

Seasonal biodiversity of pathogenic fungi in farming air area. Case study.[1]

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ABSTRACT. Poultry production proved to be a significant source of bioaerosols. The exposure to high concentration of microorganisms in the air can cause primarily irritations, infections, allergies, and toxic effects. The aim of the present study was to investigate the seasonal biodiversity of airborne fungi in the poultry house, in the surrounding area, as well as to estimate health risk. Seasonal investigations were conducted in the spring, summer, autumn and winter 2010 in the poultry house located near Wrocław in Lower Silesia (Poland). The air samples were collected with the use of a Merck MAS-100 onto nutrient Sabouraud agar and were incubated for 5 days at 26°C. Subsequently the colony-forming units (CFU) were determined. The identification of the isolated fungi was made in accordance with the standard procedures. In the summer and autumn when the weather conditions are most friendly for the spread and the development of numerous microorganisms, fungi were more abundant in the surrounding area than in early spring and winter, when both humidity and temperature were lower. The total of 26 species were analysed (10 in the poultry house and 17 in the surrounding areas). Among 12 fungal genera: *Aspergillus*, *Penicilium*, *Alternaria*, *Exophiala*, *Mycelia sterilla*, *Fusarium*, *Cladosporium*, *Scopulariopsis*, *Chaetomium*, *Acremonium*, *Candida* and *Rhodotorula* nearly everything occurred to be the potential respiratory allergens.

Key words: airborne fungi, pathogenic fungi, bioaerosol, biopollutants

Introduction

Intensive poultry production leads to the air contamination in the form of bioaerosols which may have potentially harmful effects on both human and animal health and as well on the animal productivity. On the other hand, the air emission from the farms may affect the hygiene quality of the immediate environment [1]. In animal housing, the sources of bioaerosol are: feed, manure, litter, epithelial of birds as well as the animals themselves [2]. Bioaerosols contain viruses, bacteria, fungi, fungal spores, protozoan cyst, whole cells or cell parts [1]. These numerous airborne organisms and their fragments as well as particles of biological origin passively float into the atmosphere [3].

Fungal spores constitute a significant fraction of bioaerosol microbial particles, and are often 100 to 1000 times more numerous than other bioparticles. The particulate fraction in a bioaerosol is generally 0.3–100 μm in diameter. Fungal spores larger than 10 μm are deposited in the nasopharynx and can unchain the nasal and the ocular disorders. The respirable size fraction of 1–10 μm is of primary concern. Spores and fragments smaller than 10 μm (especially those smaller than 6 μm) can be transported to the lower airways and lungs, and trigger allergic reactions or infect tissues. Bioaerosols that range in size from 1 to 5 μm generally remain in the air, whereas larger particles are deposited in the surfaces [4].

It is known that fungi grow almost everywhere and develop in wide temperature range (25°C to 50°C and greater) although individual species usually grow within much narrower range [5].

Usually, both outdoor and indoor air is dominated by the representatives of genera *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*,

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Mycelia sterilla, and by yeast [4]. The exposure to high concentration of this fungi in the air can cause several types of human health problems, primarily irritations, infections, allergies, and toxic effects, and it has been suggested that toxigenic fungi are the cause of additional adverse health effects [5].

Therefore, the aim of the present study was to investigate the health risk due to the quality and the quantity seasonal biodiversity of airborne fungi in the poultry house as well as in the surrounding area.

Material and methods

Investigations were conducted in spring, summer, autumn and winter 2010, in poultry house located near Wrocław in Lower Silesia, Poland. The farm was accommodated to 18 000 broilers, with the density of 17 chicken on 1m^2. Broilers were kept on the rye straw deep litter in buildings equipped with mechanical ventilation (inlet and outlet ventilators), heating with a central thermogen and artificial lighting with regularly distributed bulbs.

