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Short Communication

VOLUMETRIC ASSESSMENT OF AIRBORNE INDOOR AND OUTDOOR FUNGI AT POULTRY AND CATTLE HOUSES IN THE MAZANDARAN PROVINCE, IRAN

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The aim of this study was to assess the volume of airborne fungi in the indoor and outdoor environment of poultry and cattle houses in the Mazandaran Province in Iran. Indoor and outdoor air of twenty cattle houses and twenty-five poultry houses were sampled using a single-stage impactor, which draws air at 20 L min^{-1} and impacts sampled material onto Petri plates containing malt extract agar. The plates were incubated at 30 °C for seven days, after which the resulting colonies were counted. The fungi were identified and counted microscopically and macroscopically. A total of 4,662 fungal colonies were isolated from 90 plates collected from indoor and outdoor air of cattle and poultry houses. *Cladosporium* (55.3 %), yeast (10.0 %), and *Aspergillus* (9.4 %) were the most common findings. The concentration of airborne fungi in cattle and poultry houses ranged from 10 CFU m^{-3} to 1700 CFU m^{-3} in indoor and 10 CFU m^{-3} to 2170 CFU m^{-3} in outdoor environments. *Cladosporium* had the highest mean indoor (424.5 CFU m^{-3}) and outdoor (449.7 CFU m^{-3}) air concentration in the cattle houses. In the poultry houses, the highest mean concentrations were measured for *Cladosporium* (551.0 CFU m^{-3}) outdoors and yeast (440.7 CFU m^{-3}) indoors. These levels might present an occupational risk, but threshold levels for these environments have yet to be established worldwide.

KEY WORDS: Aspergillus, Cladosporium, *occupational risk, threshold levels, yeast*

Fungi are a large group of organisms naturally occurring in soil, air, water, and various organic materials. Several fungal genera have been shown to cause allergy, such as *Aspergillus*, *Alternaria*, and *Cladosporium* (1-4). A large number of fungi produce mycotoxins and/or secondary metabolites and volatile organic compounds that can affect human and animal health (5-8). In susceptible or highly-exposed individuals these can lead to invasive mycosis (9).

Many studies have shown that human exposure to airborne dust and microorganisms such as bacteria and fungi can cause respiratory diseases (10-13). Indoor air of cattle and poultry houses can be an important source of fungi (14-16) and involve high risk of occupational exposure. Epidemiological studies have confirmed an increased prevalence of respiratory symptoms and adverse changes in the pulmonary function of poultry workers (17, 18).

Mazandaran is a northern province of Iran located on the southern coast of the Caspian Sea. In the coastal plains - where we conducted our study - the humidity is high and climate temperate, favouring fungal growth and spread through air.

MATERIAL AND METHODS

Sampling sites

Twenty cattle houses and twenty-five poultry houses were randomly selected from across the coastal plains of the Mazandaran Province. Indoor and outdoor air samples were collected in the winter of 2011. We also collected indoor air samples from ten public places and households for control.

Air sampling and laboratory analysis

Air samples were taken with a SKC standard single-stage impactor (SKC Inc., UK), which draws air at 20 L min^{-1} through a stage with 400 holes and impacts the sampled material onto 90-mm diameter Petri dishes containing malt extract agar (Merck, Darmstadt, Germany). For each sample, 100 L of air were aspirated at a height of ~150 cm above the floor. The air sampler sieve plate was cleaned with 10 % formalin prior to sampling.

The Petri plates were incubated at 30 °C for seven days, after which the resulting colonies were counted. The fungi were identified by both microscopic and macroscopic observation. Fungi that could not be identified were sub-cultured on potato dextrose agar (QUELAB, Montreal, Canada), water agar (Bacto agar, USA), and/or slide cultures for further study.

Data analysis

We used positive-hole correction (19) to correct the counts of colony-forming units (CFU) for the limited number of impaction sites on the plate. We then used the following formula to get CFU per cubic meter:

$$CFU\ m^{-3} = \frac{Positive\ hole\ corrected\ CFU}{Time\ sampled} \times \frac{1\,\mathrm{min}}{Sampling\ rate\,(L)} \times \frac{1000\ L}{1m^3}$$

RESULTS

A total of 100 impacted plates were collected, of which 55 were indoor air samples and 45 outdoor. Ninety plates turned out positive. A total of 12 genera of fungi from the indoor and 13 genera from the outdoor air samples were identified from the cattle and poultry houses. Eighty-three plates were positive to *Cladosporium,* 59 to *Aspergillus*, and 51 to *Alternaria*. *Phoma*, *Trichoderma*, *Curvularia*, and *Ulocladium* had one positive plate each.

