BIODIVERSITY AND CONCENTRATION OF AIRBORNE FUNGI IN CHICKEN HOUSE

Wang, Y.[1] , Lu, G.[1], Zhang, X.[2], Ma, R.[3] and Chai, T.[2]*

*[1] College of life Science, Dalian Nationalities University, Dalian 116600, China; [2] College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Tai'an 271018,China; [3] Hospital of Shandong Agricultural University, Tai'an 271018, China; *Corresponding author: Tongjie Chai, Professor. Dr., Director of the Institute for Microbiology; Email:chaitj117@163.com*

ABSTRACT

The biodiversity and concentration of airborne fungi in chicken house were investigated. The air samples were collected by means of 6-stage Andersen sampler and common fungal counting media. 78 fungal species were identified from air samplings after mycological examination. they are mainly mitosporic fungi. *Cladosporium*, *Penicillium*, *Aspergillus*, *Candida*, *Alternaria* and *Fusarium* were the predominant fungal genera, including ten species of avian pathogenic fungi, such as *A. flavus*, *A. fumigatus, A. niger* and *F. sporotrichioides* et al. The average concentration of airborne fungi in the chicken house was 2.3×10^3 CFU/m^3. The concentrations of *A. flavus*, *A. fumigatus* and *A. niger* were relatively higher in the air of chicken house. The distribution of airborne fungi in the chicken house shows normal logarithm, the spores of *Cladosporium*, *Penicillium* and *Aspergillus* were mainly distributed within C and D stages (3.0–6.0μm), while stage A and B (>6.0μm) as well as E and F stage (<3.0μm) relatively less. On the other hand, *C. albicans*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and *Coccidioides immitis*, which occur uncommonly but pose potential threat to public health. The result provides alerting data for controlling the avian mycosis and scientific basis for making preventive and cure measures.

The sweeping of avian flu across Asia has given a warning to human being that we should give more attention to the threat. In recent years, the widespread application of broad spectrum antibiotics has resulted in the escalation of avian diseases caused by fungi, in which, mortality rate caused by aspergillosis might reach 24–94.5%. The researchers have found that *Aspergillus* species are predominant pathogens in chicken houses. Pinello etc (1977) have isolated 73 species of fungi from the chicken house air, the feed, the pad grass and chicken bodies, but the type and the density of active fungal spores (specially pathogenic fungi) inhaled by animals in the air are the key aspects which induce respiratory tract mycosis in human beings and poultry. By far there have been fewer reports on airborne fungi in chicken house.

This article takes the airborne fungi in chicken house as a factor to evaluate their harm on occupation for the first time. We have conducted systematic classification of airborne fungi in chicken house, in order to provide a scientific base for the early warning and controlling methods of avian diseases caused by fungi.

1. MATERIAL AND METHOD

1.1 Air sampler

The air samples were collected by means of 6-stage Andersen sampler(Liaoyang Application Technical Research Institute, China), its effective current interception diameter from A to F level are 8.2μm, 6.0μm, 3.0μm, 2.0μm, 1.0 μm and 0.65μm in turn. and common fungal counting media

1.2 Fungal isolation and identification

Rose Bengal Chloromycin agar (RBC) was used as the medium for the isolation of airborne fungi in chicken house. After indoor incubation, all fungal colonies were counted and purified. For accurate morphological identification of the fungal species, various media like malt extract agar (MEA), czapek yeast agar (CYA), potato dextrose agar (PDA), potato sucrose agar (PSA), Nirenberg sucrose agar (SNA) were used to grow the fungi.

2. RESULTS AND ANALYSIS

2.1 Composition and concentration of airborne fungi in chicken house

4,709 fungal colonies were obtained from 108 air samples in chicken houses. 78 fungal species were morphologically identified, including 442 isolates of yeasts and yeast-like fungi belonging to 8 species of 6 genera, 4,277 isolates of mitosporic fungi belonging to 67 species of 29 genera, 4 isolates of ascomycetes belonging to one species of one genus, and seven isolates of zygomycetes belonging to two species of two genera. Moreover, 432 isolates of 33 different colonies were not identified because of their non-sporulation. The average concentration of airborne fungi in the chicken house is 2.3×10^3 CFU/ m^3,in which the concentration of mitosporic fungi is 2.1×10^3 CFU/ m^3, accounting for 90.8%, the concentration of yeast and yeast-like fungi 2.2×10^2 CFU/ m^3, accounting for 9.4%; the concentration of zygomycetes 3.2 CFU/m^3, accounting for 0.15%; the concentration of ascomycetes 2.0 CFU/m^3, accounting for 0.1%; the concentration of unidentified fungi 2.2×10^2 CFU/m^3, accounting for 9.4%.

