## MICROBIAL CONTAMINATION IN THE ENVIRONMENT OF A CLINIC FOR SMALL ANIMALS AT THE UNIVERSITY OF VETERINARY AND PHARMACEUTICAL SCIENCES IN BRNO

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The microbial contamination of the air and defined surfaces in the waiting room and four surgeries of the clinic for small animals was studied between January and September 1989. The waiting room and surgery I, in which patients were examined to be treated immediately or referred for further examination to specialists, were taken as an example of veterinary practice. During the period studied, 8,876 animals were seen there. On 12 occasions, the air was examined by sedimentation and aeroscopic methods and the defined surfaces by swabbing. Air microflora in both the waiting room and surgery I increased between the opening and closing hours. In the other investigated rooms with different working regimens (surgery II, III, and IV), microbial counts in air samples were usually higher before than after surgery hours. The average surface contamination of tables and floors in these surgeries was higher than that in surgery I. From the surfaces tested, the lowest contamination with lactose-utilizing microbes was found on the preparation tables in all four surgeries and on the examination table in surgery I both before and after surgery hours. From the micrococci isolated from air samples, 32.8 % were found to be staphylococci positive in the plasmacoagulase test.

#### Microbial contamination, air, surface, veterinary surgery

In hospitals for small animals, microbial contamination of the air and surfaces may increase to such an extent that it may become a source of acquired infection for the animals treated there (Bech-Nielsen 1979). In both human and veterinary medicine this condition is called a hospital-acquired or nosocomial infection (Pospíšil 1978: Mayr 1983) or Krankenhaus Infektion (Grund et al. 1985). Their occurrence ranges from 2% to 20% (Daschner 1979 cited by Grund et al. 1985). Little information, however, has been published on studies in small animal practice (Silberg et al. 1967; Baker 1969; Live and Nichols 1960; Grund et al. 1985).

With a continuing privatization of veterinary practice in our country, which leads to the establishment of surgeries for small animals, data on hygienic standards will be needed. The requirements for arrangement and furnishing of private veterinary consulting rooms and surgeries have been described by Williamson (1967). The present study provides information about microbial contamination of the air and defined surfaces in selected out patient surgeries of the Internal Clinic for Small Animals at the University of Veterinary and Pharmaceutical Sciences in Brno. Some issues associated with the sanitary regimen of the new veterinary surgeries are discussed.

#### **Materials and Methods**

The period of study lasted from January 11 till September 13, 1989. During that time, 8,879 patients were treated in surgery I with an attached waiting room. These rooms were situated in building no. 6. The other three surgeries (II, III and IV) used for specialised examinations were in the rear tract of building no. 9.

The dimensions of the surgeries ranged between 59 and  $104 \text{ m}^3$  with the height being 310 cm. No automatic ventilation was installed. The coefficient of lighting ranged between 1:3.7 and 1:5.6. Cleaning and disinfection in the waiting room and surgery I were performed regularly; examination tables were cleaned after each patient, floors and the whole equipment after the end of surgery hours on the same day. In the specialised surgeries (II, III and IV) where the numbers of patients were smaller and less regular, the sanitary regimen included cleaning and disinfection of all tables after each patient but the overall cleaning was often carried out before opening hours on the following morning.

All the rooms were examined on 12 occasions. Air sampling was carried out by sedimentation (twelve times) and aeroscopic (six times) methods at 6 to 7.30 and at 12 to 14.30, i. e. before and after the surgery hours. Air samples were collected on 2 Petri dishes of each medium always in the same place of the room.

The media employed were: 4 % meat-peptone agar (MPA) for overall microbial counts, blood agar with 10 % NaCl for micrococci, Endo agar for lactose-utilizing microbes (L+ and L-), Czapek-Dox agar for overall counts of microbes and fungi. The time of sedimentation was 2 1/2 min for MPA and Czapek-Dox agar, 5 min for blood agar

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and 20 min for Endo agar. To inoculate MPA, Czapek-Dox and Endo agar, 20 l of air were collected by an aeroscope; for blood agar, 50 l of air were used. Petri dishes with MPA and Endo agar were incubated for 24 h at 37 °C, those with blood agar for 48 h at 37 °C and samples on Czapek-Dox agar were incubated for 6 to 7 days at room temperature. Colony counts on Petri dishes were converted to the number of microbes per cubic metre according to the formula by Spurný et al. (1961).

