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Mycological Air Quality at Animal Veterinary Practice

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Abstract: The objective of the study was to characterize the mycological quality of air at animal veterinary practice in Krakow. Bioaerosol measurements were performed during the summer season of 2017. The samples of outdoor and indoor air at animal veterinary practice were collected using a 6-stage Andersen`s air sampler. The highest concentration of fungal aerosol was observed in the treatment room. The analysis showed various fungal contamination in different measuring points at different measuring times of the day. Based on the analysis of bioaerosol particle size distribution it was found that the largest "load" of fungi, isolated form the air, can reach (in the human respiratory tract) to the region of the throat, trachei and primary bronchi. The predominant fungi in indoor air was *Penicillium* spp. and *Cladosporium cladosporoides.* Fungi that can cause dermatophytoses have also been isolated from indoor air: *Microsporum canis* and *Trichophyton verrucosum.* The study confirmed that the animal veterinary practice can be a workplace related to exposure to microbial agents.

Keywords: bioaerosol, fungi, air quality, animal veterinary practice

1. Introduction

In recent years, people spend between 80-95% of their time indoors. It should be emphasized that indoor air quality is one of the most significant factors affecting the human health. One of the biotic contaminants of air are fragments of fungal mycelia and fungal spores. Biological particles can be released into the air from any natural or non-natural surfaces. Fungi can get into the atmosphere from plant and soil, due to wind or thermal convection processes or after emissions from natural water reservoirs. Human activity also has a big impact on the qualitative and quantitative composition of the biological aerosol (Bowers et al. 2012, Polymenakou 2012). The main source of fungi in indoor air are living

organisms: people, animals, plants, as well as construction materials in buildings or external air getting inside the rooms. The penetration of atmospheric air into the rooms of the building is the main process that causes biological contamination of this environment with fungal spores (Chmiel et al. 2015, Małecka-Adamowicz et al. 2019).

Fungi that are part of bioaerosols can't grow during airborne transport but they are able to survive in the air for some time – it depends on their properties or environmental conditions (eg. access to nutrients, physical and chemical factors of environmental stress, the particle size – the small components of bioaerosol retain their viability in the environment longer than larger microbes). Fungal spores can survive in the air for a long time, when the most sensitive, vegetative forms of bacteria die quickly (Gatchalian et al. 2010, Menetrez et al. 2010; Puspita et al. 2012, Galperin & Yutin 2013).

The fungi present in the air can cause adverse health effects like irritations, infections, allergies, and serious toxic effects. In addition, a large number of fungi produce mycotoxins (secondary metabolites) and can affect human health (Thorne et al.1992, Kalogerakis et al. 2005, Ajoudanifar et al. 2011, Breza-Boruta 2015, Frączek et al. 2018). Biological factors can be a serious problem of occupational medicine and public health, and exposure to biological factors is related to specific professions. Environment of veterinary institutions and carrying out duties by vet or veterinary technician are considered to be related to exposure to harmful biological agents (Harper et al. 2013, Rim & Lim 2014, Grzyb & Pawlak 2020). Especially, direct contact between veterinarians and diagnosed animals is associated with the risk of biological contamination. In veterinary practice, not only animals are sources of microorganism contamination, but also people or the components of the indoor environment (Sitkowska et al. 2015).

Due to the fact that the microbiological quality of air is a very important factor in the workplace, the aim of this study was to characterize this property of air at the animal veterinary practice based on the number and species composition of the fungal population.

2. Materials and methods

The study was carried out in the summer of 2017 at the premises of the animal veterinary practice in Krakow (Poland). The veterinary practice takes care of pets, mainly dogs and cats. The samples of air were collected in two series, in duplicate at four measuring points. The selected rooms were those in which animals were housed or through which there was a regular flow of animals on a daily basis (treatment room – with a volume of 40 m^3 of air, a room with cages in which animals are housed after treatments – 30 m^3 of air, and waiting room – 36 m^3 of air) inside the building. During the measurements there were

two dogs in the room with cages (every time). Air samples were collected before opening, five hours after opening (half of the working time of the veterinary practice) and after the veterinary practice work. Five hours after opening, there were a total of five animals in the building (two dogs in the waiting room with the owners – two people, one dog in the treatment room with the owner – one person, and two dogs in the room with cages – without the owners). There are two vets working in the veterinary practice on a daily basis. All studied rooms were naturally ventilated. Also, the gravity ventilation in the building was efficient.

