MICROBIOLOGICAL AEROSOLS IN POULTRY HOUSES AND ITS AMBIENCE

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ABSTRACT

The concentration of airborne bacteria, airborne Escherichia coli and airborne enterococcus in indoor air and downwind air at the distance of 10m, 50m, 100m, 200m, 400m and 600m of the farm were detected in order to find the influence to its ambience, using the Andersen-6 stages sampler and RCS sampler in five large chicken farms. The results showed that the culturable airborne bacteria concentration in indoor air of chicken house is 3.80×10^5 2.57×10^6 CFU/m³ air. which is higher than that in downwind air at the distance of 10m, 50m, 100m, 200m, 400m and 600m of the farm. The concentration of airborne *Escherichia coli* is $0.2.36 \times 10^2$ CFU/m³air in indoor air of the farm. In the same way, we have detected the concentration of culturable airborne enterococcus in indoor air is far higher than that in the downwind air outside of the farm. We have not detected airborne *Escherichia coli* and airborne *Enterococcus* in the distance of 400m even faraway outside of the downwind air. The airborne microbe concentration of all groups between the indoor air and in the air of the neighbourhood 10m, 50m, 100m, 200m, 400m and 600m from the house exhibited statistical significance or highly statistical significance (P<0.05 or P<0.01). No statistical significance between 10m, 50m, 100m 200m, 400m or 600m was observed. The results indicated that the microbiological aerosols in the chicken houses were relatively higher and were transmitted through the atmosphere to stable surroundings and over quite considerable distance (>100 m), especially the downwind air of the farm.

Keywords: microbiological aerosols, poultry houses, airborne *Escherichia coli*, airborne *Enterococcus*, spread of aerosol

1. INTRODUCTION

With the rapid development of stockbreeding, it has provided more livestock product for human being, and made huge contribution to resolving the problem of food and nourishment of population. And the modern agricultural methods have changed the way animals are raised (Donham, K. J., et al., 1977, 1982; Olson, D. K., et al., 1996; Steven M. Wolinsky, 2006). To increase production with minimum labor, chickens have been fed in confinement buildings, which are mainly enclosed structures densely stocked with chickens. A mechanical ventilation system and a system for handling animal wastes are usually set up to maintain the health status of chickens indoors.

However, the intensive livestock farming pollutes the environment of livestock farming itself, and affects the level of livestock farming. In the meantime, it pollutes the environment around,

and affects the living environment and living quality, restricts the positive and continuance development of stockbreeding. Microorganisms and their components or products, resulting from chickens dander, fecal matter, and feed materials, are easily accumulated and aerosolized in such densely populated and enclosed buildings (C. W. CHANG, et al., 2001). Due to exposure, chicken workers may experience upper respiratory irritation, chronic bronchitis, organic dust toxic syndrome, or other respiratory symptoms (Hagmar-L, et al. 1990; Wiegand-B, et al. 1993; Zucker-BA, Muller-W, 2000; Dennis Normile, 2004; Steven M. Wolinsky, 2006).

The kind and concentration of the microbiological aerosols is the indicative of the sanitations in animal house (Dutkiewicz-J, et al., 1994; Zucker-BA, S Trojan, et al., 2000; Zucker-BA, Muller-W, 2000; Kaliste-E, et al., 2002). The aim of this study was to detect the airborne bacteria, including the concentration of airborne aerobic bacteria and airborne *Escherichia coli*. and airborne *enterococcus* with Andersen-6 stages sampler and RCS in five chicken farms and their surroundings. We have detected the concentration of airborne bacteria, airborne *Escherichia coli*. and airborne *enterococcus* in the indoor air and the downwind air at the distance of 10m, 50m, 100m, 200m, 400m and 600m of the farm in order to find the influence to surroundings of the indoor airborne microorganisms.

2. MATERIALS AND METHODS

2.1 Animal houses studied

Air samples were collected during normal work periods in all poultry houses. Animal disturbance during sampling was strictly avoided. Five poultry houses were studied in this experiment. A description of these animal houses is given in table 1.