2.2 Concentration and constitution of predominant airborne fungi

The predominant airborne fungi in chicken houses are *Cladosporium*, *Penicillium*, *Aspergillus*, *Candida*, *Alternaria* and *Fusarium*, their concentrations and constitutions are shown in table 1

2.3 Distribution, composition and concentration of predominant airborne fungi

The distribution of airborne fungi in chicken house is shown in normal school. The spores of *Cladosporium*, *Penicillium* and *Aspergillus* were mainly distributed within C and D stages (3.0–6.0μm), while stage A and B (>6.0μm) as well as stage E and F (<3.0μm) relatively less. The predominant airborne fungal species are
Cladosporium cladosporioides, C. macrocarpum, *C. herbarum*, and *P. chrysogenum* (table 1).

2.4 Concentration and distribution of common avian pathogenic fungi

The potential harm of fungal aerosol is mainly decided by the concentration and the distribution of the pathogenic fungi. More than 10 species of common pathogenic fungi were isolated in

chicken house air, including *Aspergillus* species which can cause avian aspergillosis. The finding of the high concentration of such highly pathogenic fungi as *A. flavus*, *A. fumigatus* and *A. niger* is of epidemic importance. On the other hand, it was found that the isolation rate of mycotoxin-producing isolates like *Fusarium sporotrichioides*, *F. graminearum* and the *F. moniliforme*, is very high, their secondary metabolites may cause trichothecene toxonosis in poultry, *Candida albicans* is the pathogen of thrush, *Histoplasma capsulatum* and *Cryptococcus neoformans* may cause infection of depth tissues of human beings and poultry, *Microsporum gallinae* is the pathogen of favus, but *Penicillium islandicum* and *Aspergillus ochraceus* may cause avian toxicosis.

The predominant populations of these pathogenic fungi are distributed on different stages in the air sampler, the species on stage A are primarily represented by *F. graminearum* and *A. flavus*, *A. niger* and *C. albicans* are inferior; the species on stage B primarily by *A. niger* and *A. flavus*, the inferior is *F. sporotrichioides* and *P. islandicum*; the species on stage C primarily by *A. fumigatus*, the inferior *A. flavus*, *A. niger* and *F. moniliforme*; the species on stage D primarily by *A. fumigatus*, the inferior *P. islandicum*, *A. niger* and *A. flavus*; the species on stage E primarily by *F. sporotrichioides*, inferior *Microsporum gallinae*; the species on stage F primarily by *A. flavus*, inferior *C. albicans* (table 2).

A. ochraceus which appears less frequent, is mainly distributed on stage A, its distribution on stage B is inferior. *Cryptococcus neoformans* is mainly distributed on stage C, its distribution on stage A and B inferior; *Histoplasma capsulatum* is mainly distributed on stage D, its distribution on stage E inferior (table 2).

2.5 The airborne fungi with lower frequencies

Besides the above airborne fungi, the following fungi were infrequently isolated in the chicken houses, *Absida corymbifera*, *A.melleus*, *A. spinosus*, *Coccidioides immitis*, *Eurotium herbariorum*, *Exophiala spinifera*, *Fusarium larvarum*, *F. nivale*, *F. oxysporum*, *Mucor angulisporus*, *Penicillium cyclopium*, *P. paxilli*, *Sporothrix schenckii*, *Torulopsis glabrata*, their average concentration is 2.0 CFU/m^3.

