

Organic dust exposure in veterinary clinics: a case study of a small-animal practice in Portugal

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Literature about occupational health in small-animal veterinary practices is scarce, but most of it has recognised a number of risks to be considered, including organic dust exposure. The aim of this pilot study was to assess organic dust, bacterial, and fungal contamination in the indoor environment of a typical Portuguese veterinary clinic but also to screen for azole-resistant fungi. To complement these findings we also analysed workers' nasal exudates for resistant bacteria. Particles measurements included mass concentrations (PMC) of five particle sizes (PM_{0.5}, PM₁, PM_{2.5}, PM₅, PM₁₀) and their counts (PNC). Indoor air samples were obtained from six locations as well as before and during cat dental cleaning and cultured on four media for bacterial and fungal assessment. An outdoor sample was also collected for reference. Surface samples were taken from the same indoor locations using swabs and we also use electrostatic dust cloths as passive methods. PM₁₀ showed the highest concentrations across the locations. Indoor air fungal loads ranged from 88 to 504 CFU m⁻³. The azole-resistant *Aspergillus* section *Nigri* was identified in one sample. Indoor air bacterial loads ranged from 84 to 328 CFU m⁻³. Nasopharyngeal findings in the 14 veterinary clinic workers showed a remarkably low prevalence of *Staphylococcus aureus* (7.1 %). Our results point to contamination with organic dusts above the WHO limits and to the need for better ventilation. Future studies should combine the same sampling protocol (active and passive methods) with molecular tools to obtain more accurate risk characterisation. In terms of prevention, animals should be caged in rooms separate from where procedures take place, and worker protection should be observed at all times.

KEY WORDS: *Aspergillus*; azole resistance; bioburden; particles; *Staphylococcus aureus*

Literature about occupational health in small-animal veterinary practices is scarce, but most of it has recognised a number of risks to be considered, including organic dust exposure (1-3). The most common bioburden present in the organic dust reported in veterinary clinics includes the bacteria of the *Acinetobacter* spp., *Pseudomonas* spp., and *Staphylococcus* spp., and the fungal *Aspergillus* spp. and *Penicillium* spp. (4).

The nasal cavity is the point of entry for inspired air and therefore the first to come into contact with bioburden (5). One way to assess exposure risks is to determine the bioburden in the nasal exudate, as it reflects the bioburden of a specific occupational environment. In addition, nasopharyngeal microbiota are the first to get colonised by *Staphylococcus aureus* (6), infamous for its resistance to antibiotics, methicillin in particular (hence the name methicillin-resistant *S. aureus* or MRSA). If animals such

as dogs and cats happen to be carriers of *S. aureus*, veterinarians run a real risk of occupational exposure (7).

Resistance to azoles has also been reported for several fungal strains present in animals and their environment (8, 9), which only adds to the health risks veterinarians face and should also be addressed in occupational exposure assessments to fungal burden (10-12).

The aim of this study was to assess organic dust, bacterial, and fungal load in the indoor environment of a typical Portuguese veterinary clinic but also to screen for azole-resistant fungi. To complement these findings we also analysed the workers' nasal exudates for resistant bacteria.

MATERIALS AND METHODS

The measurements and samples were taken from a typical veterinary clinic in Lisbon, Portugal in October 2017. Measurements were taken from the places where the workers spent most of their time at work (see Table 1 for details). Cat dental cleaning (plaque removal) was also assessed separately in the treatment room, since the

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procedure was performed frequently and involved a higher risk of exposure to bioburden through aerosols. Ventilation at the clinic combined mechanical and natural, with a minimum of three to six air changes per hour. Some of the clinical staff used surgical masks during dental cleaning and other surgical procedures.

Particles in five sizes ($PM_{0.5}$, PM_1 , $PM_{2.5}$, PM_5 , and PM_{10}) were measured with direct-reading handheld particle counter (model 3016/5016; Lighthouse, Fremont, CA, USA) that gives information about mass concentrations ($mg\ m^{-3}$) (PMC). The same equipment was also used to count the number of particles (PNC) by six diameter sizes, namely 0.3 μm , 0.5 μm , 1 μm , 2.5 μm , 5 μm , and 10 μm . Particles were measured in each location and also before and at the beginning of cat dental cleaning, which adds up to nine separate measurements of PMC and PNC.

