Science of the Total Environment 408 (2010) 1446–1450

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

ERIC-PCR identification of the spread of airborne *Escherichia coli* in pig houses

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ARTICLE INFO

Article history:
Received 18 September 2009
Received in revised form 27 November 2009
Accepted 10 December 2009
Available online 13 January 2010

Keywords:
Microbial aerosols
Airborne *Escherichia coli*
Pig houses
ERIC-PCR
Similarity

ABSTRACT

To understand the spread of microbial aerosols in pig houses, with *Escherichia coli* (*E. coli*) as indicator, the airborne *E. coli* in 4 pig houses and their surroundings at different points 10, 50 m upwind and 10, 50, 100, 200 and 400 m downwind respectively from the pig houses were collected, and the concentrations were calculated at each sampling point. Furthermore, the feces of pigs were collected to separate *E. coli*. The ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction) technology was used to amplify the isolated *E. coli* DNA samples, then the amplified results were analyzed by NTSYS-pc (Version 2.10) to identify the similarity of isolated *E. coli*. The results showed that the airborne *E. coli* concentrations in indoor air of the 4 pig houses (21–35 CFU m^{-3} air) were much higher than those in upwind and downwind air ($P<0.05$), but there were no significant differences ($P>0.05$) at downwind distances. The ERIC-PCR results also showed that 52.4% of the fecal *E. coli* (four houses being respectively 2/4, 50%; 2/4, 50%; 3/6, 50%; 4/7, 57.1%) were identical to the indoor airborne *E. coli* isolates, and there was more than 90% similarity between the majority of *E. coli* (50%, 21/42) isolated from downwind air at 10, 50, 100 and 200 m and those from indoor air or feces. It could be concluded that the aerosols in pig houses can spread to the surroundings, and thus effective measures should be taken to control and minimize the spread of microbial aerosols.

1. Introduction

The aerosols in animal houses may cause environmental pollution, affect the health and productivity of animals (Gross, 1994) and even trigger the prevalence of airborne epidemics. In many cases, the prevalence of epidemics comes closely along with environmental problems and the air pollution of breeding farms. Many airborne pathogenic microorganisms including bacteria and viruses can spread a long way via air, leading to the prevalence of epidemics (Mims and Mims, 2004; Lee et al., 2007). In 1981, for example, *Foot and Mouth Disease Virus* (FMDV) spread quickly in air at a striking speed to the southern part of England from Brittany, France (Donaldson et al., 1982). In 2001, a large number of people in US died from airborne *Bacillus anthracis* (Centers for Disease Control and Prevention, 2003). And pneumonia-associate *Klebsiella* can travel in air (Prazmo et al., 2003). Numerous cases showed that such pathogenic microbes could spread in form of aerosols.

Airborne microbial source tracking has been studied mostly by concentration differences of the total number of aerobic bacteria (Seedorf et al., 1998; Zucker et al., 2000; Chai et al., 2001; Kim and Kim, 2007), and the differences of antibiotic resistances of bacteria (Chai et al., 2003; Yao et al., 2007). The methods mentioned above are difficult to trace the origin of airborne microorganisms. However, The ERIC sequences are highly conserved at the nucleotide sequence level but their chromosomal locations and numbers differ among species, which can serve as a valuable and sensitive tool for genetic differentiation of *E. coli* isolated from different sites (Hulton et al., 1991; Jurkovič et al., 2007). Molecular genotyping by ERIC-PCR is faster and more cost-effective than pulsed-field gel electrophoresis (PFGE) or multilocus sequencing for generating information about the genetic similarity of bacterial strains. For many organisms, it possesses a higher discriminatory ability than that of other quick-typing techniques, leading to its increased frequency of use (Weijtens et al., 1999; De la Puente-Redondo et al., 2000; Saxena et al., 2002; Ben-Hamouda et al., 2003).

Therefore, in this experiment four pig houses were chosen randomly and the *E. coli* concentrations collected from different sampling sites were calculated. Based upon bacteriology identification methods, ERIC-PCR technology was adopted to identify the homology of *E. coli* isolated from different sampling sites and the fingerprint chromatogram of the ERIC fragments of *E. coli* were established (Versalovic et al., 1991). By analyzing the relationship between the amount and distribution of the fragments in bacterial genome and comparing their genetic similarity, the genetic distance and the source of bacteria could be found out, and consequently the spreading of *E. coli* aerosols was identified.

2. Materials and methods

2.1. Pig houses studied

Four pig houses investigated in Zaozhuang and Tai'an city, Shandong Province, China, from April 2007 to February 2008 were

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doi:10.1016/j.scitotenv.2009.12.019

located 5 km away from the villages in the suburb, where there were no buildings, other farms, and tall plants around the pig houses. The pig houses were cleaned twice and disinfected once per day. The manure was then discharged into drainage and automatically came into the ferment pond without polluting the ambience biologically. Description of the four pig houses studied was listed in the Table 1. When the air samples were collected, the meteorological indexes were recorded simultaneously (Table 2).

2.2. Analysis of airborne E. coli

Andersen-6 sampler (Andersen, 1958) was used to collect airborne *E. coli* in the indoor air of the pig houses. The sampler was placed in the middle of the stable about 70 cm above the ground (Zhong et al., 2008). Samples were collected for 0.5–8 min at airflow rate 28.3 L min^{-1}. Outdoor airborne *E. coli* samples were collected, 1.5 m above the ground (Lee et al., 2004), with the airflow rate 40 L min^{-1} for 0.5–8 min using the RCS (Reuter Centrifugal Sampler), which was placed at upwind 10 and 50 m and downwind 10, 50, 100, 200 and 400 m. Each site was sampled 5 times. The sample media was MacConkey medium (Oxoid, CM0115).