Table 1. Concentration and constitution of dominant airborne fungal species in chicken house

No.	*Fungal species*	*Concentration CFU/m^3*	*No.*	*Fungal species*	*Concentration CFU/m^3*
1	*Cladosporium cladosporioides*	347.5	18	*C. tropicalis*	23.6
2	*C. macrocarpum*	164.9	19	*Trichosporon beigelii*	23.6
3	*C. herbarum*	141.3	20	*C.albicans*	21.6
4	*Penicillium chrysogenum*	109.9	21	*F. graminearum*	21.6
5	*Candida pseudotropicalis*	108.0	22	*P. islandicum*	17.7
6	*Aspegillus flavus*	106.0	23	*Rhodotorula mucilaginesa*	17.7
7	*A. fumigatus*	92.3	24	*P. roqueforti*	15.7
8	*A. niger*	86.4	25	*F. moniliforme*	13.7
9	*P. rubrum*	58.9	26	*Monilia sitophila*	13.7
10	*P. tardum*	56.9	27	*P. citrinum*	13.7
11	*P. implicatum*	39.3	28	*P.oxalicum*	13.7
12	*Paecilomyces varioti*	37.3	29	*Scopulariopsis brevicaulis*	13.7
13	*P. citrea-viride*	35.3	30	*Trichoderma viride*	13.7

Table 1. Continuation

No.	*Fungal species*	*Concentration CFU/m³*	*No.*	*Fungal species*	*Concentration CFU/m³*
14	*P. multicolor*	31.4	31	*P. rugulosum*	11.8
15	*A. versicolor*	29.4	32	*Cryptococcus neoformans*	9.8
16	*P. urticae*	29.4	33	*Curvularia lunata*	7.9
17	*Fusarium sporotrichioides*	25.5		Total	1752.9

3. DISCUSSION

3.1 The effect of sampling methods on concentration of airborne fungi

The air samples used in this study were made by means of 6-stage Andersen sampler, and it is concentrated on the active airborne fungal spores which are easily inhaled by poultry or human beings. However the traditional medium-exposing method is applicable to the sedimentation particles of microorganisms in the air. Therefore, it is incomparable between the concentration of airborne fungi in the article and that acquired by medium-exposing method.

The concentrations of airborne fungi in different conditions are different although the same air sampler was taken. The present study indicates that the concentration of airborne fungi in chicken houses is higher than in human living room (1167CFU/m^3; Hu Xiao-xuan et al., 1999) by 1147 CFU/m^3, and higher than in the space outside by 867 CFU/m^3. The present results are similar to those acquired by means of CD-I air sampler and JWL-I micro-organism sampler in duck farm (2.1×10^3CFU/m^3; Ho Zhi-hui, 1994). All these results showed that the poultry raising farms have imposed a threat on surrounding environment.

3.2 The biodiversity of airborne fungi in chicken house

The airborne fungi in chicken house are mainly composed by mitosporic species; it is identical with the rule of fungal distribution in wild nature. It is indicated that there is no special vent between the chicken house and the surrounding environment. Because the doors of the chicken houses are frequently opened, there is no air-filtration instalment; it therefore leads to the evident enhancement in chicken house of the fungi which are typical in outdoors environment, such as *Cladosporium* spp. The lower concentration of such common zygomycetes as *Mucor* and *Rhizopus* in chicken houses is due to the limit of the sampling method and RBC which may suppress fungal growth.

The fungal population inspected in the present study is different from those tested in the atmospheric environment by means of medium-exposing method (Huang Jiang-ju et al., 2002) and tested in the hospital environment by means of LWC-1 air sampler (Huo Yun-yan et al., 1994), and it is also different from the results obtained in atmospheric environment by means of 2-stage Andersen air sampler (Chen Mei-ling et al., 2000) but the difference is not evident. Because different sampling methods are designed for different objectives, the obtained fungal populations are naturally different. Moreover, the species of airborne fungi in different environments are apt to the influence of the sampling microclimate. The fungal species are affected by their origin, animal-raising concentration and the structure of raising houses. The distribution of airborne fungi in chicken houses is of interest to microorganism researchers.

Aspergillus flavus, *A. fumigatus*, *A. niger* and other conditional pathogenic fungi are commonly isolated in the chicken houses; however the most virulent airborne fungus *A. terreus* which was reported to cause avian aspergillosis failed to be isolated in the present study. It may be due to the less fortune or the small size of samples limited by factitious factors.