All air sampling and measurements were taken at the height of the worker's nose while they performed their duties. Air measurements related to the cat dental cleaning were taken in the treatment room before and at the beginning of the procedure to evaluate contamination with particles, since the veterinarian surgeon wore only the mask and no other protection was in place. All measurements had the duration of five minutes.

For active sampling we took six 250 L air samples (one from each site) (Table 1) at a flow rate of 140 L min^{-1} and the height of 1 m onto four culture media (2 % malt extract agar (MEA) (Frilabo, Lisbon, Portugal) with 0.05 g/L chloramphenicol media; dichloran glycerol (DG18) (Frilabo, Lisbon, Portugal) agar based media; tryptic soy agar (TSA) with 0.2 % nystatin (Frilabo, Lisbon, Portugal); and violet red bile agar (VRBA) (Frilabo, Lisbon, Portugal) for detection and enumeration of lactose-fermenting coliform microorganisms). We also took an outdoor sample for reference and as a potential contamination source.

Passive sampling included swabbing the floors of the same indoor locations as described elsewhere (13) (Table 1).

At the locations where we expected higher bioburden we also used the passive sampling method with electrostatic

dust cloths (EDC) following the procedure described elsewhere (11, 14).

The prevalence of azole-resistant fungi was determined for all EDC samples in azole-supplemented media by seeding 150 μL of the wash suspension on Saboraud agar supplemented with 4 $mg\ L^{-1}$ itraconazole, 1 $mg\ L^{-1}$ voriconazole, or 0.5 $mg\ L^{-1}$ posaconazole according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (15).

After incubation on the MEA, DG18, and azole-supplemented media plates at 27 °C for five to seven days for fungi and on the TSA and VRBA media at 30 °C and 35 °C for seven days for mesophilic bacteria and coliforms, were calculated bioburden densities per volume or per area using the colony-forming units ($CFU\ m^{-3}$ and $CFU\ m^{-2}$, respectively). Fungal species were identified microscopically using tease mount or Scotch tape mount and lactophenol cotton blue mount procedures. Morphological identification was achieved through macro and microscopic characteristics (16).

Participants and nasal sampling

Before enrolment, all participants were informed about the scope and aim of this study and signed a consent form in full accordance with the Declaration of Helsinki (17).

Nasopharyngeal samples were taken from 14 volunteers (all day shift workers) with sterilised cotton swabs (Frilabo, Lisbon, Portugal), which were immediately taken to the laboratory for analysis. The swabs were rotated several times against the internal anterior walls of the nostril along the septum up to 2.5 cm from the entry (that is, up to where we encountered a slight resistance) and then placed in a transport tube with a Stuart transport medium (Frilabo, Lisbon, Portugal). Further inoculation procedure was the same as for the surface swabs taken from the floors.

For the identification of *S. aureus* in the microbiology laboratory, biological samples were immediately cultured on flat Petri dishes containing Columbia agar with 5 % sheep blood (Frilabo, Lisbon, Portugal) and incubated at 37 °C for 24 h. After incubation, the obtained colonies were

Table 1 Number of samples collected in each sampling site

Sampling site/Task	N of air samples (impaction)*	N of surfaces samples (floors)	N of EDC
Reception area	1	1	
Examination room	1	1	
Treatment room	1	1	1
Imaging room	1	1	1
Chemotherapy room	1	1	1
Canteen	1	1	
Cat dental cleaning procedure	2#	2#	
Outdoor	1		

* In each working site were taken 4 air samples (one for each media - MEA, DG18, TSA and VRBA)

Sampled before and during the activity

evaluated based on cultural characteristics (non haemolytic and/or with B type haemolysis, white and/or deep golden-yellow, catalase positive colonies) and the suspicious ones isolated in the same culture medium and incubated for at 37 °C for another 24 h. *S. aureus* was identified with Slidex Staph Kit (Thermo Fisher Scientific, Waltham, MA, USA). MRSA strains were identified with a Slidex MRSA detection Test Kit (Biomerieux, Lisbon, Portugal; ref. no. 73117). For comparison we used MRSA-positive (MRSA laboratory collection) and negative strains (*S. aureus* ATCC 25923).