	Ν	Layout		Inside		Outside			
			T(℃)	RH(%)	WS(m/s)	T(°C)	RH(%)	WS(m/s)	
1	6000	Floor unit	26	40	0	21	50	1.0-3.0	
2	2200	Cage unit	26	34	0	29	50	1.0-3.1	
3	3000	Cage unit	31	44	0	35	36	1.5-3.0	
4	3500	Cage unit	31	60	0	32	75	0-1.5	
5	4500	Cage unit	30	70	0	31	65	0-2.0	

Table 1. Description of five poultry houses studied

Note: N=Number of poultry; T=Temperature; RH=Relative Humidity; WS=Wind Speed;

2.2 Airborne aerobic bacteria, Escherichia coli and Enterococcus

Six-stage Andersen samplers (Andersen, 1958) and RCS (Reuter Centrifugal Sampler, Biotest, Frankfurt) were used to collect airborne *E. coli* in animal houses and its surroundings (upwind 10m, 50m and downwind 10m, 50m, 100m, 200m, 400m). The samplers were located near the middle of the stable about 1.0m above the ground. The rate of airflow of Anderson sampler and RCS is 28.3L min⁻¹ and 40L min⁻¹ respectively. The samplers were equipped with MacConkey Agar No.3 (OXOID. LTD., BasingStoke, Hampshire, England) and 5% sheep blood agar and operated for 1 to 10 min according to the sanitation condition. The exposed agar plates were incubated at 37° C for 48 h. Bacteria were identified by Gram staining and then by using the API

system (Bio Merieux, Marcy-I'Etoile, France). Then the number of grown colonies were counted and the positive hole correction (Andersen, 1958) was applied.

Furthermore the concentration of airborne aerobic bacteria was determined outside of the poultry houses as described above. The samples were taken windward at a distance of 10m, 50m and leeward at a distance of 10m, 50m, 100m, 200m, 400m from the animal houses.

3. RESULTS

Table 2 shows that the total number of aerobic bacteria in the poultry houses was in the range of 3.80×10^5 2.57×10^6 CFU/m³air; *E. coli* was in the range of $0 \sim 236$ CFU/m³air; *Enterococcus* was 0 1131 CFU/m³ air.

The total number of aerobic bacteria of upwind 10m and 50m from the poultry houses floated in the range of 480 to 7080CFU/m³air; *E. coli* floated in range of 0 to 27CFU/m³air; *Enterococcus* was 0 80CFU/m³air.

The total number of aerobic bacteria of downwind 10m to 400m from the poultry houses was in the range of 684 to $1.17 \times 10^6 \text{CFU/m}^3 \text{air}$; *E. coli* was 0 80CFU/m³air; *Enterococcus* was 0 240CFU/m³air.

The concentration and difference of airborne bacteria between the hen house and different distance in its neighborhood of 10, 50 and 100m were significant or highly significant (p<0.05 or p<0.01), while those in the neighborhood between 10, 50 and 100m showed no statistical significance (p>0.05).

Poultry house		Airborne aerobic bacteria			Airborn	e Escheri	chia coli	Airborne Enterococcus		
		Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median
1	Upwind 50m	2534	510	1786	7	0	1	0	0	0
	Upwind 10m	3640	480	2184	11	0	2	0	0	0
	Indoor	216466	37951	89952	134	0	37	1131	0	230
	Downwind 10m	21760	2800	13952	49	0	12	71	0	14
	Downwind 50m	7560	1867	4297	24	0	6	0	0	0
	Downwind 100m	4400	1216	2263	11	0	3	0	0	0
	Downwind 200m	4302	894	1865	3	0	1	0	0	0
	Downwind 400m	3876	684	1708	0	0	0	0	0	0
2	Upwind 50m	1236	482	843	0	0	0	0	0	0
	Upwind 10m	1344	520	1030	0	0	0	0	0	0
	Indoor	82580	48975	80018	59	0	13	142	0	26
	Downwind 10m	29920	10320	17733	35	0	8	20	0	4
	Downwind 50m	6560	4880	5587	12	0	2	10	0	3
	Downwind 100m	6760	3160	4730	0	0	0	10	0	3
	Downwind 200m	2453	1220	1994	0	0	0	10	0	2
	Downwind 400m	1480	720	1118	0	0	0	0	0	0