The collected samples were cultured at 37 °C for 24–48 h. All colonies were tested for "KOH reaction" to determine whether they were Gram-negative or Gram-positive. The Gram-negative colonies were subcultured in MacConkey agar and were identified by the API 20 E system (Bio Merieux, Marcy-I'Etoile, France). The number of colonies was calculated (Andersen, 1958) and expressed as CFU m^{-3} air. All isolates were stored at −20 °C with 50% glycerin.

2.3. Analysis of E. coli in feces

Fresh fecal samples were collected after the pigs defecated (10 samples from each house). Approximately 1 g of feces was transferred to a sterilized glass homogenizer containing 9 mL of 0.9% sodium chloride solution. The diluted samples were spread onto the surface of eosin methylene blue(EMB) agar (Tianhe, Hangzhou, China) which were incubated at for 18–24 h. Colonies with a metallic sheen on EMB agar were picked and streaked onto MacConkey agar (Oxoid, CM0115). After overnight at 37 °C, one or two typical pink colonies were selected from each MacConkey agar plate and the isolates were tested based on the above-mentioned method in Section 2.2.

2.4. Template preparation

The *E. coli* strains were inoculated into 5 mL liquid Luria-Bertani (LB) broth (Oxoid) with shaking for 12–18 h. 1.5 mL LB broth was taken out and centrifuged for 2 min at 10,000×g. The cell pellet was resuspended in 100 μL sterile double distilled water for 10 min. The culture was then cooled down by putting on ice for 5 min. The mixture was centrifuged at 12,000×g for 2 min. The supernatant was removed and stored at −20 °C and used as the DNA templates for ERIC-PCR analysis later (Cheng et al., 2006).

Table 1
Description of the four pig houses studied.

Pig houses	Housing system	Feeding system	Ventilation system	Manure system (twice/day)	Number of pigs
A	Semi-enclosed	Automatic	Natural ventilation	Manual	110–120
B	Completely enclosed	Automatic	Mechanical ventilation	Manual	110–120
C	Completely enclosed	Automatic	Mechanical ventilation	Manual	216
D	Completely enclosed	Automatic	Mechanical ventilation	Manual	216

Table 2
Meteorological indexes of the four pig houses studied.

Pig houses	Inside			Outside		
	Temperature (°C)	Relative humidity (%)	Wind speed ($m\ s^{-1}$)	Temperature (°C)	Relative humidity (%)	Wind speed ($m\ s^{-1}$)
A	20	58	0.8	18	50	1.5
B	19	50	0.6	16	46	2.0
C	21	60	1.0	19.5	50	1.0
D	22	65	0.5	19	56	1.5

2.5. ERIC-PCR

The primers ERIC1(5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC2(5′-AAGTAAGTGACTGGGGTGAGCG-3′) (Versalovic et al., 1991) were synthesized by Shanghai Biological Engineering Technology & Services Co., Ltd. The PCR reaction (25 μL) contained 10×buffer 2.5 μL, 0.2 mM dNTPs, 0.5 U TaqDNA polymerase, 2 mM $MgCl_2$, primers 400 pM each (10 μM) and template DNA 3 μL. The amplification was carried out by incubation of the mixture for 7 min at 95 °C for pre-denaturation, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and extension at 68 °C for 8 min. A final extension was performed at 65 °C for 10 min.

The PCR products were resolved by 1.2–1.5% agarose gel electrophoresis (1×TAE, EB staining, 1–1.5 h electrophoresis at 3 V cm^{-1}). Gel image was photographed with Tanon-2500 (Shanghai, China). To reduce the variation of experiment, all isolates from the same pig house were analyzed by a single experiment.

2.6. ERIC-PCR fingerprints analysis

ERIC fingerprints of amplified DNA fragments obtained from the agarose gel electrophoreses were recorded. The observed bands in the gels were evaluated based on the presence (encoded 1) or absence (encoded 0) of polymorphic fragments for the ERIC primers. Cluster analysis was performed with NTSYS-pc (Rohlf, 2000), a numerical taxonomy and multivariate analysis software package, based on Dice's similarity coefficient (S_D) with a 1% position tolerance and the unweighted pair group method using arithmetic averages (UPGMA). In addition, each isolate was considered as an operational taxonomic unit (OTU). In order to reduce the number of OTUs in the dendrogram, to facilitate interpretation, isolates of more than 90% similarity were treated as a single isolate (Borges et al., 2003).

2.7. Data statistics and analysis

Median values (CFU m^{-3} air) were expressed as the concentration of airborne *E. coli*. The maximum and minimum values were used to indicate concentration fluctuation range of collected samples. Experimental data were analyzed and processed using Excel 2003 and Spss 11.5 (Statistical Package for the Social Science, Chicago, IL, USA).

3. Results

3.1. Concentrations of airborne E. coli in houses and at different points outdoor

The concentrations of airborne *E. coli* in the four pig houses were 35, 23, 27 and 21 CFU m^{-3} air respectively. At the upwind sites 10 and 50 m away from the pig houses the concentrations of airborne *E. coli* were 4 and 2 (House A), 2 and 1 (House B), 1 and 0 (House C) and 1 and 1 CFU m^{-3} air (House D) respectively. The concentrations of airborne *E. coli* for the four pig houses were 18, 18, 14 and 9 CFU m^{-3} air at 10 m downwind respectively; at 50 m downwind, the