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Size-related bacterial diversity and tetracycline resistance gene abundance in the air of concentrated poultry feeding operations☆

Min Gao, Ruizhi Jia, Tianlei Qiu, Meilin Han, Xuming Wang*

Beijing Agro-Biotechnology Research Center, Beijing Key Laboratory of Agricultural Genetic Resources and Biotechnology, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

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ABSTRACT

Concentrated animal-feeding operations (CAFOs) are considered a source of airborne human pathogens and antibiotic resistance genes. Although bacterial abundance and diversity have been well studied, limited information on the size distribution of bioaerosols has prevented a clear understanding of the health effects of exposure to bioaerosols from CAFOs. Here, different sizes of particles were sampled from the inside and outside of atmospheric environments of layer and broiler feeding operations using 8-stage Andersen samplers. The quantitative real-time polymerase chain reaction (qPCR) and 16S rDNA-based sequencing were used to analyze the characteristics of biological abundance and diversity, respectively, according to size. The results indicated that size-related differences occurred in terms of airborne bacterial richness, diversity, and concentration at poultry-feeding operations. The richness of biological genera in the urban atmospheric environment was lower than in concentrated poultry-feeding operations. The biological diversity of airborne bacterial genera, including genera associated with potential pathogens, varied according to size. The bacterial lineages of bioaerosols present in the 7 size stages for layers clustered apart from those for broilers, suggesting that the type of poultry house is a more important factor than the particle size in shaping the microbial communities. In most cases, the concentrations of the 16S rDNA, *Escherichia coli*, *tetW*, and *tetL* genes increased as the particle size increased, with the geometric mean diameters varying from 4.7 to 5.8 μm. These results regarding the size-related differences in the diversity and abundance of bioaerosols will facilitate a better understanding of the potential health impact on both poultry and humans working in such environments.

1. Introduction

China, with a population of over 1.36 billion, has an ever-increasing demand for meat products. In 2014, the poultry industry in China produced over 28.94 million tons of products including poultry and egg (Poultry and Egg Association; http://data.stats.gov.cn/easyquery.htm?cn=C01&zb=A0D0Q&sj=2014), and it has ranked the first worldwide for nearly 30 consecutive years (http://www.moa.gov.cn/govpublic/XMYS/201109/t20110921_2292641.htm). One factor contributing to this high level of products is the wide use of concentrated animal-feeding operations (CAFOs) in China due to its high efficiency and low production costs, although they are also associated with many environmental concerns.

In recent years, CAFOs have been viewed as significant point sources of outdoor air contamination that can potentially pose serious problems to public health (Hong et al., 2012). Because livestock are concentrated in small confined buildings, an enormous amount of animal waste is produced daily that contains high concentrations of microorganisms, including potential zoonotic pathogens, and this waste can be aerosolized during the processing of manure and moving of animals (Skóra et al., 2016; Dungan, 2010). Prolonged exposure to these airborne contaminants can cause many health issues for both animals and humans through different exposure routes (Ko et al., 2008). Poultry workers have been reported to have a higher prevalence of work-related eye, respiratory, and skin symptoms than other agricultural workers (Just et al., 2011). In addition, the indoor air of CAFOs is routinely ventilated to the outdoor environment, allowing transfer of biotic

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* Corresponding author. Beijing Agro-Biotechnology Research Center, Beijing Academy of Agriculture and Forestry Sciences, No.9 Shuguang Middle Road, Haidian District, Beijing 100097, China.
E-mail addresses: wangxuming@baafs.net.cn, wangxm413@163.com (X. Wang).

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contaminants generated from these confined buildings and leading to deleterious effects on surrounding ambient air quality and human health (Hong et al., 2012).

To better understand the possible health effects on humans and livestock of exposure to contaminated environments, the characteristics of the bioaerosol components have been investigated using different approaches. Caroline Duchaine and her group performed a systematic and comprehensive study on the microbial concentration and diversity of bioaerosols found in livestock buildings (Just et al., 2011, 2012; Nehme et al., 2008). Their results demonstrated the influence of airborne bacterial diversity on the differences in respiratory symptoms between workers from floor-housed and cage-housed poultry operations (Just et al., 2011). Bacterial diversity in poultry houses has been evaluated using several molecular biology-based techniques, including bacterial tag-encoded flexible amplicon pyrosequencing (Nonnenmann et al., 2010), denaturing gradient gel electrophoresis (Just et al., 2011; Nehmé et al., 2009), 16S rRNA gene clone libraries (Brooks et al., 2010; Ko et al., 2008), and 16S rRNA-based pyrosequencing (Hong et al., 2012). Although gram-positive organisms have been commonly reported as the predominant bacterial populations in poultry houses (Brooks et al., 2010; Just et al., 2011), bacterial diversity differs among different types of poultry operations (Just et al., 2011), and the genera representing the prevalent airborne bacteria differ between different regions.