The contamination of defined surfaces (4 to 8) on the floor and preparation and examination tables was assessed by swabbing an area of  $100 \text{ cm}^2$ . Each swab was subsequently extracted by shaking for 15 min with 10 ml of sterile buffered saline in a tube. From this the swab was transferred to a tube with Savage's medium containing a gas collector and 1 ml of the extract was added to Savage's medium in another tube. The presence of L+ microbes was indicated by a change in colour and the development of gas. The number of L+ microbes and overall microbial counts were assessed on Petri dishes with Endo agar and 4 % MPA, respectively, inoculated with 0.2 ml aliquots of the extract and incubated for 24 h at 37 °C. The results were converted to counts per surface area of 1 cm<sup>2</sup>.

From the colonies of micrococci growing on blood agar with 10 % NaCl, staphylococci were identified by the plasmacoagulase test. Altogether 68 colonies were isolated to be further tested.

### Results

The microbial contamination of the air in surgery I and the attached waiting room is shown in Figs 1 and 2. This area, whose function is primary admission of patients followed by further detailed internal or surgical examination, if indicated, can be regarded as an example of regular veterinary practice. The microbial contamination of surfaces in surgery I is compared with surface contamination in surgeries II, III and IV in Tables 2 and 3.

Fig. 1 indicates that microbial contamination of the air in the waiting room and surgery I, as measured by sedimentation, was lowest before opening hours and highest after the end of the working day. There was a difference in the findings on Endo agar and MPA between the surgery and the waiting room.

The microbial contamination assessed by the aeroscopic method (Fig. 2) suggests that even in the waiting room an increase in the number of L- microbes in the air occurred at the end of working hours.

In surgery  $\overline{I}$ , the assessment of L+ microbes on Endo agar after air sampling by sedimentation showed that from 12 examinations before the opening hours there were 3 (25.0 %) positive results compared to 7 out of 12 samples (58.3 %) after the end of working hours. For L- microbes, positive results were found on 4 out of 12 (33.4 %) and 9 out of 12 (75.0 %) occasions, respectively. Positive findings for both L+ and L- microbes after air sampling with the aeroscopic method were similar; before opening the frequencies were 0.0 % and 16.7 %, respectively, after closing the frequency of both microbial types was 60.0 %.

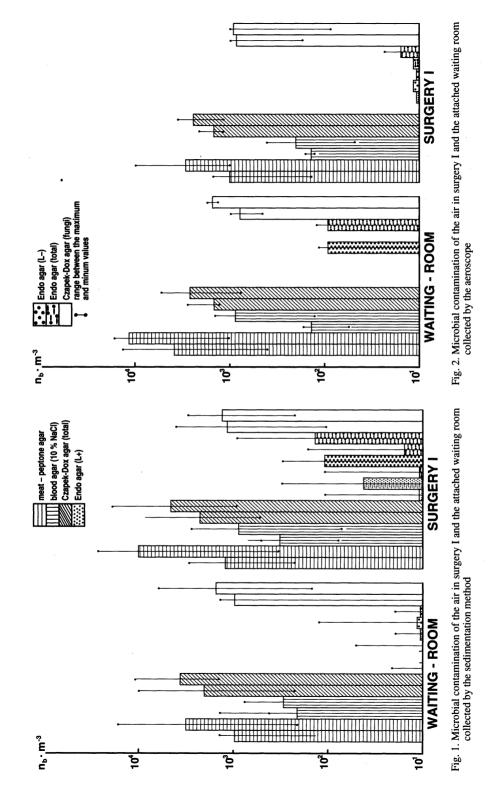
Figs 1 and 2 show that in both surgery I and the waiting room, the persistent contaminating factor in the air was the presence of fungi. These constituted a major proportion of all microbes detected on Czapek-Dox agar. Their occurrence was in the order of  $10^3$  microorganisms per m<sup>3</sup> both before and after surgery hours.

Table 1 indicates that the average counts of microbes in the air of surgeries II, III and IV were generally higher before than after surgery hours with the use of either method. The only exceptions were concentrations of fungi on Czapek-Dox agar and, in one case (II S), of microbes collected on Endo agar.

