plan view contour (Banhazi *et al.*, 2011).

RESULTS AND DISCUSSION

In Figure 2 the variation in average daily growth rate is presented. The starting average weight of this group of animals was 64.9 kg. During the first 3 days the weight of the animals stagnated. A gradual 972 g/day weight gain is observed from the 4th to the 14th day, animals reaching 76.3 kg on average. A significant decrease in ADG was noted from the 15th to the 18th day when animals reached 78.8 kg on average. During this 3 day period the ADG of the animals dropped to 518 g/day. From the 19th to the 25th day

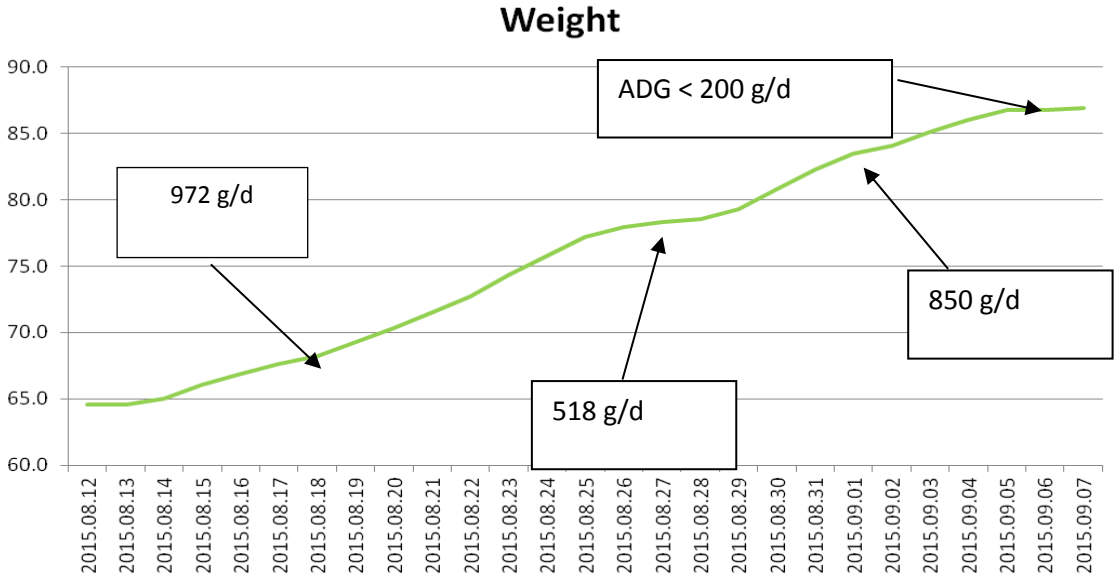

Figure 2. Data collected at the Australian farm over 28 days period (The arrows point at the different time periods when vastly different growth rates were observed. Further explanation about these distinctive periods are given in the text below) .

the ADG of the animals picked up to 850 g/day and in the last 3 day the ADG of the animals was less than 200 g/day, reaching on average the final weight of 86.7 kg. The ADG of the animals during this 28 days period can be broken into 5 stages:

1. Day 1 to day 3: Animal's weight stagnated probably due to the stress that they were moved to a new pen; therefore their weight stagnation was understandable and foreseeable.
2. Day 4 to day 14: Animals performed best during the 2nd stage. After the initial 3 day interim period the ADG of the animals' reached 972 g/day.
3. Day 15 to day 18: An unexpected drop in ADG occurred that lasted over 4 days. During this period the ADG of the animals decreased by 47% from 972 down to 518 g/day. This episode was out of ordinary and would need a further investigation. One possible explanation was a slight change in diet composition. However, the next stage of this study will focus on the identification of underlining causes of these ADG depressions.
4. Day 19 to day 25: Animals seemingly recovered from the unknown stress they experienced during the 3rd stages and they ADG climbed back to 850 g/day. Although animal's performance did not reach the previous 972 g/day perhaps indicating that the stress they endured during the 3rd stages was still influencing them to a some extent.

5. Day 26 to day 28: Animals' weight stagnated in the last 3 days and gained less than 200 g/day. Such stagnation during this time of the growing period is rather typical and most likely does not indicate any out of ordinary event.

Weight-Detect™ pinpointed a 4 day period within a 28 day cycle that potentially had a major economic effect on the profitability of the farm. These 4 days were probably unnoticed by farm workers and manager due to its shortness and because the animals continued gaining weight even if to a lesser extent. It is probably impossible to detect a smaller than 0.5 kg less daily weight gain by the naked eye, especially if the farm workers are less experienced and if the event lasts for a short period only, or if it occurs over the weekend. Nevertheless, 47% drop in average daily gain can have a major impact on the farm's profitability even if the episode last for only 4 days (Willis *et al.*, 2016). The fact that the animals were unable to revert to their original 972 g/day ADG indicates further losses, as animals took longer to reach their final weight, causing extra expenses in feed, energy consumption and labour costs. Regular use of Weight-Detect™ enables farm managers to monitor and profile their management practices. Continuous weight monitoring empowers them to detect subtle changes in the conditions of the animals therefore allowing them to investigate and respond in time. Better understanding, quicker and more appropriate management responses most certainly save time and money for producers both on short and long term.

CONCLUSION

The new technology, Weight-Detect™, provided practical information to livestock producers that empowered them to make management changes based on scientific data to enhance the profitability of their production. Weight monitoring pinpointed problem areas and checked the effectiveness of management style of the livestock producers.

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DEWORMING PRACTICES IN SHEEP: SELECTIVE DEWORMING, BENEFITS?

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SUMMARY. Gastrointestinal parasites cause losses in animal production even cause deaths in young animals. In intensive and extensive production units has become a common practice dewormed periodically, even stabled animals the parasitic load of gastrointestinal nematodes (NGI) in three herds were identified stabled. Three flocks of sheep females over one year of age, state of Mexico, one of Zumpango (1) 40 females) and two Temascaltepec (2) 10 females, 3) 6 females) were used. Faecal samples were collected from the rectum; Mc Master technique was performed, the morphological characteristics were observed, counted eggs per gram (epg), the frequency and intensity was obtained. With positive stool stool culture was performed. NGI in the three flocks were recorded: 1) 20% (394 epg); 2) 100% (255 epg) and 3) 100% (3867 epg). It was suggested deworm 5%, 0 and 100% respectively. The genera identified were *Haemonchus, Cooperia* and *Trichostrongylus Chabertia ovina*. Thus, it was found that before a deworming should make a comprehensive evaluation of the herd supported a diagnosis to identify animals that needed to integral deworming for to improve the animal, human and environmental health.

Key words: gastrointestinal parasites, selective deworming, sheep.

