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Microbiology of the canine nasal cavities

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SUMMARY

The anterior and superior regions of canine nares yielded two distinct microbial populations consisting of 25 microbial species. Streptococci predominated in the anterior nose; clostridia and staphylococci were also quite prevalent. Al least two distinct microorganisms were cultured from most anterior sites. In contrast, sterile conditions prevailed in the superior nasal regions of at least 50% of the ninety-two animals. Various Bacillus *sp. were recovered from the remainder.*

Interest in the endogenous microbiota of domestic dogs has resulted in studies of the canine skin, pharynx, gastrointestinal tract and rectum (Brennan and Simkins, 1970; Clapper and Meade, 1963; Davis et al., 1977; Krogh and Kristensen, 1976). Only few attempts have been directed at the normal flora of the canine nose and only a limited number of subjects have been screened (Abramson et al., 1976; Balish et al., 1977; Clapper and Meade, 1963). Such cultures were taken from the external nares only, ignoring the microbiota of the dogs complex nasal structures. Recently, Abramson et al. (1976) described the microbial diversity of the canine nose. That study suggested a selective role for different structural compartments of the canine nares. The investigation reported here summarizes our analyses of the left and right superior and anterior regions of the dogs' nose.

MATERIAL AND METHODS

Ninety-two healthy, predominantly male, mongrel dogs were the test objects of this study. Cultures of 92 right and 92 left anterior nares as well as 63 right and 86 left superior nares were taken in the following manner: All animals were fasted overnight, anesthetized with intravenous sodium pentobarbital (10-12 mg/lb). Using separate, sterile, six inch cotton applicators, nasal swabs of the right and left anterior regions were taken approximately 4 cm distal to the tip and along the ventral (floor) surface. Nasal swabs of the right and left superior (dorsal) regions were procured as described previously. (Abramson et al., 1976).

The applicators were placed into 10 ml semi-solid broth which contained per 1000 ml, 30 gram trypticase soy broth (BBL*), 0.2 gram yeast extract (BBL), 10 g

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agar (BBL), 10 ml 2% heme solution supplemented with menadione. Also, each tube contained two pieces of boiled beef liver. These media were autoclaved under standard conditions. After inoculation, all cultures were incubated for 48 hours at 35°C.

Following incubation, each swab, removed with sterile forceps, was inoculated onto 5% sheep blood agar with a trypticase soy agar base (BBL) which permitted the growth of most obligately and facultatively aerobic bacteria and yeasts, eosin-methylene blue (agar BBL) useful in the isolation of aerobic and facultatively anaerobic gram negative rods, mitis-salivarius agar (Difco) which aids in the separation and presumptive identification of viridans streptococci, enterococcus agar (BBL) which permits the selection of enterococci and non-enterococcal Lancefield group D streptococci as well as *Listeria monocytogenes*, and staphylococcus 110 agar (BBL) which sequesters staphycocci from polymicrobic specimens. The swabs used for the inoculation of these media were transferred to a fresh broth described above. All inoculated agars and the broth were incubated at 35°C for 18 and 48 hours. For the isolation of anaerobic bacteria, a prereduced sheep blood agar plate was inoculated and incubated under properly controlled anaerobic conditions in a GasPak anaerobic system (BBL) at 35°C for 72 hours.

Identification tests were performed on isolated colonies after appropriate staining according to the established tenets of diagnostic microbiology (Cowan, 1974; Lennette et al., 1974). Thus, staphylococci here examined for their ability to produce coagulase, mannitol fermentation, hemolysin production, salt tolerance and catalase production. The streptococci were grouped according to their colonial morphology on specific isolation media and tested for their ability to produce acid from arabinose, glycerol, lactose, maltose, mannitol, raffinose, salicin, sorbitol, sucrose and trehalose, esculin hydrolysis, survival after 30 minutes exposure to 60°C, growth in 6.5% NaCl, at pH 9.6, in 10% and 40% bile, optochin susceptibility, hippurate and arginine hydrolysis and, where indicated, polysaccharide antigen analysis. *Bacillus* spp. were identified by their Gram reaction in young cultures, motility, spore shape and position, growth at pH 5.7, citrate utilization, anaerobic growth in glucose broth, acid production from glucose, arabinose, mannitol and xylose with ammonium chloride as nitrogen source, hydrolysis of gelatin, starch and casein, production of acetylmethylcarbinol, indole and nitratase.