To control our findings we also took nasal swabs from healthy individuals uninvolved in veterinary practice using the same procedures as for fungal (38 individuals) and bacterial assessment (30 individuals).

Statistical analysis

The data were analysed with the statistical software IBM SPSS Statistics version 24.0 for Windows, (IBM, NY, United States). Univariate descriptive statistics included frequency (n; %) and medians. The results were considered significant at the 5 % level. Since the Shapiro-Wilk test did not confirm normal distribution, PMCs and PNCs were compared between the locations using the Friedman test, and when statistically significant differences were detected we used the Friedman multiple comparison test.

To study the relationship between PMCs and the concentrations of the fungi (MEA and DG18) and bacteria (TSA and VRBA) we used Spearman's correlation coefficient, because the assumption of normality was not verified.

RESULTS

PMC and PNC

Figure 1 shows PMC and PNC distribution by locations and particle sizes. The highest PMCs were measured in the treatment room, and PM₁₀ significantly dominated across all particle sizes. As for PNCs, the treatment room again

showed the highest counts, and particles with the diameter <0.3 µm significantly dominated across all sizes.

As expected, cat dental cleaning led to an increase in PNC in the treatment room, particularly of smaller particles (below 5.0 µm) (Figure 2), which is probably related to abrasion resulting in high particle emission.

Fungal loads

Figure 3A shows that the fungal load in indoor air ranged from 88 to 504 CFU m⁻³ on MEA and was the highest in the examination room. However, what raises greater concern is that five of six sampling locations had fungal loads higher than the WHO limit (150 CFU m⁻³) (21). Similar results were obtained for fungi incubated on DG18 and ranged from 124 to 512 CFU m⁻³. Again, the examination room had the highest load and five of six locations exceeded the WHO limit. As the outdoor location was overloaded with the Mucorales order and *Penicillium* spp., it was impossible to count colonies and compare it with indoor loads (data not shown). Figure 3B shows the fungal contamination in surface samples by locations.

Eight fungal species were found in the air samples on MEA and seven on DG18. *Penicillium* spp. was the most prevalent in both media (90.7 % MEA; 47.3 % DG18), followed by the *Cladosporium* spp. on MEA and *Paecilomyces variotii* on DG18. Besides the most common *Fusarium graminearum* complex, the *Aspergillus* sections *Circumdati* and *Nigri*, *Aureobasidium* spp., *Chrysosporium* spp. and *Chrysonilia* spp. on MEA and *Alternaria* spp., *Aspergillus* section *Circumdati*, *Chrysosporium* spp., and *Chrysonilia* spp. on DG18 were also found in lower counts (Table 2).

In the surface samples on MEA, the *Cladosporium* genus was the most prevalent (90.7 %), followed by the *Penicillium* spp. and *Trichothecium roseum*. EDC sampling revealed the presence of *Fusarium equisetii* and the *Cladosporium* spp. on MEA and DG18, respectively (Table 2).

During dental cleaning the air fungal load dropped from 32 CFU m⁻³ (measured before) to 20 CFU m⁻³ on MEA and from 80 (before) to 20 CFU m⁻³ on DG18 (Figure 3A). The