INTRODUCTION

Currently, as part of veterinary medical practices, it is customary to periodically deworming all the animals of a flock to eradicate parasites, sometimes using drugs indiscriminately without considering the management that is given to them, however, it is not always necessary. When administering anthelmintic treatment an economic cost is generated, in addition the residues are stored in tissue and when removed contaminate the environment. Another consequence is that the use of anthelmintics repeatedly or under dosed promotes the selection of nematode populations resistant (Figueroa and Acevedo, 2011). Therefore, it is necessary to have studies on population and parasitic dynamics to carry out a selective and integral control. The objective was to identify the parasitic burden of gastrointestinal nematodes (NGI) in three flocks stabbed to identify animals with high parasitic loads and to perform selective deworming.

MATERIAL AND METHODS

The study was carried out in Temascaltepec and Zumpango, State of Mexico, with temperate climate. Three flocks of adult ewes between 1 and 3 years old were studied, most of them were pregnant. Two flocks (10 and 40 ewes) with permanent housing and one flock (6 ewes) with grazing in implanted prairies, nocturnal housing and with food supplementation. Faecal samples were collected directly from

the rectum in plastic bags individually. The samples were labelled and transferred to the Parasitology laboratory of FMVZ-UNAM. They were kept at 4 ° C until processing. The Mc Master technique was performed, the intestinal parasites were identified and counting was performed. With stools positive to gastrointestinal nematodes, they were homogenized in plastic containers, the mixture was moistened without excess water, incubated at 27 ° C for 10 days and oxygenated daily (Liébano, 1989). Third larvae (L_3) were collected by means of the larval migration technique for 24 hours. The larvae migrated from the faeces and, through gravity, passed through a sieve, appearing as sediment at the bottom of the funnel and the first drops were taken (Thienpont et al., 1979). A drop of the collected liquid was taken from the Baermann funnel and a drop of lugol was placed to fix the L_3. By means of the microscopic observation the taxonomic identification of the different genera of NGI was realized (Niec, 1968, Vega and Romero, 1983; van Wyk, et al., 2004).

Statistical analysis: Egg counts per gram of faeces (epg), frequency (percentage of positive samples) and mean intensity were obtained: mean eggs of the positive samples (Eckert et al., 1984).

RESULTS

In the three flocks, gastrointestinal nematodes were identified, however, the conditions were different, since two herds do not grazing, their parasitic loads are low. It is worth mentioning that in two flocks, 100% of the sheep were positive for gastrointestinal nematodes. In two flocks, the epg was less than 500, while in flock 3 the average intensity was 3867 epg (Figure 1). Therefore, according to parasitic loads, only some animals need to be dewormed (Table 1).
The genera identified were *Haemonchus, Cooperia* and *Trichostrongylus*.

DISCUSSION

Although flocks are located in different geographical areas, two herds are permanently housed, only one grazing in the same pasture, however, all receive periodic anthelmintic treatment. From this analysis, it can be concluded that from the herd 1 only 5% of the animals are required to be dewormed, since they have 1000 epg beads. While the herd 2 does not require deworming since although all the sheep were positive, the beads are less than 750 epg. By contrast, herd 3 requires deworming of all its animals, due to its high beads, which is the result of its brief grazing in the same meadow. In this way it was shown that it is not always necessary to deworming the entire herd, since according to the management to which they are subjected only some animals are infected and of those a low percentage will have high epg. Therefore, before carrying out a deworming, it is necessary to make a comprehensive diagnosis, so that a selective deworming can be done, only administering medication to those who require it, thus reducing costs and contamination of animal products and the environment.

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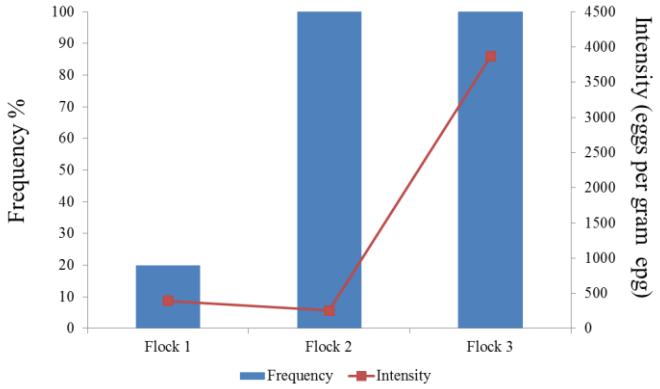

Figure 1. Frequency and intensity of gastrointestinal nematodes in three flocks of housing ewes from Mexico.

Table 1. Percentage of sheep candidates for deworming.

Flock	% to deworm
1	5
2	0
3	100

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EFFECT OF ANTHELMINTIC TREATMENTS IN HORSES

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SUMMARY. Parasites in horses cause significant losses; however, to reduce its effects, sometimes periodic clinical manifestations result deworming performed without laboratory diagnosis. The frequency and intensity of gastrointestinal parasites of horses in Culiacan, Sinaloa in three periods (February (two blocks, 45 horses), September and December 2014 (one block, 17 horses)) was determined. Faeces were collected from the rectum in plastic bags, were kept refrigerated and were taken to the laboratory of Parasitology FMVZ-UNAM. Mc Master technique was performed, intestinal parasites were identified, egg counts were performed per gram (epg), the frequency and intensity was obtained. With positive stool cultures were performed, genera were identified. Positive animals were dewormed with fenbendazole (in February and September) and ivermectin (in December). A faecal samples were collected seven days after treatment. Results in February, block 1: 15% (116 epg) *Anoplocephala*, 50% (1375 epg) *Strongyloides*, 5% (50 epg) *Parascaris equorum* and 5% (50 epg) *Oxyuris equi;* block 2: 36% (700+1801 epg) stongylides and 4% (200 hphg) *Parascaris equorum*. In September, two samples were taken with 43 and 57% stongylides (825-425 epg), 7.1 and 14.3% (50-1475 epg) *P. equorum*, and a reduction of epg was apparent after treatment - 62.4 and 0% in the frequency and 44.5 and 93.2% in the intensity of epg, respectively. In December, 58% (535 epg) the stongylides and 47% (406 epg) *P. equorum*. A reduction of 87.5 and 100%, respectively, was obtained. Thirty days later an increase in epg was recorded, which indicated reinfection. We identified the *Strongylus vulgaris* (27%), *S. edentatus* (21%), *S. equinus* (6%), *Cyathostomum* sp. (25%), *Trichostrongylys axei* (16%) and *Strongyloides westerii* (5%). Resistance to fenbendazole and ivermectin is suspected. Diagnosis is necessary to perform selective deworming and reduce the occurrence of anthelmintic resistance and environmental damage.

Key words: gastrointestinal parasites, deworming, horses, anthelmintic resistance.

