

Composition and variability of airborne fungi in an enclosed rabbit house in China

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Abstract Sampling was conducted from June 2007 to May 2008 in an enclosed rabbit house to investigate composition and variability of airborne fungi. Samples were collected using an Andersen-6 sampler, with Sabouraud culture medium as sampling medium. The results showed that monthly mean concentration was $2.79\text{--}5.46 \times 10^3$ colony forming unit/m³ air (CFU/m³ air), with the maximum level in October, and the minimum level in January. Within a day, the maximum level occurred at 09:00, followed by 17:00 and then 13:00. A total of 6,523 fungal colonies, belonging to 17 genera and 36 species, were obtained. The predominant genera included *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*, comprising 71.45% of the colony count. The obtained fungi of the year were mainly centralized in the stage D of the sampler (2.0–3.0 µm), accounting for 37.8% of the colonies. The minimum value occurred at stage F (<0.65 µm), accounting for 1.10% of the colonies.

Keywords Enclosed rabbit house · Airborne fungi · Monthly mean concentration · Predominant genera

1 Introduction

Bioaerosols are small particles, such as bacteria, fungi, viruses, dust mites, pollens, spores, and decomposers of cracked animals or plants. They are mainly derived from soil, water bodies, animals, plants, and human beings (Ariya and Amyot 2004). Diffused and transmitted through the air, bioaerosols spread human diseases, such as infectious diseases, allergies, or poisoning, as well as animal and plant diseases (Ho and Duncan 2005; Kodama and McGee 1986). Fungal aerosols produced in livestock and poultry industry may threaten caretakers, animals and external environment. Modern livestock and poultry industry has adopted enclosed breeding mode with high density of animals and therefore higher fungal aerosol concentration. Respiratory infection or damage may occur in caretakers as well as livestock and poultry with prolonged exposure to the environment at high microorganism levels (Christiani et al. 1993; Heederik et al. 1991). In the environment of a horse house, fungal aerosols at high level may result in chronic obstructive pulmonary diseases (COPD) in horses, and such diseases may be exacerbated with increased level of fungal aerosols (Robinson et al. 1996). Microbial aerosols of high levels are also associated with allergy and asthma in caretakers (Ostro et al. 2001; Ross et al. 2000). In recent years, air fungi have much attention from medical researchers as well as environmentalists (Liu et al. 2008; Marchisio and Airaudi 2001; Shelton et al. 2002; Zhao and Yang 2007).

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An enclosed rabbit house with high breeding density is favorable to growth and reproduction of various fungal aerosols. However, little research report on fungal aerosols in the environment of enclosed rabbit house has been published. This study was intended to detect composition, predominant genera, and particle size distribution of airborne fungi in an enclosed rabbit house.

2 Materials and methods

2.1 Sampling site and time

The sampling site was a rabbit-breeding farm of Qingdao Kangda Food Co., Ltd., 15 km from the downtown area. Continuous sampling was carried out in an enclosed rabbit house randomly selected from the rabbit-breeding farm. Rabbits were all in good health. The rabbit house was 60 m × 6 m × 3 m in volume; 2,000 adult rabbits were bred with feed particles in 2-tier cages, under positive-pressure ventilation, at temperature 19–29°C and relative humidity 73–78%. Six caretakers carried out daily management. This study was conducted in a period from June 2007 to May 2008. Samples were collected on four working days each month, at three sampling time points each working day, i.e. 09:00, 13:00, and 17:00, respectively.

2.2 Collection of samples

The international standard Andersen-6 air sampler was placed in the center of the rabbit house, 1.40 m above the ground. Sabouraud culture medium was used as sampling medium. Air flow was 28.3 L/min during the sampling. The driving time was 5 min (Andersen 1958; Mart and Jacek 2006). Temperature and relative humidity were recorded during the sampling.

2.3 Treatment of samples

The air samples collected were placed in an incubator at 28°C and incubated for 5 days, then the colony count was recorded; the colony count was recorded again in 7 days, and then fungal aerosol concentration (CFU/m³ air) was calculated. Identification of fungal colonies was based on morphological and

physiological characters (Ainsworth et al. 1973; Hawksworth et al. 1995).

2.4 Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni test. The Pearson correlation coefficients between CFU/m³ air and meteorological data (temperature, relative humidity) were also analyzed according to the method of Gabriella et al. (2004).

3 Results

3.1 Monthly changes of airborne fungi

The CFU in the environment of the rabbit house was from 0.76×10^3 to 9.23×10^3 CFU/m³ air, and the monthly mean concentration was from 2.79×10^3 to 5.46×10^3 CFU/m³ air, with the maximum level in October and the minimum level in January (Table 1). The variation in monthly concentrations of CFU was statistically significant ($P < 0.01$). The monthly mean concentration was correlated to the temperature ($P < 0.05$), but not to the relative humidity ($P > 0.05$).