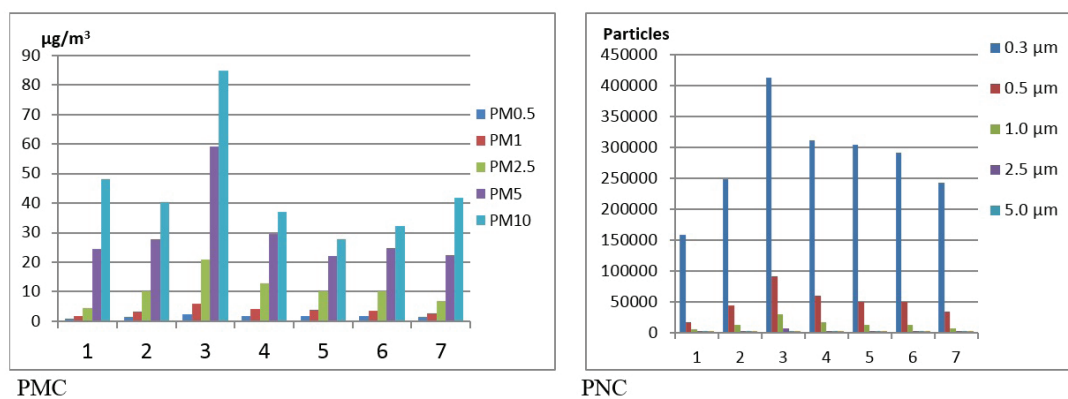


Figure 1 Particle mass concentration (PMC) and particle number count (PNC) distribution by location. Measurement locations: 1 – reception area; 2 – examination room; 3 – treatment room; 4 – imaging room; 5 – chemotherapy room; 6 – equipment cleaning room; 7 – canteen

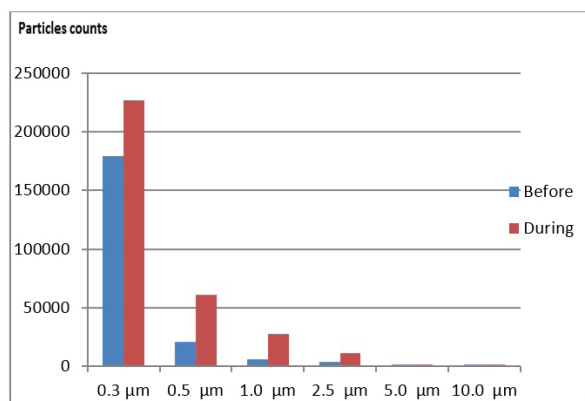


Figure 2 Particle counts by size before and during dental cleaning

Cladosporium spp. prevailed (75 % on MEA; 80 % on DG18) before and during the procedure (80 % on MEA; 60 % on DG18). On the surfaces we found only the *Penicillium* spp. before the procedure in both media and only the *Cladosporium* spp. on DG18 during the procedure. The azole-resistant *Aspergillus* section *Nigri* (100 CFU m⁻² of EDC) non-susceptible to 1 mg L⁻¹ of voriconazole was identified in one sample taken from the treatment room (location 3).

Bacterial loads

Outdoor air loads (216 CFU m⁻³) were higher than in the canteen and the imaging and chemotherapy rooms and had no Gram-negative bacteria.

Figure 4A shows the air bacterial load by locations. Total mesophilic Gram-positive bacterial loads ranged from 84 to 416 CFU m⁻³ and were the highest in the treatment room. Gram-negative bacterial load in the air was only found in the reception area and during dental cleaning. Similar to fungal loads, dental cleaning reduced total bacterial load (Figure 4A).

The total mesophilic bacterial contamination on the surface samples ranged from 2×10⁴ to 388×10⁴ CFU m⁻² and was the highest in the reception area. Gram-negative bacteria ranged between 2×10⁴ and 4×10⁴ CFU m⁻², but there were none in the treatment and chemotherapy rooms or during dental cleaning (Figure 4B).

The only significant correlation between PMCs and fungal or bacterial loads was between PM₁₀ and the bacteria load on TSA (r_s=0.886, p=0.019) (Table 3).

Nasal findings

Of all the samples taken from the veterinary clinic staff (n=14) only those inoculated on DG18 showed fungal isolates and only the *Cladosporium* genus in eight people. For comparison, of the 38 controls, in 17 we isolated *Penicillium* spp., in eight *Cladosporium* spp., in one *Mucor* spp., and in one *Geotrichum* spp. None of them had more than one isolate.

Total Gram-positive bacteria were found in the samples of 12 out of 14 staff and Gram-negative bacteria only in three.

All participants (veterinary staff and controls) had normal commensal flora, namely *Staphylococcus* spp. and *Streptococcus* spp., but the prevalence of *S. aureus* in the veterinary staff was particularly low (1 of 14) in comparison to controls (10 of 30). The identified *S. aureus* strain from the veterinary clinic sample was susceptible to methicillin (MSSA), whereas in the control group three participants had the methicillin-resistant type (MRSA).

