World Rabbit Sci. 2016, 24: 71-75
doi:10.4995/wrs.2016.4170

TECHNICAL NOTE: CONCENTRATION AND COMPOSITION OF AIRBORNE AEROBIC BACTERIA INSIDE AN ENCLOSED RABBIT SHED

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Abstract: Numerous studies have been conducted to analyse bacterial aerosols in animal houses, which is beneficial for the control of animal diseases. However, little information on aerosols in enclosed rabbit sheds was available. An FA-1 sampler was employed to collect air samples in an enclosed rabbit house in the Qingdao region of China. Concentration, composition, and aerodynamics of bacterial aerosols inside the enclosed rabbit shed were systematically analysed. The concentration of airborne bacteria inside the rabbit shed was 2.11-6.36×10^4 colony forming unit/m^3 (CFU/m^3). Seventeen species of bacteria belonging to eight genera were identified. Among these, there were 11 species belonging to 4 genera of gram-positive bacteria, and 6 species belonging to 4 genera of gram-negative bacteria. The dominant species of bacteria were, in descending order, *Micrococcus luteus* (49.4%), *Staphylococcus epidermidis* (25.5%), and *Alcaligenes odorans* (10.2%). A total of about 76.3% of airborne bacteria was distributed in stages C-F of the FA-1 sampler (that ranges from A to F), with aerodynamic radii <3.3 µm in diameter. These particulates could enter lower respiratory tracks and even alveoli, posing a potential threat to the health of both animals and breeders.

Key Words: airborne aerobic bacteria, rabbit, dominant species, opportunistic pathogens, aerodynamics.

INTRODUCTION

Aerosol is a dispersed system formed by solid and liquid particulates stably suspending in gaseous medium. The gaseous medium is called the continuous phase, and is usually air. The particulates are known as the dispersed phase, and their composition is complex. Particulates vary in size, with diameters from 0.001 to 100 µm. Particulates <10 µm in diameter can be inhaled into the respiratory systems of humans and animals, and are thus known as inhalable particles. Moreover, their light weight enables a longer suspension time in the air (Bakutis *et al.,* 2004). Aerosols incorporating microbe-containing particulates are known as microbial aerosols (Cercasov *et al.,* 1998; Hameed *et al.,* 2012). In places where animals are concentrated, sneezing and coughing of animals generates an aerosol with saliva and mucus as the main components, known as droplets. Upon evaporation of the water content, the residual mucus and microbes of these aerosols are known as droplet nuclei. Over 90% of droplets are <5 µm in diameter, averaging 1-2 µm. Droplets that can be suspended in the air for extended periods are an important route of transmission of animal diseases. Usually, aerosol particulates in animal sheds contain a higher proportion of biological substances, including microorganisms, and feed debris (Hameed *et al.,* 2012). Aerosol particulates, because of their persistent and diffusible nature, can affect multiple sites of an animal through various routes, including the respiratory tract, digestive tract, mucosa, and skin (Banhazi *et al.,* 2008).

Modern animal farms have a high density of reared animals, and ventilation inside the housing sheds is often poor. Together with the organic particulates generated from feeding, sweeping, and animal activities, high humidity and

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doi:10.4995/wrs.2016.4170

lack of direct sunshine contribute to the survival and proliferation of microbes inside animal sheds. Thus, animal sheds are abundant in bio-aerosols that are rich in variety and difficult to control. The last decade has seen an increase in research into animal shed aerosols, which has contributed to the control of animal diseases and protection of breeders (Golbabaei and Islami, 2000; Just *et al.,* 2011; Laube *et al.,* 2014). However, there has been little investigation into microbial aerosols in enclosed rabbit sheds. Therefore, this study aimed at enhancing our understanding of the composition, quantity, and aerodynamics of microbial aerosols inside enclosed type rabbit sheds, providing primary information for improvement of the environment.

MATERIALS AND METHODS

Conditions of the rabbit shed

The rabbit shed examined in this study was an enclosed-type shed, in which 5 air conditioners were used to alter the air properties (primarily temperature and humidity) to achieve more comfortable living conditions for rabbits. During sampling, the room temperature was kept at 19±3°C and relative humidity was maintained at 68±5%. There were 2 rows of rabbit cages and each row was 3 stories in height. The length, width, and height of the shed were 30, 8 and 3 m respectively. Faeces were cleared once daily. A total of 1200 healthy adult meat rabbits were housed inside the shed.

Sample collection

An FA-1 similar to Andersen-6 sampler was placed in the centre of the shed, 150 cm from the floor. The sampling culture medium was blood agar containing 5% male sheep's blood. Flow rate during sampling was 28.3 L/min, and operation time was 1 min (Chen *et al.,* 2008). During the sampling process, sampling personnel and animal breeders were not in the vicinity of the sampler. In March 2014, 10 samples were collected daily from 09:00 to 10:00 a.m., consecutively for 1 wk. Upon completion of each sample collection, the agar plates were transferred in an ice box to the laboratory within 6 h of collection, and incubated under aerobic conditions at 37°C for 24-48 h.

Quantification and identification of airborne aerobic bacteria

The number of bacterial colonies on the agar plates was counted following incubation. Upon calibration using the FA-1 sampler calibration table, the quantity of aerobic bacteria was calculated based on sampling time and sampling flow rate (Rosas *et al.,* 2001). The colonies on agar plates were isolated and purified, and the identities of isolated bacterial strains were verified using a conventional Analytical Profile Index (API) system.