Table 2 shows that there were no considerable differences in microbial counts per floor area (1 cm<sup>2</sup>), as assessed by cultivation on MPA, before and after surgery hours in all surgeries examined (I, II, III, IV). However, the contamination of examination tables was occasionally found to have decreased in surgeries I, II and III after working hours. The average contamination of floors and tables was generally higher in surgeries II, III and IV that in surgery I.

Table 3 shows that the proportion of samples which gave positive findings of L+ microbes on the defined surfaces was high in surgery I both before and after surgery hours. However, the average values for corresponding surfaces in surgeries II, III and IV were even higher.

The lowest contamination of examined surfaces with L+ microbes in surgery I was found



The first column refers to the examination period between 6 and 7.30 h, the second to the collection time between 12 and 14.30 h

on the preparation table and one of the examination tables both before and after the surgery hours. Similar situation was observed in surgeries II, III and IV. The average counts of L+ organisms on 1 cm<sup>2</sup> of defined surfaces in surgery I were 14 before and 15 after the surgery hours. In surgeries II, III and IV these values were 5.0, 9.5 and 124.5, respectively, before opening, and 8.5, 13.5 and 17.0, respectively, after closing. The value of 124.5 for L+ microbes in surgery IV was a surprising finding.

Table	1
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Average microbial counts per cubic metre in the air of surgeries II, III and IV collected by aeroscopic and sedimentation methods before and after the surgery hours

		Meat-peptone		Blood agar		Endo agar					Czapek-Dox agar				
Surgery	agar		with 10% NaCl		L+				total		overall counts		fungi		
Sur		В	Α	В	Α	В	Α	В	Α	В	Α	В	Α	В	A
п	AE	6100	3700	1000	580	17	11	130	0	137	11	7900	4100	3900	1900
	SE	3400	1900	480	370	8.3	13	19	56	27.3	69	3900	5200	1400	1900
ш	AE	11600	1200	850	170	84	5	230	10	314	15	2700	2600	1200	1700
] .	SE	2500	1900	320	250	88	1.5	180	16	268	17.5	4700	3900	800	1500
IV	AE	9500	2400	1600	530	88	11	240	15	328	26	8900	3100	4700	2100
	SE	4500	1500	690	630	200	41	210	4.1	410	45.1	3800	4100	1200	1700

AE, aeroscopic method; SE, sedimentation method; B, before opening; A, after closing

 Table 2

 Average microbial contamination of defined surfaces, assessed on MPA, in surgeries I, II, III, and IV before and after the surgery hours.

 The values relate to areas of 1 cm<sup>2</sup>.

Surgery	Flo	or		Preparation table				
	В	A	В	А	В	Α	В	А
I	196.3	204.8	77.5	26.9	11.4	56.7	13.4	194.7
II III IV	554.0 381.5 379.2	205.0 369.6 485.7	159.4 139.6 278.4	47.2 103.0 371.0	- - -	- - -	78.9 108.7 38.9	141.3 59.3 24.6

B, before opening; A, after closing

 Table 3

 Positive findings of L+ microbes (in %) in the samples collected by swabbing from all the defined surfaces in each surgery

Surgery	sampling									
		before opening hours		at closing time						
	S/S	S/E	E/E	S/S	S/E	E/E				
I	72.4	55.7	17.7	85.2	62.5	23.8				
II III IV	85.5 97.5 86.4	82.0 83.3 85.0	24.4 27.8 30.0	84.0 88.9 90.0	68.9 84.4 75.0	6.0 34.4 27.8				

L+ microbes were assayed in Savage's medium inoculated with swabs (S/S) and 1 ml extract (S/E), and on Petri dishes with Endo agar inoculated with 0.2 ml extract (E/E)

From micrococci collected on blood agar with 10 % NaCl, 68 colonies were isolated and identified. There were 22 staphylococcus strains with a positive plasmacoagulase test (32.8 %) and 3 staphylococcus strains were plasmacoagulase negative. The colonies of Gram-positive micrococci produced white, yellow or, occasionally, ochrous pigment.

### Discussion

The microbial contamination of the environment, as assessed by sedimentation and aeroscopic methods, showed different results in surgery I and the attached waiting room and in the other surgeries (II, III and IV).

In the surgery I-waiting room complex, which was considered to represent a typical veterinary practice, the microbial concentration was considerablly lower before opening than after the end of working hours. This was observed repeatedly in both rooms in spite of regular cleaning and disinfection routines, because the number of treated patients was very high.

