SEASONAL CHANGES OF ZOONOTIC AGENTS PRESENCE IN DAIRY MANURE OF MODERN AND TRADITIONAL FARMS

Santorum, P.[1], García, R.[1] and Fernández, B.[2]

[1] Centro de Investigación y Formación Agrarias, Consejería de Ganadería, Agricultura y Pesca del Gobierno de Cantabria, C/ Héroes 2 Mayo, 27. 39600 Muriedas (Cantabria, Spain); [2] Servicio de Agricultura y Diversificación Rural, Consejería de Ganadería, Agricultura y Pesca del Gobierno de Cantabria, Edificio Europa, C/ Gutierrez Solana, s/n. 39001 Santander (Cantabria, Spain)

SUMMARY

Two clusters were investigated, one with upscaled waste facilities and another with traditional ones. The microorganisms looked for were zoonotic agents (protozoa, bacteria and viruses). Upscaled facilities produced more diluted and more saline manures than traditional ones. Results showed summer and spring as the seasons when the higher number of manures presented zoonotic agents, followed by autumn. The presence of each zoonotic agent depended on season and on farm type. Total aerobic bacteria and faecal coliform counts varied in upscaled farms and faecal streptococcus counts in traditional farms, depending on season.

Keywords: dairy, zoonotic agents, manure, wastes, spreading, indicators

OBJECTIVE OF THE WORK

Organic amendment of soil is the widest reuse practice in manure management. Current practices in most of dairy farms in Cantabria (Northern Spain) involve spreading of all the manure produced and stored as fertilizer, without composting or another treatment. In the present economical, geographical and structural conditions in this region, dairy farms' size is increasing, just as volume of manure does. Product price based on quality standards and related legislation forced farmers to install or upscale the milking machinery. Taking into account that this updating is not always accompanied by waste stores upscaling, very different types of farms can be found. No updating of waste facilities or machinery parallel to the rise in waste volume makes the frequency of waste spreading becomes higher. Consequently, risk in pathogens' dissemination could be increased and should be evaluated.

MATERIAL AND METHODS

We have grouped dairy farms by clustering in four types, according to farm size, manure store type and manure spreading system. The second cluster included middle size farms that have constructed covered stores and own the tanker. The third one groups small farms with uncovered tanks or piles to storage manure and that use manual spreading, pumping or solid spreaders (Santorum, 2007). The two extreme situations were investigated in relation to health risk: whole

adaptation of waste management facilities and machinery (second cluster) and traditional waste management (third cluster). The updated farms' size fluctuates between 100.000 and 500.000 kg of milk production per year, while the traditional farms produce less than 100.000 kg. Five facilities of each cluster were selected in order to evaluate the physicochemical composition and the number of pathogens present in manure sampled in spring, summer and autumn.

The physicochemical composition of the manures to be fertilized was ascertained regarding to pH, conductivity and dry matter. The first and the second parameters were measured by pH meter equipped with a salinity meter. Dry matter was calculated as the loss of weight until it is constant, while subsamples were maintained in drying oven at 75ºC for at least 48 hours. Flow injection analysis provided ammonia content in manure subsamples.

The detection of protozoan cysts was performed by a modification of Ziehl-Neelsen acid-fast method. The separation included centrifugation followed by flotation in saline solution (d=1.22) and the identification of the cysts was confirmed after staining *C. parvum* oocysts with Kingyou kit (MAIM) and with iodine lugol for *Giardia* cysts. The presence of typical cysts was assessed in 10 randomly selected fields at 400x and 1000x magnification for *C. parvum* oocysts and in the whole cover slip at 400x for *Giardia* cysts.

