AEROBIC BACTERIAL GINGIVAL FLORA OF THE DOG

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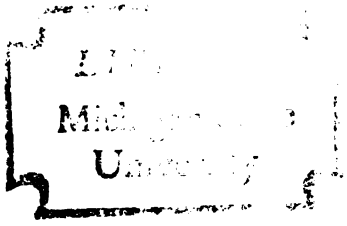

ABSTRACT

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By

Dennis Armin Saphir

Gingival scrapings from dogs were collected in order to isolate and identify species of the aerobic gingival flora. Scrapings were inoculated into selective and general purpose media and placed in a CO_2 incubator for a minimum of 48 hours. Various tests were then used to identify the organisms isolated. Members of the following genera were found: *Streptococcus, Staphylococcus, Actinomyces, Escherichia, Corynebacterium, Pasteurella, Caryophanon, Mycoplasma, Acinetobacter, Moraxella, Neisseria, Enterobacter,* and *Bacillus.* Of particular interest was the frequent recovery of three unclassified groups of aerobic gram-negative bacteria, IIj, EF-4, and M-5, previously associated with human infections resulting from dog bites. Although no set pattern seemed to exist between the variability and consistency of gingival microbiota as related to age, sex and breed of dog, a certain characteristic flora may be predicted in the gingiva of the healthy dog.

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By

Dennis Armin Saphir

A THESIS

Submitted to
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Dedicated to my wife Karen,

my mother and father, and

Barbara and David

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INTRODUCTION

The study of bacterial flora isolated from the oral cavity of domestic animals is virtually an unexplored field compared with the data available on human oral flora. Researchers investigating the gingival microbiota in humans faced two problems, one of sampling, and the second involving the "technical task of enumerating a diverse, poorly described, difficult to cultivate, complex group of microorganisms" (84). Those organisms which were successfully isolated from the gingival crevice region could not always be categorized into acceptable classification tables.

Studies of gingival flora in the dog are of particular value since this animal has a gingival crevicular epithelium similar to that found in humans (31). Few investigators, however, have concentrated their efforts on describing gingival flora in dogs. Most certainly the problems of sample collection are enhanced with these animals, and many of the organisms which can be isolated are not recognizable using present classification schemes (personal observation).

The organisms found within the oral cavity of domestic animals consist of many potential pathogens and commensals (77,84,88). Disease may occur as a result of local infection by the microorganisms which usually inhabit the mouth in large numbers. However, for an

organism to produce lesions, dynamic alterations involving the host and/or the microorganisms must occur. These may include a local or general reduction of vitality of the host, a lessened resistance of the host's tissue, physiological changes in the host, an increase in the number of organisms, or an increase in the virulence of the microorganisms. Irritation, trauma, penetration of foreign bodies such as bones or sticks, carious teeth, and neoplasms may all contribute to bacterial invasion (30). It has also been shown that certain organisms of the oral cavity have been recovered in wounds resulting from animal bites. For example, *Pasteurella multocida* infections in humans have for some time been associated with animal bites (2,19,20,28,43,44,47,49,60,70,89,92).

The microorganisms within the oral cavity consist of the resident and transient flora. Resident flora can be continuously demonstrated by culture, staining, and immunologic techniques. The transient flora represent those organisms which are occasionally isolated from the oral cavity. Knowledge of the resident and transient oral flora is essential in determining which organisms cause or are associated with different clinically recognized oral diseases of man and animals (69).

The purpose of this investigation was to isolate and identify to species, where possible, the aerobic bacterial flora found in gingival scrapings of dogs. Particular attention was given to those organisms previously reported to be associated with human infections resulting from dog bites.

REVIEW OF THE LITERATURE

PART I: THE ORAL MICROBIAL FLORA AND THEIR HOSTS

The Development of the Indigenous Oral Microbiota of Man

Research workers investigating the oral flora in man are in disagreement as to when bacteria first appear in the mouth. Some believe the oral cavity is basically sterile at birth (46,66) with no evidence of microbial growth seen prior to the initiation of the respiratory process (1). Other reports indicate the newborn first comes in contact with the microbial flora of the mother's vagina, and then with the organisms of the external environment (11,69), where the bacterial count increases rapidly (10,59,61).

During the first few days of the child's life, his mouth is highly selective towards the type of bacteria that can establish themselves. Subsequently, bacterial composition is quite diversified within this time period (11,88). Aerobic and facultative aerobic bacteria dominate the oral flora prior to the appearance of teeth (1,66,69,75). Some of the representative flora include: streptococci, pneumococci, micrococci, staphylococci, coliforms, *Bacillus subtilis, Neisseria, Actinomyces,* and *Corynebacterium* (10,11,66,69,75). Although the anaerobe *Veillonella alcalescens* has been frequently recovered from infants over one week old (66,69), other anaerobes are seen in low numbers in the child's initial year (5,10,51,61,66,71).

There are both qualitative and quantitative changes in the oral cavity with the appearance of teeth. Such anaerobes as *Leptotrichia*, spirochetes, fusiform bacilli, spiral forms, and *Vibrio* increase in number significantly (69). Dental carious lesions, which frequently appear in adolescents, create an entirely new environment in the oral cavity for new microbial growth. These lesions provide new attachment sites for organisms which previously had difficulty in adhering to surfaces in the mouth (88).

The bacterial flora in man is subjected to changes in diet and habitats through tooth extractions and use of dentures. Subsequently, bacterial flora remains diversified, and competition among organisms with respect to nutrients and attachment sites is high. For example, *Streptococcus salivarius* is provided with an adequate nutritional environment in the mouth of an infant. This organism primarily resides on the tongue rather than teeth (10,15,66,69). In contrast, spirochetes are not found in the edentulous infant. Spirochetes have strict nutritional demands and require the gingival crevice area as their habitat (88). However, Carlsson *et al.* (15) have found that *S. salivarius* cannot compete successfully with other organisms in the adult mouth unless dietary sucrose is provided. The percentage of *S. salivarius* in the oral cavity, therefore, decreases with a decrease in dietary sucrose.

Due to individual variation, it is difficult to describe a basal flora for the oral cavity of all healthy dentulous humans. However, a broad pattern of the oral bacterial flora may still be predicted. A review of the literature indicates at least 29 different species

of microbes found within the oral cavity (11). Table 1 depicts the composition and distribution of oral flora in the adult.

The proportion of diphtheroids and gram-negative anaerobic rods increases in plaque and gingival sulcus. *Neisseria, Bacteroides melaninogenicus,* and spirochetes are frequently seen. Lactobacilli, staphylococci, and filamentous forms comprise approximately 1% of the population. The incidence of *Candida,* coliforms, and *Mycoplasma* varies. *Mycoplasma* is not seen in the edentulous mouth which would indicate that these organisms inhabit the gingival sulcus (11). In addition, the edentulous mouth shows a considerable reduction in the number of spirochetes, lactobacilli, yeasts, *Streptococcus mutans* and *Streptococcus sanguis* (9,16,62,81). Although *B. melaninogenicus* and spirochetes are regular inhabitants of the adult gingival crevice region, neither organism is seen with any degree of regularity in children (25). A list of cultivable organisms in the gingival crevice area and the genera commonly found in this site is shown in Table 2.