DISCUSSION

Airborne particles can carry contaminants such as bioburden and chemicals (18), and the smaller ones can reach the alveoli and act in the entire organism, depending on their chemical and biological composition (19). Our PMC results have shown that particles tend to agglomerate, and that explains higher PM₁₀ results. Additionally, we need to consider that larger particles, even at lower counts, can result in higher mass concentration.

Higher PMCs and PNCs in the treatment room are quite likely related to the higher number of workers and animals occupying the area for a longer time than in the areas where animals stay for a short while (such as the reception and examination room) or not at all (canteen). In addition, the increase in PNC during dental cleaning in the treatment room confirms that particle contamination also depends on activity (18).

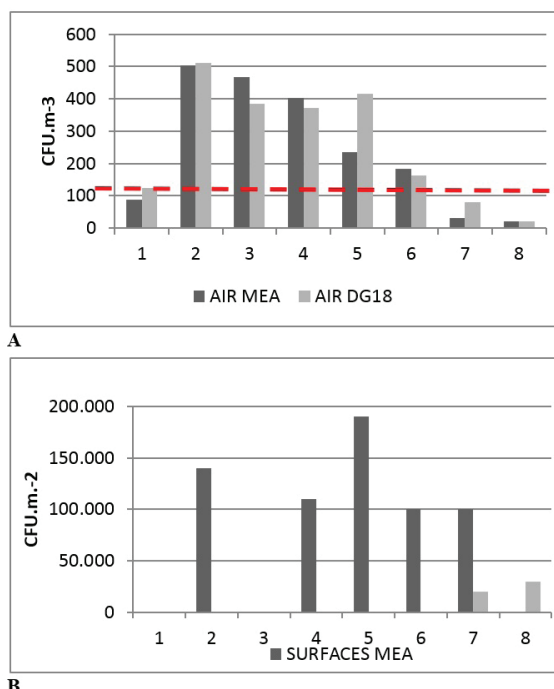


Figure 3 Fungal load in air (A) and surface (B) samples. Dashes denote the WHO limit (21). Measurement locations: 1 – reception area; 2 – examination room; 3 – treatment room; 4 – imaging room; 5 – chemotherapy room; 6 – canteen; 7 – before cat dental cleaning; 8 – during cat dental cleaning

Table 2 Fungal distribution in environmental samples after inoculation onto MEA and DG18

MEA		DG18	
Air	(CFU m ⁻³) (%; n)	Air	(CFU m ⁻³) (%; n)
<i>Penicillium</i> spp.	90.7; 1676	<i>Penicillium</i> spp.	47.3; 932
<i>Cladosporium</i> spp.	6.1; 2700	<i>Paecilomyces variotii</i>	41.8; 824
Others	3.2; 60	Others	10.9; 216
Surfaces	(CFU m ⁻²) (%; n)	Surfaces	(CFU m ⁻²) (%; n)
<i>Cladosporium</i> spp.	90.7; 4.9x10 ⁵	-	-
<i>Penicillium</i> spp.	5.6; 3x10 ⁴	-	-
<i>Trichothecium roseum</i>	3.7; 2x10 ⁴	-	-
EDC	(CFU m ⁻²) (%; n)	EDC	(CFU m ⁻²) (%; n)
<i>Fusarium equisetii</i>	100; 50	<i>Cladosporium</i> spp.	100; 50

Our sampling approach followed the current scientific trend – parallel use of active and passive methods – to ensure a more precise assessment of occupational exposure to bioburden (20), since each technique has unique advantages and disadvantages. Active methods provide information about the bioburden load, while passive methods such as surface swabs and EDC provide a more detailed scenario regarding occupational exposure to bioburden (11-14).

The bioburden was characterised with culture-based methods. As viable bioaerosol particles constitute a small percentage of the total bioburden (21), to properly interpret the obtained results, one needs to take into account bias as well as seasonality (3). Culture-based methods provide information about viable bioburden and potential infection (22). By combining them with molecular tools that target specific harmful species we can overcome their limitations (23). Air fungal load followed the same trend as PMC and PNC, with the exception of the examination room, which had a higher load. Higher loads in the treatment, imaging, and chemotherapy room were expected, as these are more frequented by staff and animals as carriers of fungi (19).

