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Bacteriological Air Quality at Animal Veterinary Practice

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1. Introduction

In recent years, biological factors are becoming a serious problem of occupational medicine and public health. Hazards caused by these factors are related to specific professions, as well as to the presence and properties of individual factors. The microorganisms present in the air, such as bacteria and fungi can cause adverse health effects (infections, immunotoxic diseases and allergies) (Thorne et al. 1992, Kalogerakis et al. 2005, Frączek et al. 2018). The disease effect, ensuing from the inhalation of various molecules in the air, depends primarily on their size, chemical composition, microbiological properties and the place of their depositing in the human respiratory tract (Kelly & Fussell 2012). Carrying out duties by vet or veterinary technician during contact with the animals and work with products of animal origin are considered to be related to exposure to harmful biological agents (Rim & Lim 2014). Despite the relatively low prevalence of serious zoonotic diseases, veterinarians must remain vigilant. Small animal veterinary practitioners must be aware of the risks of zoonotic diseases for their own safety. The reservoir for microorganisms that can cause disease are wild, farm and home animals. The cause of the disease may be contact with an sick animal as well as contact with its excretions and secretions, such as: faeces, urine, mucus or contact with material of animal origin (Weese et al. 2002).

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 small animal veterinary practice based on the number and species composition of the bacterial population.

2. Materials and methods

The study was carried out at the premises of the animal veterinary practice in Krakow (Poland) in 2017, in two measuring rounds, during the contractually agreed „summer" season (6-month period from April to September), with average outside air temperature above 10°C persisting for at least 7 days (in the summer period, in the temperate climate zone, a higher concentration of fungal aerosol is observed, both in the indoor and outdoor air). The veterinary practice takes care of pets, mainly dogs and cats. The tests were carried out at one veterinary practice, which was selected as a „model" object (on the basis of previous studies). The samples of air were collected in duplicate at four measuring points. A total of 96 air samples were analyzed. 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, a room with cages in which animals are housed after treatments and waiting room) inside the building. Air samples were collected before opening, five hours after opening and after the veterinary practice work. All studied rooms were naturally ventilated.

Additionally, the air samples were collected at a point situated outside the building (as the "background"). The air samples were collected by 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. Bacteria were collected on tryptic soy agar (TSA LAB-AGARTM, Biocorp), EMB medium (Biocorp) for Gram-negative bacteria and Chapman's medium (Biocorp) for mannitol-positive staphylococci. During sampling, the air temperature and relative humidity were measured using a hygrometer Kestrel 4000. The TSA plates were incubated for 24 hour at 37°C, then 3 days at 22°C and another 3 days at 4°C. The EMB and Chapman's plates were incubated for 24 hours at 37°C. The prolonged incubation of samples for culturing of bacteria or fungi enables the growth of slowly growing strains at a lower temperature range. After incubation, the bacterial colonies were counted. The concentration of bioaerosol was calculated as the number of colony forming units per cubic meter of air ($cfu \cdot m^{-3}$) (Wlazło et al. 2008, Bulski 2017, Frączek et al. 2018).

Due to the specificity of the studied environment, randomally selected bacterial strains, differing from each other macroscopically, were preliminary identified by Gram staining for their morphology 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 parametric distribution (Shapiro-Wilk test). The analysis of variance (one-way and two-way ANOVA) was performed and the significance of differences between means was verified by the Tukey's test ($\alpha = 0.05$). 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 Pearson's correlation.

3. Results

The concentrations of bacterial aerosol are presented in Table 1, Table 2 and Figure 1. Concentrations of total number of bacteria in the studied premises ranged from 256 to 1123 cfu·m^{-3}. The results showed that the highest average concentration value of bacterial aerosol was observed in the treatment room and the lowest concentration was observed in the waiting room. The analysis (one-way ANOVA) showed a significant differences in the concentrations of bacterial aerosol between the treatment room and outdoor air (Tukey's test: $p < 0.05$). The concentrations of bacterial aerosol were higher in the indoor air than in the outdoor air, but the differences between waiting room, room with cages and outdoor air were not statistically significant (Tukey's test: $p > 0.05$). The analysis of two-way ANOVA showed that the higher concentration of total number of bacteria in the studied rooms was observed in the treatment room after veterinary practice work and the lowest concentration was observed in the waiting room, five hours after opening. The analysis showed that there were no significant differences in the concentration of total number of bacteria in treatment room taking into account the measuring time (Tukey's test: $p > 0.05$). The same results were observed in waiting room. There were significant differences in concentration of total number of bacteria only in room with cages before opening and five hours after opening (Tukey's test: $p < 0.05$).