Table 2. Concentrations of airborne aerobic bacteria, *Escherichia coli and Enterococcus* in indoor air and outdoor air of the 5 poultry houses (CFU/m³ air). (n=5)

Poultry house		Airborne aerobic bacteria			Airborn	e Escherie	chia coli	Airborne Enterococcus		
		Max.	Min.	Median	Max.	Min.	Median	Max.	Min.	Median
3	Upwind 50m	1896	583	1023	0	0	0	0	0	0
	Upwind 10m	2451	826	1894	0	0	0	0	0	0
	Indoor	182862	110530	143173	71	0	14	671	0	143
	Downwind 10m	28646	7654	22760	12	0	3	35	0	7
	Downwind 50m	7560	3864	5684	0	0	0	0	0	0
	Downwind 100m	6760	3160	3750	0	0	0	80	0	16
	Downwind 200m	2556	1234	2994	0	0	0	0	0	0
	Downwind 400m	2480	976	2019	0	0	0	0	0	0
4	Upwind 50m	5760	890	3248	10	0	2	0	0	0
	Upwind 10m	7080	3100	5027	27	0	7	80	0	16
	Indoor	257279	84488	148919	236	0	63	424	0	87
	Downwind 10m	29040	23440	26688	80	0	24	80	0	16
	Downwind 50m	8400	3920	5888	40	0	16	10	0	3
	Downwind 100m	11560	6880	9632	10	0	2	0	0	0
	Downwind 200m	10246	5810	8764	10	0	2	0	0	0
	Downwind 400m	4123	2947	3278	0	0	0	0	0	0
5	Upwind 50m	2480	1520	2080	0	0	0	0	0	0
	Upwind 10m	2982	1630	2458	0	0	0	0	0	0
	Indoor	192721	158021	170389	35	0	9	495	0	108
	Downwind 10m	24480	5680	14293	18	0	5	240	0	48
	Downwind 50m	7840	2840	5800	0	0	0	0	0	0
	Downwind 100m	116960	91120	100747	0	0	0	144	0	31
	Downwind 200m	43520	25387	32338	0	0	0	10	0	2
	Downwind 400m	5120	3547	4276	0	0	0	0	0	0

Table 2. Continuation

4. DISCUSSION

It is well known that there are many types and strains of E. coli, a few of which are potentially pathogenic. Various strains may cause illness by a variety of infective and toxin-producing mechanisms. In poultry, E. coli can cause many diseases such as septicemia, swollen head syndrome, omphalitis, cellulitis, yolk-sack infection and respiratory tract infections (Sojka and Carnaghan, 1961; Morley and Thomson, 1984; Randall et al., 1984; Dho-Moulin and Fairbrother, 1999). The resulting morbidity and mortality have led to serious economic losses to the poultry industry (Gross, 1994). And it can cause many diseases to human beings, such as hemolyticuremic syndrome (HUS), haemorrhagic colitis (HC), neonatal meningitis and bloody diarrhea (Marjut Eklund, et al., 2001; Norval J.C. et al., 2005; S.K. Manna, et al., 2006; Marilda C. Vidotto, et al., 2007). In addition, Enterococcus is a kind of common bacteria in the air of animal house environment too(Cormier Y, et al., 1990; Crook B, et al., 1991; Predicala BZ, et al., 2002). And Enterococcus, particularly some of the species including E. faecalis and E. faecium, is indigenous flora in the human bowel and has emerged as one of the leading causes of nosocomial bacteremias, urinary tract infections, central nervous system destroy, and wound infections (Uttley et al., 1998; Satoshi Takahashi, et al., 1999; Gambarotto et al., 2000; Soltani et al., 2000; NNIS 2001; É.J. Kaszanyitzky, et al., 2007).