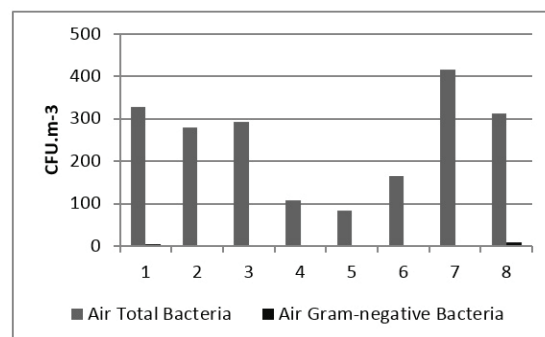
Only the reception area did not surpass the WHO guideline (24), even with the door kept closed. In addition, almost all species of the *Aspergillus* genus have a toxigenic potential (25).

The identification of fungal species only in the samples collected with the passive methods, namely *Trichothecium roseum* (on surface swabs) and *Fusarium equisetii* (on EDC), reinforces the need to apply different sampling methods to get a more complete picture of bioburden exposure (14, 20). Along the same lines, different findings with MEA and DG18 clearly show that using both media will give a more accurate assessment of fungal exposure. DG18 restricts the colony size of fast-growing genera, allowing a more complete and accurate characterisation of fungal contamination (14).

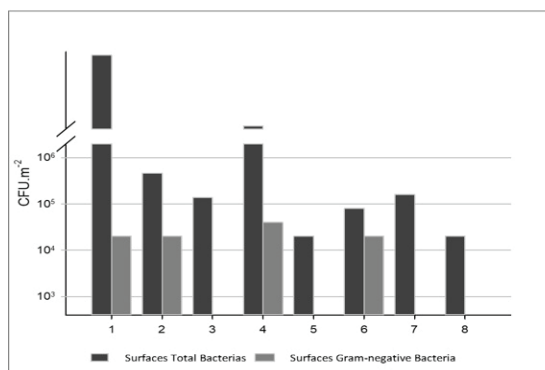
The reception area showed a higher bacterial load, probably due to the bacteria present on the skin, mouth, mucous membrane or hair of animals and their owners and also staff circulating in a small area (26, 27), but also due to competition reasons between microorganisms (28), since

fungi followed the opposite tendency regarding load. The mean bacterial load (248 CFU m⁻³) was similar to other reports (25), whereas the Gram-negative bacterial load was lower than reported elsewhere (26, 29).

Higher bacterial loads may also depend on the type of activity, as demonstrated by the results obtained before and during dental cleaning (27). The correlation found between PM₁₀ and bacterial load on TSA corroborates this, since particles can act as a vehicle of bioburden dispersion (18). However, the fungal and bacterial loads were lower during dental cleaning even though particle counts were higher. This could be explained by the fact that aerosols released comprise mainly nonviable bioburden, whereas culture-



A



B

Figure 4 Bacterial load in air (A) and surface (B) samples. Measurement locations: 1 – reception area; 2 – examination room; 3 – treatment room; 4 – imaging room; 5 – chemotherapy room; 6 – canteen; 7 – before cat dental cleaning; 8 – during cat dental cleaning

Table 3 Relationship between the PMCs and fungal and bacterial loads

	Fungi (MEA) CFU m ⁻³	Fungi (DG18) CFU m ⁻³	Bacteria (TSA) CFU m ⁻³	Bacteria (VRBA) CFU m ⁻³
PM _{0.5}	0.657	0.486	-0.314	-0.655
PM ₁	0.657	0.486	-0.314	-0.655
PM _{2.5}	0.657	0.486	-0.314	-0.655
PM ₅	0.600	0.086	0.429	-0.131
PM ₁₀	-0.086	-0.429	0.886*	0.393

* $p < 0.05$ (2-tailed)

based methods applied in this study measure only the viable part and yield underestimations (21).

We found azole resistance in one environmental sample with one isolate of the *Aspergillus* section *Nigri* non-susceptible to 1 mg L⁻¹ voriconazole. It is a common clinical isolate in humans and animals. The susceptibility of these cryptic species to antifungal treatment is not known (9), but a recent study suggests intrinsic resistance (29).

