THE AIRBORNE FUNGI FROM INDOOR AIR OF ANIMAL HOUSES

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ABSTRACT

The airborne fungal concentrations, sizes and compositions as well as the dominant genera in animal houses were investigated. Andersen-6 stage microbe sampler and RBC as medium were used to collect fungi aerosol from indoor air. The total number of airborne fungi was determined and their species were identified by morphological characteristics. At same time the aerodynamic analysis of the airborne fungi was also conducted. Altogether 7.77×10^3 CFU cultivable fungal particles were incubated. The concentrations of fungi aerosol in chicken, pig, rabbit and cow houses were 2.39×10^3, 2.51×10^3, 1.764×10^3 and 1.66×10^3 CFU/m^3 air respectively, with the mean value of CMD (count median diameter) 3.02, 3.52, 3.29 and 3.39 μm, GSD (geometric standard deviation) 2.03, 1.71, 1.70 and 1.69 respectively. The predominant fungi in all sampling animal houses were *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium* and *Fusarium* among the 21 genera of identified fungi. It is concluded that the airborne fungal concentrations in the animal stables surroundings were much higher than those of wild fields and common rooms. The fungal particles are easy to be inhaled into deep respiratory tract. The dominant genera of aerosol fungi in animal stables are closely related with fungal infection and mycotoxicoses.

Keywords: animal houses; airborne fungi; dominant fungi; harm assessment

INTRODUCTION

Fungi aerosol which is procreated continually in animal raising house is not only endangered to the feeders and domestic animals, but also cause environment pollution[1]. Some veterinarians and feeders are very easily infected respiratory diseases who were exposed in the fungal aerosol, 13% veterinarians in Argentina are reported be infected in this way[2]. Now the total number of 318 pathogenic fungi and 420 metabolized mycotoxins, which have been identified, would make human and animals grow slowly, immunosuppressant, organ function let down, even death of mycotoxins[3].

Air fungal composition, concentration and particle sizes were the three key harm of fungal aerosol. Many reports, such as Lanzhou Veterinary Institute[3], showed that the harm were closely correlation with fungal concentration. The airborne fungi size was very closely correlated with the infection and endangerment of airborne fungal particles as well. 50% young turkeys were dead of *Aspergillus fumigatus* and none by *A. flavus* when two groups young turkeys were infected by same dose were found by Richard[4] and other investigations. The isolation rate of *A. fumigatus*

was 4 times of *A. flavus* in the infection turkey lungs, and more serious pathological symptom in lung in extent. The fact has been testified by Gao etc[5]. that *A. fumigatus* was more harmful than *A. flavus* and *A. niger*, therefore it deeper distance in aspiratory tract. In general, the smaller size of the fungal particles was more endangered than the bigger size by same dose of inhalation[2].

Recently there are many study reported about active fungal particle harm in some hospitals, living rooms and public areas, but through new searched results, there is no reports about fungi aerosol in raising farms as professional disease pathogens.

1. MATERIALS AND METHODS

1.1 Sampler and culture medium

International standard ANDERSEN grade-6 sampler, air ventilation 28.3 L/min[6], and RBC(rose Bengal chloromycetin)[7] for culture medium.

1.2 Sample collection

Inhalation fungal amount by human and animal were expressed by fungal CFU/min, which human or animal respiration amount (m^3/min) multiply fungal concentration inhalation into mini-bronchia and alveolus. Fungal particles on stage-6 ANDERSEN from A to B stage (>6μm) could be invaded in mini-bronchia, and from C to F (<5μm) in alveolus. Fungal concentration invaded in mini-bronchia or alveolus equal percentage from A to B or C to F multiply the total concentration of sample.

Samples were collected at 50cm height from ground and for 2–4 minutes in three different structure blocked houses of chickens and pigs, one of rabbit, two semi-blocked houses of rabbit, and three opened houses of cow respectively in Shandong province. According to three times sampling in every house, 3–5 samples collected each time per week, 15 samples were gained from chicken houses and 9 samples from other animal house.

1.3 Incubated methods

Samples were incubated for 72 h at 25°C, and taken account of CFU(colony forming unit), and corrected the account of CFU after 7 days, then that is the real number of fungal aerosol particles on every grade of sampler.

1.4 Factors detection of sampling environment

Thermoscope and hygrometer (made in China) were used to detect the temperature and humidity in sampling environment.