The flow of patients and the cleaning routines were different in surgeries II, III and IV. In each of the rooms, the number of patients seen was different, generally being lower than in the surgery I-waiting room complex. Full cleaning and disinfection were done after the examination of each animal. These results are in agreement with the studies performed in 1982–1983 in a new hospital for small animals at the Free University in Berlin. Grund et al. (1985) investigated the microbial contamination in the policlinic and surgery wards. The counts and spectrum of microbes were found to be related to the flow of treated patients, weekly and daily opening and closing hours, the degree of ventilation and the standard of sanitary procedures.

Opinions on the highest acceptable amount of microbes in the closed rooms of hospitals differ considerably. B o u r d ill o n et al. (1948), cited by Halačka and Blažek (1960), require for operating theatres of human hospitals not more than 70 microbes in 1 m<sup>3</sup> of air in neurosurgery, 300 per m<sup>3</sup> in large operating theatres and 700 per m<sup>3</sup> in small operating theatres. Pekárek and Eisnerová (1960), in internal instructions, permit in 1 m<sup>3</sup> of air up to 1500 microbes for delivery rooms, nurseries and post-operation units, up to 2500 microbes for puerperal wards and up to 1000 microbes for operating theatres, if the aeroscopic method is used. Our results show that the values recorded in the surgeries of the veterinary teaching hospital in Brno were up to the order of  $10^4$  microbes in 1 m<sup>3</sup> of air, collected on MPA, as contrasted with the range of 1.0 to 2.5x10<sup>3</sup> required by the above mentioned authors.

G r u n d et al. (1985) used the Anderson aeroscope for their examinations at the Free University in Berlin and found that, in the policlinic, the limit value of 200 microorganisms in  $1 \text{ m}^3$ , recommended by R u d e n (1978), was exceeded only in one of 30 samples examined. Air contamination in operating theatres exceeded the recommended limit of 30 microorganisms per m<sup>3</sup> in 4 samples. The authors, however, found severe contamination of the surfaces of examination and operation tables (7,985 to 12,000 CFU/16 cm<sup>2</sup>) with a high proportion of Gram-negative rods (35 % to 45 %) and pointed out the importance of regular disinfection.

Our results can be compared with those concerning microbial contamination of the environment in a piglet cage rearing station of the piggeries with minimal morbidity (Fišer 1978a, b). The strict sanitary routine used in a piglet rearing unit in Břeclav (Kukla et al. 1978) gives a basis for comparison with the results recorded in the veterinary teaching hospital. Air contamination was at the same level in the surgeries and, by some criteria (counts on MPA and Endo agar), even lower in the waiting room. Similarly, microbial contamination on the surfaces of preparation tables in surgeries II, III and IV (78.9 microbes/cm<sup>2</sup> before opening; 59.3 microbes/cm<sup>2</sup> after closing; 38.9 before and 24.6 microbes/cm<sup>2</sup> after surgery hours) is comparable with the contamination of indicated places in the piglet cage rearing station after disinfection (55.17 to 66.20 microbes/cm<sup>2</sup>) (Fišer 1978b). In surgery I, the results on the surfaces of examination tables before opening and after closing (11.4 to 77.5 microbes/cm<sup>2</sup>) and on the preparation table before opening (13.4 microbes/cm<sup>2</sup>) were satisfactory.

The average overall contamination, assessed on MPA, on the defined floor areas in all the surgeries and on the surfaces of examination tables in surgeries II, III and IV (with the exception of surgery II after closing) was higher than the average value of 79 microbes/cm<sup>2</sup> required as a measure of the efficiency of disinfection in pig sheds (Hojovec et al. 1977).

The proportions of L+ microbes on the surfaces of examination and preparation tables were particularly high in all rooms both before opening (72.4 % to 97.5 %) and after clo-

sing (84.0 % to 90.0 %) as compared with the limit given by Hojovec et al. (1977), which is 1.57 % of positive findings after efficient disinfection, tested by swabbing. However, when checking for the efficiency of disinfection in 8 departments of the piglet cage rearing station, this strict demand was met only immediately after disinfection (Fišer 1978a).

Based on the indicative results presented, it is necessary to emphasize the importance of repeated thorough disinfection of all surfaces with which patients have come into contact in the surgery. These results also suggests that the current cleaning and disinfecting routine for the exposed surfaces is not adequate. The considerably high proportion (32.8 %) of plasmacoagulase-positive staphylococci isolated from the micrococci in air samples and the counts of fungi per  $m^3$  of air in the surgeries call for a better decontamination of the air either by increased aeration of the rooms during surgery hours or by application of UV radiation during closing hours.