Variability

Socransky and Manganiello (88) have suggested that oral bacterial flora differ between host species, within the same species, between sites in the same oral cavity, and within the same site in the same individual. Gordon and Jong (41), reporting on bacterial flora from human saliva, indicated that gram-positive facultative cocci represented the largest single group (46.2%), with streptococci comprising 41% of the salivary isolates. Gram-negative anaerobic cocci made up 15.9% of the total isolates, with most strains belonging to the genus

Table 1. Composition and distribution of oral flora in the adult

	Gingival crevice (%)	Dental plaque (%)	Tongue (%)	Saliva (%)
Gram-positive facultative cocci	29	28	45	46
Streptococcus spp.	27	28	38	41
S. mutans	L**	L-H	L	L
S. sanguis	M	H	M	M
S. salivarius	L	L	H	H
Enterococci	M	L	L	L
*Staphylococcus salivarius****	L	L	H	H
Related nonpathogenic staphylococci	L	L	H	H
Gram-positive anaerobic cocci				
Peptostreptococcus, Peptococcus	7	13	4	13
Gram-negative facultative cocci				
Neisseria spp.	<1	0	3	1
Gram-negative anaerobic cocci				
Veillonella spp.	11	6	16	16
Gram-positive facultative rods and filaments				
*Nocardia, Rothia, Corynebacterium, Bacterionema****, *Lactobaccillus*	15	24	13	12
Gram-positive anaerobic rods and filaments				
Actinomyces, Propionibacterium, Leptotrichia	20	18	8	5
Gram-negative facultative rods	1	<1	3	2

Table 1 (continued)

	Gingival crevice (%)	Dental plaque (%)	Tongue (%)	Saliva (%)
Gram-negative anaerobic rods	16	10	8	5
Fusobacterium	2	4	<1	<1
Bacteroides oralis	6	5	5	2
Bacteroides melaninogenicus	5	<1	<1	<1
Vibrio sputorum	4	1	2	2
Spirochetes				
Treponema microdentium, T. denticola, T. oralia	1	<1	<1	<1

Modified from *Microbiology*. Davis *et al.* (eds.), Harper and Row, Maryland. 1973. (24)

* Approximate percentage of total cultivable flora present in each area.

** Approximate proportions found: L=low, M=moderate, H=high.

*** This organism is not a member of the *Staphylococcus* genus. It grows poorly and ferments glucose weakly (6). Bergan *et al.* (4) have suggested it is a micrococcus.

**** Belonging to the family *Actinomycetaceae*.

Table 2. Organisms of the human gingival crevice region

Group	Approximate percentage of cultivable microbiota	Genera and/or species commonly found in this site
Gram-positive facultative cocci	28.8	Staphylococci, enterococci, *S. mutans, S. sanguis, S. mitis*
Gram-positive anerobic cocci	7.4	*Peptostreptococcus*
Gram-positive facultative rods	15.3	*Corynebacterium, Lactobacillus, Nocardia, A. viscosus, B. matruchotii*
Gram-positive anaerobic rods	20.2	*A. bifidus, A. israelii, A. naeslundii, A. odontolyticus, P. acnes, L. buccalis, Corynebacterium*
Gram-negative facultative cocci	0.4	*Neisseria*
Gram-negative anaerobic cocci	10.7	*V. alcalescens V. parvula*
Gram-negative facultative rods	1.2	
Gram-negative anaerobic rods	16.1	*B. melaninogenicus, B. oralis, V. sputorum, F. nucleatum, S. sputigenum*
Spiral organisms	1 to 3	*T. denticola, T. oralis, T. macrodentium, B. vincentii*

Modified from S. S. Socransky (84).

Veillonella. Gram-positive facultative rods comprised 11.8% of the total number of organisms.

Bowen (7) showed that the salivary flora of monkeys is very similar to humans although quantitative differences do exist. Streptococci comprised 72% of the total aerobic population. *Veillonella* represented only 3% of the total anaerobic organisms or approximately one-seventh of that found in human saliva. The appearance of coliform bacteria was greater in monkeys than humans, and the lactobacilli count was ten times greater than that reported from human saliva. *Actinomyces* was not cultured from the saliva of any of the monkeys (7), in contrast to its occasional recovery from human saliva (41).

MacDonald *et al.* (64) observed that the flora in the rice rat periodontium is composed primarily of aerobic, gram-positive bacteria comprising mostly enterococci and diphtheroids. A limited number of actinomycetes were found while no spirochetes were reported. Of note was the infrequent recovery of *Bacteroides* or *Fusobacterium*, organisms indigenous to man.

Courant *et al.* (21) indicated similarities in the relative abundance of bacteria from gingival debris recovered from beagle dogs and humans. However, significant differences were apparent following limited bacterial identifications. Coliform organisms were found to be minor components of dog crevicular flora. However, a comparison between Gibbons' *et al.* (38) report on gingival crevice flora in man with Courant's *et al.* (21) survey indicates that *B. melaninogenicus* is twice as prevalent in the dog's gingival flora.

Table 1 supports Socransky's and Manganiello's report (88) on the variability of oral flora among different sites within the oral cavity. There is considerable evidence which supports their findings on variability of microbial flora within the same site in the oral cavity at different periods of time. The flushing action of saliva results in the swallowing of 1 to 2.5 grams of bacterial cells each day (33). Actions performed by the tongue, lips, mucous membranes of the cheeks, and the mechanism of chewing can remove bacteria from dental surfaces. Organisms from the gingival crevice areas are removed by fluids which originate from the submucosal capillaries. Epithelial cells are constantly being shed, resulting in the removal and subsequent swallowing of microorganisms in the saliva (69). Carlsson (12), Ritz (75), and Socransky and Manganiello (88) examined various sites in the oral cavity at different periods of time. Ritz reported that as plaque development occurs, an anaerobic environment emerges which establishes favorable conditions for the significant increase of such organisms as *Actinomyces, Corynebacterium,* and *Fusobacterium*. Subsequently, the proportion of streptococci and *Neisseria* decrease with increasing plaque formation (75).

The difficulty in establishing a new species of the oral flora in the mouths of older animals may be due to a failure of the organism to break into an established ecosystem (88). During the first few years of life, the oral cavity is exposed to a myriad of microorganisms. As physiologic changes occur in the host, certain organisms become established in their own niches. As more organisms become established, a certain degree of stability may be seen in the adult and there are

less openings for the establishment of other species. The later appearing organisms must be able to compete with the already established flora, or a major alteration as may occur through antibiotic treatment, must take place. This explains why, although the oral cavity is continuously exposed to such a variety of microbes, many are not able to find permanent residency there. Those organisms for which there is no permanent niche make up the transient oral flora. Generally, the organisms best adapted to a given site and circumstance will survive (88).

Mechanisms of Retention

If an organism is to survive in the oral cavity, a mechanism of retention as well as certain types of nutritional and physicochemical environments are required. Retention in the mouth usually occurs through adhesion or mechanical entrapment (88).

1. Adhesion

Microorganisms have the capacity to adhere either to dental surfaces and/or to each other (37,88). Gibbons and Nygaard (37) tested 23 strains of representative plaque bacteria for their ability to agglutinate. Noting that 18 of the 23 strains agglutinated, they concluded that there are plaque bacteria which can adhere to the surface coatings of specific strains of other plaque species. They reasoned that this may explain how some species are able to adhere together in dental plaque. Dental plaque has been defined as a product of microbial growth, tenaciously attached to the surfaces of teeth and exhibiting a definite histological architecture (31).