Members of Enterobacteriaceae were identified by lack of oxidase production and reactions elicited with the EnteroTube (Roche Diagnostic, Nutley, NJ) and API 20E (Analytab Products Inc., Hicksville, NY).

The nonfermentative gram-negative rods were identified by the oxidase test, Gram stain, flagellar distribution of motile, oxidative acid production in glucose, xylose, mannitol, lactose, sucrose, maltose, fermentation of glucose, growth on MacConkey's agar, citrate utilization, urease and nitratase production, gelatin

and esculin hydrolysis, 6% NaCl tolerance, production of water-soluble pigments, and action litmus milk.

Aerobic gram-positive rods which tolerated potassium tellurite examined for catalase-production, motility, esculin and arginine hydrolysis nitrate reduction, acid production from glucose, lactose, maltose, mannitol, salicin, starch, sucrose, trehalose, xylose, gelatin liquefication and the elaboration of urease.

All anaerobic bacteria were shown to be unable to grow under aerobic and microaerophilic conditions, examined by Gram stain and analyzed with the Minitek System (BBL).

RESULTS

Table 1 summarizes the microbiota recovered during this investigation. A total of 25 different bacterial species were isolated; 5 of these were obligately anaerobic, 20 facultatively or obligately aerobic. By far, the most common anaerobic bacterium was *Clostridium perfringens*, recovered from 57.6 and 52.2% of the anterior right

Table 1. Microorganisms isolated from the canine nose.

MICROORGANISMS ISOLATED FROM THE CANINE NOSE

ORGANISMS	RIGHT ANTERIOR total no.-92		LEFT ANTERIOR total no.-92		RIGHT SUPERIOR total no.-63		LEFT SUPERIOR total no.-86	
	NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	NO.	PERCENT
ANAEROBES								
Bifidobacterium longum	1	1.1	—	—	—	—	—	—
Clostridium perfringens	53	57.6	48	52.2	2	3.2	1	1.2
Clostridium sphenoides	1	1.1	—	—	—	—	—	—
Eubacterium lentum	—	—	1	1.1	—	—	—	—
Lactobacillus sp	1	1.1	2	2.2	—	—	—	—
AEROBES								
Acinetobacter anitratus	—	—	1	1.1	—	—	—	—
Bacillus circulans	—	—	1	1.1	—	—	—	—
Bacillus laterosporus	—	—	—	—	—	—	1	1.2
Bacillus lentus	5	5.4	6	6.5	3	4.8	6	7.0
Bacillus pulvifaciens	—	—	1	1.1	—	—	—	—
Bacillus pumilus	12	13.0	8	8.7	21	33.3	27	31.4
Corynebacterium sp	2	2.2	—	—	—	—	—	—
Escherichia coli	9	9.8	6	6.5	1	1.6	1	1.2
Klebsiella pneumoniae	2	2.2	3	3.3	—	—	—	—
Pasteurella sp	1	1.1	2	2.2	—	—	1	1.2
Proteus mirabilis	—	—	—	—	1	1.6	—	—
Pseudomonas alcaligenes	—	—	—	—	—	—	1	1.2
Staphylococcus aureus	28	30.4	17	18.5	—	—	1	1.2
Staphylococcus epidermis	24	26.1	29	31.5	—	—	2	2.3
Streptococcus durans	—	—	1	1.1	—	—	—	—
Streptococcus equi	—	—	1	1.1	—	—	1	1.2
Streptococcus faecalis	2	2.2	—	—	1	1.6	—	—
Streptococcus faec. var. liq.	—	—	3	3.3	—	—	—	—
Streptococcus faecium	6	6.5	2	2.2	—	—	—	—
Streptococcus mitis	1	1.1	3	3.3	—	—	1	1.2
Streptococcus spp	55	59.8	62	67.4	1	1.6	2	2.3
No growth	—	—	—	—	35	55.6	43	50.0

and left specimens. It was the only anaerobe recovered from the posterior nares, albeit on but few occasions, twice from the right and once from the left. All cultures from both anterior compartments yielded bacteria. The most frequently isolated group of organisms is designated in Table 1 as *Streptococcus* sp. These streptococci could not be identified on the basis of their biochemical reactions and physiological activities. They did not react with any of the available streptococcal group carbohydrate specific antisera. While the laboratory responses of these 120 streptococci suggested that they were identical or closely related, further studies are required for such a conclusion. The staphylococci, both *Staphylococcus aureus* and *S. epidermidis* were also represented in both anterior sites frequently. These bacteria were classified on the basis of coagulase production, salt tolerance, manitol as the major carbon and gelatin as the major nitrogen sources. It is, therefore, possibble that other species of the genus may be included as *S. epidermidis*. The paucity of serogroup D and Q streptococci in this investigation was surprising in view of earlier findings (Abramson et al., 1976). Only a few gram-negative rods were recovered from the anterior nares.