INTRODUCTION

Equine production is produced equines for meat supply, livestock activities, sports and exhibition. Parasitic infections are undervalued and seldom treated properly. Gastrointestinal parasites are very common in grazing animals and decrease the quality of animals, decrease in work, performance, discomfort and skin lesions and hair loss as a result of scratching, which translates as economic losses. Generally, deworming is administered to all animals even to animals that do not require it since no previous diagnosis is made, which promotes the development of anthelmintic resistant parasites (Anziani and Catanzaritti, 2005). The objective was to determine the frequency and Intensity of gastrointestinal parasites of horses in Culiacan, Sinaloa in three periods (February (two blocks, 45 horses), September and December 2014 (one block, 17 horses) and measure the effectiveness of the treatment administered.

MATERIAL AND METHODS

The study was conducted in flocks from Culiacan, Sinaloa, Mexico, with a temperature of 20.6 ° C - 30.4 ° C, and annual precipitation of 688.5mm. The study included three samplings during the year 2014.

In February 2014, two-grazing horses were analyzed. Block 1 with 25, 2 with 20 horses, which was followed on two other occasions, although the number of horses decreased to 17. The positive horses in block 2 were dewormed with febendazole (February). In August, animals with clinical signs were observed, mainly lesions in response to pruritus in the anal region, so they were dewormed with febendazole, nematode nematodes and *P. equorum* were identified; At day 28 postreatment,

Anoplocephala was identified. In December, high counts of nematodes and *P. equorum* were recorded, all animals were dewormed with ivermectin. After administration of anthelmintics, samples were collected at 8 and 21 days postreatment and eggs counted per gram of faeces were collected. Faeces were collected from the rectum in plastic bags, were kept refrigerated and were taken to the laboratory of Parasitology FMVZ-UNAM. Mc Master technique was performed, intestinal parasites were identified, egg counts were performed per gram (epg), the frequency and intensity was obtained.

Gastrointestinal nematode (GI) positive faeces were homogenized with sterile sawdust in plastic containers, the mixture moistened without excess water, incubated at 27 ° C for 10 days, and oxygenated daily (Liébano, 1989). The third stage larvae (L_3) were collected by the Baermann technique (Thienpont et al., 1979). It was observed under the microscope and the taxonomic identification of the different genera of NGI (Niec, 1968; Vega and Romero, 1983).

Statistical analysis: Egg count per gram of faeces (epg) was obtained, frequency (percentage of positive samples) and intensity (average of oocysts or eggs of positive samples). The percentage of identified genera or species was obtained. The reduction percentage was calculated with a 95% confidence interval using the arithmetic mean (Coles et al., 1992). The percent reduction was obtained as follows: 100 (1 -Xt / Xc) where t is the treated group, ie at day 8 postreatment, group c is epg at day 0.

If at least one of the following criteria is met: 1) egg reduction percentage was less than 90%, 2) confidence level less than 90%, suspected resistance.

RESULTS

In both blocks intestinal parasites were recorded. In block 1, cestodes of the genus *Anoplocephala*, strongylides nematodes, *Parascaris equorum* and *Oxyuris equi* (Figures 1) were identified. In block 2, oocysts of the genus *Eimeria* were also recognized only in February. Positive horses were dewormed with febendazole in February and December. In August, animals with clinical signs were observed, so they were dewormed with ivermectin, nematode trichostrongylid nematodes and *P. equorum* were identified and at day 28 post-treatment *Anoplocephala*. In December, strongyloide nematodes and *P. equorum* were recorded.

The most frequent species were *Strongylus vulgaris* (27%), followed by *S. edentatus* (21%), *S. equinus* (6%), *Cyathostomum* sp. (25%), *Trichostrongylus axei* (16%) and *Strongyloides westerii* (5%) (Figure 2).

The parasite load was decreased by the administration of anthelmintic, however, it can be thought that a reduction of 48% was obtained after 8 days postreatment of the second administration of febendazole, however, after 21 days postreatment, a parasitic load of strongylides more low. Finally, in the administration of ivermectin, it also decreased until it had scores of zero, however, at 21 days, the epg increased. Regarding *P. equorum*, the reduction of eggs was 88% and at 21 days increased parasite load (Table 1).

DISCUSSION

Gastrointestinal parasites are present in pastured horses of Culiacan, Sinaloa, Mexico, there are no records on anthelmintic resistance. The resistance of small strongylides (cyatostomids or *Triconema*) to anthelmintics is a documented fact in the 60's in Europe, Oceania and the USA, Brazil, Chile and Argentina (Anziani and Catanzaritti, 2005). *P. equorum* has been reported to have resistance to ivermectin (von Samson, 2012).

According to the results observed in this study, it was observed that in the first administration of febendazole it had acceptable efficacy against strongylides, of 95%, however, in the second

administration, the reduction was only 48% Which, according to Coles et al. (1992), could be suspected to have a reduction of eggs of less than 95%.
With regard to ivermectin administered at the end of the year, the percentage of egg reduction at day 8 was 100% in strongylides, but *P. equorum* only had a reduction of 88%, so anthelmintic resistance could also be suspected. On the other hand, at day 21, the parasite load increased, which demonstrated low residual efficacy.

The phenomenon of resistance to benzimidazol has been reported in several countries (Coles et al., 2006); however, in this study, the percentage reduction with febendazole was acceptable, higher than 90%, except in the case of *P. equorum*. Resistance against macrocyclic lactones is more difficult to identify, it has not been widely described (Mathews, 2014), in this work, a reduction of 88% of *P. equorum* was identified at 8 days after treatment, which is indicative of resistance. On the other hand, at 21 days, the reduction was 50% and 22% for strongylides and *P. equorum* and although it could be thought of reinfection, it is a fact that the residual effect of ivermectin was low, which differs with the follow-up Of Wayne et al. (2002).
In February, only horses that were positive were given treatment, so that the economic expenditure in the investment of anthelmintic was lower, so that it is necessary to make the diagnosis to conduct selective deworming in order to reduce the risk of development Resistance and lessen negative effects on animal and environmental health.