A second mechanism for interbacterial adhesion has been demonstrated using strains of *Streptococcus mutans*, an organism which produces an extracellular polymer in the presence of sucrose. This extracellular polysaccharide, similar in structure to dextran (24), is involved in the attachment of the organisms to the teeth, and has been shown to hold the organisms together both *in vivo* (58) and *in vitro* (35,57). Fitzgerald *et al.* (29) demonstrated that dextranase, the enzyme responsible for dextran degradation, can prevent plaque formation in a hamster model system. It was observed that this enzyme did not inhibit the growth or survival of the streptococci. Therefore, they concluded that the anti-caries effect was brought about by the use of dextranase, which prevented the streptococci present in the oral cavity from colonizing on the tooth surfaces. Kelstrup and Gibbons (58) reported that a strain of *Streptococcus salivarius* produced large quantities of levan and smaller amounts of a glucose-containing polysaccharide when grown in the presence of sucrose. Since plaque formation was not observed from those cells grown in sucrose broth supplemented with dextranase, they concluded that the dextran-like polysaccharide, rather than levan, was essential for the development of plaque formation *in vivo* and *in vitro*. In addition, it has been shown that levan is rapidly hydrolyzed by levanase, an enzyme produced by many human plaque streptococci (22,93).

Gibbons and Fitzgerald (36) noted that although *S. mutans* was capable of agglutinating with dextran, other bacterial species, including other dextran-forming organisms, could not. They reasoned that *S. mutans* agglutinated in the presence of dextran because of

specific receptor sites on the surface of the organism which are capable of binding dextran molecules. Agglutination would be observed if the molecule was large enough to allow several cells of *S. mutans* to bind to the same polymer.

A third method of interbacterial adhesion can take place through the production of polymers by the host. Although the exact mechanism of this process is still under investigation, such organisms as *Streptococcus sanguis, Actinomyces naeslundii,* and *Actinomyces viscosus* agglutinate when mixed with salivary polymers (39,88).

In addition to interbacterial adhesion, oral microorganisms can be retained by attaching to the epithelium or dental surfaces (88). However, the attachment of a microbe to a particular site in the oral cavity is highly specific. Note from Table 1, the preference of certain species for specific sites in the human oral cavity. Observe, for example, how *S. mutans* and *S. sanguis* prefer hard surfaces, while *S. salivarius* has an affinity for epithelial cells (88).

2. Mechanical Entrapment

Another mechanism of retention in the oral cavity is through mechanical entrapment. Possible sites of nonadhesive retention of bacteria include carious lesions, gingival crevices, and periodontal pockets (88). Spirochetes such as *Treponema denticola,* and various gram-negative anaerobic rods such as *Bacteroides melaninogenicus,* have been recovered primarily from periodontal pockets or gingival crevices (24). A greater number of lactobacilli have been recovered from denture-wearers than from edentulous adults. This suggests that dentures mechanically retain organisms.

Spirochetes are reduced in numbers in the edentulous adult with or without dentures (59,78), and in infants without teeth (10, 59,61). Spirochetes do not appear in plaques, on teeth, or on tissue surfaces. Loesche (63) showed that spirochetes' unique nutritional requirements for growth could only be met by retention in the gingival crevice region. This shows how nutrition plays a dominant role in combination with mechanical entrapment (88).

Physiology

Nutritional sources for organisms residing in the oral cavity include the host's diet, the host's tissues or secretions, and/or secretions by other microorganisms (88). Both the quantity of flora and kinds of microbial populations are influenced by the diet of the host (8,12,14,26). De Stoppelaar *et al.* (26) reported that human subjects put on a carbohydrate-free diet for 17 days showed significant decreases in the percentage of *Streptococcus mutans* in dental plaque. Simultaneously, the percentage of *Streptococcus sanguis* increased. Reinstitution of the normal diet resulted in the return of these organisms to their original proportions. The presence and increase of *S. sanguis* during the carbohydrate-free period indicates that this organism does not require the synthesis of extracellular polysaccharides and the presence of sucrose for its establishment on tooth surfaces. The importance of sucrose in the establishment of *S. mutans* in dental plaques of humans and animals has already been discussed (24,35,57,58). The amount of plaque in man and animals increases with the sucrose rather than glucose content in the diets (12,14,38). Carlsson and Sundstrom (17) observed alterations in

population densities and carbohydrate to nitrogen ratios due to the production of extracellular polysaccharides by certain plaque organisms.

Socransky *et al.* (85) reported on the differences in microbial flora in rats fed high protein diets or Purina lab chow diets. The rats on high protein diets showed twice as many gram-positive facultative pleomorphic rods. It was believed that these rods were responsible for the increased incidence of calculus formation in these rats.

Studies using dogs given a soft diet showed a higher incidence of gingivitis than those on a hard diet (27). Subsequent studies by Carlsson and Egelberg (13) revealed no differences in the fairly rapid accumulation of plaque in dogs whether a soft diet composed of protein or fat was used with or without added sucrose. Polysaccharide-producing streptococci, as found in human mouths, are not found in the oral cavities of dogs. This may account for the differences in plaque formation in response to sucrose in the two hosts.

Many organisms residing in the oral cavity are not influenced by the host's diet. Such organisms as ***Bacteroides melaninogenicus*** and ***Treponema denticola*** obtain their nourishment directly from the saliva, gingival crevice fluid, or mammalian tissue cells (88). ***Treponema denticola*** requires alpha 2 globulin compounds for survival. This compound is found only within the oral cavity in mammalian tissues or secretions (86). Loesche (63) has postulated that other sources of nutrition for bacterial growth may come from local tissue cell destruction.

Theilaide *et al.* (91) described variations in microbial flora which occurred as a result of alterations in the normal flow of gingival crevice fluid. They described a close correlation between the amount of plaque development and gingivitis, and concluded that plaque formation influences gingivitis. Socransky (88) has postulated that the reverse may be true; i.e., inflammation of the gingiva may encourage plaque formation.

Some bacteria can survive in the oral cavity only if certain other microbes which would supply them with their essential nutrients are present. For example, *Treponema microdentium* is capable of surviving in the oral cavity only if organisms secreting isobutyrate and polyamines are present (87). *Bacteroides melaninogenicus* requires a vitamin K-like substance for growth which can be provided by *Staphylococcus aureus* (69).

Different organisms have varying oxygen-tension requirements. The oral cavity can house aerobic, microaerophilic, and anaerobic organisms. As the amount of oxygen present within a particular site in the oral cavity changes, so do the microorganisms which inhabit that location. Plaque formation simulates an anaerobic environment and, subsequently, the number of aerobes such as *Neisseria* and streptococci decrease and anaerobes such as *Actinomyces* and *Fusobacterium* increase (75).

Microbial Relationships

Microbial populations in the oral cavity are directly influenced by the interrelationships among its members and the oral environment. The associations between different microorganisms and their hosts are

described as symbiotic, commensal, opportunistic, synergistic, or pathogenic. Microorganisms can establish similar types of relationships among themselves (69).

Many organisms found within the oral cavity resemble potential pathogens. Alpha-hemolytic streptococci, staphylococci, and spirochetes of the canine mouth resemble well known potential pathogens. Evolutionists have postulated that the human normal flora was initially pathogenic but in time established a passive relationship with man (24).

Loesche (63) and Socransky *et al.* (87) reported that the presence of certain organisms which secrete isobutyrate and polyamines, as well as the type of environment provided by the gingival sulcus region, are required for the growth of *Treponema microdentium*. Certain aerobic organisms utilize atmospheric oxygen and subsequently reduce the oxidation-reduction potential, thus favoring the growth of anaerobes (69). Ritz (75) reported that the growth of anerobic organisms such as *Neisseria* and *Nocardia*, while high initially, declined with plaque development. Anaerobes such as *Fusobacterium* and *Veillonella* increased in proportions as plaque grew. *Bacteroides melaninogenicus* was dependent upon organisms in the oral cavity which secrete a vitamin K-like substance. This commensal-type relationship can be seen on blood agar with *B. melaninogenicus* growing as a satellite colony within a *Staphylococcus aureus* colony (69).