Concentrations of Gram-negative bacteria in the studied premises ranged from 0 to 398 cfu·m^{-3}. The results showed that the highest average concentration value of Gram-negative bacteria was observed in the treatment room and the lowest concentration was observed in the room with cages. The concentrations of Gram-negative bacteria aerosol were higher in the outdoor air than in the indoor air, but the analysis (one-way ANOVA) showed a non-significant differences in the concentrations of Gram-negative bacteria between indoor and outdoor air (Tukey's test: $p > 0.05$). The analysis of two-way ANOVA showed that the higher concentration of Gram-negative bacteria in the studied rooms was observed in the treatment room five hours after opening and the lowest concentration was observed in room with cages, after practice work. The analysis showed that there

were no significant differences in the concentration of Gram-negative bacteria in indoor air taking into account the measuring time (Tukey's test: $p > 0.05$).

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

Environment		Total number of bacteria		Gram-negative bacteria		Mannitol-positive staphylococci	
		Range	Median	Range	Median	Range	Median
Indoor air	Treatment room	567-1123	699	9-398	27	151-1404	474
	Room with cages	468-1016	628	0-27	9	196-804	469
	Waiting room	256-725	469	18-115	45	159-645	381
Outdoor air		397-496	447	9-239	124	159-447	318

Table 2. Average bacterial aerosol concentration (cfu·m^{-3}, ±SD) in indoor air at animal veterinary practice (two-way ANOVA)

Measuring time x Measuring point		Average±SD		
		Total number of bacteria	Gram-negative bacteria	Mannitol-positive staphylococci
Before opening	Treatment room	580ab*±18	27a±0	826b±817
	Room with cages	526a±82	14a±6	491a±331
	Waiting room	452a±125	111a±6	323a±232
Five hours after opening	Treatment room	699ab±25	208a±269	935b±335
	Room with cages	1003b±19	18a±13	500a±430
	Waiting room	415a±225	27a±13	495a±212
After work	Treatment room	1012b±157	18a±13	160a±13
	Room with cages	628ab±25	5a±6	354a±161
	Waiting room	562ab±231	36a±25	469a±88

* averages marked with the same letters are not significantly different by Tukey's test ($\alpha = 0.05$)