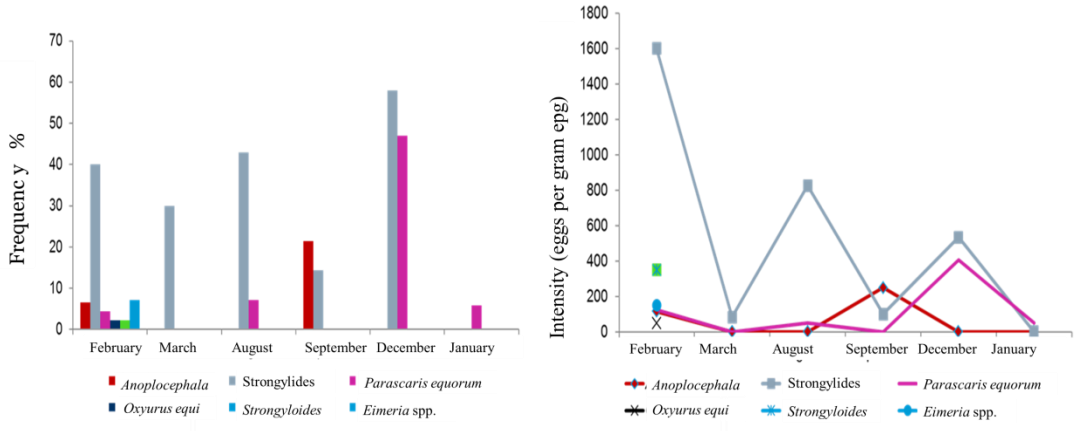

Figure 1. Frequency and intensity of gastrointestinal parasites identified on horses two blocks from Culiacan, Sinaloa.

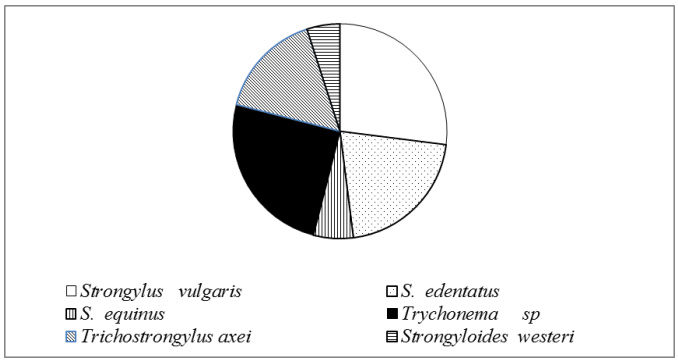

Figure 2. Percentage of vigorous nematodes in horses from Culiacan, Sinaloa.Table 1. Efficacy of anthelmintic treatments in the reduction of egg intensity per gram of faeces

	February: febendazol	August: febendazol				December: ivermectin			
	8 post tx	8 post tx		21 post tx		8 post tx		21 post tx	
	Strongylus spp.	*Strongylus spp.*	*P. equorum*	*Strongylus spp.*	*P. equorum*	*Strongylus spp.*	*P. equorum*	*Strongylus spp.*	*P. equorum*
Intensity reduction %	95	48.5	0	88	100	100	87.5	50.9	22

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TECHNICAL PERFORMANCES INFLUENCED BY INFECTIOUS AND NON-INFECTIOUS FACTORS: A STUDY IN 41 SWINE HERDS

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SUMMARY The study aimed at identifying infectious and non-infectious factors associated with the technical performances of 41 French swine herds without vaccination against porcine circovirus type 2 (PCV2) in piglets and without clinical signs related to PCV2 associated diseases. Data related to management, biosecurity, husbandry and the main technical performances (average daily weight gain, feed conversion ratio and mortality from 8 to 115 kg and carcass slaughter weight) were collected by a questionnaire. Blood was sampled from 20 pigs from two batches (10 to 12 weeks old and at least 22 weeks old). Infections by *Lawsonia intracellularis, Mycoplasma hyopneumoniae,* porcine reproductive and respiratory syndrome virus (PRRSV), PCV2 and swine influenza viruses were detected by specific ELISAs. Two groups of herds were identified by a clustering analysis: a cluster of 24 herds with the highest technical performances and a cluster of 17 herds with the lowest performances. Multiple correspondence analysis was used to identify factors associated with the level of technical performances. Infection by PRRSV, *M. hyopneumoniae* and PCV2 were factors associated with the cluster having the lowest performances. This cluster was also characterised by herds of farrow-to-finish type, having a short interval between successive batches of pigs (≤3 weeks) and mixing the pigs in the growing or/and finishing steps. Inconsistency between the nursery and the fattening building management was another feature of the cluster having the lowest performances. Herd management and respiratory infections significantly influenced the performance levels of the swine herds included in this study.

Key words: Herd technical performances, management, respiratory infections

INTRODUCTION

Swine farm profitability and efficiency partly rely on the technical performances of the pigs. Several respiratory or digestive infectious pathogens may reduce swine performances during the growing-finishing steps, like porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), swine influenza viruses (SwAV), *Mycoplasma hyopneumoniae* (Mhp) or *Lawsonia intracellularis* (LI). Environmental factors also directly drive the herd performances through the feeding and climatic conditions applied or indirectly by affecting the diseases occurrence and severity (Fablet, 2009). Many recommendations concerning the improvement of the technical performances of a herd are based on the results of studies assessing the effect of one or a limited number of pathogens or environmental factors. To date, few studies investigated both type of factors on swine herd performances. The aim of our study was thus to identify infectious and non-infectious factors associated with the technical performances of the growing and finishing steps of 41 French herds.

MATERIAL AND METHODS

Data and sera used were collected in 41 pig farms involved in a study on PCV2 course of infection in PCV2 sub-clinically infected herds without vaccination against this virus in piglets (Western France

2014-2015). Blood was sampled from 20 pigs from two batches in each herd (10 to 12 weeks old and at least 22 weeks old). Antibodies against LI (BioScreen Ileitis Antibody ELISA), Mhp (IDEIA™ MYCOPLASMA HYOPNEUMONIAE EIA KIT), PRRSV (IDEXX PRRS X3 Ab Test) and PCV2 (SERELISA® PCV2 Ab Mono Blocking) were searched in all sera. SwAV antibodies (ID Screen® Influenza A antibody competition) were detected in the sera of the oldest pigs (n=6 samples/herd). Data related to management, biosecurity, husbandry and the main technical performances (average daily weight gain [ADWG], feed conversion ratio [FCR], mortality from 8 to 115 kg [MORT] and carcass slaughter weight [CSW] in 2014) were collected by a questionnaire. The four parameters describing the technical performances of the herds were included in a clustering analysis to identify the underlying groups differing in performances. A multiple correspondence analysis was then used to identify factors associated with the level of technical performances.

RESULTS

Two groups of herds were identified by the clustering analysis: a cluster of 24 herds with the highest technical performances (mean ADWG= 781.1g/day +/-26.3 ; mean FCR= 2.5kg/kg +/-0.1; mean MORT=4.1% +/- 0.9; and mean CSW=121.2kg +/-5.2) and a cluster of 17 herds with the lowest performances (mean ADWG=715.8 g/day +/-26.5 ; mean FCR=2.6kg/kg +/-0.1; mean MORT=6.8% +/-2.0; and mean CSW=117.7kg +/-3.6). Infections by PRRSV, Mhp and PCV2 were associated with the cluster having the lowest performances. This cluster was also characterised by herds of farrow-to-finish type and having a short interval between successive batches of pigs (≤3 weeks). Mixing the pigs in the growing or/and finishing steps and inconsistency between the nursery and the fattening building management were other features of this low performing cluster.