The superior nares, although assayed in slightly fewer animals, displayed an entirely different microbiota. At least 50% of these compartments had no recoverable organisms. The majority of specimens yielding organisms contained *Bacillus* sp. of which the majority were *B. pumilus*, a bacterium present in lower but definite quantities in the anterior portion. *B. lentus* on the other hand, was distributed equally between the anterior and superior portions. The predominating bacteria of the anterior nares were recovered infrequently from the posterior nose cultures.

Table 2 addresses the frequency of polymicrobic colonization of the nasal compartments. Two bacterial species were encountered most frequently anteriorly followed closely by monomicrobic and 3 component recoveries. No anterior spec-

Table 2. Microbial colonization of nasal sites.

Nasal Site	Total no. of dogs	NUMBER OF ORGANISMS						
		0	1	2	3	4	5	6
RIGHT ANTERIOR	92	0	21.7	46.7	22.8	7.6	0	1.1
LEFT ANTERIOR	92	0	18.5	52.2	26.1	2.2	1.1	0
RIGHT SUPERIOR	63	55.6	41.3	3.2	0	0	0	0
LEFT SUPERIOR	86	50.0	46.5	3.5	0	0	0	0

numbers represent percent of dogs yielding their bacteria from sites indicated

imen was sterile and only a few dogs had an excess of three different bacteria in this location. Contrariwise, the superior nares were usually sterile and when bacteria were recovered, monomicrobic constitution was the rule. Only approximately 3% of the animals had two different bacteria in the superior portion and none harbored more.

It was of interest to compare the microbial species found in the various sites. The right and left anterior nares harbored the same species of bacteria most frequently (46.7%). Differences in microbial carriage by only one organism occurred with almost the same frequency (40.2%) while in 10.5% differences with two species were encountered. The sterile conditions of both superior compartments is reflected in that 63.3% of 60 dogs did not show any variations in the type of bacteria recovered from the superior site. Of course, 50 and 55.6% of the superior nares were without microorganisms indicating the diminished opportunity for microbial colonization of the upper canine nose.

The comparison between the anterior and superior portion of each side reflects this paucity of microorganisms in the superior location. Thus, on the left side 10.5% of 86 dogs showed no difference in the recovered bacteria; but 33.7% differed by one species, 38.4% by 2, 15,1% by 3 and 2.3% by 4 microbial species. On the right side, very similar observations obtained. No difference was demonstrated in 6.3% while 36.5% differed each by 1 and 2 microorganismes, 17.5% by 3 and 1.6% by 4 protista.

These differences are underlined further when the distribution of the microorganisms between the 4 compartments are considered. Both the anterior nares always carried organisms. However only 4.7% of 43 dogs yielded one identical species only. One different species of bacteria was found in 11.6% when only a single organism was recovered. When the anterior nares cultures grew 2 different organisms they showed the same bacteria at a level of 16.3%, differed by one of the 2 species 34.9% and both bacteria were completely different in the right and left anterior nares in 9.3% of the animals.

The left and right superior nares differed far less. The same organism was recovered in 42.1% of the animals in both locations; a different species was isolated in 2.6% of the dogs.

The anterior and superior sites on the left side differed in 83% of the subjects, on the right side in 75%.

DISCUSSION

The isolation of anaerobic bacteria in the anterior regions of canine nares is of interest since past investigations have claimed only a minimal occurrence (Abramson et al., 1976; Clapper and Meade, 1963; Lennette et al., 1974). In this study, *Clostridium perfringens* colonized over half the population. Smith and Holdeman (1968) report that this organism's principal habitats are soil and the intes-

tinal tracts of man and animals. Canine habits would indicate ample opportunity for such acquisition. The ability of *C. perfringens* to be aerotolerant would make it an ideal candidate for survival in the anterior region. However, this explanation must be evaluated in view of the paucity of facultatively anaerobic gram-negative rods found in the anterior as well as superior compartments of the canine nose in this study.