Additionally, the air samples were collected at a point situated outside the building (as the "background"). The air samples were collected using a six-stage Andersen cascade sampler (model 10-710, Graseby-Andersen, Inc., Atlanta, GA). The sampler was placed at a height of 1.5 m above the floor or ground (outdoor measurements) to simulate the aspiration from the human breathing zone. A 5-minute sampling period and the flow rate of 28.3 $dm^3 \cdot min^{-1}$ were applied for the collection of air samples. Fungi were collected on malt extract agar (MEA LAB-AGAR^{TM}, BioMaxima, Poland). During sampling, the air temperature and relative humidity were measured using a hygrometer Kestrel 4000. The MEA plates were incubated for 4 days at 30°C, then 4 days at 22°C. The prolonged incubation of samples for culturing of fungi enables the growth of slowly growing strains at a lower temperature range. After incubation, the fungal colonies were counted. The concentration of fungal aerosol was calculated as the number of colony forming units per cubic meter of air ($cfu \cdot m^{-3}$).

Due to the specificity of the studied environment, isolated fungal strains were identified on the basis of macroscopic and microscopic features using diagnostic keys and, finally, by the mass spectroscopy (MALDI TOF MS), using laser desorption/ionization, with matrix-assisted and time-of-flight analyzer, by using MALDI Biotyper analyzer (Bruker).

The results were statistically analysed using Statistica 13.1 (StatSoft, Inc., Tulsa, OK, USA). The collected data was characterized by non-parametric distribution (Shapiro-Wilk test). The significance of differences between means was verified by the Kruskal-Wallis test. The results showing the effect of microclimatic parameters (temperature and relative humidity) on the prevalence of airborne microorganisms were evaluated using the R coefficient of the Spearman's correlation.

3. Results

The concentrations of fungal aerosol are presented in Table 1 and Table 2. Concentrations of fungi in the studied premises ranged from 1052 to 2739 $cfu \cdot m^{-3}$. The results showed that the average highest concentration of fungal aerosol was observed in the treatment room and the lowest concentration was observed in

the room with cages. The statistical analysis showed a significant differences in the concentrations of fungal aerosol between the treatment room and room with cages (Kruskal-Wallis test). Also, the statistical analysis showed a significant differences in the concentrations of fungal aerosol between the treatment room and outdoor air. The concentrations of fungal aerosol in room with cages were higher than in the outdoor air, but the differences between them were not statistically significant. There were no statistical significant differences in the concentration of fungi between treatment room and waiting room. Concentration of fungal aerosol was significantly higher in the waiting room than in the room with cages.

Table 1. Fungal aerosol concentrations (cfu·m^{-3}) at animal veterinary practice and outdoor air

Environment		Fungi concentration	
		Range	Median
Indoor air	Treatment room	1052-2739	2306
	Room with cages	1203-1944	1463
	Waiting room	1626-2500	2055
Outdoor air		1184-1370	1277

Table 2. Average fungal aerosol concentration (cfu·m^{-3}, ±SD) in indoor air at animal veterinary practice including the time of measurement

Measuring time	Measuring point	Fungi concentration
Before opening	Treatment room	1264 ±299
	Room with cages	1296 ±131
	Waiting room	2160 ±481
Five hours after opening	Treatment room	2576 ±231
	Room with cages	1428 ±156
	Waiting room	2055 ±107
After work	Treatment room	2439 ±337
	Room with cages	1812 ±187
	Waiting room	1980 ±500

Results of microclimate parameters measurments are presented in Table 3.

By using a 6-stage Andersen's air sampler, it was possibile to get information about the size distribution of air fungal biota in the investigated measuring points at the animal veterinary practice (Figure 1). Based on the analysis of fungal aerosol particle size distribution it was found that in all investigated rooms the fungi concentration had a maximum value mainly in a range of diameters 3.3-7.0 μm.

Table 3. Temperature and relative humidity of indoor and outdoor air at animal veterinary practice

Environment		Temperature [°C]		Relative humidity [%]	
Indoor air	Treatment room	Range	Median	Range	Median
		22.5-24.1	23.6	52.5-64.5	60.7
	Room with cages	22.7-22.9	22.7	54.5-62.3	60.9
	Waiting room	22.5-25.7	23.2	49.2-59.1	57.0
Outdoor air		20.2-21.8	20.7	50.3-62.6	58.2