1.5 Result express and relation numeration

1.5.1 Airborne fungal concentration expression:

Fungal clone forming unit (CFU) in the air per steer (CFU/ m^3) were used to express the airborne fungal concentration as follow:

$$\text{CFU/ m}^3 = \frac{\text{Total amounts of 6 flat plates}}{28.3\text{L/m}^3 \times \text{sampling time(min)}} \times 1000$$

1.5.2 Airborne fungi size expression:
Total CFU of 6 stages of sampler divided by amount of CFU in every grade are every grade percentage.

1.5.2.1 Airborne fungal particle size was expressed by count median diameter(CMD).
Percentages of every stage added up stage by stage from F to A were accumulation percentage of every stage. Then linearity regression equation was calculated through accumulation percentage as x-axis and effective capture diameter (ECD, μm) as y-axis, Y' value is CMD when X equal 50%[1,8].

1.5.2.2 Airborne fungal particles disperse degree were expressed by geometric standard deviation (GSD). That is to say Y' value was divided by CMD when X equal 84.13% in the linearity regress equation[1,8].

2. RESULTS

2.1 Sampling environment condition, fungal particle size, distribution and concentration.

2.1.1 Fungal concentration and environment factors:
Fungal concentration in closed chicken house were $1.8\sim3.0\times10^3$ CFU/m^3 when temperature changed less than 3°C, raising animal density as 5.9–10.2 ones per m^2, humidity as 47~73%. Fungal concentration in closed pig house were$2.3\sim2.7\times10^3$ CFU/m^3 when raising density as 5–10 m^2 per one. Especially in semi-opened rabbit house, fungal concentration were $1.1\sim2.7\times10^3$ CFU/m^3 when raising density as 0.3~2.7 ones per m^2 and little change in temperature and humidity. Fungal concentration in opened cow house was $1.6–1.8\times10^3$ CFU/m^3 when raising density as 10~15 m^2 per one(table1).

2.1.2 Airborne fungal particle characters:
Fungal particles distribution apex in sampling sites was at stage-D (1.0~2.0μm) with 23.4~36.3% excepted pig house at stage-C. CMD in every sampling site were 2.9~4.1μm, and GSD as 1.7~2.3. No significant between different sampling sites(t=0.06, P>0.05)(Table1).

Table 1. The concentration of aerosol fungi as well as the characteristic of sampling environment and fungal particles ($\times10^3$ CFU/ m^3)

	Close house						semi-close house			open house		
	chicken 1	chicken 2	chicken 3	pig1	pig2	pig3	rabbit1	rabbit2	rabbit3	cow1	cow2	cow3
Fungal concentration	1.8	3.0	2.4	2.3	2.5	2.7	2.7	1.5	1.1	1.6	1.8	1.6
raising density	5.9	10.2	8.3	10	8	5	0.3	3	2.7	10	15	12
temperature(□)	25.5	24	22.5	23	25	22.5	21.5	19	21	30	25	28
humidity (%)	47	73	51	52	53.5	50	65	46	48	40	45	43
CMD(μm)	4.1	3.3	2.6	3.6	3.6	3.4	2.9	3.3	3.7	3.5	3.4	3.3
GSD	1.6	1.8	2.3	1.7	1.7	1.8	1.8	1.7	1.6	1.7	1.7	1.7

(chicken: n=15,others: n=9); raising density (chicken and rabbit: ones/m^2; pig and cow: m^2 /one)

2.1.3 Concentration and fungal particles distribution:
According to the fungal distribution on different stage, concentration of 1.0–2.0μm fungal particles was 5.3–6.8×10^2 CFU /m^3. Concentration of less than 5μm fungal particles that could invade directly into alveolus was 2 times than concentration of more than 6μm fungal particles invaded mini-bronchia. Percentage of less than 8.2μm (from stage-B to F) fungal particles that could invade respiration under nose were 79.5~97.6%, and into alveolus as 58.1~73%,and into mini-bronchia as 27.0~47.9% (Table2).