Aerodynamic analysis

To identify the deposition sites of microbial particulates in animal and human respiratory tracts, bacterial colony numbers on each plate at the different stages of the FA-1 sampler were calibrated according to the conversion table, and were subsequently used to calculate total bacterial numbers and percentage contribution at each stage of the sampler.

RESULTS AND DISCUSSION

Despite the lack of evidence for a correlation between quantity of airborne aerobic bacteria and disease incidence, there has been substantial research suggesting that increasing airborne aerobic bacterial load can impair immunity, retard growth and lower the productivity of animals (Simpson *et al.,* 1998). In the current study, the concentration of airborne aerobic bacteria inside the enclosed-type rabbit shed was calculated to be between 2.11-6.36×10^4 colony forming unit (CFU)/m^3. This number is lower than corresponding figures reported from pig and chicken sheds (10^5-10^6 CFU/m^3) (Just *et al.,* 2011; Liang *et al.,* 2013). This may be a result of the stricter husbandry practices in enclosed-type rabbit sheds compared with the other examples.

Table 1: Species and concentration of aerobic bacterial aerosol collected in the enclosed rabbit shed.

	Aerial bacteria in rabbit house	
Category	The number of isolates	Percentage (%)
Gram-positive bacteria	1056	81.5
Staphylococcus		
S. epidermidis	330	25.5
Micrococcus		
M. luteus	640	49.4
M. varians	5	0.4
M. roseus	16	1.2
Bacillus		
B. cereus	7	0.5
B. laterosporus	28	2.2
B. coagulans	4	0.3
B. pseudomycoides	7	0.5
B. megaterium	2	0.1
Corynebacterium		
C. aquaticum	13	1.0
C. xerosis	4	0.3
Gram-negative bacteria	187	14.5
Flavobacterium		
F. odoratum	4	0.3
Alcaligenes		
A. odorantlon	132	10.2
A. faecalis	10	0.8
A. xylosoxidans	3	0.2
Escherichia		
E. coli	10	0.8
Pseudomonas		
P. alcaligenes	28	2.2
Unidentified	52	4.0
Total	1295	100

A total of 1295 bacterial isolates were obtained following incubation. Upon verification, we found 17 species of bacteria belonging to 8 genera across the different sampling stages. There were 11 species belonging to 4 genera of gram-positive bacteria, and 6 species belonging to 4 genera of gram-negative bacteria. Gram-positive bacteria accounted for 81.5% of the total bacterial population. The dominant species of airborne bacteria inside the shed were, in descending order, *Micrococcus luteus*, *Staphylococcus epidermidis*, and *Alcaligenes odorans* (Table 1). Although no pathogenic bacteria were detected in the current study, some of the isolated gram-positive and gram-negative bacteria are known opportunistic pathogens. All of the 6 species of gram-negative bacteria are opportunistic pathogens for both humans and animals, and except for *Bacillus cereus*, all gram-positives have been implicated in

Table 2: Pore diameter and collected particle size of different stages of the FA-1 sampler.

Stages (top to bottom: A-F/1-6)	Pore diameter (mm)	Particle range (µm)
A/1	1.18	>7.0
B/2	0.91	4.7-7.0
C/3	0.71	3.3-4.7
D/4	0.53	2.1-3.3
E/5	0.34	1.1-2.1
F/6	0.25	0.65-1.1

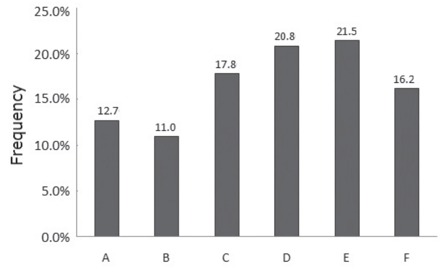

Figure 1: Frequency (%) of aerobic bacterial aerosol in 6 stages (top to bottom: A-F/1-6) of the FA-1 sampler (see Table 2).

respiratory tract infection (Chen *et al.,* 2008). This implies that raising animals under the studied conditions could result in the spread of opportunistic pathogens among the animals, while also posing a potential threat to the health of their human caretakers.

Airborne aerobic bacteria were mainly concentrated in stages C-F of the FA-1 microbial sampler (76.3%), indicating that they were <3.3 μm in diameter (Table 2 and Figure 1). Particulates of this size can enter the lower respiratory tract and even alveoli of humans and animals. Particulates >5 μm in diameter can be filtered by the nasal cavity, and thus cannot enter the respiratory tracts of animals or humans. Particulates larger than 1 μm cannot enter pulmonary alveoli. The smaller the particulate, the deeper it can penetrate into the respiratory tract (Roumeliotis and Van Heyst, 2007). Our study showed that around 76.3% of airborne aerobic bacteria were captured at stages C-F, with aerodynamic radii <3.3 μm in diameter. These particulates could even enter the alveoli and represent a potential threat to the health of both animals and breeders (Chapin *et al.,* 2005; Létourneau *et al.,* 2010).

CONCLUSIONS

The current study showed that a large number of airborne aerobic bacteria were present inside the enclosed rabbit shed ($2.11\text{-}6.36\times10^4$ CFU/m^3). This type of breeding environment may pose a serious threat to the health of exposed animals and individuals.

Acknowledgments: This study was supported by the National Natural Science Foundation of China (81501357).

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