DISCUSSION

In the current study, the herds were classified according to four technical parameters: daily weight gain, feed conversion ratio, mortality rate and carcass weight of slaughtered pigs. Feed-conversion efficiency, daily weight gain and mortality are recognized as the most important production-performance factors on fattening farms (Heinonen et al., 2001). All these parameters were therefore retained to describe the herds according to their technical levels. Carcass slaughter weight was also taken into account because of the potential economic impact of this parameter on herd income; farmers being partly paid according to the carcass weight. Growth performances were negatively correlated with the feed conversion ratio and mortality rate in our study. Pig growth and feed conversion efficiency were previously found to be correlated (Heinonen et al., 2001).

The study was carried out in a non-negligible but limited number of herds without vaccination against PCV2 in piglets and without clinical signs related to PCV2 associated diseases. The results of the study may therefore only apply to this kind of herds. This survey should therefore be considered as an exploratory one that help gain insight into factors associated with reduced herd performances in the growing finishing steps and design further large scale studies.

Respiratory infections, particularly PRRSV and Mhp, but also PCV2 infection, were found to be associated with decreased performances in the current study. All these pathogens are involved in the porcine respiratory disease complex (PRDC) one of the most costly disease for the swine industry worldwide. These results are in line with another study showing that herds severely affected by lung lesions had reduced growth, lower feed efficiency and higher mortality rate (Aubry et al., 2009). Digestive troubles were not found to be associated with lower performances at the herd level in our study. Only LI infection was considered whereas several other pathogens may disturb the gut health. Further studies involving the main frequent digestive pathogens in growers and finishers are needed to better assess the impact of these infections on the herd performances.

Not only infectious factors but also non-infectious ones related to farms characteristics and management were found to influence the level of technical performances of the herds. The effects of these non-infectious factors may be linked to their impacts on swine health and pathogen transmission. Indeed, Fablet et al. (2012) showed that a short interval between successive batches of pigs was at risk for pneumonia severity. Several studies indicated that the lack of all-in all-out management and mixing pigs during the production stages negatively impacted the respiratory health or favored respiratory infections (Cleveland-Nielsen et al., 2002);(Fablet et al., 2013; Fablet et al., 2016). On the other hand, moving is usually associated with the practice of regrouping pigs and hierarchical fights generally occur after mingling. All these conditions are sources of stress to the animals (Blecha et al., 1985), which may then be responsible for immune response alteration and increased disease susceptibility. Regrouping also enhances the probability of pathogen transmission and frequent moving in subsequent facilities increases the opportunities for exposure to residual infectious agents. Furthermore herd type and management policy are interrelated and their specific effects are not always easy to identify and evaluate. These intermingled non-infectious factors strongly influence disease transmission pattern and severity and in turn the herd performances.

Risky herd profiles were therefore identified as regard to the technical performances of swine herds. Herd management and respiratory infections significantly influenced the performance levels of the swine herds included in this study. To conclude, improvement of management and reduction of the occurrence of respiratory infections should significantly contribute to increase herd performances levels.

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HYGIENIC STATUS OF ORGANIC ENRICHMENT MATERIALS IN PIG PRODUCTION

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SUMMARY. In the EU permanent access to enrichment material has to be provided for pigs. The new Commission Recommendation (EU) 2016/336 demands these materials to be edible so that only organic materials are employable. However, according to an EFSA Scientific Opinion, this may pose risks and has to be further investigated. The aim of the present study is to evaluate the hygienic status of different organic enrichment materials and the possible risks of pathogen introduction.
In total 21 organic materials were examined. Most of them were commercially available in Germany, only three were produced on-farm. The materials consisted of wood and partly compressed straw and hay. Additionally six miscellaneous materials were analysed. All materials were tested for total viable count (TVC), coliform count, *Escherichia coli*, *Klebsiella* spp., *Yersinia* spp., *Salmonella* spp., fungi, methicillin resistant *Staphylococcus aureus* (MRSA), and *Mycobacterium* spp.. In addition, a high-performance liquid chromatography-mass spectrometry based multi-mycotoxin analysis was performed.
Escherichia coli, *Klebsiella* spp., *Yersinia* spp., *Salmonella* spp. and MRSA were not detected in any material. The only finding of *Mycobacterium* spp. was *M. smegmatis* in hemp litter. The TVC ranged from no detection of colonies to 7.7×10^7 colony forming units per g dry matter. Wood and compressed straw and hay products showed a lower microbial load than loose straw and hay. The analysis of mycotoxins revealed a high mycotoxin load in some materials, especially products made of maize.
The tested materials differed in their hygienic status widely. Important pathogens such as *Escherichia coli*, or zoonotic agents such as MRSA or *Salmonella* spp. were not found in any material. Some materials contained high amounts of mycotoxins which might pose a health risk for pigs. In conclusion, most tested materials are suitable as enrichment material for pigs, some with restrictions.

Key words: Biosecurity, Environmental enrichment, Pathogen introduction

INTRODUCTION

In the EU permanent access to enrichment material has to be provided for pigs (Council Directive (EC) 2008/120). The exemplary mentioned materials are all organic materials and according to the recent Commission Recommendation (EU) 2016/336 optimal materials have to be edible which limits optimal enrichment to organic materials. However, the mentioned Recommendation demands the materials to be concurrently safe, clean and hygienic. The European Food Safety Authority (EFSA) mentioned possible risks by those materials and points out the need for further research (EFSA, 2014). The aim of the present study is to evaluate the hygienic status of different organic enrichment materials and the risks of pathogen introduction this type of material might pose.

MATERIAL AND METHODS

A total of twenty-one different organic materials were examined in this study. Eighteen of those materials are commercially available in Germany, three materials were taken from a farm which produces its own straw, hay and silage. Four materials are made of wood, i.e. sawdust and wood

shavings of different sizes. Eleven enrichment materials consist of straw or hay originating from different plant species. Four of the preceding materials are compressed into pellets, small bales or cylinders. The remaining six materials are: beet pulp treated with molasses, pellets made of maize, peat for piglets, lick blocks made of molasses, litter material made of lignocellulose and maize silage.
After the weighted samples were grinded a suspension was prepared for microbiological examination. All materials were tested for total viable count (TVC), coliform count, *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Yersinia* spp., *Salmonella* spp., fungi, methicillin resistant *Staphylococcus aureus* (MRSA), and *Mycobacterium* spp.. The examination for *Salmonella* spp., MRSA and *Mycobacterium* spp. was qualitative the other tests were quantitative with a limit of detection between 423 and 1438 colony forming units per gram dry matter (cfu/g DM). In addition, a high-performance liquid chromatography-mass spectrometry based multi-mycotoxin analysis was performed.