The oral flora may benefit the host in several ways. Components may compete with certain pathogens in such a way as to restrict the number of the latter in the mouth. This has not been the case in

those individuals whose normal flora has been altered through the use of various antibiotics. The normal microbial populations may also act as an antigenic stimulus as these organisms commonly enter the blood stream in small numbers. This results in the host having low levels of circulating antibodies which may cross-react with various pathogens (24).

The antibodies in saliva are almost all of the secretory IgA class of immunoglobulins, which function especially well on mucosal surfaces (31). As a result of antibody interaction with plaque microbial antigens, certain processes such as enhanced phagocytosis, bacterial lysis, neutralization of enzymes or toxins of the bacteria, or interference with bacterial metabolism or growth may result (31).

The oral flora may also play a substantial role in initiating many serious diseases. These microbes can gain access to tissues in man and cause abscesses in the lungs, brain, and extremities. Actinomycosis, candidiasis and subacute bacterial endocarditis are examples of infections initiated by oral flora (24,31,69). The accumulation of certain types of oral bacteria in the mouth can have serious consequences such as carious lesions or periodontal diseases, e.g., gingivitis and periodontitis.

Dental plaque can induce pathological changes in periodontal tissues as a result of microbial products and components such as enzymes and endotoxins, immunopathologic processes, and the release of endogenous histolytic enzymes from the host tissues as a result of microbial action (31). Endotoxin has been associated with pathologic changes in oral tissues, but may also induce nonspecific resistance

to local and systemic infections involving human oral bacteria. Endotoxin-stimulated polymorphonuclear cells have increased capacities for intracellular killing of oral bacteria *in vitro* (54). Therefore, it is thought that endotoxin-stimulated polymorphonuclear leukocytes could be involved in phagocytosis of the gingival sulcus (42). Jenson *et al.* (55) have reported on the capacity of the *Veillonella* endotoxin to induce inflammation as well as enhance phagocytosis. Gustafson *et al.* (42) have found that the endotoxin produced by *Leptotrichia buccalis* has a greater potency than the endotoxin of *Escherichia coli*. This organism has potent lethal, pyrogenic, and leukopenic activity in rabbits. Endotoxins from gram-negative plaque bacteria may cause the rupture of polymorphonuclear leukocytes. This could result in the release of lysozomes from leukocytes which release free acid phosphatases, esterases, and other enzymes that affect host gingival tissue and may cause periodontal disease (69). Baboolal *et al.* (3) pointed out that the quantity of bacterial endotoxin and the clinical degree of inflammation are directly correlated.

Past studies have indicated that spontaneous periodontitis and gingivitis are not widespread in animals. Studies of periodontal disease in monkeys and dogs are of particular value since these species have a gingival crevicular epithelium similar to that found in humans (31). Saxe *et al.* (79) have reported that gingivitis and destruction of the deeper periodontal tissues in the beagle dog were correlated with the accumulation of dental plaque. However, plaque formation increased in response to sucrose in the human diet, whereas in the dog there was no difference in plaque formation in response to diets with or without sucrose (13).

Periodontal disease is the main cause of tooth loss in the adult and has been attributed to certain organisms residing in the gingival crevicular area (31). Actinomycetes, *Rothia, Nocardia, Corynebacterium,* and certain streptococci all have the ability to form large quantities of subgingival plaque. The exact mechanism for this disease is unknown, but it has been shown that removal of microbial plaque may help prevent periodontal disease (11).

No specific organism has been shown responsible for gingivitis. The types of organisms isolated from healthy gingiva have already been listed (Table 2). In gingivitis, there appears to be a marked increase in the number of organisms present, as well as a shift in the prevalent types. Gram-negative cocci and bacilli, fusiform bacilli, spirochetes, and vibrios all increase in number, but invasion by new organisms does not occur (80).

A number of products produced by various microorganisms are responsible for the breakdown of normal tissues. Table 3 lists some of the microbial products that may contribute to the invasiveness and virulence of the organisms, and those organisms which may be associated with pathogenicity in gingival disease.

PART II. ORGANISMS IN THE ORAL CAVITY WHICH MAY BE ASSOCIATED WITH DISEASE

Pasteurella

Pasteurella multocida has been known as an important animal pathogen for many years and its occurrence as a human pathogen has been increasingly noticed (19). *Pasteurella multocida* is now considered a commensal usually found in the upper respiratory and

Table 3. Microorganisms and products which may be associated in gingival diseases

Products	Action	Microorganisms producing these products
ENDOTOXIN	A) interferes with essential metabolic functions necessary to maintain cellular integrity B) may cause extensive tissue destruction C) causes generalized physiologic changes in the host D) appears to have no tissue selectivity	Gram-negative bacilli
HEMOLYSINS	A) breakdown of red blood cells and periodontal tissue ultimately resulting in the spread of infection	Streptococci and staphylococci, *et al.*
STREPTODORNASE (deoxyribonuclease)	A) liquifies purulent exudates	Gram-positive cocci
HYALURONIDASE	A) breaks down the intracellular substance hyaluronic acid B) facilitates the spread of infection through tissues	Staphylococci, streptococci, pneumococci, diphtheroid bacilli, *et al.*
PROTEASES	A) aid in invasion of normal tissues	Anaerobic streptococci

Table 3 (continued)

Products	Action	Microorganisms producing these products
COLLAGENASES	A) hydrolyzes collagen B) destroys collagen fibers	*Clostridium* sp. *Bacteroides* sp.
EDEMA-PRODUCING FACTOR	A) causes edema	Pneumococci
LEUKOCIDIN	A) destroys polymorphonuclear leukocytes	Staphylococci, streptococci

Modified from *Oral Microbiology*. William A. Nolte (ed.). C. V. Mosby Co., St. Louis, Mo. 1973; and Scopp, I. W. *Oral Medicine. A Clinical Approach With Basic Science Correlation*. C. V. Mosby Co., St. Louis, Mo. 1973.

digestive tracts of domestic animals. It can be readily transmitted by animal bites (83). Smith (82) studied the bacterial flora in the nose and tonsils of 111 healthy dogs, and found that 54% of them harbored *P. multocida* in their tonsils and 10% in noses. He also reported that *P. multocida* was frequently the dominant bacterium recovered from the tonsils of dogs. Hawkins (43) reported on a survey regarding the incidence of *P. multocida* in tonsils and gums of healthy dogs and cats. Fifty-two percent of these cats and 14% of the dogs harbored these organisms. Lee and Buhr (60) noted that *P. multocida* was the most common infecting organism in their 1960 report of 69 patients who had been bitten by dogs. Hubbert and Rosen (49) reported that the infection rate resulting from cat bites was approximately ten times greater than that for dog bites. Cats may initiate this infection by biting and/or scratching. The sharpness of the cat's claws, coupled with its habit of grooming by licking its paws, appears to be of significance in infections (92).

Variations in populations of *P. multocida*, like the pneumococci, tend to increase during the cold and damp seasons. In some dogs, *P. multocida* may function as a secondary invader, resulting in penetration of the mucous membrane, with secondary pneumonia following recovery from such viral diseases as distemper (82). Lee and Buhr (60) and Smith (82) reported an increase in the incidence of *P. multocida* infections from dog bites during the winter months. This was attributed to the greater incidence of respiratory diseases such as canine distemper in the winter. Smith (82) reported a high incidence of *P. multocida* in the nose and tonsils of dogs between 1 and 3

years of age, with a tendency towards nonfatal cases of viral infections associated with the respiratory tract. Smith did not recover *P. multocida* in dogs over 8 years of age and he thought this reflected a general simplification of the nasal and tonsillar flora with increasing age in dogs. Put forward as possible explanations were changes in the mucous membranes due to senility and the development of full immunity against certain organisms (82). *Pasteurella multocida* seems to have developed a high degree of adaptation for a commensal existence as evidenced by its spread and abundant occurrence in dogs without usually causing disease. The dog accommodates a large number of these organisms in its throat and yet is usually able to withstand invasion of tissues (82).