The frequent isolation of *C. perfringens* may also be the result of immediate inoculation of a medium well-suited for its preversation. Collee et al. (1974) underline the importance of the time interval between procurement of the specimen and its inoculation on appropriate substrates. Bartlett et al. (1976) report that when various transport media were used, isolation rates varied, but delayed processing had little impact on the yield. Dowell (1974) stressed the adverse effect of exposure to oxygen between procurement of specimens and its inoculation into appropriate media. This investigation used sterile cotton swabs as a means to sample at random the microbiota of the canine nose. The time between actual procurement of all specimens and inoculation of a complete rather than a transport medium was a matter of seconds, followed by immediate incubation. The medium employed was quality controlled invariable with *C. novyi B* as well as *Bacteroides* and *Fusobacterium* sp (Isenberg and Berkman, 1966).

The most striking result of this investigation is the demonstration of two entrirely different microbial populations in the anterior and posterior regions of the canine nasal pasages. Previous reports addressed bacteria isolated but did not record distinctions between left and right nares nor anterior and superior regions (Abramson et al., 1976; Clapper and Meade, 1963; Smith, 1961; Smith and Holdeman, 1968). In describing the whole nasal structure, Clapper and Meade (1963) found *S. epidemidis*, corynebacteria and *Bacillus* sp. dominant organisms. Smith (1961) and Abramson, et al. (1976) reported the prevalence of *Staphylococcus* sp. and *Escherichia coli* while Balish, et al. (1977) and Abramson, et al. (1976) noted the frequency of *Streptococcus* sp. This study reported *C. perfringens, Streptococcus* sp. and *Bacillus* sp. as frequent inhabitants of the anterior regions but occurring at different rates on the right and left side of individual dogs.

Abramson et al. (1976) studied the superior compartments in 13 dogs and isolated non-fermentative gram-negative rods most frequently. In this study we examined a much larger dog population and found both the right and left superior regions sterile in half of the animals. When bacteria were present, *Bacillus* sp., usually by themselves, were isolated. There is, of course, no proof that the bacilli existed in a vegetative rather than spore stage. It is conceivable that the frequency of their recovery may be related to their ability to form spores. We would assume that clostridia would then be encountered with equal frequency unless the upper nasal passages provide more favorable attachment opportunities for the aerobic spore formers in their vegetative and/or resting phase. This hypothesis must be

investigated further. We must point out as well that the animals in this study all were housed in the same environment during quarantine and the actual study. Therefore, dogs in the community may not present the same type of bacteria but rather those reflecting their own environment.

Variations exist between left and right regions. While Smith (1961) procured specimens with one swab from both nostrils, this study required separate swabs from each sub-compartment in situ to ascertain the consistency of the microbial populations in their respective anatomic sites. Evidently, the canine nasal structure is an ecosystem that is a self-contained unit with different niches colonized by particular organisms.

Although self-contained, it naturally interacts with other external and internal systems. It is not as yet clear, however, which habits and habitats of the canine host select which particular groups of protista and if the canine nares encourage attachment because of cellular receptor sites favoring certain microorganisms or the opportunities for microbial glycocalyces to interact with cellular counterparts. This investigation emphasizes the need to consider divisions of anatomic compartments as well as the external environment. Finally, this study underlines the general lack of information concerning the microbiota of the canine nose under differing environmental conditions. This paucity of information makes it difficult to draw conclusions. The microbial flora of animals living in a human home environment as compared to those running free in open country must also be studied before meaningful conclusions can be drawn. It is, therefore, imperative that the findings reported here be viewed as reflecting unnatural conditions for the dog and the conclusions drawn await their general appliction until surveys of dogs in their natural habitat are concluded.

ZUSAMMENFASSUNG

Die vorderen und hinteren Nasenabschnitte des Hundes enhielten 2 verschiedene mikrobielle Populationen, die 25 Bakterienspezien umfassten. In der vorderen Nase wurden hauptsachlich Streptokokken gefunden sôwie Clostridien und Staphylokokken. Im allgemeinen wurden zummindest 2 verschiedene Bakterienarten angezuchtet. Dagegen waren die hinteren Nasenabschnitte bei 50% der Tiere steriel. Bei den restlichen Hunden wurden verschiedene Bazillusarten isoliert.

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