The biopollutants were determined on the base of the airborne fungi. Air samples were taken using a MAS-100 air sampler (Merck KgaA, Darmstadt, Germany). This instrument is based on the principles described by Andersen, and aspirate air through a perforated plate onto the nutrient Sabouraud agar [6]. The speed of the air flow through the sampler was about 11 m/s, air volumes were 1–100 liters (depending on expected contamination level) and the sampling rate was 100 l/min. The samples were taken at the central point of poultry houses 1.3 m from the ground level. The emission level outside farming objects was determined similarly, i.e. 1.3 m with sampling points situated 10 m, 50 m, 100 m and 200 m (background) from the farming buildings. At the same time both humidity and temperature were evaluated by a termohigrometer (Label).The classifications of the isolated microorganisms were made in accordance with the standard procedures [7]. Microbiological studies of the air samples were used to determine the number of fungi, which were determined with the usage of Sabouraud (Merck) medium. Colonies were counted after 5 days at 26°C and subsequently the colony-forming units (CFU) were determined. After the fungal growth, the most dominant species of moulds were passed onto fresh Chapek-Dox medium and the cultured colonies were described macroscopically. Slides stained with lactophenol were made to identify the microscopic features. Fungi belonged to *Aspergillus* and *Penicilium* genera were identified with the usage of the Raper and Fennell keys as well as Raper et. al. [8,9]; the *Fusarium* species was on the base of key Kwaśna [10], and other species with the usage of the „Atlas of Clinical Fungi" [11]. The biochemical properties of yeasts were characterized on the base of bio–Mérieux API-tests (API 20C AUX).

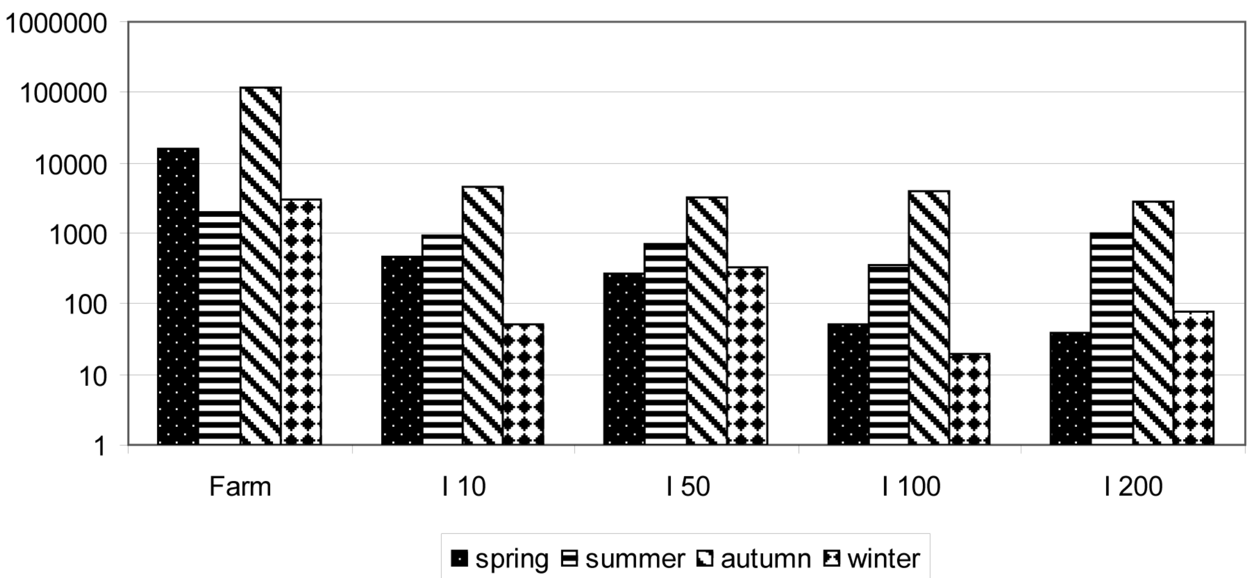

Fig. 1. Seasonal differences in quantity of fungi

Table 1. Fungal species isolated from the poultry houses and from surroundings area during study period (SP – spring, SM – summer, A – autumn and W – winter 2010)