Three clinical patterns of *P. multocida* infection in man are usually seen. These include: a) local infection following animal bites or scratches; b) chronic pulmonary infection with *P. multocida*, either as the primary pathogen or in association with other organisms; and c) systemic infections with meningitis or bacteremias (28,43,89, 92). Tindall *et al.* (92) reported that the most common type of human infection stemmed from injuries caused by animals, especially cat and dog bites as well as cat scratches. Within 18-24 hours, the resultant infection may be quite severe and painful to the touch, swollen, and red, with a gray-colored serous or purulent discharge emanating from the puncture wound. Considerable cellulitis is usually found in the surrounding area (28,44). If the wound infection is deep, osteomyelitis, an inflammation of the underlying bone caused by pus-forming bacteria, may result (44,67,89,92). Tissue damage may

be extensive and human infections are characteristically slow in healing as granulation is delayed. Frequently, infections are aggravated by the use of sutures in the wounds, thus requiring prolonged treatment, and resulting in unsightly scars (60). These organisms may also invade the blood stream of debilitated individuals and persons with a general lowered resistance, producing a septicemia followed by chills, high fever, severe prostration, and occasionally death (44,70,92).

Pasteurella multocida is a small, gram-negative rod or coccobacillus, nonmotile, nonsporeforming, and fermentative (20). However, some differences in cellular morphology, although not diagnostic, have been noted in strains from different animals. *Pasteurella multocida* recovered from dogs varied from small coccobacilli to long filaments. Bovine strains tended to be pleomorphic as compared to the nonpleomorphic, porcine strains (83). Weaver (94) described characteristics of *P. multocida* which differentiate it from various aerogenic *P. multocida*-like organisms (Table 4). Forty-four strains of *Pasteurella*-like organisms were described as producing some gas from glucose (strains designated P. "gas"). Of these, 17 strains had been recovered from humans bitten by dogs and 2 from humans bitten by cats (94). Talbot and Sneath (89) reported isolating a strain of *P. multocida* recovered from a human wound resulting from a dog bite which produced small amounts of gas from sucrose, maltose, mannitol, and trehalose. Rogers and Eldes (76) isolated an aerogenic *P. multocida* strain associated with purulent leptomeningitis in a dog. Winton and Mair (95) isolated what was reported to be the first strain of *Pasteurella pneumotropica* recovered from a dog bite wound.

Table 4. Differential characteristics of *Pasteurella multocida* and aerogenic *Pasteurella multocida*-like organisms

	Pasteurella multocida	*Pasteurella* "gas"
Gas from glucose	-	- or +
Beta hemolysis?	-	-
Carbohydrate base used	F	F
Glucose	A	A
Xylose	V	-
Mannitol	V	-
Lactose	V	-
Sucrose	A	A
Maltose	V	A
Levulose	A	A
Catalase	+	V
Oxidase	+	+
MacConkey	-	-
Gelatinase	-	-
TSI sl/butt	A/A	A/A
gas/H_2S	-/-	V/-
Motility	-	-
Indole	+	+
Nitrate	-	-
Urea	-	+
NO_3 reduction	+	+

Modified from R. E. Weaver (94).

+ = positive
- = negative
A = acid
V = variable
F = fermentative

Miscellaneous Gram-Negative Bacteria

The late Elizabeth O. King and her colleagues at the Center for Disease Control (CDC) in Atlanta, Georgia, have made considerable progress in establishing the identity of over 35,000 cultures from human clinical specimens between 1949 and 1973. Of these, over 4,000 organisms have been classified into what is today known as the "miscellaneous gram-negative bacteria" (90). Particular attention is directed in this literature review to those organisms frequently associated with human infections resulting from dog bites.

1. Group EF-4

This group of bacteria consists of small gram-negative, short, rod- to coccoid-shaped organisms. Their action on carbohydrates is fermentative. Colonies average 1-2 mm in diameter and appear circular, entire, opaque, convex, and mucoid. They are not hemolytic although an occasional greening on blood agar has been observed after 24 hours. Some of the typical biochemical reactions are shown in Table 5. CDC has reported isolating 85 strains of this type; 66 of these were recovered from humans and, of these 66, 32 were from humans who had been bitten by dogs or cats. EF-4 has also been isolated from extra-intestinal sources in humans; 11 isolates were reported from gums, mandibles, and lungs of dogs (90).

2. Group IIj

Following an incubation period of 24 hours, colonies of this type are 0.5 mm in diameter, circular, entire, translucent, smooth, glossy, and butyrous. No hemolysis is seen in blood agar plates,

Table 5. Characteristics of unclassified gram-negative aerobic oral flora as reported in the literature (90)

Test or substrate	EF-4	IIj	M-5
Catalase	+ (100%)*	+ (94%)	+ (100%)
Oxidase	+ (100%)	+ (100%)	+ (100%)
Growth on MacConkey	($+^{w}$) or - 0(65%)	- (0%)	+ or - (85%)
Urease	- (0%)	+ (100%)	I (100%)
Indole	- (0%)	+ (100%)	- (0%)
Simmon's citrate	- (0%)	- (0%)	- (0%)
Motility	- (0%)	- (0%)	- (0%)
Gelatin	- or (+) 0(25%)	+ (94%)	- (0%)
Oxidation-fermentation	F (100%)	I or NG (100%)	I (100%)
Glucose	+ or (+) 82(18%)	- (0%)	- (0%)
Maltose	- (0%)	- (0%)	- (0%)
Lactose	- (0%)	- (0%)	- (0%)
Mannitol	- (0%)	- (0%)	- (0%)
Sucrose	- (0%)	- (0%)	- (0%)
NO_3 reduction:			
$NO_3 \rightarrow NO_2$	+ or - (26%)	- (0%)	- (0%)
$NO_3 \rightarrow$ amine	- or + (15%)	- (0%)	- (0%)
Unreduced nitrate	- (4%)	+ (100%)	+ (100%)

Table 5 (continued)

Test or substrate	EF-4	IIj	M-5
Pigment	- (0%)[a]	+ (94%)	+ or - (44%)
TSI sl/butt	K or A'w/A or N'	K[7]/N or N/N	K/K or K/N
H_2S on TSI agar	- (0%)	- (0%)	- (0%)

*(%) = percent positive based on 85 cultures of EF-4, 35 cultures of IIj, and 41 cultures of M-5.

w = weak positive reaction or light growth
- or + = most strains negative, some are positive
+ or - = most strains positive, some are negative
I = inactive NG = no growth + = positive - = negative
(+) = most strains positive
K = alkaline
A = acid

[a]Some discrepancies in the literature.

although occasional greening may be observed. These bacteria are most often medium length rods, although some filamentous forms have been reported. Of the 36 cultures isolated at CDC, 17 had been recovered from infected human lesions resulting from bites or scratches of dogs or cats. Eleven cultures, obtained from human spinal fluid, blood, and sputa, were of unknown host origin. Seven isolates were recovered from lower animals, including dogs and cats (90). Two strains were isolated in the Clinical Microbiology Laboratory at Michigan State University, one from the conjunctiva of a dog, and the second from the periodontium of a cat. The biochemical characteristics of IIj are listed in Table 5.