In addition, the complexity of the exposure phenomena in CAFOs necessitates a more comprehensive characterization of the factors that contribute to human exposure (Meklin et al., 2002). Particle size is one critical factor that affects the dose of inhalable particles and the resulting health effects (Meklin et al., 2002; Yamamoto et al., 2012). For example, coarse particles are mainly deposited in the extra-thoracic region, whereas fine particles are more likely to penetrate into and be deposited deeper in the tracheal, bronchial, and alveolar regions (Kawanaka et al., 2009). To date, only a few studies have been performed to evaluate the size distribution of bioaerosols from animal-feeding operations. The results from research on bioaerosols from dairy barns indicated that the majority of microorganisms sampled had an aerodynamic diameter of over 2.1 μm (Blais Lecours et al., 2012). Culturable bacteria of respirable bioaerosols were investigated in poultry (Gao et al., 2015) and swine houses (Cormier et al., 1990), respectively. However, few studies have been performed on the size-related biological diversity of total airborne bacteria from CAFOs.

In recent years, the unmonitored use of antibiotics on swine farms has expanded the diversity and abundance of the antibiotic resistance reservoir in the farm environment (Zhu et al., 2013), and airborne particulate matter (PM) derived from cattle feed yards facilitated the dispersal of microbial communities containing antimicrobial resistance genes (McEachran et al., 2015). Potential health concerns related to biological air pollutants from the growing number of concentrated poultry-feeding operations necessitate investigating the characteristics of bioaerosols at these sites. The aims of this study were to (1) determine the size-related biological abundance and diversity of airborne bacteria, (2) analyze the geometric aerodynamic diameters of particles containing 16S rDNA, *tetW*, *tetL*, and *Escherichia coli* from poultry-feeding operations, and (3) compare the size-related characteristics of bioaerosols between broiler and layer feeding operations.

2. Materials and methods

2.1. Characteristics of animal-confinement buildings

Two types of poultry-housing facilities are commonly used in China: layers are raised in cages for egg production, and broilers are raised on netted floors for meat production. The manure cleaning cycles of both types of facilities were similar in that the feces are dropped on the ground directly and are removed every 2–3 d. The broiler operations process approximately 5–6 flocks per year, whereas the layer flocks are kept for about 400–500 d. Both types of birds remained in the houses until they were sent to the slaughterhouse. The two types of poultry operations have other differences, as reported for operations in Canada (Just et al., 2011), in terms of worker time spent in direct contact with birds, the predominance of female birds in layer facilities, the presence of eggs in layer operations, and housing management practices (including antibiotic use). No disease outbreaks were reported during our visits to the facilities. In addition, no significant differences were noted in the temperature and relative humidity between the layer and broiler houses because all confinement buildings were mechanically ventilated with fans. The characteristics of the animal-confinement buildings are shown in Table 1.

2.2. Sample collection and DNA extraction

The study was conducted from April to May 2015 on 4 commercial layer and 4 broiler farms located in the Pinggu and Huairou districts in Beijing (the upwind area), respectively. For comparison, bioaerosols were also collected on the roof of a 20-m high building at the Beijing Academy of Agriculture and Forestry Sciences at the Western 4th Ring Road in Beijing, which was approximately 83.9 and 58.4 km away from the Pinggu and Huairou districts, respectively. Air sampling was conducted both inside and outside of the layer and broiler houses (approximately 5 m out of the houses) at a height of 1.5 m above the floor. The terms O, LI, LO, BI, and BO represent the samples from outside the office, inside the layer house, outside the layer house, inside the broiler house, and outside the broiler house, respectively. Eight-stage non-viable Andersen samplers (aerodynamic diameter: > 9.0, 5.8–9.0, 4.7–5.8, 3.3–4.7, 2.1–3.3, 1.1–2.1, 0.7–1.1, and 0.4–0.7 μm; TE-20-800, TISCH, Cleves, OH, USA) were used to collect airborne particles on quartz-fiber substrates and were operated continuously for 48 h for each sampling site. The cutoff diameters used represented particle diameters with 50% collection efficiency. Aerosol samples were collected at an air flow rate of 28.3 L min^{-1}, with calibration before each sampling.

The sampling process was performed based on a previous study (Cao et al., 2014), but a few modifications were made. All filters were baked in a Muffle furnace at 500 °C for 5 h. Each sterilized filter was kept in a sterilized plastic box until being loaded into the sampler. All related tools were cleaned with 75% ethanol or autoclaved after each use. After sampling, the filters were stored at −80 °C.

One fourth of filter was accurately cut off with the weight difference among the 4 parts of filter within ±1 mg. DNA was extracted from all samples at the same time by the same person. DNA extraction of the loaded quartz-fiber filters was performed using the Power Max Soil DNA Isolation Kit (Mobio Laboratory, Carlsbad, CA, USA) with previously described modifications (Jiang et al., 2015). DNA samples of the 7 stages (0.4–0.7, 0.7–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7, 4.7–5.8, and 5.8–9.0 μm) from the 4 broiler and from the 4 layer houses were pooled based on previous results, indicating that the microbial communities exhibited similar clustering patterns based on the livestock type (Hong et al., 2012). Therefore, 35 samples (7 indoors for layers, 7 outdoors for layers, 7 indoors for broilers, 7 outdoors for broilers, and 7 outdoors for the office) were analyzed.

2.3. Illumina sequencing and data processing

The 338F and 806R primers were used to amplify the V3V4