In the case of *Salmonella* spp., resuscitation and selective media cited in UNE-EN ISO 6579:2002 were choosen. *L. monocytogenes* detection was achieved following UNE-EN ISO 11290-1:1996/Amd. 1:2004. Nutrient broth was employed for recovering *Campylobacter* spp, to which lamb blood (Biomerieux) and Preston Campylobacter selective supplement were added. Loops of liquid cultures were streaked on the selective medium for *Campylobacter* spp. (recovering broth plus agar). Further confirmation of the typical colonies was achieved with API Campy (Biomerieux) and with oxidase tests and Gram staining. Two bacteria were isolated directly from manure subsamples on agar selective media: *Mycobacterium* spp. on MGIT agar, and *Brucella* spp. on Farrell's medium (Oxoid). All culture media, unless otherwise stated, were from Merck. Rotavirus and coronavirus were diagnosed using ELISA kit developed by Institut Pourquier (ref. P00603). When all the incidences in manures were grouped, the number of manures that presented at least one pathogen (positive manures) was estimated for each season and cluster.

Total aerobic bacteria as well as bacterial indicators that are linked to faecal contamination were enumerated (UFC/g). Manure subsamples were serially diluted in Ringer solution and volumes of 1 ml were mixed with melted agar. Total aerobic bacteria were counted on nutrient agar, faecal coliforms on VRB agar and faecal streptococcus counts were determined on KF Streptococcus agar (Oxoid) supplemented with TTC (Sigma).

Statistical analysis was performed with SPSS 12.0. Analysis of variance was used when data were distributed in a normal function (Shapiro-Wilk test, significance > 0,200) and homogeneity of variances assumption was accepted regarding to Levene's test (significance < 0,050). When differences were found, Tukey test was conducted to compare the three seasons. In the case that distribution was not normal and variances were heterogeneous, Welch's robust test of equality of means was applied (Reed and Stark, 1988). The number of positive manures or farms was compared by non parametric tests (chi-squared tests).

EXPERIMENTAL DATA AND RESULTS

The results related to physicochemical composition are expressed as: mean ± standard error. The upscaled facilities produced more diluted organic wastes (percentage of dry matter: 11.75 ± 1.05) than traditional ones (14.48 ± 1.46), with significance < 0.050. More saline manures were founded in upscaled farms (4.280 ± 1.489 *vs* 1.184 ± 0,148) with significance < 0.100.

Seasonal changes on composition of manures were also observed, as can be seen in Table 1. The pH and conductivity were different in autumn compared with summer or spring values, in the second cluster studied. On the other hand, in the third cluster, pH, conductivity and ammonia concentration showed differences in summer, when compared with spring or autumn.

Table 1. Physicochemical composition of manures, by season and cluster. Mean ± standard error. ^{a,b}: Values with different superscript letters are significantly different. ***: significance < 0.050 and *: < 0.200

PARAMETER	SEASON	CLUSTER 2	CLUSTER 3
pH	Spring	8.06 ± 0.27^{a}	7.95 ± 0.24^{a}
	Summer	7.88 ± 0.16^{a}	$8.68 \pm 0.08^{b\ ***}$
	Autumn	$8.22 \pm 0.13^{a,b\ *}$	8.23 ± 0.13^{a}
Conductivity	Spring	5.596 ± 2.767^{a}	1.212 ± 0.105^{a}
	Summer	6.247 ± 3.394^{a}	$0.651 \pm 0.067^{b\ ***}$
$N\text{-}NH_3$ (%)	Autumn	$0.995 \pm 0.100^{b\ *}$	1.285 ± 0.261^{a}
	Spring	0.14 ± 0.03^{a}	0.14 ± 0.02^{a}
	Summer	0.14 ± 0.02^{a}	$0.09 \pm 0.03^{b\ *}$
	Autumn	0.13 ± 0.01^{a}	$0.13 \pm 0.03^{a,b}$