3. Group M-5

Members of this group resemble bacteria of the genus *Moraxella*. They produce either a yellow or tan pigment and are rod-shaped or coccoid, and usually occur in pairs or chains. Filamentous forms have also been observed. Table 5 lists some of the biochemical reactions of this group. Of 41 cultures studied at CDC, 25 had been isolated from infected wounds caused by dog bites. Four strains were recovered from the tongue, gums, and trachea of dogs. The other 12 isolates recovered from wounds in humans were of unknown origin (90).

Actinomycetes

Members of this genus are gram-positive, irregularly staining bacteria which are nonacid-fast, nonsporeforming, nonmotile, and anaerobic or microaerophilic. Many species exhibit filaments with true branching (6). A catalase positive, aerobic, nonacid-fast

actinomycete isolated from hamster dental plaque has been extensively studied. Howell *et al.* (48) officially named this organism *Odontomyces viscosus*; it was later renamed *Actinomyces viscosus* (6,32). This "hamster organism" was shown to induce periodontal disease (48). *Actinomyces viscosus* has been isolated from the oral cavity of hamsters, rats, and man, but its pathogenicity for man has not been established (6).

Actinomycosis is a chronic, suppurative infection, most often found involving the oral cavity and cervicofacial region of domestic animals as well as man (69). The principal causative agent of human actinomycosis is *A. israelii* (72,80), although *A. naeslundii* and *A. eriksonii* have occasionally been implicated (69). *Actinomyces viscosus* was the infective agent in 6 cases of actinomycosis diagnosed in dogs (23). *Actinomyces israelii* is a gram-positive anaerobic to microaerophilic organism, normally found in the human mouth (6,80). It is unable to penetrate intact mucosa and requires a break in the tissues to establish itself (69). Actinomycosis may result from tooth extractions or injury to the mouth or throat (65), surgical or accidental trauma, e.g., a compound jaw fracture (52), periodontal disease (18), or an infected root canal of a carious tooth (40). Actinomycetes may also be aspirated into the lungs causing pulmonary actinomycosis, or be swallowed and invade the intestinal mucosa resulting in abdominal actinomycosis (69,94). Transmission by human bites, although rare, has been reported (69). *Actinomyces israelii* gains entrance to the soft tissues and, as the lesion grows, multiple abscesses develop. These may break through the skin and cause a

characteristic pattern of multiple sinuses. Within these abscesses are found sulfur granules consisting of microcolonies of filamentous and branching actinomycetes (72).

Currently attempts are being made to answer the question, why do some actinomycetes, which are normal inhabitants of the oral cavity, become pathogenic? In cases involving cervicofacial actinomycosis in particular, actinomycetes are found in association with organisms such as streptococci, fusobacteria, *Bacteroides corrodens*, or *Actinobacillus actinomycetemcomitans*. Some researchers think it is the association of aerobes and anaerobes acting synergistically which contributes to the pathogenicity of the actinomycetes (45). Others hypothesize that an allergic reaction may have a role in the development of lesions (69).

Staphylococci

Micrococci occur universally in the human oral cavity, but are not usually dominant numerically (73). Coagulase positive *Staphylococcus aureus* may be found regularly in the oral cavity of man (56). *Staphylococcus epidermidis* is found in greater frequency than *S. aureus* in the healthy mouth. However, *S. aureus* predominates over *S. epidermidis* in cases involving open suppurative lesions such as periodontitis (53).

The pathogenicity of staphylococci may relate to their ability to produce such extracellular factors as hemolysins, leukocidin, enterotoxins, coagulase, and hyaluronidase (69). Staphylococci have been implicated in a number of oral and dental lesions such as osteomyelitis of the jaw, parotitis (an infection characterized by a

discharge of purulent exudate), and facial cellulitis (69). Tooth extractions may result in bacterial endocarditis under certain circumstances (74).

Streptococci

Streptococcus species comprise approximately 30 to 60% of the bacteria residing on the surfaces of teeth, cheek, tongue, and saliva. The majority of these organisms belong to the viridans or alpha-hemolytic group (34). *Streptococcus mutans* appears to be the most virulent of the alpha-hemolytic streptococci and has been shown to have the capacity to initiate dental decay affecting smooth enamel surfaces. The cariogenic potential of this organism may be associated with its ability to adhere on tooth surfaces forming large microbial dental plaque deposits as previously described (24,31,34, 35,57,58). The two species found most frequently in the oral cavity are *S. mitis* and *S. salivarius*. These have been found in abscesses, ulcerative stomatitis, root canals, periodontal pockets, calculus, and carious lesions (69).

The appearance of beta-hemolytic streptococci in the oral cavity is of considerable interest because of their possible role in the spread of infection. Group A beta-hemolytic streptococci have been implicated in causing septic sore throat, scarlet fever, and rheumatic fever in human beings. Certain extracellular products such as hyaluronidase, streptokinase, deoxyribonuclease, and hemolysins (69) contribute to the pathogenesis of streptococcal diseases.

Neisseria

Neisseria species have been found in the oral cavity and upper respiratory tract of healthy humans (6); however, some of these organisms have been identified as the only microorganisms present in some serious infections. Hudson (50) isolated *N. pharyngis* from a patient who had developed subacute bacterial endocarditis. *Neisseria catarrhalis (Branhamella catarrhalis)* has been implicated in an infection of the parotid gland following a blow from a fist (69). Additional evidence suggests that pigmented dental plaque may be produced by certain oral chromogenic *Neisseria* organisms (69). Carious mouths have a characteristic population of nonpigmented *Neisseria* organisms which contain catalase and do not produce a copious amount of polysaccharide on 5% sucrose agar. Noncarious mouths, in contrast, have been shown to contain *Neisseria* organisms which do not produce catalase but do produce a copious amount of polysaccharide on 5% sucrose agar (68).

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ARTICLE

AEROBIC BACTERIAL GINGIVAL FLORA OF THE DOG

By

D. A. Saphir and G. R. Carter

SUMMARY

Gingival scrapings from dogs were collected to isolate and identify species of the aerobic, bacterial gingival flora. Of particular interest was the frequent recovery of three unclassified groups of aerobic gram-negative bacteria, IIj, EF-4, and M-5, previously associated with human infections resulting from dog bites. Although no set pattern existed between the variability and consistency of gingival microbiota as related to age, sex, and breed of dog, certain characteristic flora can be predicted in the healthy canine gingiva. Members of the following genera were found: *Streptococcus, Staphylococcus, Actinomyces, Escherichia, Corynebacterium, Pasteurella, Caryophanon, Mycoplasma, Acinetobacter, Moraxella, Neisseria, Enterobacter,* and *Bacillus*.

INTRODUCTION

Studies of gingival flora in the dog are of particular value as this animal has a gingival crevicular epithelium similar to that found in humans (7). Few investigators, however, have concentrated their efforts on describing gingival flora in dogs.

The organisms found within the oral cavity of domestic animals consist of many potential pathogens and commensals (14,17,18). Disease may occur as a result of local infection by microorganisms which usually inhabit the mouth in large numbers. However, for an organism

to produce lesions, dynamic alterations involving the host and/or the microorganisms must occur. Irritation, trauma, penetration of foreign bodies such as bones or sticks, carious teeth, and neoplasms contribute to bacterial invasion (6). It has been shown that certain organisms of the oral cavity were recovered in wounds resulting from animal bites. For example, *Pasteurella multocida* infections in humans have for some time been associated with animal bites (2,5,10).