Genus	Species	Sampling site				
		Farm I	I 10	I 50	I 100	I 200
Aspergillus	*flavus*	$+^{SP}$	$+^{SP}$			
	fischerii				$+^{SP}$	
	candidus	$+^{SP}$				
	versicolor	$+^{SP}$				
	fumigatus			$+^{SM}$		
Penicilium	*chrysogeum*	$+^{SM}$		$+^{SM}$	$+^{SM}$	
	sp. 1			$+^{SP, SM}$	$+^{SP, SM}$	$+^{SP}$
	granulatum				$+^{SP}$	$+^{SP}$
	solitum	$+^{SM}$				$+^{SM}$
	verruculosum			$+^{W}$	$+^{W}$	
	commune		$+^{W}$	$+^{W}$		$+^{SM}$
	funiculosum			$+^{SM}$		
Alternaria	*chlamydospora*		$+^{SP}$	$+^{SP}$	$+^{SP}$	
	alternata		$+^{W}$			$+^{SP}$
Exophiala	sp.	$+^{W}$	$+^{W}$			
Mycelia	*sterilla*		$+^{A}$	$+^{SM, A}$	$+^{SP, SM}$	$+^{SP, SM}$
Fusarium	*graminearum*		$+^{SP}$	$+^{SP}$	$+^{SP}$	
Cladosporium	*cladosporoides*	$+^{A}$	$+^{SM}$	$+^{A}$	$+^{SM, A}$	$+^{A}$
	oxysporum	$+^{A}$	$+^{SM}$			$+^{A}$
Chaetomium	*murorum*				$+^{SM}$	
	artrobrunneum				$+^{SM}$	
Scopulariopsis	*brevicaulis*	$+^{SM}$				
Acremonium	*strictum*	$+^{W}$				
Candida	*albicans*	$+^{SM}$	$+^{SM}$	$+^{SM}$		
	sp.	$+^{SP, SM}$	$+^{SP, SM}$			
Rhodotorula	sp.	$+^{SP}$	$+^{SM}$	$+^{SP}$	$+^{SM}$	$+^{SP, W}$

Results and discussion

During the studies, the temperature of the atmosphere was 15.5°C, 23°C, 8°C and –8°C, respectively for the spring, summer, autumn and winter 2010; the inside temperature of the poultry houses varied from 22°C to 27°C. Indoor relative air humidity was 49.3% (spring), 69.8% (summer), 80.4% (autumn) and 73% (winter); outdoor humidity was ca. 56–85%. Weather conditions had a huge influence on the count of microorganisms in the air (Fig. 1). High humidity and optimal temperature can lead to a sudden increase in the concentration of microorganisms in the air. Consequently in summer and autumn when the weather conditions are most friendly for the spread and development of numerous microorganisms, fungi were more abundant in the surrounding area than in early spring and winter, when humidity and temperature were lower.

In Poland there are no standard regulations concerning the permitted number fungi indoor air [12]. In the case of farm environments, Krzysztofik's proposals with regard to the acceptable numbers of moulds were less than 2×10^3 CFU/m^3. In our studies the allowed number of moulds in poultry house was higher in each season. (Fig. 1). According to the regulations of the Polish Norms [13] the number of fungi in the surrounding areas did not exceed 5.0×10^3 CFU/m^3, thus air can be classified as low-contaminated.