3.2 Diurnal changes of airborne fungi

The monthly mean concentrations of CFU at the three sampling time-points, i.e. 09:00, 13:00, and 17:00, were 3.10 – 6.28×10^3 CFU/m³ air, 2.47 – 4.72×10^3 CFU/m³ air, and 2.79 – 5.38×10^3 CFU/m³ air, respectively (Table 1). The concentration at 09:00 was significantly higher than that at the other two time-points ($P < 0.05$). There was no significant difference between the concentrations at 13:00 and 17:00 ($P > 0.05$).

3.3 Composition of airborne fungi

A total of 17 genera and 36 species of CFU were identified for the entire period of observation. The predominant genera were *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* accounting for 20.35, 16.64, 25.79, and 8.67% of the colony count, respectively (Table 2).

Table 1 Concentrations of CFU in enclosed rabbit house during the year (June 2007–May 2008)(unit: CFU/m³ air)

T ^b M ^a	June (n = 4)	July (n = 4)	August (n = 4)	September (n = 4)	October (n = 4)	November (n = 4)	December (n = 4)	January (n = 4)	February (n = 4)	March (n = 4)	April (n = 4)	May (n = 4)
9: 00 Mean	4,292	4,508	4,475	4,729	6,283	3,965	3,469	3,099	3,267	3,987	4,669	4,598
9: 00 Median	4,123	4,528	4,396	4,608	6,213	4,096	3,498	3,003	3,249	3,907	4,652	4,308
13: 00 Mean	3,390	3,533	3,378	3,840	4,716	3,097	2,988	2,471	2,652	2,985	3,170	3,907
13: 00 Median	3,207	3,651	3,421	3,789	4,651	3,196	2,685	2,208	2,799	2,688	3,011	4,009
17: 00 Mean	3,877	4,002	3,901	4,209	5,377	3,381	3,201	2,790	2,970	3,391	4,007	4,233
17: 00 Median	3,809	3,925	4,000	4,220	5,209	3,290	3,217	2,788	2,899	3,286	3,971	4,228
Monthly mean concentrations	3,853	4,014	3,918	4,260	5,458	3,481	3,219	2,786	2,963	3,454	3,948	4,246

n Number of days in which samples were collected

^a Months of sampling

^b Time of sampling

3.4 Features of fungal particle size distribution

The Andersen-6 sampler used in this study has six stages from top to bottom (A–F/1–6); different stages can collect different size particles (A: >8.2 µm; B: 6.0–8.2 µm; C: 3.0–6.0 µm; D: 2.0–3.0 µm; E: 1.0–2.0 µm; F: <0.65 µm). The particle size distribution of CFU in the environment of the rabbit house displayed similar pattern throughout the year. The maximum value occurred at stage D (2.0–3.0 µm), and the minimum value occurred at stage F (<0.65 µm). The percentage at the six stages of the Andersen-6 sampler was 14.20, 17.20, 19.30, 37.80, 10.40, and 1.10%, respectively (Table 3). Particle size distribution of CFU also displayed similar pattern throughout the day: 15.00, 16.30, 20.00, 35.50, 11.80, and 1.14%, respectively, at the six stages of the Andersen-6 sampler (Table 4).

4 Discussion

Fungal aerosol is an important component of the environment in livestock and poultry house. Fungal aerosol concentration in the enclosed rabbit house is much higher than air fungi concentrations in the indoor and outdoor environments, as well as in the ward environment of hospitals (Moustafa and Kamel 1976; Lawande and Onyemehkwe 1984; Kasprzyk et al. 2004; Wu et al. 2000), and also higher than air fungi concentrations in the environments of the investigated hen house, pig house, and cow house (Wang et al. 2005). Although caretakers and rabbits were in good health at the sampling site, prolonged exposure to such environment would readily result in occult infection or develop to chronic nosomycosis and lead to predisposition to other diseases. At present, there are no safe levels of airborne fungi concentration in breeding environments, but high concentration would result in threats to the health of human beings and animals.

Aspergillus fumigatus in rabbit house is a major pathogen and may cause allergic alveolitis, asthma, pulmonary aspergillosis, and mycotoxicosis (Lacey and Crook 1988; Lacey and Dutkiewicz 1994). *Penicillium* may cause penicilliosis, often secondary to leukemia and lymphoma, leading to cerebral or pulmonary lesions. *Alternaria* may cause skin alternariosis, and also allergic pneumonia and asthma, and