RESULTS

E. coli, *Klebsiella* spp., *Yersinia* spp., *Salmonella* spp. and MRSA could not be detected in any of the tested materials. The only finding of *Mycobacterium* spp. was *Mycobacterium smegmatis* in the hemp litter. The mycotoxins Deoxynivalenol (DON) and zearalenone (ZEA) were detected in seven and five out of ten tested materials respectively (table 1). However, only in the maize products they exceeded the EU guidance value (Commission Recommendation (EC) 2006/576).

Table 1. Level of deoxynivalenol (DON) and zearalenone (ZEA) in the tested organic materials in µg/kg relative to a material with a moisture content of 12%.

Material	**DON**	**ZEA**
Wood granulate	< LOD	< LOD
Wheat, rye, triticale	497	30
Alfalfa/Lucerne	15	0.4
Rye	196	< LOD
Straw (from farm)	132	32
Hay pellets	< LOD	< LOD
Maize pellets	5054	1220
Peat	< LOD	< LOD
Lignocellulose	2	< LOD
Maize silage	1673	201
Guidance value[1]	900	100/250

[1] according to Commission Recommendation (EC) 2006/576 for complementary and complete feedingstuffs for pigs (DON) and piglets/gilts and sows/fattening pigs respectively (ZEA)
LOD = limit of detection

The results for TVC, coliform count and fungi differed considerably as shown in table 2.

Table 2. Microbial count of the tested organic material in cfu/g DM.

Material	**TVC**	**Coliform count**	**Fungi**

Wooden materials:			
Wood granulate	2.0×10^3	< LOD	2.0×10^3
Wood shavings	< LOD	< LOD	6.4×10^3
Sawdust	1.1×10^3	< LOD	1.6×10^3
Millings	5.4×10^3	1.5×10^3	9.8×10^2
Loose straw and hay:			
Flax	1.1×10^7	6.0×10^5	1.1×10^3
Wheat, rye, triticale	9.8×10^6	1.6×10^6	< LOD
Alfalfa/Lucerne	4.7×10^5	6.4×10^3	< LOD
Rye	6.4×10^6	1.7×10^6	1.2×10^4
Hemp	5.8×10^6	2.7×10^5	5.7×10^2
Hay (from farm)	3.5×10^7	2.3×10^6	7.0×10^4
Straw (from farm)	4.3×10^7	9.2×10^5	3.4×10^3
Compressed straw and hay:			
Compressed straw cylinder	5.2×10^4	< LOD	< LOD
Straw pellets	2.3×10^5	< LOD	< LOD
Hay pellets	9.9×10^6	< LOD	< LOD
Miscanthus cylinder	2.0×10^5	5.2×10^2	4.1×10^3
Miscellaneous:			
Beet pulp, molasses	< LOD	< LOD	< LOD
Maize pellets	2.0×10^4	< LOD	< LOD
Peat	4.5×10^6	< LOD	2.2×10^5
Lick block	3.0×10^3	< LOD	< LOD
Lignocellulose	< LOD	< LOD	< LOD
Maize silage	7.7×10^7	< LOD	< LOD

TVC = total viable count; LOD = limit of detection

DISCUSSION

The tested organic materials showed a wide difference in their hygienic status. Multiple studies show a contamination of unused sawdust and wood shavings with coliforms and *Klebsiella* spp. (Hogan and Smith, 1997; Zadoks et al., 2011) as well as *Mycobacterium* spp., especially *Mycobacterium avium* subsp. *hominissuis* (Matlova et al., 2004; Álvarez et al., 2011). This data does not correspond to the results of our study since we could detect coliforms only in the millings. The common detection of *Mycobacterium* spp., again especially *Mycobacterium avium* subsp. *hominissuis*, in unused peat (Matlova et al., 2012; Agdestein et al., 2014) also contradicts the results of this study.

Neither important pathogens like *E. coli* and *Klebsiella* spp., nor zoonotic agents such as *Salmonella* spp. and MRSA were detected by our methods. However, the amounts of the mycotoxins DON as well as ZEA in the maize pellets and maize silage exceeded the EU guidance value (Commission Recommendation (EC) 2006/576). In the maize silage the ZEA value only exceeds the guidance value for piglets and gilts but not for sows and fattening pigs. DON can lead to the reduction of feed intake and growth in pigs (Dersjant-Li et al., 2003) while ZEA causes hyperestrogenism (Fink-Gremmels and Malekinejad, 2007). Even though enrichment material is ingested in lower amounts than feed, it should

be considered as a source for mycotoxin intake. If the feed used on a farm has already mycotoxin values close to the EU guidance value, maize products should not be used as additional enrichment material.

The results of this study, however, are based on the examination of a single batch of the different materials. Further studies are required to get thorough knowledge about the contamination of organic enrichment material. In conclusion, most tested materials are suitable as enrichment material for pigs, the maize products with restrictions concerning mycotoxins.

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Comfortable housing of dairy cows – basis for health, welfare and biosecurity

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SUMMARY. Cow comfort is a function of the cow's management of environment. The housing for dairy cattle must provide a comfortable, clean, well-drained and dry lying area together with a shelter to protect them from adverse weather, space to allow the animal to move, lie down and rise freely as well as access to adequate food and water. The study is focused on the analysis of the importance of individual components of housing environment (microclimate, lying places, corridors, feeding, watering, milking, lighting and ventilation) paying attention to the welfare, health and biosecurity of dairy cows based on the result of monitoring that took place in two farms for dairy cows with similar free-stall bedding cubicle housing technology. The parameters were evaluated by statistics software using the non-parametric tests. The impact of heat stress on a lactating cow, its comfort and productivity makes significant changes in the cattle behaviour. During the hot summer period (t > 25°C) in the relation to the spring period (t=8-12°C) we proved the feeding time got decreased by 20 % (range 15-25), rumination by 16 % (range 12–20) and locomotion time by 2 % (range 1–3); and the standing time got increased by 30 % (range 22–38) so did the drinking time by 3 % (range 2–5). Higher producing cows are more sensitive to heat stress than lower producing cows, especially from the point of resting and standing activity. Overstocking (25 %) reduces the lying time by 10 % (range 8–12) and simultaneously increases the frequency of aggressive interactions by 2 % (range 1–4), and increases standing time by 12 % (range 9-15). Adequate light intensity (200 lx) for 16 hours/day increase milk production by 8 % (range 5–16) and time of feed intake by 5 % (range 3–7) compared to cows exposed to 13 hours or less of light/day. Adequate level of a roof insulation and air exchange should prevent high humidity in winter and excessive heat in summer in barn. We proved the 10°C (range 6-12) of air temperature difference measured under the insulated and uninsulated roof. A clean, dry and comfortable resting place is associated with greater resting time, better health and improved productivity. A stable design is a very important factor the quality of cows' welfare, health and biosecurity.