Table 2. The concentration (×10^2 CFU /m^3)of fungi and fungal particle distribution (%) in the sampling place (chicken houses: N=15, others: N=9)

	Stages of sampler						bronchia	alveolus
	A	B	C	D	E	F	A□B	C□D□E□ F
chicken house	3.3(14.8)	4.4(18.4)	5.1(21.6)	5.5(23.4)	4.0(16.1)	1.5(5.7)	7.8(33.2)	16.1(66.8)
pig house	4.6(20.5)	4.8(21.4)	5.9(25.9)	6.8(19.0)	2.5(11.1)	0.5(2.1)	9.5(41.9)	15.7(58.1)
rabbit house	2.2(12.4)	2.5(14.6)	3.8(22.0)	6.5(36.3)	2.4(13.1)	0.3(1.6)	4.7(27.0)	13.0(73.0)
cow house	2.3(18.0)	2.8(21.3)	4.7(24.6)	5.3(27.7)	1.4(7.6)	0.2(0.8)	5.1(39.3)	11.5(60.7)

2.2 Fungal amount invaded into different respiration tract

Human respiration energy were calculated by 6.94E-03 m^3 per min (10m^3/24h[2]), and of chicken, pig, rabbit, and cow by 23.5,12,12.5 and 20 ones per min under quietude respectively, and aerate amount per minute by 8.46E-04, 2.88E-02, 6.0E-04 and 1.44E-01m^3 per min [14]. Living fungal particle amount that invaded into human mini-bronchia exposure sampling sites were 3.3~6.6 CFU, and into alveolus 7.9~11.1 CFU, and into deep respiration ducts 11.4~17.7 CFU per minute (Table3).

Table 3. The amount of aerosol fungi arrived in the different respiratory tracts of human and animals in sampling places (CFU/ min)

	chicken house		pig house		rabbit house		cow house	
	worker	chicken	worker	pig	worker	rabbit	worker	cow
bronchia	5.4	0.7	6.6	27.4	3.3	0.3	3.5	73.0
alveolus	11.1	1.3	10.9	45.2	9.0	0.8	7.9	165.6
deep respiration tract	16.5	2.0	17.7	72.6	12.3	1.1	11.4	238.6

2.3 Dominant fungi in the farming environment

Fungal aerosol of 12 animal houses was detected. 7773 CFU were captured from 252 flat plates of 42 samples after isolation and purification according to genus identification standards[15, 16, 17]. Total 21 geniuses were isolated. *Aspergillus*, *Penicillium*, *Alternaria*, *Cladsporium*, *Fusarium* et al. (Table 4) Which were found as domination fungi in sampling environment, and the others were *Acremonium*, *Bipolaris, Acremonium*, *Botrytis*, *Coniothyrium*, *Curvularia*, *Graphium*, *Mucor*, *Rhizopus*, *Myrothecium*, *Paecilomyces*, *Phoma*, *Rhodotorula*, *Saccharomycess*, *Scopulariopsis*, *Scytalidium* and *Trichoderma*.

Table 4. The categories and amount or CFU (comparison%) of the advantage fungi genera in different farming environment (n=42)

	The amount of fungal colonies CFU (percentage)				
Fungi name	chicken house	pig house	rabbit house	cow house	total
Penicillium spp.	304(12.7)	384(22.3)	204(10.1)	345(20.9)	1237 (15.9)
Aspergillus spp.	405(17.0)	196(11.4)	547(27.1)	139(8.4)	1287 (16.6)
Fusarium spp.	153(6.4)	68(3.9)	134(6.7)	95(5.8)	450 (5.8)
Cladsporium spp.	287(12.0)	0(0)	340(16.9)	178(10.8)	805 (10.4)
Alternaria spp.	334(14.0)	265(15.4)	236 (11.7)	289(17.5)	1124 (14.5)
Tichoderma spp.	90(3.7)	47(2.7)	134(6.7)	128(7.8)	399 (5.1)
Rhodotorula spp.	230 (9.6)	0(0)	25(1.2)	54(3.3)	309 (4.0)
Paecilomyces spp.	24(1.0)	114(6.6)	104(5.2)	119(7.2)	361(4.6)
Saccharomycess spp.	0(0)	121(7.0)	86(4.3)	129(7.8)	336(4.3)
Curvularia spp.	25(1.0)	66(3.8)	0(0)	0(0)	91(1.2)
Others	533(22.3)	464(26.9)	205(10.2)	172(10.4)	1374 (17.7)
Total	2385(30.7)	1725(22.2)	2015(25.9)	1648(21.2)	7773

3. DISCUSSION

Aerosol fungal concentrations were influenced by many factors such as animal habit, weather, sanitation condition and illumination. As results, aerosol fungal concentration was controlled by man-made measure, such as house structure, raising density, temperature and humidity. The higher raising density, the higher fungal concentration, this is same as the report by Huang (1977) for the readers in Library. The reason why concentration is highest in rabbit house with lowest density was temperature and humidity in blockhouses in favour of fungi increasing, bad air-conditioned. It was obvious by the cooperation to find the fungal concentration in blocked chicken and pig house was higher than in semi-blocked rabbit house and opened cow house, this result is consistent with B.W Karnick 's report which death rate of avian aspergillosis could be reached 50% in a farming house and little death as outdoor breeding. Pinello[13] improved that fungi group concentration could be reduced by window opening in blocked chicken house in spring, and incidence of avian mycosis was dropped 75% by less dust and better ventilation. so fungal pollution in breeding environment could be improved by human measures.