Totally, we detected 26 species (10 in the poultry house and 17 in surrounding areas) representing 12 fungal genera: *Aspergillus* (*A. flavus, A. fischerii,* *A. candidus, A. versicolor* and *A. fumigatus*), *Penicilium* (*P. chrysogeum, P. granulatum, P. solitum, P. verruculosum, P. commune, P. funiculosum and Penicilium* sp.), *Alternaria* (*A. chlamydospora, A. alternaria*), *Exophiala*, *Mycelia sterilla*, *Fusarium* (*F. graminearum*), *Cladosporium* (*C. cladosporoides, C. oxysporum*), *Chaetomium* (*Ch. murorum, Ch. artrobrunneum*), *Scopulariopsis* (*S. brevicaulis*), *Acremonium* (*A. strictum*), *Candida* (*C. albicans, Candida* sp.) and *Rhodotorula* – Table 1. The majority of these species are known as the potential respiratory allergens. Furthermore, the presence of opportunistic pathogens from the genus of *Aspergillus* poses the risk of invasive aspergillosis in farm workers and those living in the proximity of the farm [16]. For example, according to published data, the inhalation of a large amount of the *Aspergillus candidus* spores caused organic dust toxic syndrome. On the other hand, *A. versicolor* was identified as a cause of allergic alveolitis. Whereas, *Aspergillus fumigatus* is capable of causing severe infections such as aspergilloma and allergic bronchopulmonary aspergillosis [17]. However, amongst several secondary metabolites produced by *A. flavus* are aflatoxins, the most toxic and potent carcinogenic natural compounds ever characterized. In addition, *Penicilium* and *Cladosporium* are recognized as a causative agents of fungal allergy [18]. Furthermore, the *Cladosporium* species was also involved in a pulmonary infections [11]. We should also emphasize that *A. alternata* is one of the most common fungi associated with asthma. Not only due to the presence of asthma but also due to its persistence and severity, asthma has been strongly associated with sensitization and exposure to *A. alternaria* [19].

The most common airborne fungi, inside the poultry house, as well as in the surrounding areas, were *Penicilium* sp., *Aspergillus* sp., *Cladosporium* sp. and *Alternaria* sp. The yeast were mostly the dominant fungus of the indoor air (in spring) in comparison with the outdoor air, where this group of fungi occurs very occasionally. The following genera were represented by both indoors and outdoors minority: *Exophiala*, *Fusarium*, *Acremonium*, *Chaetomium*, *Scopulariopsis* and *Rhodotorula.* Among fungi identified from the poultry house, the distinctly dominant species belonged to genera *Candida* spp. which constituted on average, over 90% in spring and 66.7% in summer. This is a large group of potentially pathogenic species. They are usually an etiological factor in mycoses and less frequently, in mycoallergies. As they can produce toxins (e.g. candotoxins), the species can cause mycotoxycoses and increase the microorganism's sensitivity to some bacterial infections [15]. In comparison with the predominated yeast inside the poultry houses, the most frequent species in surrounding area in spring were moulds *Alternaria chlamydospora* and *Aspergillus flavus* (92.2% and 20% in sampling point I 10 and I 50, respectively), *Penicilium funiculosum* (66% in summer), *Cladosporium cladosporoides* (80.2% in autumn) and *Penicilium commune* (97% in winter). In relation to the outdoor environments, indoor air typically display lower biodiversity.

According to the data obtained by the Lugauskas et al. [20], *Aspergillus oryzae* and *A. nidulans* were prevailing species and constitute of 15.1% and 9.7% of all the identified species from the farming air. Moreover, *Penicilium* was represented by 12 species, amongst which *P. expansum*, *P. claviforme* and *P. viridactum* were dominant. The same authors isolated species belonging to *Rhizopus* genus: *R. oryzae, R. stolonifer, R. nodosus* as well as to keratinophylic genus *Trichophyton.* The air quality of the Australian poultry buildings has been described by Agranovski et al. [21]. Their study showed the concentrations of airborne fungi ranging from 4.4×10^3–6.2×10^5. The following genera were recorded: *Cladosporium*, *Aspergillus*, *Penicillium*, *Scopulariopsis*, *Fusarium*, *Epicoccum*, *Mucor*, *Trichophyton*, *Alternaria*, *Ulocladium*, *Basidiospores*, *Acremonium, Aurobasidium*, *Drechslera*, *Pithomyces*, *Chrysosporium*, *Geomyces and Rhizomucor.* Romanowska-Słomka and Mirosławski described the occurrence of the moulds and yeast *Aspergillus* sp., *Penicillium* sp., *Candida* sp. *and Cryptococcus* sp. in poultry houses [22]. However, Karwowska isolated moulds that belonged to genera: *Aspergillus* (*A. niger*, *A. nidulans*, *A. ochraceus*), *Penicilium* (*P. notatum*, *Penicillium* sp.), *Cladosporium* and *Alternaria*.