In conclusion, our preliminary observations indicate the need for further work to define adequate guidelines for general sanitary regimens and for their extension to specific clinical situations.

# Mikrobiální kontaminace prostředí na klinice pro malá zvířata fakultní nemocnice Vysoké školy veterinární a farmaceutické v Brně

V období od ledna do září 1989 byla ve 12 odběrových dnech zjišťována mikrobiální kontaminace ovzduší a indikátorových ploch čekárny a čtyř ordinací kliniky pro malá zvířata Vysoké školy veterinární a farmaceutické v Brně. Za modelové prostředí byla považována čekárna a ordinace I, kde přicházející pacienti byli vyšetřeni, ošetřeni přímo na místě nebo odesláni k dalšímu speciálnímu vyšetření. Průchodnost pacientů čekárnou a ordinací I činila za sledované období 8 876 zvířat.

V každém odběrovém dni bylo ve všech sledovaných místnostech provedeno vyšetření ovzduší sedimentační a aeroskopickou metodou a stěrovou metodou vyšetřeny indikátorové plochy před zahájením a po skončení ordinačních hodin. Výsledky ukázaly v čekárně a ordinaci I vzestup vzdušné mikroflory mezi začátkem a koncem provozu, přičemž počty plísní byly vysoké trvale. V ostatních sledovaných ordinacích, které měly odlišný provozní režim, byla kontaminace ovzduší obvykle vyšší před zahájením, než na konci provozu. Průměrná kontaminace indikátorových míst podlah a odkládacích a vyšetřovacích stolů byla v ordinacích II, III a IV pravidelně vyšší než v ordinaci I. Výskyt mikrobů zkvašujících laktózu, které jsou považovány za indikátory fekálního znečištění, byl nejnižší na odkládacích stolech všech ordinací a na vyšetřovacích stolech ordinace I na počátku i na konci provozu. Isolované vzdušné mikrokoky byly dále identifikovány a ve 32,8 % byly prokázány plasmakoagulázo-pozitivní stafylokoky.

# Микробиальное заражение помещений клинических кабинетов малых животных факультетской больницы Высшего ветеринарного института в Брно

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С января до сентября 1989 проводили исследования микробиальной контаминации и индикаторных поверхностей зала ожидания и четырех кабинетов клиники малых животных Ветеринарного института в Брно. Модельной средой считали зал ожидания V и кабинет I, в котором исследовали прибывающих пациентов и непосредственно лечили по поставленному диагнозу или отправляли для дальнейшего исследования в другие кабинеты. Залом ожидания и упомянутым кабинетом в течение исследуемого периода прошли 8 876 животных.

Для исследования атмосферы применяли седиментационный и аэроскопический методы,

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для исследования индикаторных поверхностей - метода мазков. Из питательных сред применяли 4% MPA, кровяной агар с 10% NaCl, агар Энде, агар Чапек-Докса и среду по Саважу. Изолированных воздушных микрококков исследовали, определяя род Стафилококкус с позитивным плазмокоагуляционным тестом.

В зале ожидания V и приемной I было установлено постепенное увеличение воздушной микрофлоры с начала до конца дообеденного рабочего времени. Увеличилась также частота захвата 1+ и 1- микробов в конце рабочего времени на 33,3 % или на 41,6 %, а также на 60,0 % и на 43,3 %. Постоянно высокими были данные по численности плесени, следовательно, около 10<sup>3</sup> на 1 м<sup>3</sup> воздуха до и в конце работы в зале ожидания V и в кабинете I. В остальных исследуемых кабинетах с отличающимся режимом работы численность выявляемых седиментацией и аэроскопическим методом микробов была большей частью выше до начала, чем в конце работы. Средняя контаминация индикаторных мест полов и процедурных столов была всегда больше в приемных II, III и IV по сравнению с кабинетом I.

Самая низкая частота наличия 1 + микробов индикаторных поверхностей была установлена до и в конце работы на столах для откладывания вещей всех исследуемых кабинетов и на процедурных столах кабинета І. Исследованием изолированных воздушных микрококков было установлено в 32,8 % плазмокоагуляза-положительных Стафилококков.

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