3.3 The distribution characteristic of airborne fungi in chicken houses

The distribution of airborne fungi in the chicken houses shows normal logarithm, it is basically consistent with the distribution rule of airborne fungi in Nanjing (Chen Ming-xia, 2001) and Beijing (Fang Zhi-guo, 2004). Especially the pathogenic fungi, such as *Aspergillus fumigatus* distributed on stage C with a peak, while *A. niger* and *A. flavus* mostly on stage B. The fungi float in the air mainly in the form of single spores, the spore size is the key factor to discriminate all levels of aerosols. In the current study *A. flavus* is the predominant group of fungi on stage F, it is an accidental result (only once), and it is possibly due to artificial factor.

3.3 The latent harm and warning of pathogenic fungi in chicken houses

The current research work is different from other studies; it is focused on the concentration and diversity of the airborne fungal spores which can be inhaled by animals and human beings. It is known that the fungal spores larger than 8.2 μm in diameter are usually detained outside the nose cavity, and the larger spores may fall down by gravity, only the active fungal spores which can be inhaled into the depth tract of respiratory system impose a threat to the peoples' and animals' health.

The fungal spores collected by means of 6-stage Andersen Air Sampler within stage A–B (> 6 μm) may get down to the small bronchus, the spores within stage C–E (1–5 μm) may invade the pulmonary alveolus directly, the spores at stage F are extremely thin granule(<0.65μm). If the fungal spores are less than 0.4μm in diameter, they are easily expired with current. The concentration of active fungal spores within stage C and E is of biological importance. The results of the current study indicate that *A. fumigatus* is a latent threat to chickens in the investigated houses because its concentration peak is just on stage C and it is much higher than *A. flavus* and *A. niger*.

Although the concentration of *Aspergillus fumigatus* is very high in the investigated chicken houses, aspergillosis did not happen within the chickens. It may be due to the following reasons: firstly, the concentration of *A. fumigatus* is much lower and has not reached to the concentration that can cause aspergillosis with 3 magnitudes less, but the long-term contact of chickens with lower dosage of the pathogen inevitably results in slower sub-clinic symptoms, such as losses of appetite, slow-growing, low quantity egg production, immunity failure and so on, these aspects remain to be further studied. Secondly, the investigated chickens are grown-up ones (to be eliminated); they may have acquired certain immunity to the pathogenic fungus. These results provide a warning to the owner that the air disinfection in chicken houses should be tightened; otherwise it may affect the young chickens' survival. It has been found that the happening of avian aspergillosis is associated with the structure of chicken houses. Richard et al. (1984) had proven that the fungal concentration in the enclosed chicken houses could be reduced by opening the window to ventilate in spring. Reece et al. (1986) reported that the fungal diseases in chicken houses could be decreased by 75% if the methods such as reducing dust and forcing ventilation in the chicken houses were taken. Therefore, these results could provide as the scientific basis for how to control the air quality of enclosed chicken houses or those without ventilation facilities.

It is well known that *Candida albicans*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and *Coccidioides immitis* can cause infection of the depth tissue which human beings and poultry can suffer from. Although their inspection rate is very low, they have a particular significance in public sanitary. They should be paid with enough attention because they impose a latent harm to the health of the related persons engaged in poultry industry.

4 EXPECTATIONS

4.1 Strengthening the formulation of pollution criterion of airborne fungi

By far there has been no pollution criterion of airborne fungi in chicken houses which can be referenced to evaluate the air quality. By comparison of the fungal concentrations in wild environment (1.0×10^3 CFU/m^3) and in chicken houses ($1.3\sim3.4\times10^3$CFU/m^3), it is found that the concentration or airborne fungi in chicken houses is very high. It still needs further research to formulate the detailed pollution criterion for poultry raising environment

4.2 Strengthening the research on the harm of *Fusarium* and its mycotoxins to poultry

Richard & Debay (1995) discovered that if turkeys were infected by *Aspergillus fumigatus*, a mycotoxin called gliotoxin toxin was produce in the infection process, and the concentration of gliotoxin in partial tissues is more than 6×10^{-6}, they thought there was relationship between aspergillosis and gliotoxin. This viewpoint reminds us that the dominant population of *Fusarium* in chicken houses should be given enough attention. Recently there are more and more reports about avian toxicosis related to *Fusarium*. For example, one report from one American State University (Guo Ji-ying, 1994) found that the toxins produced by *Fusarium* could change the chicken's productivity and immunity. It also indicates that mycotoxins could trigger the infection of poultry to some diseases. Therefore, the finding of the high concentration of *Fusarium* in chicken houses and the harm of their toxins should be given enough attention.