Conclusions

Particles emitted in the poultry house may form the real health risk. The majority of the identified fungal species were characterized as the potential allergens; and exposure to their spores may provoke the immune response in the susceptibility of the individuals. The standard regulations concerning the permitted number of fungi in indoor air should be created in order to assure the proper hygienic condition of farming environments as well as to prevent the emission of the potentially harmful bioaerosols into the atmospheric air.

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Sezonowa bioróżnorodność patogennych grzybów w otoczeniu ferm. Studium przypadku.

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Mikrobiologiczne zanieczyszczenie środowiska spowodowane emisją bioaerozolu generowanego przez fermy drobiarskie jest narastającym problemem ściśle związanym z postępującą intensyfikacją produkcji zwierzęcej. Ze względu na zróżnicowany skład mikroorganizmów bioaerozol zawarty w powietrzu może być przyczyną wielu niekorzystnych objawów chorobowych tj. podrażnień układu oddechowego, jak też reakcji alergicznych i toksycznych. Celem niniejszej pracy było wykazanie sezonowej bioróżnorodności grzybów izolowanych z powietrza kurnika, jak również z okolicy fermy. Sezonowe badania przeprowadzono wiosną, latem, jesienią i zimą 2010 r. na fermie drobiu zlokalizowanej w pobliżu Wrocławia na Dolnym Śląsku (Polska). Próbki powietrza pobierano na agar odżywczy Sabourauda, za pomocą specjalistycznej

aparatury Merck MAS-100. Płytki inkubowano przez 5 dni w temperaturze 26°C, a następnie określano stężenia grzybów w jednostkach cfu/m^3. Identyfikację przeprowadzono w oparciu o standardowe obserwacje makro- i mikroskopowe wyrosłych na podłożach kolonii grzybów.

W rocznym okresie badań wykryto 26 gatunków (10 w kurniku i 17 w okolicy fermy). Większość, spośród 12 wyizolowanych przedstawicieli rodzajów *Aspergillus*, *Penicilium*, *Alternaria*, *Exophiala*, *Mycelia*, *Fusarium*, *Cladosporium*, *Scopulariopsis*, *Chaetomium*, *Acremonium*, *Candida i Rhodotorula*, uważanych jest za czynniki etiologiczne alergicznych i mikotoksycznych chorób układu oddechowego. Wśród grzybów izolowanych z powietrza kurnika, dominującymi gatunkami byli przedstawiciele rodzaju *Candida* (ok. 90% wszystkich rodzajów wiosną i 66,7% latem). Natomiast, w otoczeniu fermy najczęstszymi gatunkami były *Alternaria chlamydospora* i *Aspergillus flavus* (92,2% i 20% wiosną), *Penicillium funiculosum* (66% latem), *Cladosporium cladosporoides* (80,2% jesienią) i *Penicillium commune* (97% zimą). Powietrze zewnętrzne, w porównaniu z wnętrzem kurnika, charakteryzowało się większą bioróżnorodnością gatunków grzybowych. Identyfikacja jakościowa drobnoustrojów w powietrzu dowiodła występowania wielu potencjalnie patogennych grzybów, stanowiących zagrożenie nie tylko dla ludzi i zwierząt, ale także dla środowiska naturalnego. Ogólnie, odnotowano wyższe stężenia grzybów w okresie letnim, niż wczesną wiosną i zimą, gdyż jak wiadomo wysoka wilgotność i wyższa temperatura doprowadzają do gwałtownego wzrostu stężenia mikroorganizmów w powietrzu.

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