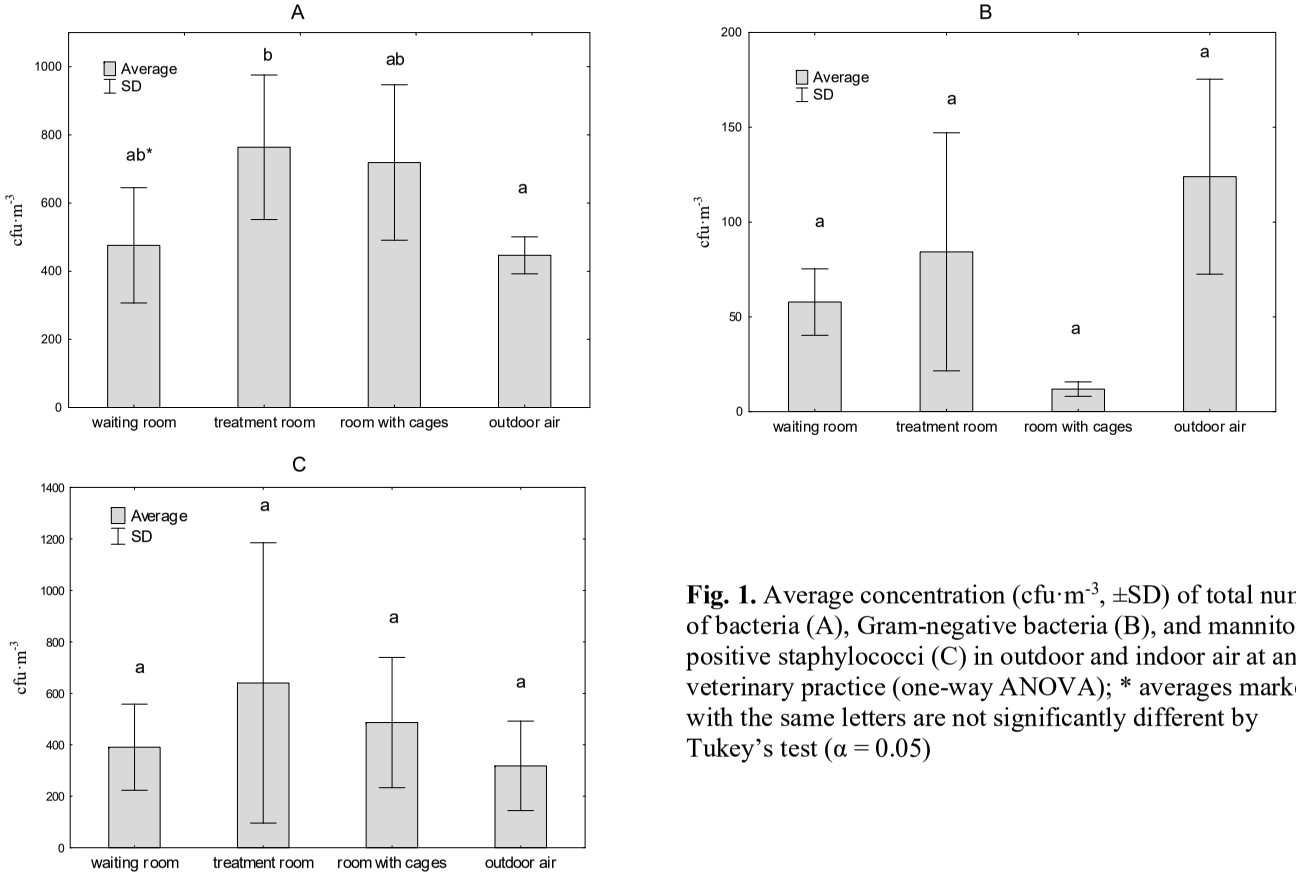

Fig. 1. Average concentration (cfu·m^{-3}, ±SD) of total number of bacteria (A), Gram-negative bacteria (B), and mannitol-positive staphylococci (C) in outdoor and indoor air at animal veterinary practice (one-way ANOVA); * averages marked with the same letters are not significantly different by Tukey's test ($\alpha = 0.05$)

Concentrations of mannitol-positive staphylococci in the studied premises ranged from 15 to 1404 cfu·m^{-3}. The results showed that the highest average concentration value of mannitol-positive staphylococci was observed in the treatment room and the lowest concentration was observed in the waiting room. The concentrations of mannitol-positive staphylococci aerosol were higher in the indoor air than in the outdoor air, but the analysis (one-way ANOVA) showed a non-significant differences in the concentrations of staphylococci between indoor and outdoor air (Tukey's test: $p > 0.05$). The analysis of two-way ANOVA showed that the higher concentration of mannitol-positive staphylococci in the studied rooms was observed in the treatment room five hours after opening and the lowest concentration was observed also in treatment room but after practice work. The analysis showed that the concentrations of mannitol-positive staphylococci in treatment room before opening and five hours after opening were significantly higher than concentrations in treatment room after veterinary practice work (Tukey's test: $p < 0.05$).

Microclimate conditions may affect the number of microorganisms and their spread in the air (Katial et al. 1997). Results of microclimate parameters measurments are presented in Table 3. Analysis of the impact of the temperature and relative humidity on the observed bacterial aerosol showed a significant correlation between the concentration of total number of bacteria and temperature ($R = 0.45$, $p < 0.05$) and relative humidity ($R = 0.37$, $p < 0.05$). There were no significant correlations between the concentrations of Gram-negative bacteria or mannitol-positive staphylococci and studied microclimate parameters ($p > 0.05$).