The purpose of this investigation was to isolate and identify to species, where possible, the aerobic bacterial flora found in gingival scrapings of dogs. Particular attention was given to those organisms previously reported to be associated with human infections resulting from dog bites.

MATERIALS AND METHODS

Collection of material. Gingival scrapings were taken, using sterilized gauze pads, from 50 dogs in the East Lansing, Michigan, area. Care was taken to select only healthy dogs.

Cultural and identification procedures. Immediately following collection, gauze pads were immersed in sterile flasks containing 10 ml of nutrient broth (Difco). All samples were thoroughly agitated, then streaked 15 minutes after collection for isolation on blood agar plates, 4% blood agar, MacConkey plates, and PPLO agar (Difco). Plates were incubated in a 4% CO_2 incubator for 24 h, examined, and reincubated for an additional 24 to 48 h. Each different colonial type was subcultured. Colonial and cellular morphologies of all strains were recorded and biochemical characteristics of the organisms

were determined. Each organism was identified, when possible, using present classification tables (1,19).

Antibiotic susceptibility tests. Antibiotic susceptibility tests were done on several isolates of Group IIj and Group EF-4 using the standard Kirby-Bauer procedure (19). Group EF-4 was placed on Mueller-Hinton agar media (Difco), and antibiotic susceptibility patterns for Group IIj were done using a tryptose agar (Difco) plus yeast extract (Difco) base.

RESULTS

Fifty dogs were used in this survey. The organisms isolated from gingival scrapings included Group IIj, Group EF-4, *Moraxella* (including *M. phenylpyruvica, M. nonliquefaciens,* M-4, and M-5), *Enterobacter aerogenes, Escherichia coli, Acinetobacter calcoaceticus (*var *lwoffi* and *anitratus), Pasteurella* (including *P. multocida* and *Pasteurella*-like organisms), *Neisseria, Staphylococcus aureus, Staphylococcus epidermidis,* and other *Micrococcaceae,* α-, β-, and γ-hemolytic streptococci, *Corynebacterium, Actinomyces* (including *A. viscosus), Bacillus, Caryophanon,* and *Mycoplasma*. Only those organisms capable of growing aerobically on blood agar and/or MacConkey agar plates, and PPLO medium (Difco) within 72 h at 37 C in a 4% CO_2 incubator were reported.

Group EF-4

Bacteria of this type consisted of small gram-negative, short rod- to coccoid-shaped organisms. Colonies averaged 1-2 mm in diameter and appeared circular, entire, opaque, convex, and mucoid, sometimes

producing a yellow pigment. They were nonhemolytic, although an occasional greening of blood agar was observed. Typical biochemical reactions are shown in Table 1. Thirty percent of the dogs examined harbored these organisms (Table 2).

Group IIj

Colonies of this group were circular, entire, translucent, smooth, glossy, and butyrous, and very sticky, making them difficult to remove from solid media. Growth in broths was poor. No hemolysis was seen on blood agar plates, although greening was occasionally observed around colonies. Organisms were gram-negative, medium length rods. Much of the viability of this organism was lost within one week. Table 1 lists the differential reactions of Group IIj. A relatively large inoculum on Christensen's urea agar showed rapid hydrolysis. Group IIj was seen in 38% of the dogs examined.

Moraxella

Members of this genus were rod-shaped or coccoid, usually occurring in pairs or chains. Of the 40% of the canine samples harboring bacteria of this type (Table 2), M-5 was the most frequent isolate (Table 3). M-5 resembled other bacteria of this genus. They produced a yellow or tan pigment, were nonhemolytic, and were identified by the biochemical reactions shown in Table 1.

Pasteurella and *Pasteurella*-like organisms

These gram-negative coccoid to small rod-shaped organisms were found in 22% of the dogs (Table 2). Half of these isolates identified from the reactions shown in Table 1 consisted of organisms typical of

Table 1. Summary of identification reactions of some unusual aerobic microorganisms found in gingival scrapings from dogs

ORGANISMS	catalase	oxidase	carbohydrate base used	glucose	lactose	sucrose	maltose	mannitol	TSI sl/butt	TSI gas/H_2S	urea	Simmon's citrate	MacConkey	gelatin	motility	indol	nitrate reduction	hemolysis
Group IIj*	+	+	NG	-	-	-	-	-	N^{V}/N	-/-	+	-	-	+	-	+	-	
Group EF-4*	+	+	F	A	-	-	-	-	K/A^{V}	-/-	-	-	$+^{W}$	$-^{V}$	-	-	$+^{V}$	
M-5*	+	+	I	-	-	-	-	-	K/K^{V}	-/-	I	-	$+^{W}$	-	-	-	-	
Corynebacterium "species"	+		F	A	V	A^{V}	A				-		-		-		-	
Actinomyces viscosus	+		F	(A)	A^{V}	A	A	-			-			-		-	+	

Table 1 (continued)

ORGANISMS	catalase	oxidase	carbohydrate base used	glucose	lactose	sucrose	maltose	mannitol	TSI sl/butt	TSI gas/H_2S	urea	Simmon's citrate	MacConkey	gelatin	motility	indol	nitrate reduction	hemolysis
Acinetobacter calcoaceticus																		
var *lwoffi*	+	-	I	-	-		-^{v}	-	K/N	-/-	V	V^{v}	+	-^{v}	-	-	-	V
var *anitratus*	+	-	O	+	(+)		-^{v}	-	K/N	-/-	V	+^{v}	+	V	-	-	-	V
Pasteurella multocida	+	+	F	A	(-)^{v}	A	(-)^{v}	V	A/A	-/-	-	-	-	-	-	+	+	
Pasteurella-like	+	+	F	A	-	A	A^{v}		A/A	-/-	-	-	-	-	-	-	+	
Unknown**	+	+	OF	w	w	w	w	w	N/N	***	-	-	-	+^{d}	-	-	-	

*Modified from H. W. Tatum, *et al.* (19); **An organism frequently observed but not identifiable at present; ***H_2S - 4+ reaction on lead acetate paper, no gas in TSI; NG = no growth; N = neutral; F = fermentative; O = oxidative; v = occasionally variable; K = alkaline; A = acid; w = weak positive; V = variable; (+) = most strains positive; (-) = most strains negative; d = delayed.

Table 2. Frequency of isolation of aerobic bacteria recovered in gingival scrapings from 50 dogs

GRAM-NEGATIVE	No. of + canine samples	Percent of incidence	GRAM-POSITIVE	No. of + canine samples	Percent of incidence
1) *Moraxella*	20	40.0%	1) Streptococci	41	82.0%
2) Group IIj	19	38.0%	2) *Micrococcaceae*	30	60.0%
3) Group EF-4	15	30.0%			
4) *Escherichia coli*	11	22.0%	3) *Corynebacterium*	13	26.0%
5) *Pasteurella*[a]	11	22.0%	4) Actinomycetes	7	14.0%
6) *Caryophanon*	10	20.0%	5) *Bacillus*	6	12.0%
7) *Neisseria*[b]	10	20.0%			
8) *Acinetobacter calcoaceticus*	5	10.0%			
9) *Enterobacter*	1	2.0%			

OTHER

1) *Mycoplasma* in 35 out of 41 dogs 85.4%

[a]Includes *Pasteurella*-like organisms.

[b]Includes *Branhamella catarrhalis*.