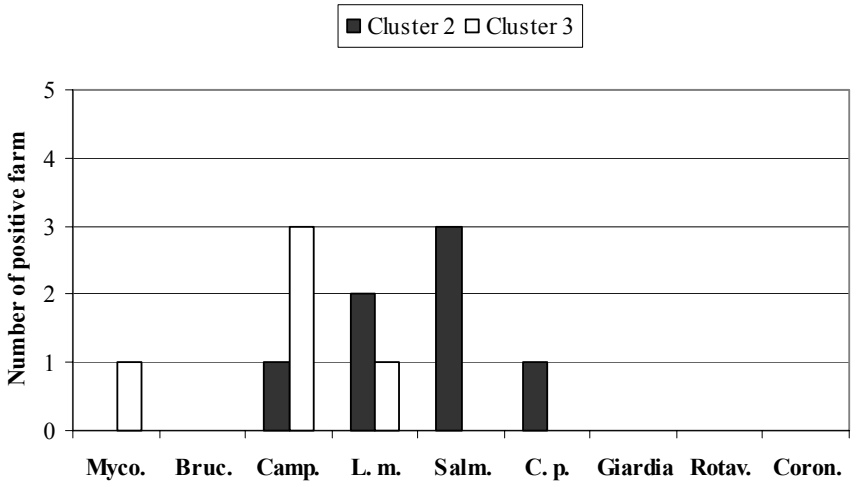
Regarding to bacterial indicators which are linked to faecal contamination, corresponding data are indicated in Table 2. When upscaled farms' manures were tested, the means of total aerobic bacteria and faecal coliforms' counts were significantly different in autumn *vs* summer values. On the other hand, traditional farms' manures presented seasonal differences in the mean of faecal streptococcus counts when comparing summer with spring or autumn.

When positive manures were estimated for each season, results revealed spring (5 positive manures out of 10) and summer (5 out of 10) as the seasons in which the higher number of manures presented zoonotic agents, followed by autumn (3 out of 10). Although in autumn, second cluster included two positive farms compared with only one positive farm in third cluster, neither the number of pathogens nor the number of positive manures or farms gave enough frequency in each cell of Chi tests to ascertain the influence of season alone or season combined with cluster. Nevertheless, statistical analysis showed that the upscaled farms had similar incidence of positive manures (7 positive manures out of 15) than the traditional ones (6 out of 15), when the data of all the seasons were grouped. The corresponding Chi test obtained a significance of 0,713, giving that none of the cells had less than 5 observations and that the minimum expected frequency was 6,50.

Table 2. Logarithm of bacterial counts, by season and cluster. Mean ± standard error. [a,b]: Values with different superscript letters are significantly different. [***]: significance < 0.050, [**]: < 0.100 and [*]: < 0.200

PARAMETER	SEASON	CLUSTER 2	CLUSTER 3
Total aerobic bacteria	Spring	7.48 ± 0.25[a]	7.56 ± 0.30[a]
	Summer	7.39 ± 0.18[a]	7.59 ± 0.19[a]
	Autumn	7.93 ± 0.13[a, b ***]	7.58 ± 0.17[a]
Faecal coliforms	Spring	4.60 ± 0.52[a]	5.68 ± 0.26[a]
	Summer	4.36 ± 0.49[a]	4.46 ± 0.98[a]
	Autumn	5.32 ± 0.32[a, b *]	5.74 ± 0.07[a]
Faecal streptococci	Spring	5.46 ± 0.56[a]	6.05 ± 0.25[a]
	Summer	4.62 ± 0.87[a]	4.18 ± 0.76[b] **
	Autumn	5.54 ± 0.37[a]	6.15 ± 0.20[a]

Graph 1 represents the frequency for each of the zoonotic agents investigated in the two clusters. It is necessary to remark that the zoonotic agent present in each season and farm type was different. So, upscaled farms had higher frequency of these pathogens: *L. monocytogenes*, *Salmonella* spp. and *C. parvum* while the zoonotic agents that prevailed in traditional farms were *Campylobacter* spp. and mycobacteria. Moreover, the presence of *L. monocytogenes* in upscaled farms' manures was not detected in summer, while it did not appear in traditional manures in autumn. *Campylobacter* spp. was present in the summer manures of second cluster, but in summer and autumn manures of third cluster. Manures positive for *Salmonella* spp. were sampled in spring, summer and autumn.



Graphic 1. Number of positive farms for each of the zoonotic agents, out of five farms by cluster

DISCUSSION

Regarding to manures' composition in the two clusters studied, the traditional farms tended to produce manure with higher solid content and lower saline content. So, the quality of traditional manures for fertilizing is better than the one of second cluster manures and the spreading of their manures could be presented as more environmentally friendly. Experimental data showed autumn as the season in which upscaled farms produced less saline manure and with higher pH, while traditional manures in summer were less saline, had lower ammonia content and higher pH. So, the use of these wastes as fertilizer could be encouraged in autumn for the second cluster farms and considered in early summer for the third cluster farms.