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
Outdoor air		20.2-21.8	20.7	50.3-62.6	58.2

By using a 6-stage Andersen's air sampler, it was possibile to get information about the size distribution of air bacterial biota in the investigated measuring points at the small animal veterinary practice (Figure 2 A-C). Based on the analysis of bioaerosol particle size distribution it was observed that in the treatment room the bacteria concentration had a maximum value in a range of diameters 2.1-3.3 μm. It shows that these microorganisms were present in the air as single cells and small aggregates and can be deposited in the human respiratory

tract in primary bronchi. In the treatment room, Gram-negative bacteria concentration had a maximum value in a range of diameters 0.65-1.1 μm, so these microorganisms were present in the air as a single cells (and can be deposited in the human respiratory tract in secondary bronchi and brionchioles). Mannitol-positive staphylococci concentration had a maximum value in a range of diameters 4.7-7.0 μm (medium aggregates), so they can be deposited in the human respiratory tract in throat and trachea (Owen & Ensor 1992, Wlazło et al. 2008). In room with cages for animals, the total number of bacteria and Gram-negative bacteria concentration had a maximum value in a range of diameters 1.1-2.1 μm. Mannitol-positive staphylococci concentration had a maximum value in a range of diameters 4.7-7.0 μm. The analysis showed that in the waiting room the total number of bacteria concentration had a maximum value in a range of diameters 4.7-7.0 μm, Gram-negative in a range of diameters 1.1-2.1 μm and the mannitol-positive staphylococci concentration had a maximum value in a range of diameters 3.3-4.7 μm.

The percentage shares of identified bacteria in the examined veterinary practice are presented in Figure 3 (A-B). A total of 11 species of bacteria have been identified. In the indoor air, the predominant groups of microorganisms were rods of the *Bacillus* genus and Gram-positive cocci from the *Micrococcus* and *Staphylococcus* genus. *Bacillus thuringiensis*, *Staphylococcus saprophyticus*, *Micrococcus luteus*, *Staphylococcus Arletta*, *Bacillus jeotgali*, *Bacillus pumilus*, *Bacillus vallismortis*, *Paenibacillus amylolyticus*, *Lysinibacillus fusiformis*, *Enterococcus faecium* and *Pseudomonas putida* were isolated. From the outdoor air only 3 bacterial species were isolated (*Bacillus thuringiensis*, *Micrococcus luteus* and *Pseudomonas putida*).