A total of 4662 fungal colonies were grown on the 90 positive plates impacted by indoor and outdoor air samples taken from cattle and poultry houses. The most common were *Cladosporium* (55.3 %), yeast (10.0 %), and *Aspergillus* (9.4 %). *Cladosporium* (63.8 %), *Aspergillus* (13.5 %), and *Penicillium* (12.9 %) were the most frequent in indoor air of control places.

Table 1 shows the concentration of airborne fungi in outdoor and indoor air of cattle and poultry houses and control places from Mazandaran Province, Iran. It ranged from 10 CFU m^{-3} to 1700 CFU m^{-3} indoors and from 10 CFU m^{-3} to 2170 CFU m^{-3} outdoors (not shown in Table 1). The highest mean concentration in indoor and outdoor air of cattle houses was found for *Cladosporium* (424.5 CFU m^{-3} and 449.7 CFU m^{-3}, respectively). In poultry houses, yeast (440.7 CFU m^{-3}) had the highest indoor and *Cladosporium* (551.0 CFU m^{-3}) the highest outdoor mean concentration.

The highest mean concentrations in control places were found for *Cladosporium* (683.0 CFU m^{-3}), *Penicillium* (143.7 CFU m^{-3}), and *Aspergillus* (143.4 CFU m^{-3}).

DISCUSSION

Occupational environments with high temperature humidity and organic material levels such as poultry and cattle houses favour fungal growth and release of spores. The involved risk of adverse effects on the health of workers and animals has been addressed by a number studies from different countries (10, 20-23).

Our finding that *Cladosporium*, yeast and *Aspergillus* were the most prevalent fungi in cattle and poultry houses is in line with some studies (20, 24). Other researchers (10, 15, 23, 25) reported the dominance of *Aspergillus* and *Penicillium* in indoor air of cattle or poultry houses, while *Cladosporium* ranked below these genera. Differences between these findings may be due to different sampling methods, different sampling seasons, different geographical

conditions, and different culture media. For instance, Khattab and Levetin (26) have shown that the concentration of airborne fungal spores is also related to sampling height. Concentrations of some types of airborne fungal spores were higher at the ground level than at the ceiling level.

In contrast to cattle and poultry environment, yeast in indoor air of control places such as mosques, households, and schools had the lowest prevalence while *Cladosporium*, *Aspergillus*, and *Penicillium* prevailed. Our previous study (27) and some other studies from different countries (28-31) have also shown that *Cladosporium*, *Aspergillus*, and *Penicillium* are common in indoor and outdoor air of human dwellings. Of all observed environments, yeast had one the highest occurrences indoors of poultry houses (48 %) (Table 1).

Indoor air fungal concentrations in our study are significantly lower than in some other studies (13, 21, 23, 33). Our study was conducted in the winter, when the concentrations of fungal spores are usually lower, because of the most fungi cannot grow and sporulate properly at lower temperatures, which drop even in indoor environments (14). Matković et al. (33) suggested that the total fungal count in barn air depends on animal species, housing conditions, and animal feeding and grooming. Ventilation system can also play an important role in indoor environment. Investigators from different countries who used sampling methods similar to ours have reported diverse concentration ranges of airborne fungi in cattle and poultry houses (10, 15, 20-23). This may be due to variations in climate, season, and sampling time.

Our results show an obvious increase in mean *Scopulariopsis* CFU in indoor air of cattle and poultry houses compared to outdoor air. Similar results were seen for *Fusarium* and *Trichoderma* in cattle houses and for *Ulocladium* in poultry houses, even though *Trichoderma* and *Ulocladium* were isolated from one collected sample each. We cannot offer an explanation for these differences, but they may be related to indoor conditions of poultry and cattle houses. The reason for differences in the *Fusarium* levels seems to be more obvious. *Fusarium* is a grain-associated fungus and grain is used indoors as feed for both poultry and cattle.

According to other authors (28, 34-36) and our previous study (27), general outdoor environments usually have higher levels of airborne fungi than indoor places. In addition, outdoor levels highly contribute to concentrations indoors (37). In our study, airborne fungi had higher concentrations outdoors of

Table 1 *Concentrations of airborne fungi indoors and outdoors of cattle and poultry houses and control places in Mazandaran Province, Iran*