Key words: Dairy cows, housing, comfort

INTRODUCTION

Cow comfort is a function of the cow's management of environment. Dairy cows that live in comfortable housing and environmental condition, have less stress, eat more, have fewer health problems and, of course, produce more milk. Housing conditions have a significant impact on the dairy cattle welfare. Cattle should be housed under conditions, which are in the harmony with the health, welfare a biosecurity (Rushen et al., 2008).

Animal productivity decreases in high stress factors of housing conditions. Animal spends a part of their energy to overcome stress. The effects of these factors should be taken in consideration when making a design of comfortable barns and animal houses (Uzal and Ugurlu, 2008). All technological systems should allow the housing animals express natural behaviour and be designed to avoid suffering from pain, fear, injury or distress (NFACC, 2009).

Environmental factors include temperature, humidity, air movement and solar radiation. Temperature is one of the primary factors affecting the physiological requirements and feed intake of a dairy cow. Thermal stress has decreased feed intake by 3–4 kg per day in mid-lactation dairy cows. Heat stress may slow the rate of increase in dry matter intake postpartum and exaggerate the pre-partum decline in dry matter intake of dairy cows (Grant and Albright, 1995). During the heat stress period the cows reduced feed intake, increased water intake, simultaneously changed metabolic rate and maintenance requirements (Armstrong, 1994). Any imbalance between a metabolic heat production inside the animal body and its dissipation to surroundings results of the heat stress under high air temperature condition during hot summer days (Das et al., 2016). The sensitivity of dairy cattle to the heat stress increases with an increase of milk production (Kumar, 2011). The housing for dairy cattle must provide a comfortable, clean, well-drained and dry resting area together with a shelter protecting from an adverse weather, space to allow the animal to move, lie down and rise freely as well as access to an adequate food and water.

MATERIAL AND METHODS

The study is focused on the analysis of the importance of individual components of housing environment (microclimate, lying places, corridors, feeding, watering, milking, lighting and ventilation) respecting the welfare, health and biosecurity of dairy cows based on the results of monitoring that took place in two dairy cow farms with similar free-stall bedding cubicle housing technology during hot summer (outside air temperature > 25°C) and spring (outside air temperature from 8 to12°C) period of the year. The basic data of the outside and inside environment (air temperature and relative humidity) were obtained from dataloggers, daily cows activities (feeding, rumination, standing, lying were recording by pedometers and vitalimeters installed on cows legs and necks. The milk production data were recovered from the milking parlour software. The ambulatory measuring of environment condition were measured by TESTO 435-2 (air temperature humidity and movement, lighting intensity) and the quality of a roof thermal insulation were measured by Thermocamera Flir C2. The parameters were evaluated by Statistica software using the non-parametric tests.

RESULTS

In Table 1 are summarised the basic analysis of selected activities of lactating dairy cows during hot summer and spring climatic condition.

Table 1. Selected daily activities of lactating dairy cow during hot summer and spring period.

	Hot summer period (t > 25°C)		Spring period (t=8-12°C)	
Daily activity	Mean (hours per day)	Range (hours per day)	Mean (hours per day)	Range (hours per day)
Feeding time	3.60	3.00 – 5.10	4.50	3.00 – 6.00
Rumination time	6.90	5.60 – 8.00	8.20	7.00 – 10.00
Locomotion time	2.55	2.43 – 3.40	2.60	2.50 – 3.50
Standing time	2.86	2.00 – 4.14	2.20	1.70 – 3.00
Drinking time	0.52	0.21 – 1.60	0.50	0.20 – 1.50

The impact of heat stress on lactating cow comfort and productivity makes changes in a behaviour of the cattle. During the hot summer period (t > 25°C) in the relation to the spring period (t=8-12°C) we

proved that a daily feeding time **decreases** by 20 % (range 15-25), rumination by 16 % (range 12–20) and daily locomotion time by 2 % (range 1–3); and the standing time **increases** by 30 % (range 22–38) and drinking time by 3 % (range 2–5). Higher producing cows are more sensitive to heat stress than lower producing cows, especially from the point of resting and standing activity.
We proved, that the overstocking of pens (25 %) reduces the cow's ability to practice natural behaviours, reduces the lying time by 10 % (range 8–12) and simultaneously increases the frequency of aggressive interactions by 2 % (range 1–4), and increases standing time by 12 % (range 9-15).
Light is a primary condition of life and as such is an important environmental factor for dairy cows. We found that appropriate lighting level (200 lx) for 16 hours of light per day increases a milk production by 8 % (range 5–16) and time of feed intake by 5 % (range 3–7) compared to cows exposed to 13 hours or less of light per day.
The ideal ventilation depends on the type of barn its size, the number of animals to be housed and the season. Natural ventilation is the most efficient and cheapest system of air exchange which is able to provide an optimum environment condition in stables for dairy cows, remove the excess heat (hot summer climate) and water vapour (cold winter climate) from stables. An adequate quality of a roof insulation and an air exchange should prevent high humidity in winter and excessive heat in summer in a barn. We proved 10°C (range 6-12) of air temperature difference measured between the insulated and uninsulated roof. During hot weather climatic condition is suitable to use the mechanical ventilation in front of the cubicles because otherwise a cow will not lie down. Also fans near the feed table help to decrease the heat stress and keep the cows eating.

DISCUSSION

Some factors of dairy cow breeding need continuous attention such as temperature and humidity control, stall hygiene, food and water supply; others, like free-stall design, facility design and feeding fence are continual factors in a stable so they do not need frequent monitoring (Van Eerdenburg, 2009).
Dairy cattle will spend between 4.2 and 6.5 hours per day eating, 7.7 to 9.6 hour per day ruminating and have 10–17 rumination periods per day (Albright, 1993).
Thermal comfort and good air quality is very important for the health and well-being of the dairy cow. In general, the dairy cow is far more tolerant to cold than to heat stress (Cook et al., 2007). Tapki and Sahin (2006) found that, increasing the air temperature from 25 to 40°C, decreased eating (46%), locomotion (19%) and rumination (22%) and increased standing by 34%. Higher producing cows (>32 kg/d) were more sensitive than lower producing cows, especially for lying and ruminating activities.
Blowey (1994) found out that dairy cows spend 45% of 24 h resting and with respect to different housing systems their resting behaviour changed between values 46-50%.
If stocking density within a pen increases, the frequency of aggressive interactions is higher, cows spend less time lying down and more time standing outside the free-stall, they consume feed up to 25 percent faster and take less time to lie down after milking (Fregonesi et al., 2007). Overstocking may also suppress rumination activity, lower a milk fat percentage and increase a somatic cell count in milk samples (Hill et al., 2009). Schefers et al. (2010). We recorded a reduction of conception rates in relation with the higher stocking densities. Hill (2006) proved that commingling primiparous and multiparous cows in one pen leads to loss of resting activity, rumination and milk production yield.
There are several levels of lighting required for a general inspection and welfare. Enhanced lighting levels may be required for higher stimulation of milk yield and for more visually demanding tasks of stockmen. In such cases, higher lighting levels may have to be provided in some areas for a specific time (EFSA, 2009; Rushen, J. et al. 2008). Dairy cows consumed food most frequently at the beginning and the end of the daylight period (Albright, 1993). Shaded feed bunks located in outside lots during

heat stress conditions increased feeding activity of periparturient cows by 63% versus unshaded feed bunk areas during daylight hours (Grant and Albright, 1995).