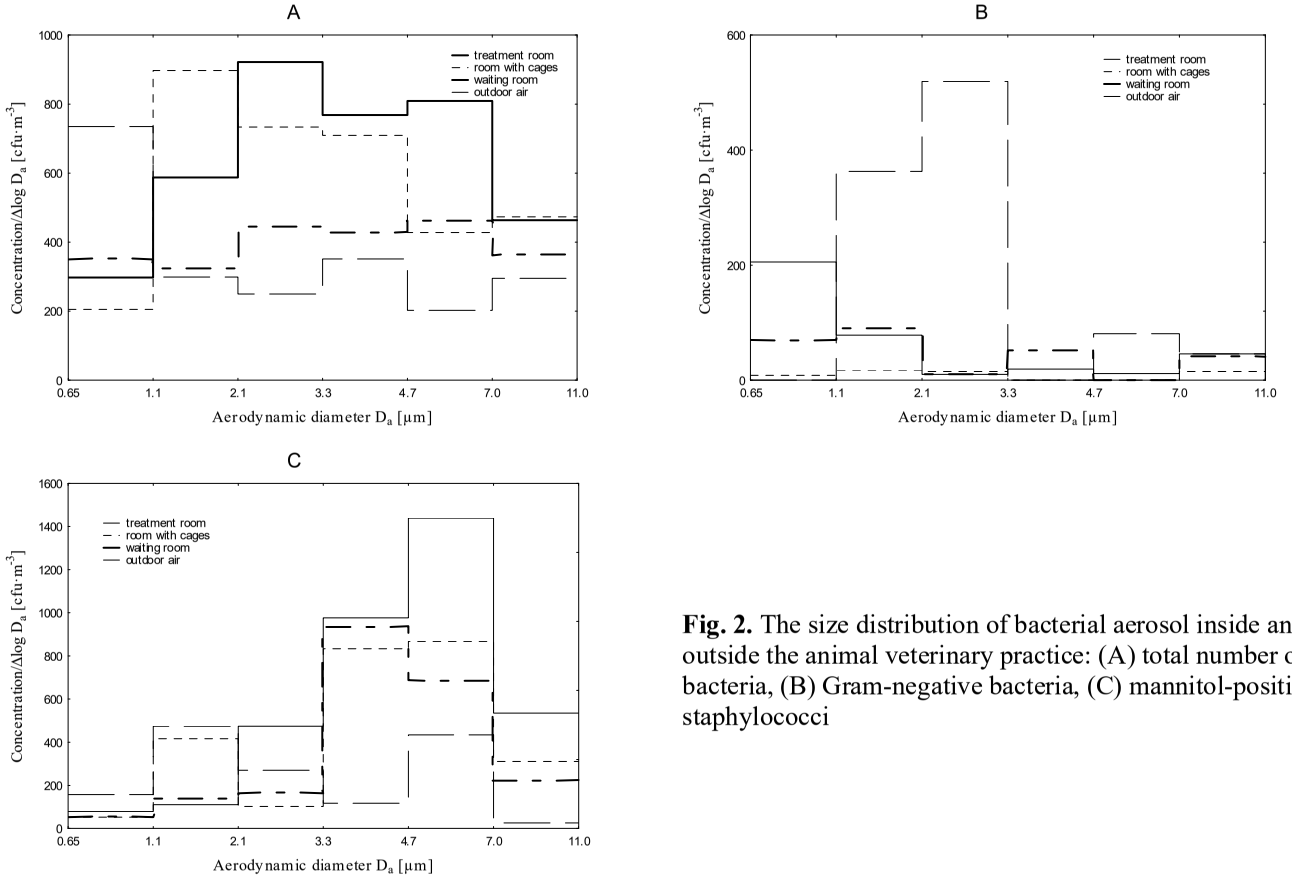

Fig. 2. The size distribution of bacterial aerosol inside and outside the animal veterinary practice: (A) total number of bacteria, (B) Gram-negative bacteria, (C) mannitol-positive staphylococci