ACKNOWLEDGEMENTS

This study was financially supported by NSFC grant (30571381) “The spreading mode of microorganism aerosol in animal farms”

REFERENCE

Chen H W, 1999. Airborne microbiological survey in Jinan Tai’an and Qufu. *Environment and Exploitation*. **14** (4): 43–45 (in Chinese)

Chen M L, Hu Q X, Xu X Z, Zhai J H, Sun Z H, Lu J C, Sun R Q, 2000. Investigation of air pollution by microorganism in Nanjing city. *China Public Health*, **16** (6): 504–505(in Chinese)

Chen M X, Jin L S, Sun Z H, Lu J C, Sun R Q, Li Z K, 2001.The vertical distribution and diurnal variation of bioaerosol concentration in Nanjing city. *China Environmental Science*, **21** (2): 97–100 (in Chinese)

Fang Z G, Ouyang Z Y, Hu L F, Lin X Q, Wang X K, 2004. Granularity distribution of airborne microbes in summer in Beijing. *Environmental Science*, **25** (6): 1–5(in Chinese)

Guo J Y, 1994. *Fusarium* toxin can affect chicken immunity. *Shandong Poultry*, **4**: 44 (in Chinese)

Hou Z H, Wang X R, Liu X H, Wang Q E, Liu Q, Ge J C, Dong L, Liang X F, Jin C G, 1994. Effect of city duckery on environmental air. *Journal of Environment and Health*. **11** (4): 167–168 (in Chinese)

Hu Q X, Cai Q L, Lu Z X, 1999. Preliminary approach on the status of indoor and outdoor airborne fungal particles. *Shanghai Environmental Science*, **18** (10): 454–456(in Chinese)

Huang J J, Hong S L, Zhou W, Yang Y C, 2002. Investigation of airborne allergic fungi in Chongqing city. *Journal of Chongqing Medicine*, **31** (8): 701–703(in Chinese)

Huo Y Y, Han Y P, Suo J J, Chen S P, 1994. Investigation of fungal distribution and sterilizing experiment of *Aspergillus flavus* in hospital air. *Journal of Chinese Nurse*, **29** (3): 131–133 (in Chinese)

Katoch R C, Bhowmik K B, Katoch B S, 1975. Preliminary studies on mycoflora of poultry feed and litter. *Indian Veterinary Journal*. **52**: 759–762

Li G Q, Cao G R, 2000. Advance on poultry aspergillosis epidemiology. *Shandong Poultry*, **6**: 30–32 (in Chinese)

Li G Q, Cao G R, 2000. Exhibiting types of poultry aspergillosis. *Journal of Animal Science and Veterinary Medicine*. **19**: 9–12 (in Chinese)

Lovett J, Messer J W, Read R B, 1971. The microflora of southern Ohio poultry litter. *Poultry Science*, **50**: 746–751

Pinello C B, Richard J L, Tiffany L H, 1977. Mycoflora of a turkey confinement brooder house. *Poultry Science*, **56**: 1920–1926

Raine J, Peinter U, Poeder R, 2001. Biodiversity and concentration of airborne fungi in a hospital environment, *Mycopathologia*, **149** (2): 87–97

Reece R, Taylor L K, Dickson D B, Kerr P J, 1986. Mycosis of commercial Japanese quail ducks and turkeys. *Australian Veterinary Journal*, **63**: 196–197

Richard J L, Debey M C, 1995. Production of gliotoxin during the pathogenic state in turkey poultry by *Aspergillus fumigatus* Fresenius. *Mycopathologia*, **129**: 111–115

Richard J L, Thurston J R, Peden W. M, Pinello C, 1984. Recent studies on aspergillosis in turkey poults. *Mycopathologia*, **87**: 3–11

Sauter E A, Peterson C F, Steele E E, 1981. The airborne microflora of poultry houses. *Poultry Science*, **60**: 569–574

ThiSo D, Dick J W, Holleman K A, 1978. Mold spore populations in bark residues used as broiler litter. *Poultry Science*, **57**: 870–874

Zhu R L, Tang K X, Niu Z X, 1995. Aspergillosis broking out in American rainbow chicken. *Chinese Journal of Animal and Poultry Infectious Diseases*, **5**: 41–42 (in Chinese)