Table 2 Fungal species and quantity relations

Groups	Number of colonies	Percents of total fungi
<i>Acremonium strictum</i>	12	0.18
<i>Alternaria alternaria</i>	396	6.07
<i>Alternaria tenuissima</i>	169	2.60
<i>Aspergillus carbonarius</i>	419	6.42
<i>Aspergillus flavus</i>	90	1.38
<i>Aspergillus fumigatus</i>	12	0.18
<i>Aspergillus niger</i>	136	2.08
<i>Aspergillus oryzae veffusus</i>	159	2.44
<i>Aspergillus oryzae varoryzae</i>	93	1.43
<i>Aspergillus sydowii</i>	69	1.06
<i>Aspergillus tamarii</i>	18	0.28
<i>Aspergillus versicolor</i>	32	0.49
<i>Aspergillus</i> spp.	293	4.49
<i>Aureobasidium pullulans</i>	11	0.17
<i>Bipolaris</i> spp.	4	0.06
<i>Bipsora betulina</i>	97	1.49
<i>Botryotrichum piluliferum</i>	78	1.19
<i>Cladosporium herbarum</i>	694	10.63
<i>Cladosporium macrocarpum</i>	393	6.02
<i>Cladosporium</i> spp.	596	9.14
<i>Emericella nidulans</i>	59	0.89
<i>Epicoccum nigrum</i>	266	4.08
<i>Fusarium</i> spp.	147	2.25
<i>Gliocladium roseum</i>	225	3.45
<i>Mucor plumbeus</i>	17	0.26
<i>Mucor</i> spp.	9	0.14
<i>Paecilomyces lilacinus</i>	263	4.03
<i>Penicillium canescens</i>	151	2.31
<i>Penicillium daleae</i>	90	1.38
<i>Penicillium expansum</i>	9	0.14
<i>Penicillium funiculosum</i>	43	0.66
<i>Penicillium griseofulvum</i>	184	2.82
<i>Penicillium nigricans</i>	121	1.85
<i>Penicillium purpurogenum</i>	81	1.24
<i>Penicillium restrictum</i>	24	0.36
<i>Penicillium rugulosum</i>	96	1.47
<i>Penicillium tardum</i>	157	2.41
<i>Scolecobasidium</i> spp.	10	0.15
<i>Trichodema harzianum</i>	121	1.85
<i>Trichodema pilulifem</i>	65	0.99
<i>Trichodema polysporum</i>	101	1.55
<i>Trichodema viride</i>	22	0.34

Table 2 continued

Groups	Number of colonies	Percents of total fungi
<i>Trichodema</i> spp.	39	0.60
Unidentified fungi	452	6.93
Total	6,523	100

possibly esophageal cancer. *Fusarium* is a common contaminant of cereals and feedstuff, and under certain environmental conditions, it may produce toxins, leading to poisoning of human beings and animals. Fungal spore B-glucan is an important proinflammatory agent that causes pulmonary infection (Johannes et al. 2000). *Aspergillus niger* and *Aspergillus flavus* may also infect the respiratory systems of human beings and animals (Eduard et al. 1993). Different fungal species may have different pathogenicities; and meanwhile, body resistance is also the key against fungal infection. Therefore, it needs in-depth investigation of aerosol fungal pathogenicity and body immunity to determine harms of fungal aerosols to human beings and animals.

The concentrations in summer (June–August) and autumn (September–November) are higher than those in winter (December–February) and spring (March–May), consistent with the research results about the environment of hen houses conducted by Wang et al. (2005). The concentration of CFU may also change in a day. This may be related with feeding time (08:00), rabbits' activities, personnel flow, as well as disinfection operation (10:00). The correlation between monthly mean concentrations and meteorological factors (temperature and relative humidity) was in agreement with the study conducted in Spain (1992).

The Andersen-6 sampler used in this study simulates the structure of human respiratory system and the aerodynamic performance and is capable of collecting a large number of active particles with high sensitivity (Andersen 1958). The collected particles of A and B stage are stopped in nasal cavity, and a small part of such particles are settled in the mucosal surface of the upper respiratory tract of human beings and animals. Due to gravity, such particles float in air for a shorter time. Fungal particles of C–F stage may enter bronchiole or alveoli. Aerosol particles of such sizes move in

Table 3 Distribution percent of CFU on the A–F stage of the Andersen-6 sampler of 1 year

Stage of Andersen-6	A (>8.2 µm)	B (6.0–8.2 µm)	C (3.0–6.0 µm)	D (2.0–3.0 µm)	E (1.0–2.0 µm)	F (<0.65 µm)
Percent of distribution	14.20	17.20	19.30	37.80	10.40	1.10

Table 4 Distribution percent of CFU on the A–F stage of the Andersen-6 sampler of the day

Stage of Andersen-6	A (>8.2 µm)	B (6.0–8.2 µm)	C (3.0–6.0 µm)	D (2.0–3.0 µm)	E (1.0–2.0 µm)	F (<0.65 µm)
Percent of distribution	15.00	16.30	20.00	35.50	11.80	1.14

Brownian motion in the air and float for a longer time, thus leading to increased opportunities to be absorbed by human beings or animals (Khattab and Levetin 2008). These absorbable fungal aerosols pose greater threats to human beings and animals. The particle size distribution in our study is consistent with the results obtained by Ho and Duncan. (2005).

The results of airborne fungal study is highly influenced by the method of sampling, the type of sampler and collecting media, frequency of collecting, location, and many other factors (Andreas et al. 2007). For example, in this study, non-cultivable fungal spores were not determined. As sampling at night causes stress to animals, no sampling was conducted at night in this study.

5 Conclusion

The results from the current study indicate that *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria* are predominant genera in enclosed rabbit house. The concentration of CFU changes significantly during the day and also varies throughout the year. The particle size distribution of CFU was maximal at stage D (2.0–3.0 µm) and minimal at stage F (<0.65 µm). Better understanding of airborne fungal composition and variability in enclosed rabbit house would help us to regulate the rabbit farm and control fungal diseases.

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