Moreover, second cluster manures presented in autumn higher number of total aerobic bacteria and of faecal coliforms, while faecal streptococcus counts remained constant. So, it would be more useful to choose total aerobic bacteria or faecal coliforms as bacterial indicators of faecal contamination. In third cluster manures, on the contrary, the outstanding indicators are faecal streptococci, which counts are higher in summer.

The pathogens selected in this work affect not only to dairy cows but to humans, and are listed as zoonotic agents to be monitored (Directive 2003/99/CE). The pathogens isolated in upscaled farms could be related to in-house stocking density, as Hutchison *et al.* (2005) found lower levels of *Cryptosporidium* oocysts in pig wastes when stocked at densities lower than 0.4 animal m^{-2}. Pathogens identified in both clusters are hygienic conditions dependant, for instance Nightingale *et al.* (2005) indicated that maintaining adequate animal hygiene prevented against listeriosis in cattle farms. The presence of *Salmonella* spp. in the upscaled farms' manures is also underlined by the fact that it was the only pathogen detected in the three seasons studied. In this way, Venglovský *et al.* (1998) related literless technology and liquid manures to *salmonellae* ideal conditions for survival.

CONCLUSIONS, SCIENTIFIC AND/OR PRACTICAL IMPLICATIONS OF THE WORK

These evidences show that changes in dairy facilities and manure management must be carefully tested so that health risk is not compromised. The higher numbers of cows, the dilution of manure or the reduced time of storage associated with upscaled farms could be related to the isolated pathogens. Anyhow, traditional management influences the type of pathogens being present, as well as it produces a fertilizer of higher quality which is more environmentally friendly, including smaller energy consumes. The type of pathogens isolated in upscaled farms could be the ones related to big herds and liquid slurry and the ones identified in both clusters could be hygienic conditions dependant. The high incidence of *Salmonella* spp. in second cluster and of *Campylobacter* spp. in third cluster should be further investigated, so that proper manure management or reducing of risk factors is achieved and the dissemination of these relevant pathogens is avoided. The detection of *Salmonella* spp. from spring to autumn in upscaled farms should be corrected and no incidence of *L. monocytogenes* in autumn should be researched in traditional farms.

ACKNOWLEDGEMENTS

We thank INIA for supporting the project number RTA04-093. Manure analysis was coordinated by A. Alonso, and mycobacteria identification by G. Aduriz, both in Neiker. Pathogen identifications were leaded by G. Gradillas in S. Laboratorio y Control (Gob. Cantabria). We are greatfull for assistance of I. Tejero and R. Collado, from the U. Cantabria and P. Cavero, from S. Sanidad Animal (Gob. Cantabria).

REFERENCES

Directive 2003/99/EU of 17 November 2003. DOCE L325, of 12 December 2003.

Hutchison, M.L., L.D. Walters, S.M. Avery, F. Munro and A. Moore. Appl. Environ. Microbiol. (2005) 71 (3): 1231–1236.

Nightingale, K. E.D. Fortes, A.J. Ho, Y.H. Schukken, Y.T. Grohn and M. Wiedmann. JAVMA (2005) 227 (11): 1808–1814.

Reed, J.F. 3rd and D.B. Stark. Comput Methods Programs Biomed. (1988) May-Jun; 26(3): 233–7.

Santorum, P, R. García and B. Fernández. 20th Anniversary Sardinia Symposium, Eleventh International Waste Management and Landfill Symposium (2007). Cagliari, Italy.

UNE-EN ISO 11290–1: 1996 and Amendment 1: 2004. Horizontal method for the detection and enumeration of *Listeria monocytogenes*.

UNE-EN ISO 6579: 2002. Microbiology of food and animal feedings stuffs. Horizontal method for the detection of *Salmonella* spp.

Venglovský, J., I. Plachá and N. Sasáková. 8th Int. Conference on management strategies for organic wastes use in agriculture. 1998, Cemagref, France.