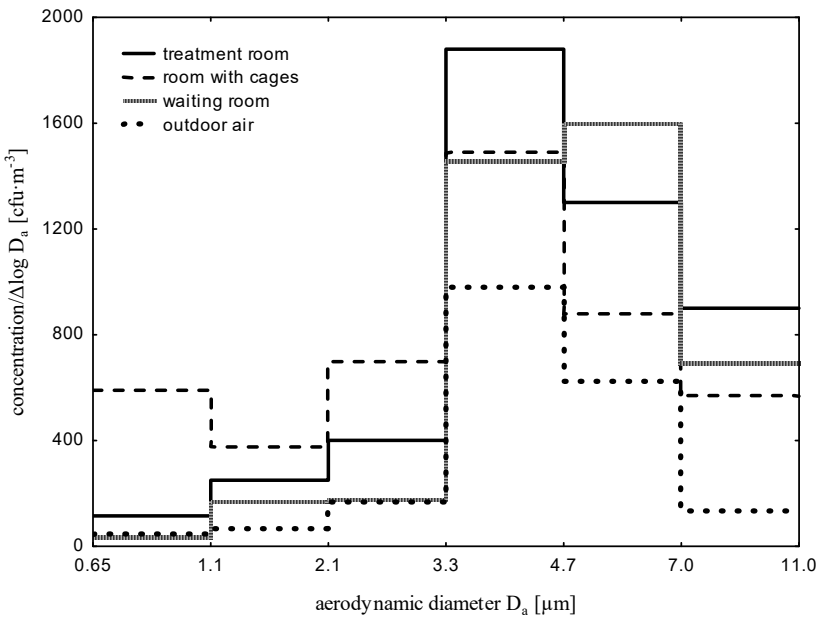

Fig. 1. The size distribution of fungal aerosol inside and outside the animal veterinary practice

The percentage shares of identified fungi in the examined veterinary practice and outdoor air are presented in Table 4.

Table 4. Species of fungi (%) isolated from the air at the studied animal veterinary practice: indoor and outdoor air

Environment	Species of fungi	Fraction [%]
Indoor air	*Penicillium* spp.	44.85
	Penicillium pinophilum	1.21
	Penicillium chrysogenum	3.75
	Cladosporium cladosporoides	14.21
	Alternaria alternata	2.53
	Alternaria spp.	1.21
	Fusarium spp.	2.05
	Scopulariopsis brevicaulis	4.05
	Aspergillus sydowii	4.80
	Aspergillus clavatus	0.05
	Aspergillus niger	0.05
	Aspergillus fumigatus	0.19
	Microsporum canis	9.05
	Rhizopus spp.	2.05
	Acremonium strictum	4.70
	Trichophyton verrucosum	4.85
	Ulocladium spp.	0.40
Outdoor air	*Penicillium* spp.	26.15
	Penicillium digitatum	4.60
	Penicillium chrysogenum	4.80
	Cladosporium cladosporoides	19.85
	Aspergillus spp.	13.95
	Scopulariopsis brevicaulis	7.55
	Alternaria alternata	9.10
	Fusarium spp.	9.55
	Rhizopus spp.	4.45

4. Discussion

Due to the specificity of work, veterinary staff come in contact with microorganisms present on the skin, mucous membrane or animal hair. Veterinarians and veterinary technicians can also be exposed to other infectious factors (e.g. animal excreta and body fluids). Occupational exposure to zoonotic diseases is a risk in veterinary medicine (Weese et al. 2002, Sitkowska et al. 2015). In these studies an assessment of the mycological quality of air in animal veterinary practice was made. Concentrations of fungi in the studied premises ranged from 1052 to 2739 cfu·m^{-3}. The obtained results of indoor measurements of fungal aerosol concentrations were compared with the Polish proposals for threshold limit values, which are $5 \cdot 10^3$ cfu·m^{-3} for fungi in indoor and outdoor environments. It was found that the average concentrations of fungi obtained in this study (Figure 2) were lower than reference values for fungi concentrations in residential and public buildings recommended by the Polish Panel of Experts of Biological Factors (Górny 2010). There are a few available works describing the problem of microbiological contamination of air at small animal veterinary clinics, veterinary hospitals and pet stores, where similar values of fungal aerosol concentration have been observed – the mean concentration of fungal aerosol in that types of facilities was 700 to 8068 cfu·m^{-3} (Jo & Kang 2006, Bulski 2017, Bulski & Korta-Pepłowska 2017, Chen et al. 2017).

In indoor air, total of 17 species of fungi have been identified. The predominant fungi was *Penicillium* spp. and *Cladosporium cladosporoides.* Fungi that can cause dermatophytoses have also been isolated from indoor air: *Microsporum canis* and *Trichophyton verrucosum.* From the outdoor air, 9 fungal species were isolated. The predominant fungi in outdoor air was *Penicillium* spp., *Cladosporium cladosporoides* and *Aspergillus* spp. The fungal genera and species identified in this research mostly are associated with allergic respiratory diseases (especially in people with impaired immune systems); some of them can be a source of polysaccharides such as the β(1→3)-glucans (e.g. *Cladosporium* spp., *Alternaria* spp.). Some of fungi species, isolated from indoor air at veterinary practice, are important producers of mycotoxins, secondary metabolites, with neurotoxic and carcinogenic properties (e.g. *A. niger*, *A. clavatus*) (Pitt 2000). In tested air, the presence of *Microsporum canis* and *Trichophyton verrucosum* was found. *M. canis*, a species widespread throughout the world, it is characterized by a significant degree of adaptation to various animal species and a wide range of pathogenicity. It causes a dermatophytoses, especially in cats or dogs and can be dangerous for people by causing mycosis of skin of head (Wawrzkiewicz et al. 1994). *T. verrucosum* is a dermatophyte largely responsible for fungal skin disease in dogs. Infection to humans is largely zoonotic and can cause the scalp ringworm. The majority of infections are occupational, and this includes veterinarians and veterinary technicians (Kane