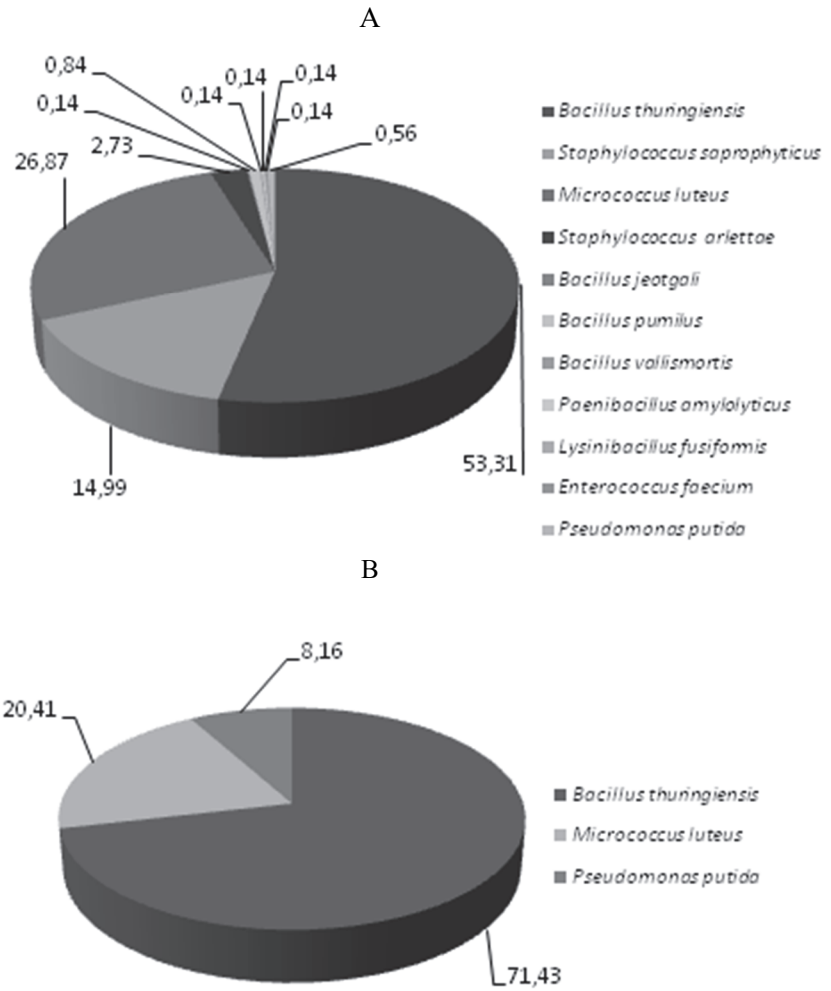

Fig. 3. The results of the identification of bacteria (%) isolated from the air at the studied animal veterinary practice: (A) indoor air, (B) outdoor air

4. Discussion

Occupational exposure to zoonotic diseases is an ingerent risk in veterinary medicine (Weese et al. 2002). In these studies an assessment of the microbiological quality of air in a small animal veterinary practice was made. Concentrations of total number of bacteria in the studied premises ranged from 256 to 1123 cfu·m^{-3}. The obtained results of indoor measurements of bioaerosol concentrations were compared with the Polish proposals for threshold limit values, which are $5 \cdot 10^3$ cfu·m^{-3} for bacteria in indoor and outdoor environments. It was found that the average concentrations of total number of bacteria obtained in this study were lower than reference values for total number of bacteria in residential and public buildings recommended by the Polish Panel of Experts of Biological Factors (Górny 2010). Low bioaerosol concentrations and similar results were obtained in studies in small animals veterinary hospitals (Harper et al. 2013). The inhalation of airborne Gram-negative bacteria can cause respiratory diseases (for example a chronic lungfunction impairment) (Zucker et al. 2000). In indoor air, the highest concentration of Gram-negative bacteria was observed in treatment room. The average concentrations of these microorganisms were also lower than reference values for Gram-negative bacteria in residential and public buildings ($2 \cdot 10^2$ cfu·m^{-3}) (Górny 2010). Because exsposure to endotoxins is associated with respiratory and systemic pathologies, endotoxin measurements should be carried out in future research (Samadi et al. 2010, Dequenne et al. 2013). In the indoor air, one of the predominant groups of microorganisms were Gram-positive cocci from the *Micrococcus* and *Staphylococcus* genus. It confirms the results obtained in bioaerosol studies in veterinary teaching hospitals in Taiwan (Chen et al. 2017). *Staphylococcus* was the third most frequently isolated genus from the air. It should be emphasized that the environment, such as small animal veterinary practice, may be an important source of MRSA infection (Hanselman et al. 2008). Some of the isolated bacteria such as *Pseudomonas putida* or *Staphylococcus arlettae* can be potentially pathogenic for human (Yang et al. 1996, Dinakaran et al. 2012). In the indoor and outdoor air the dominant bacterial species was *Bacillus thuringiensis*. Bacteria of the genus Bacillus occurs mainly in the external environment (eg soil, plants) and can be transmitted by people into the interior of the rooms (eg on clothes or footwear) (Wlazło et al. 2008). Statistical analysis showed significantly higher airborne microbial contamination in different measuring points at different measuring times of the day. Taking into account the normal size of one of the dominant group of bacteria (Gram-positive cocci), with aerodynamic diameters in the 1.1-2.1 μm range, it was found that the analysis of bioaerosol particle size distribution indicates additional emission of Gram-positive cocci from their main reservoir, which is the human body (increased emission during intense breathing and abrasion of the epidermis during work). Based on

the results of this study it was found that the largest "load" of bacteria, isolated from the air, can reach (in the human respiratory system) to the region of the nasal and oral cavity, throat and trachea, as well as bronchioles (Owen & Ensor 1992). This information is very important for the assessment of the effects of biological aerosols on the human body (the place of deposition of a harmful factor usually determines the type of adverse health response). Analysis of the impact of the temperature and relative humidity on the observed bacterial aerosol showed that the increase in temperature and relative humidity affects the increase in concentration of bacteria in tested air at small animal veterinary practice, which is consistent with the observations of other authors (Li & Kendrick 1995).