Fungi	Concentration / CFU m^{-3}														
	Cattle houses (n=20)						Poultry houses (n=25)						Control places (n=10)		
	Indoors			Outdoors			Indoors			Outdoors			Indoor		
	N	Total	Mean	N	Total	Mean	N	Total	Mean	N	Total	Mean	N	Total	Mean
Cladosporium	16	6792	424.5	19	8545	449.7	23	5438	236.4	15	8265	551.0	10	6830	683.0
Aspergillus	14	932	66.6	11	780	70.9	18	2318	128.6	6	747	124.5	10	1434	143.4
Penicillium	13	726	55.8	11	1322	120.2	14	825	58.9	2	203	101.5	9	1293	143.7
Fusarium	5	712	142.4	6	261	43.5	4	151	37.7	7	332	47.4	3	50	16.7
Alternaria	9	464	51.5	9	567	63.0	16	623	38.9	9	270	30.0	8	483	60.4
Yeast	2	194	97.0	3	256	85.3	12	5288	440.7	5	1885	377.0	1	30	30.0
Sterile hyphae	8	170	21.2	6	120	20.0	12	412	34.3	8	351	43.9	7	291	41.6
Trichoderma	1	81	81.0	-	-	-	-	-	-	-	-	-	-	-	-
Scopulariopsis	2	80	40.0	-	-	-	12	2706	225.5	2	121	61.5	1	30	30.0
Rhizopus	4	50	12.5	5	50	10.0	-	-	-	5	60	12.0	-	-	-
Mucor	1	30	30.0	1	40	40.0	-	-	-	-	-	-	-	-	-
Ulocladium	-	-	-	-	-	-	1	81	81.0	-	-	-	-	-	-
Unidentified	-	-	-	-	-	-	-	-	-	-	-	-	1	71	71.0
Curvularia	-	-	-	1	20	20.0	-	-	-	-	-	-	-	-	-
Phoma	-	-	-	-	-	-	-	-	-	1	10	10.0	-	-	-

n - number of samples
N - number of positive samples

cattle houses than indoors, but the reverse is true for poultry houses. This may be owed to a substantial presence of fungal growth substrates inside the poultry houses.

Cattle and poultry houses are considered occupational environments with high levels of exposure to fungi. Indoor exposure levels are usually much higher than outdoor levels, which seldom exceed 10^4 spores per cubic meter (38). Activities in these indoor places such as cleaning and feeding animals increase occupational risk of exposure to airborne microorganisms. Spores of some type of fungi including *Cladosporium, Aspergillus, Penicillium* and *Alternaria* may carry allergens, antigens, polysaccharides such as the β(1→3)-glucans, and mycotoxins and can cause allergic respiratory disease in susceptible individuals. However, no guidelines or limit values for fungal concentrations in occupational or non-occupational environments have been set by now. A comprehensive review by Eduard (38) suggests that each fungal type should have its own limit set, as different fungal concentrations are needed for different types of fungi to cause a related syndrome in exposed workers.

The most common species in our study *Cladosporium, Aspergillus, Penicillium*, and *Alternaria* are strongly associated with allergic respiratory disease, especially asthma. *Aspergillus* and *Fusarium* are also important producers of mycotoxins and/or secondary metabolites and volatile organic compounds in nature. In addition, all of the above mentioned fungal genera can cause invasive mycosis in susceptible individuals or those exposed to extremely high levels.

Our study has determined airborne fungal levels that might present occupational risk of respiratory diseases.

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Sažetak

VOLUMETRIJSKI NALAZI LEBDEĆIH SPORA GLJIVICA U UNUTRAŠNJOSTI I IZVAN PERADARNIKA I STAJA U IRANSKOJ PROVINCIJI MAZANDARAN

Cilj je ovog ispitivanja bio utvrditi razine gljivica u zraku u unutrašnjosti i izvan peradarnika i staja u iranskoj provinciji Mazandaran. Uzeti su uzorci zraka iz unutrašnjosti i izvan prostora dvadeset staja i dvadeset i pet peradarnika s pomoću jednostupanjskog impaktora s protokom zraka od 20 L min^{-1}. Uzorkovan je zrak impaktiran na Petrijeve pločice s hranjivom podlogom od ekstrakta slada. Pločice su inkubirane sedam dana na 30 °C, a zatim su izolirane i prebrojene dobivene kolonije mikroskopski i makroskopski. Ukupno su izolirane 4.662 kolonije s 90 pločica. Najčešće su bile gljivice *Cladosporium* (55,3 %), kvasac (10,0 %) i *Aspergillus* (9,4 %). Koncentracije gljivica nošenih zrakom kretale su se od 10 CFU m^{-3} do 1.700 CFU m^{-3} u unutrašnjosti staja i peradarnika te od 10 CFU m^{-3} do 2.170 CFU m^{-3} izvan njih. Najviša srednja koncentracija u unutrašnjosti (424,5 CFU m^{-3}) i izvan staja (449,7 CFU m-3) izmjerena je za *Cladosporium*. U peradarnicima najviše su srednje koncentracije u unutrašnjosti i izvan njih izmjerene za *Cladosporium* (551,0 CFU m^{-3}) i kvasac (440,7 CFU m^{-3}). Te koncentracije mogu biti povezane s rizikom od profesionalnih respiracijskih bolesti, ali još uvijek nisu utvrđene gornje dopuštene razine za ovu vrstu okoliša bilo gdje u svijetu.

KLJUČNE RIJEČI: Aspergillus, Cladosporium, *gornje dopuštene razine, kvasac, profesionalne respiracijske bolesti*

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