et al. 1997). According to other authors, the fungi that occurred most frequently in the veterinary hospitals or other veterinary facilities were *Coriolopsis* spp. and *Microporus* spp. (Chen et al. 2017). People working in this type of environment are most often exposed to dermatologic diseases caused by *Microsporum* spp., *Trichophyton* spp., and *Blastomyces dermatitidis* (Weese et al. 2002). The species of microscopic fungi isolated from the air in this study (*Aspergillus fumigatus*, *Microsporum canis,* and *Trichophyton verrucosum*) belong to the second risk group according to the list of harmful biological agents in the work environment (may cause disease in humans, can be dangerous to employees, but their spread in the human population is unlikely; usually, there are effective methods of prevention or treatment) (Regulation of the Polish Minister of Health, 2005).

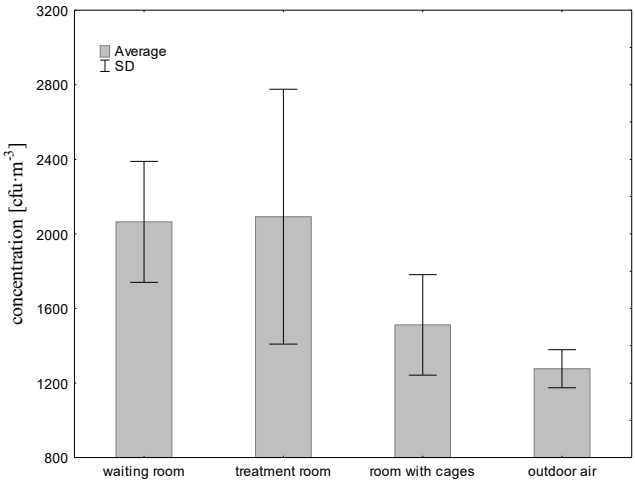

Fig. 2. Average concentration of fungi (cfu·m^{-3}, ±SD) in outdoor and indoor air at animal veterinary practice

The results showed various fungal contamination in different measuring points at different measuring times of the day, what could have been caused by the changes in the number of clients, animals or activities performed at veterinary practice. Based on the results of this study it was found that the largest "load" of fungi, isolated from the air, can reach (in the human respiratory tract) to the region of the throat, trachei and primary bronchi (Owen & Ensor 1992). This is necessary and important information for the assessment of the effects of biological aerosols on the human body – the place of deposition of a harmful biological factors determines the type of adverse health effect. Microclimate conditions may affect the number of microorganisms and their spread in the air

(Li & Kendrick 1995, Katial et al. 1997). Analysis of the impact of the temperature and relative humidity on the observed fungal aerosol showed a significant correlation between the concentration of fungi and temperature ($R = -0.73$, $p < 0.05$) and relative humidity ($R = 0.77$, $p < 0.05$).

The analysis showed that the higher concentration of fungi in the studied rooms was observed in the treatment room five hours after opening and the lowest concentration was observed in the treatment room before opening. The analysis showed that there were significant differences in the concentration of fungi in treatment room taking into account the measuring time – before opening and five hours after opening/after work, but there were no significant differences in concentration of fungi in treatment room five hours after opening and after work. There were no significant differences in concentration of fungal aerosol in other measuring points taking into account the measuring time.

5. Conclusions

Concentrations of fungal aerosol between the internal studied rooms at the veterinary practice were significantly different and were always lower than 2740 cfu·m^{-3}. The highest concentrations of fungi in the studied rooms were observed in the treatment room five hours after opening the veterinary practice. However, the results of this study showed the possible biological risks for the veterinary workers or clients of animal veterinary practice. Although the concentrations of fungi did not exceed the Polish limit values for fungal aerosol, it was found that among the detected fungi pathogenic species as: *Microsporum canis* and *Trichophyton verrucosum* were present. The presence of pathogenic microorganisms and prolonged exposure can create a health risk for allergic symptoms or dermatophytoses in veterinary staff. To protect people from occupational injuries, causes by biological factors, it is recommended to maintenance proper disinfection and sterilization procedures in workplaces where animals need adequate medical care. Also, there should be introduced a high-performance mechanical ventilation or air conditioning system, providing the appropriate microbiological quality of air. Monitoring the quality of air is also very important for assessment of the exposure to potentially pathogenic microorganisms.

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