Depending on the species, fungal spores can remain aerosolised for a long time (30), and this may explain the prevalence of *Cladosporium* spp. in the nasal samples taken from the staff.

Previous studies have reported a prevalence of 31–33.1 % for *S. aureus*, and a prevalence of 2–8 % for MRSA asymptomatic carriers in the community (31), with community prevalence of MRSA reaching 24.7 % in some contexts (32). Our control findings are in line with the literature considering both *S. aureus* (33.3 %) and MRSA (10 %). Veterinary biological samples demonstrated a remarkably low prevalence of *S. aureus* (7.1 %). These results may be due to the strictly implemented hygiene measures (33) and the use of individual masks during clinical treatment. Since *S. aureus* colonisation may be transient (34), it would be important to follow up the workers in order to evaluate potential persistent and transient carriers.

Nasopharyngeal findings have already proven their worth as indirect measures of bioburden exposure (3, 35). Although our findings of MRSA asymptomatic carriers did not corroborate this public health concern we need to pay special attention to workers in direct contact with animals (36).

To conclude, this pilot is the first study of occupational exposure to organic dust in a veterinary clinic. Our results point to contamination with organic dusts generally above the WHO limits, and to the need of better ventilation. Future studies should combine the same sampling protocol (active and passive methods) with molecular tools to obtain more accurate risk profiles. In terms of prevention, animals should be caged in rooms separate from where procedures take place, and worker protection should be observed at all times.

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Pilot-istraživanje profesionalne izloženosti organskoj prašini u veterinarskim klinikama za male životinje u Portugalu

Literature o profesionalnom bolestima u veterinarskim stanicama za male životinje iznimno je malo, ali upućuje na niz zdravstvenih rizika koji valja uzeti u obzir, uključujući izloženost organskoj prašini. Cilj je ovoga pilot-istraživanja bio ocijeniti opterećenje organskom prašinom, bakterijama i gljivama u prostorijama jedne tipične portugalske veterinarske stanice, ali i utvrditi postoji li među gljivama rezistentnih na azole. Ove smo nalaze nadopunili nalazima rezistentnih bakterija u nosnom brisu. Mjerena je masena koncentracija čestica (PMC) u pet veličina ($PM_{0.5}$, PM_1 , $PM_{2.5}$, PM_5 , PM_{10}), kao i njihov broj (PNC). Uzorci zraka uzeti su sa šest mjernih mjesta te prije i tijekom postupka čišćenja zuba mačaka na obradi. Uzorci su kultivirani na četirima medijima za bakterije i gljive. Za usporedbu je uzet i jedan uzorak vanjskoga zraka. Osim toga, uzorkovane su i površine na istim mjernim mjestima uzimanjem brisova i elektrostatskim krpama za prašinu. Čestice PM_{10} iskazale su najveće koncentracije na svim mjernim mjestima. Opterećenje gljivama kretalo se u rasponu od 88 do 504 CFU m^{-3} . *Aspergillus nigri* otporan na azole identificiran je u jednom uzorku. Bakterijsko se opterećenje u zraku kretalo od 84 do 328 CFU m^{-3} . Brisovi iz nosa 14 radnika u veterinarskoj stanici pokazali su iznimno nisku prevalenciju bakterije *Staphylococcus aureus* (7,1 %). Naši rezultati pokazali su da je kontaminacija organskom prašinom na većini mjernih mjesta bila viša od ograničenja Svjetske zdravstvene organizacije te upozorili na potrebu bolje ventilacije prostora. Buduća bi istraživanja trebala upotpuniti postojeće aktivno i pasivno uzorkovanje s molekularnim testovima, čime bi se dobila preciznija slika profesionalnog rizika. Što se prevencije tiče, kaveze sa životinjama trebalo bi držati u zasebnim prostorijama, a ne u onima u kojima se izvode postupci dijagnoze i liječenja, a radnici bi trebali nositi zaštitnu opremu cijelo vrijeme.

KLJUČNE RIJEČI: *Aspergillus*; azoli; biološko opterećenje; čestice; rezistencija; *Staphylococcus aureus*