5. Conclusions

Concentrations of bacterial aerosol between the internal studied rooms at the veterinary practice were not significantly different and were always lower than 1123 cfu·m^{-3}. The highest concentrations of total number of bacteria in the studied rooms was observed in the treatment room after veterinary practice work. However, the results of this study showed the possible biological risks for the veterinary workers or clients of small animal veterinary practice. Although the concentrations of bacterial aerosol were not exceed the proposals of limit values, it was found that among the detected bacteria pathogenic species as: *Pseudomonas putida* and *Staphylococcus arlettae* were present. To protect people from occupational injuries it is recommended to maintenance proper disinfection and sterilization procedures in workplaces with health education to the animal care workers. Therefore, there should be introduced a high-performance mechanical ventilation or air conditioning system, providing the appropriate microbiological quality of air in rooms where animals need adequate medical care.

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Abstract

Due to the nature of the work (animal treatment and care of animals) the environment of small animal veterinary practice can be contaminated by microorganisms. Therefore, veterinary practices or hospitals are facilities, where veterinarians, clients and animals can be exposed to biological agents. The objective of the study was to characterize the bacteriological quality of air in small animal veterinary practice in Krakow. Bioaerosol measurements were performed during the summer season of 2017. The samples of outdoor and indoor air at small animal veterinary practice were analyzed, using a 6-stage Andersen`s air sampler. The highest concentration of bacterial aerosol was observed in the treatment room. There were statistically significant differences in the concentrations of bacterial aerosol between indoor and outdoor air. Also, the analysis showed that there were significantly higher airborne microbial loads in different rooms at different measuring times of the day. Based on the analysis of bioaerosol particle size distribution it was found that the largest "load" of bacteria, isolated form the air, can reach (in the human respiratory system) to the region of the nasal and oral cavity, throat and trachea, as well as bronchioles. In the indoor air, the predominant groups of microorganisms were rods of the *Bacillus* genus and Gram-positive cocci from the *Micrococcus* and *Staphylococcus* genus. The study confirmed that the small animal veterinary practice can be a workplace related to exposure to microbial agents.

Keywords:
bioaerosol, bacteria, air, animal veterinary practice

Bakteriologiczna jakość powietrza w gabinecie weterynaryjnym

Streszczenie

Ze względu na specyfikę pracy (leczenie oraz opieka nad zwierzętami) środowisko gabinetu weterynaryjnego może być zanieczyszczone mikrobiologicznie. Gabinety czy szpitale weterynaryjne są placówkami, w których weterynarze, klienci i zwierzęta mogą być narażeni na działanie czynników biologicznych. Celem badań była charakterystyka bakteriologicznej jakości powietrza w gabinecie weterynaryjnym w Krakowie. Pomiary bioaerozolu wykonano w okresie lata w 2017 r. Próbki powietrza zewnętrznego oraz wewnętrznego w gabinecie weterynaryjnym pobierano przy pomocy 6-stopniowego impaktora Andersena. Najwyższe stężenie aerozolu bakteryjnego obserwowano w pokoju zabiegowym. Odnotowano istotne statystycznie różnice w stężeniach aerozolu bakteryjnego pomiędzy powietrzem wewnętrznym, a tłem zewnętrznym. Analiza wykazała również istotnie wyższe stężenia mikroorganizmów w zależności od punktu pomiarowego i pory dnia. Analizując rozkłady ziarnowe bioaerozolu stwierdzono, że najwyższy „ładunek" bakterii może dotrzeć (w układzie oddechowym człowieka) do rejonu jamy nosowej

i jamy ustnej, gardła i tchawicy, a także oskrzelików. W powietrzu wewnętrznym dominującymi grupami mikroorganizmów były laseczki z rodzaju *Bacillus* i Gram-dodatnie ziarniaki z rodzaju *Micrococcus* i *Staphylococcus*. Badanie potwierdziło, że gabinet weterynaryjny może być miejscem pracy związanym z narażeniem na czynniki mikrobiologiczne.

Słowa kluczowe:
bioaerozol, bakterie, powietrze, gabinet weterynaryjny