In this study, the concentration of aerobes, *E. coli* and *Enterococcus* in indoor air of poultry houses was much higher than it's ambient. In the open-air there was only a small amount of *E. coli* and *Enterococcus* normally (Yu, xihua and Fx. Che, 1997). Therefore, microorganism in the air of the neighborhood of the hen house came from the indoor air of the poultry house. It can be concluded that the concentration of the bacteria aerosols in the hen house was slightly higher than normal. So, such a high concentration of bacteria particles in the indoor air means that the animals must have had some diseases, were in recessive infection or were germ carriers. Animals breeding in high density will lead to building a quicker channel exchanging of pathogen bacteria within animals, and the pathogenicity of the bacteria will be raised (Melhorn and Chai, 2000). This suggested that the microbiological aerosols in the chicken house could be transmitted through the atmosphere to stable surroundings over quite considerable distance and could cause environment pollution as well as spread of epidemics. And the presence of high concentrations of airborne culturable bacteria and potentially allergic might pose health risks for workers (C. W. CHANG, et al., 2001). So, poultry house should be set up outside 400 meters away from resident at least. These results can provide important reference for the officers and farm keeper.

Although there is no statistical data which can prove the fact that there is some inherent correlation between the concentration and the incidence of the disease, from many studies we infer that the high concentration of the airborne microorganism can burden the immune system of the animals, make them grow slowly and reduce their economic value. Chai (1998) concluded that the concentration in the cowshed with straw should not exceed 10^4 CFU/m³ and in the hen house should not exceed 10⁴ CFU/m³, though there is no unitive criterion about the concentration of the airborne particles in the hen environments. In the present study, the concentration of the aerobes indoor air. upwind air and downwind air of poultry houses in were 3.80×10⁵ 2.57×10⁶CFU/m³air, 480 to 7080CFU/m³air and 684 to 1.17×10⁶CFU/m³air respectively, which are very higher than that in the normal plain (Yu and Che, 1997). So the hygienic condition of the poultry house should be improved.

ACKNOWLEDGEMENTS

This study was financially supported by NSFC grant (30571381) "The spreading mode of microorganism aerosol in animal farms"

REFERENCES

- Andersen, A. (1958): New sampler for the collection, sizing, and enumeration of viable airborne particles [J]. J. Bacterial., 76, 471–484.
- C. W. CHANG, H. CHUNG, C. F. HUANG, H. J. J. SU, Exposure of Workers to Airborne Microorganisms in Open-Air Swine Houses. Applied and Environmental Microbiology, Jan. 2001, p. 155–161.
- Cormier Y, Tremblay G, Meriaux A, Brochu G, Lavoie J. 1990. Airborne microbial contents in two types of swine confinement buildings in Quebec. Am Ind Hyg Assoc J 51: 304–309.
- Crook B, Robertson JF, Glass SA, Botheroyd EM, Lacey J, Topping MD. 1991. Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. Am Ind Hyg Assoc J 52: 271–279.
- Dennis Normile. INFLUENZA: GIRDING FOR DISASTER: Asia Struggles to Keep Humans and Chickens Apart. *Science* 15 October 2004: Vol. 306. no. 5695, p. 399.