Optimum level of dairy cow barn ventilation is followed by decreasing of amount of microorganisms, dust and gases in the indoor air together with the uniform distribution of air. In winter time, when the air exchange is too low, insufficient ventilation leads to high humidity of the animal house air, moist stall conditions, wet skin, uncomfortable conditions such as draught and poor surface and air hygiene. Therefore a concentration of airborne micro-organisms including pathogens, dust and manure gases rise in a barn. Unsatisfactory ventilation increases the likelihood of mastitis and even the spread of respiratory disorders causing poor health and loss of production (Wathes, 1992).

Ensuring the comfortable living conditions for cows in the field of welfare, health and biosecurity, improves fertility, yield, longevity and disease resistance, as well as reduction of the production costs and increases a labour productivity and economical rentability of the farm.

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INFLUENCE OF IMIDACLOPRID ON BEES PREVIOUSLY FED SYRUP WITH ADDITION OF ACTIVE COMPOUNDS

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SUMMARY. The aim of the study was to examine the motor function of bees, previously fed sugar syrup with active compounds coming from *Kamchatka honeysuckle*, treated imidacloprid. The experiment was conducted in the laboratory in controlled environmental conditions. Bees were collected and transferred into 20 x 15 x 6 cm^3 cages. Bees were divided into following groups: Cs – control one, fed only 1.8M sucrose syrup; CsP_{10} - group fed first only syrup and then 10 nM imidacloprid for 24h; CsP_{100} - group fed first with sucrose syrup and then 100 nM imidacloprid for 24h; CsJ_1- group fed sucrose syrup with 0,1% extract of active compounds; CsJ_2- fed sucrose syrup with 0,5% extract of active compounds; CsJ_1P_{10}- group fed sucrose syrup with 0,1% active compounds and on the 6th day given 10nM imidacloprid for 24h; CsJ_1P_{100} - group fed sucrose syrup with 0,1% extract of active compounds and on the 6th day given 100nM imidacloprid for 24h; CsJ_2P_{10}- group fed syrup with 0,5% active compounds and on the 6th day given 10nM imidacloprid for 24h; CsJ_2P_{100}- group fed sucrose syrup with 0,5% active compounds and on the 6th day given 100nM imidacloprid for 24h. Active compounds from honeysuckle fruits were obtained from juice, which was first purified by removing sugars and organic acids and next eluent was concentrated. Behavioral assay was evaluated the day after the period of bees feeding. Behaviour was recorded using Noldus Observer software. No differences in mortality and food intake were observed between groups. Groups fed with addition of extract and imidacloprid to the syrup spent less time on flying in comparison to the group fed syrup with only 0,5% extract addition. The study results suggest that the addition of biological active compounds into bee diet could influence on the motor function of worker bees treated with pesticides.

Vector borne diseases and vector control

HEALTH STATUS OF POLISH RED DEER – PRELIMINARY REPORT

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INTRODUCTION. Red deer (*Cervus elaphus*) is a representative of the largest species of the deer family in Europe. The scientific analysis of the this species in central Europe is very rare and extremely challenging in a wildlife as these animals have a typical timid behaviour and live in remote forested habitats. Moreover, the health status and blood composition of Polish red deer population is not well known. Therefore, the aim of the study was to investigate the impact of natural environment on the health status of these ruminants in Karkonoski National Park (Lower Silesia, Poland).

MATERIAL AND METHODS. The experiment was designed to catch and release 20 red deer (individuals of both sexes) in their natural environment. The evaluation of health status and samples collection was made during a short-term (30 min.) of pharmacological immobilization (4 mg xylazine /kg b.w. and 4 mg ketamine /kg b.w.). The animal condition was examined and the blood analysis was made. The haematological and biochemical parameters were tested using ABC Vet (Horiba ABX) and Pentra - 400 (HORIBA ABX, Canada) respectively. The presence of skin parasites was controlled and the laboratory analysis of the collected feces (~ 20 grams) was also made. Additionally, the contamination of heavy metals in pelage profile was measured in the SpectrAA 220 FS (Varian Company) to determine the environmental pollution in Karkonoski National Park.

RESULTS AND DISCUSSION. The preliminary report suggest good general condition of red deer population but the parasite presence (nematodes, tapeworms and ticks) is an essential risk for the health status for these animals.

International experiences in Curriculum about Animal hygiene and animal welfare

AN EXCHANGE PROPOSAL: JEAN MONNET MODULE: HYGIENE AND ANIMAL WELFARE

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SUMMARY. Jean Monnet is supported program by the European Commission. Autonomous Metropolitan University (UAM) has been selected to develop this proposal from 2017-2019.
The Module will concentrate on the animal welfare indicator concept of the Welfare Quality Protocol applied in Europe to the production, transportation and slaughter of animals and elucidates the development towards this concept. Mexican undergraduate and graduate students and young professionals They shall learn to assess animal health, well-being and suffering of food producing animals such as pig, poultry and cattle.
The lecturers will be professors from Germany, the Netherlands, Estonia and Mexico. Participants in the Module will benefit from the accumulated knowledge about hygiene and animal welfare coming from Europe. Each year a European renowned researcher will impart lectures seminars and workshops on hygiene and animal welfare at the and will tutor young students. The course by a European professor will be enriched with the participation of Mexican professors and Mexican government officials.
This module is designed to build up capacities at UAM in animal health and welfare, human health and associated economic aspects from a European perspective. Its chief objectives are:

- To promote research and teaching on issues of animal health, welfare, hygiene and food safety from a European perspective.
- To provider undergraduate and graduate students and professionals a keen insight into European research and teaching on animal breeding.
- To become a reference center for trade in animals and animal products between the European Union and Mexico.

The Module will cover the following areas:

- Overview of European legislation on animal welfare and the corresponding practices
- The importance of teaching animal health and welfare from a European perspective
- Applications of animal hygiene standards to dairy animals,transport, stunning and slaughter,