Stage-6 ANDERSEN sampler was made according to human respiration structure and aerodynamics characteristic of airborne particles. Capture dynamics diameters of airborne particles from A to F were >8.2μm, 6.0~8.2μm, 3.0~6.0μm, 2.0~3.0μm, 1.0~2.0μm, <0.65μm in turn[9, 19]. Capture efficiency and particle distribution of air sampler in China is the same as in America. It was well known that 20–30μm particles could invade into nose and upper respiration tract (bronchia), 6–10μm particles into mini-bronchia, 1–5μm particles into deep lung (alveolus) [10, 20]. 0.3~15μm living aerosol particles could be captured by ANDERSEN sampler which were serious harm on human and animal, especially sediment rate of 5μm particles much more higher(>90%). Bigger size and higher sediment rate of particles, which settle down in the air or block out of nose, were captured by conventional sediment method. Reported concentration was different for two sampling methods because they captured different size particles.

2.2×10^6 CFU fungi per gram were found in cultivated lung when young turkeys would die out in 5 days, and less than 5.2×10^5 CFU fungi per gram would die in 3–4 days, and death rate low down. That is to say young turkeys would die out of inhalation of 305 CFU/min. It was obvious

that chicken could not suffering mycosis at 1.3 CFU/min of sampling chicken house, but avian mycosis could be forecasted by fungal aerosol detection. As result, fungal concentration in detecting chicken house were 2.5 times than in outdoor environment (1037.5 cfu/m^3 [11]), and 2.2 times than in living room (1167 cfu/m^3[6]), that is to say, one of the important pollution of atmosphere was raising environment. Fungal concentration in sampling places could not cause human or animal urgent harm, but exposure in the low fungal concentration for a long time would suffer chronic mycosis, and susceptibility to other diseases was intensity, which need to study further more.

The difference of CMD value of each sample is possible related with different source of collected fungal particles, house temperature, humidity, illumination and animal activity. The reason why these samples average CMD value was 1.5–2.0 times smaller than bacterium (supplied in our Lab.) was that fungal particles was in existence in the air as single spore and the bacterium gathered together or adhered to dust in the air, so the fungal particles was easier to enter the depth of respiration ducts than bacterium because its GSD value is over 1.6 and its distribution was larger as well[1].

High level of biodiversity was found in all three farms. The dominant species correlate closely with fugal infections. The most frequent fungal aerosol belongs to genus *Aspergillus,* some of which are opportunistic pathogens. For example, *Aspergillus fumigatus* and *A. terrius* may infect human and animals suffering aspergillosis. Animal tests have shown that some *Aspergillus* (e. g. *A.flavus*, *A. parasiticus*, *A. versicolor*) may produce aflatoxins that induce tumour or reduce white blood cells. The second most frequent species belong to genus *Penicillium* that sometimes also infects human beings who are affected by leukaemia or lymphoma. Some species may infect brain or lung, producing ochratoxins. Third frequent genus is *Alternaria*, which may cause skin infection, hypersensitivity pneumonitis, or asthma. Some species in this genus may also produce mycotoxin that induces esophageal cancer. The fourth frequent is genus *Acremonium*, which may cause chromomycosis or phaeohyphomycosis. They commonly infect brain or skin. The fifth frequent is genus *Fusarium*, which commonly contaminates food and feed. When environment is compromised, *Fusarium* may produce mycotoxin. Some species induce skin or cornea ulcers. In rare case, *Fusarium* is associated with cancer[7]. Virulence of different species varies widely. Because resistance of the body to fungal infection also plays a crucial role, it is necessary to further study the pathogenic ability of fungal aerosol and body immunity.

4. CONCLUSION

It was found through this conclusion that the fungal concentration in breeding house is higher than outdoor and indoor environment. The concentration could be reduced by way of choosing opened or semi-blocked structure animal house. Adjusting the temperature and humidity could control fungal concentration. The airborne fungal spores can be easily inhaled into the deep respiration tract than bacterium. The fungal concentrations of environment were changed with function of the places, and the dominant fungus has close relation with mycosis and toxicosis.

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