- Dho-Moulin, M., Fairbrother, J.M., 1999. Avian pathogenic Escherichia coli (APEC). Vet. Res. 30, 299–316.
- Donham, K. J., and K. E. Gustafson. 1982. Human occupational hazards from swine confinement. Ann. Am. Conf. Gov. Ind. Hyg. 2: 137–142.
- Donham, K. J., M. Rubino, T. D. Thedell, and J. Kammermeyer. 1977. Potential health hazards to agricultural workers in swine confinement buildings. J. Occup. Med. 19: 383–387.
- Dutkiewicz-J, Pomorski-ZJH, Sitkowska-J, et al. Airborne microorganisms and endotoxin in animal houses[J]. Grana. 1994, 33: 2, 85–90.
- É.J. Kaszanyitzky, M. Tenk, et al. Antimicrobial susceptibility of enterococci strains isolated from slaughter animals on the data of Hungarian resistance monitoring system from 2001 to 2004. International Journal of Food Microbiology 115 (2007) 119–123.
- Gambarotto, K., et al., 2000. Prevalence of vanacomycin-resistant enterococci in fecal samples from hospitalized patients and nonhospitalized controls in a cattle-rearing area of France. Journal of Clinical Microbiology, 38, 620–624.
- Gross, W.B., 1994. Diseases due to Esherichia coli in poultry. In: Gyles, C.L. (Ed.), Escherichia coli in Domestic Animals and Man. CAB International, Wallingford, UK. pp. 237–259.
- Hagmar-L, et al. Health effects of exposure to endotoxins and organic dust in poultry slaughter-house workers[J]. International Archives of Occupational Environmental Health. 1990, 62(2): 159–164.
- Kaliste-E, Linnainmaa-M, et al. Airborne contaminants in conventional laboratory rabbit rooms [J]. Laboratory-Animals. 2002, 36: 1, 43–50.
- Marilda C. Vidotto, Mirela B. Queiroz, et al. Prevalence of ibeA gene in avian pathogenic Escherichia coli (APEC). Veterinary Microbiology 119 (2007) 88–89.
- Marjut Eklund, Flemming Scheutz and Anja Siitonen, Clinical Isolates of Non-O157 Shiga Toxin-Producing Escherichia coli: Serotypes, Virulence Characteristics, and Molecular Profiles of Strains of the Same Serotype. Journal of Clinical Microbiology, Aug. 2001, p. 2829–2834.
- Mehlhorn. G.und Chai, T. G. In: Mehihorn G. (Hrsg.): Lehrbuch der Tierhygiene[M].VEB Gustav Fischer Verlag Jena, S. 63–67; 117–200; Aufl. (2000) S. 5–15; 17.
- Morley, A.J., Thomson, D.K., 1984. Swollen head syndrome in broiler chickens. Avian Dis. 28, 238-243.
- NNIS. 2001. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992–June 2001. Am J Infect Control 29: 404–421.
- Norval J.C. Strachana, T, Michael P. Doyleb, et al. Dose response modelling of Escherichia coli O157 incorporating data from foodborne and environmental outbreaks. International Journal of Food Microbiology 103 (2005) 35–47.
- Olson, D. K., and S. M. Bark. 1996. Health hazards affecting the animal confinement farm worker. AAOHN J. 44: 198–204.
- Predicala BZ, Urban JE, Maghirang RG, Jerez SB, Goodband RD. 2002. Assessment of bioaerosols in swine barns by filtration and impaction. Curr Microbiol 44: 136–140.
- Randall, C.J., Meakins, P.A., Harris, M.P., Watt, D.J., 1984. A new skin disease in broilers? Vet. Rec. 114, 246.
- S.K. Manna, M.P. Brahmane et al. Occurrence, virulence characteristics and antimicrobial resistance of Escherichia coli O157 in slaughtered cattle and diarrhoeic calves in West Bengal, India. Applied Microbiology. 43 (2006) 405–409.
- Satoshi Takahashi, Takaoki Hirose, et al. Analysis of cross infection using genomic fingerprinting in nosocomial urinaty tract infection caused by Enterococcus faecalis. Japan. J Infect Chemother (1999)5: 46–48.
- Sojka, W.J., Carnaghan, R.B.A., 1961. Escherichia coli infection in poultry. Res. Vet. Sci. 2, 340–352.
- Soltani et al., 2000. Mechanisms of resistance to quinupristin-dalfopristin among isolates of Enterococcus feacium from animals, raw meat, and hospital patients in Western Europe. Antimicrobial Agent and Chemotherapy, 44, 433–436.
- Steven M. Wolinsky. PUBLIC HEALTH: Chicken Monster or Chicken Little? Science 10 February 2006: Vol. 311. no. 5762, pp. 780–781.
- Uttley et al., 1998. Vancomycin resistant enterococci. Lancet, 57-58.

- Wiegand-B, Hartung-J, et al. Air quality in Louisiana type broiler houses. Part 2: Bacteria and endotoxin levels in airborne dust [J]. Landbauforschung Volkenrode. 1993, 43: 4, 236–241.
- Yu, xihua and Fx. Che (1997): Modern air microbiology and sampling detection and identification techniques. Militeric Medicinhic Publising House, p.19–22; 272–282
- Zucker-BA, Muller-W. Airborne microorganisms in animals stables. 3. Relationship between inhaled endotoxins, dust and airborne bacteria in a poultry housing unit[J]. Berliner und Munchener Tierarztliche Wochenschrift. 2000, 113: 7–8, 279–283.
- Zucker-BA, S Trojan, et al. Airborne gram-negative bacterial flora in animal houses[J]. Journal of Veterinary Medicine Series B – Infectious Diseases and Veterinary Public Health, 2000, Vol 47, Iss 1, pp 37–46.