Table 3. Frequency of isolation of aerobic bacterial species recovered in gingival scrapings from 50 dogs

GRAM-NEGATIVE	No. of + canine samples	Percent of incidence	GRAM-POSITIVE	No. of + canine samples	Percent of incidence
1) *Moraxella* - 21 isolates			1) Streptococci - 45 isolates		
M. phenylpyruvica	3	14.3%	α-hemolytic streptococci	36	80.0%
M. nonliquefaciens	2	9.5%	β-hemolytic streptococci	5	11.1%
M-5	9	42.8%	γ-hemolytic streptococci	4	8.9%
M-4	1	4.8%			
M-"species"	6	28.6%			
2) *Pasteurella* - 12 isolates			2) *Micrococcaceae* - 35 isolates		
P. multocida	6	50.0%	*Staph. aureus*	9	25.7%
P-like	6	50.0%	*Staph. epidermidis*	16	45.7%
3) *Neisseria* - 10 isolates			Other species	10	28.6%
*B. catarrhalis**	5	50.0%			
Neisseria "species"	5	50.0%	3) Actinomycetes - 8 isolates		
			A. viscosus	5	62.5%
4) *Acinetobacter calcoaceticus* - 5 isolates			*A.* "species"	3	37.5%
var *lwoffi*	4	80.0%			
var *anitratus*	1	20.0%			

*Present classification - *Branhamella catarrhalis*
formerly - *Neisseria catarrhalis*

Pasteurella multocida. However, organisms similar in colonial and cellular morphology, but differing in key biochemical reactions from *P. multocida*, were also found (Table 1). These organisms, which appeared to belong to the *Pasteurella* genus, did not conform to any recognized species by available classification tables.

Caryophanon

This motile, filamentous, multicellular eubacterium was found in 10 out of 50 dog samples (Table 2). Some discrepancies exist in the literature relating to the gram-reaction of *Caryophanon*, but the organisms recovered in this survey were distinctly gram-negative.

Neisseria

Isolates of *Neisseria catarrhalis*, more recently reclassified as *Branhamella catarrhalis* (12), were found in 5 dogs (Table 3). The other 5 isolates identified as *Neisseria* were gram-negative, oxidase positive cocci that produced a yellow pigment. They could not be identified with known species.

Actinomyces

Five isolates of *Actinomyces viscosus* were found in gingival scrapings (Table 3). The organism was identified on the basis of delayed growth pattern (48 to 72 h), a crumbly, off-white, and dry colonial morphology, gram-positiveness, nonacid-fastness, filamentous to "Y-shaped" cellular configuration, and the biochemical reactions shown in Table 1. Three isolates with similar colonial and cellular morphology but different biochemical characteristics were also recovered.

Corynebacterium

Twenty-six percent of the canine samples contained diphtheroids. They were all nonhemolytic, 0.5 to 1.0 mm in diameter, and white, but varied in biochemical reactions. Glucose and maltose were fermented in all cases, but lactose and sucrose fermentation varied (Table 1).

Mycoplasma

These organisms, isolated on PPLO medium (Difco), had a characteristic "fried egg" appearance. Colonies were pleomorphic, extremely small on the agar medium, opaque, with a yellowish central area. These organisms were recovered in 35 out of 41 dogs sampled.

Miscellaneous unidentified bacteria

1. A number of organisms isolated from gingival scrapings in dogs produced a yellow pigment, were nonmotile, weakly or nonfermentative, and catalase positive (Table 3). These bacteria were gram-negative, small, thin, rod- to coccoid-shaped cells and seemed to resemble species of *Flavobacterium*.

2. Granular, dry, nonhemolytic, rhizoid colonies were frequently recovered from dogs. Colonies were pinpoint in 24 h, but increased in size following further incubation. These filamentous organisms stained gram-variably, and were inactive biochemically.

Streptococci and staphylococci

Alpha-hemolytic streptococci were isolated more frequently than other organisms, with the exception of *Mycoplasma*, and made up a large part of the flora of gingival scrapings from these dogs (Table 2).

Isolates of beta- and gamma-hemolytic streptococci were much less frequent in comparison (Table 3). Of the 35 isolates of *Micrococcaceae*, over 45% consisted of *Staphylococcus epidermidis* and 25% were yellow or white pigmented, beta-hemolytic *Staphylococcus aureus* (Table 3).

Twenty-three female and 27 male dogs were examined. No differences were found in gingival scrapings between males and females. Also, no differences in aerobic flora were observed when dogs were grouped according to the American Kennel Club classification of breeds. The results of the antibiotic susceptibility tests on Groups IIj and EF-4 are presented in Tables 4 and 5, respectively.

DISCUSSION

The gingival crevicular epithelium in man and dogs is quite similar (7). Perhaps the establishment of the oral flora in dogs and man is also similar. If an organism is to survive in the oral cavity of an animal, some mechanism of retention, as well as a suitable nutritional or physicochemical environment are usually required (18). The development of teeth provides a new attachment site for bacteria in carious lesions and gingival crevice areas. The oral cavity can sustain aerobic, microaerophilic, and anaerobic organisms. As the amount of oxygen present within a particular site in the oral cavity changes, so do the microorganisms which inhabit that location. Plaque formation simulates an anaerobic environment and, subsequently, the number of aerobes such as *Neisseria* and streptococci decrease and anaerobes such as *Actinomyces* and *Fusobacterium* increase (13).

Table 4. Results of antibiotic susceptibility tests on Group IIj

Antimicrobial agents	a	b	c	d	e	f	g
Ampicillin	S	S	S	S	S	S	S
Chloramphenicol	S	S	S	S	S	S	S
Gentamycin	S	S	S	S	S	S	S
Lincomycin	S	S	S	S	S	S	S
Neomycin	S	S	E	E	R	S	S
Nitrofurantoin	S	S	S	S	S	S	S
Penicillin	S	S	E	E	S	S	S
Polymyxin B	R	R	R	R	S	S	R
Sulfadimethoxine	R	R	R	R	S	S	S
Tetracycline	S	S	S	S	S	S	S

S = sensitive

R = resistant

E = equivocal

Table 5. Results of antibiotic susceptibility tests on Group EF-4

Antimicrobial agents	a	b	c	d	e
Ampicillin	S	S	S	S	S
Cephalothin	S	R	S	S	R
Chloramphenicol	S	S	S	S	S
Gentamycin	S	S	S	S	S
Lincomycin	R	R	S	S	R
Neomycin	R	R	R	R	R
Nitrofurantoin	S	S	S	S	S
Novobiocin	S	S	S	S	S
Penicillin	R	R	S	R	R
Polymyxin B	S	S	S	S	S
Sulfadimethoxine	S	S	S	S	S
Tetracycline	S	S	S	S	S

S = sensitive

R = resistant

The gingival flora seems to be as diversified or even more variable in the puppy than in the adult dog. Variability in the puppy, as in the human child, may be due to failure of a particular organism to find a suitable attachment site, the absence of an essential nutrient required for growth, or an unsatisfactory oxygen relationship to mention only several possibilities. Older animals develop an established ecosystem, making it more difficult to incorporate a new bacterial species. During the first few years of life, the oral cavity is exposed to a myriad of microorganisms. As physiologic changes occur in the host, certain organisms seem to establish themselves in particular locations. As organisms settle in these niches, a certain degree of stability ensues, especially in the adult, and there are fewer vacancies for new species. The latter must compete with the resident flora, and they frequently have difficulty breaking into an established ecosystem (18).

Based on the data of this survey, indications are that the young dog acquires gingival flora, similar to that found in the adult, at an early age. Most of the younger dogs examined had only limited contact with humans and were frequently housed among other members of their own species. It would therefore seem likely that the puppy acquires its adult-like gingival flora by association with other dogs, including its mother.

The Center for Disease Control in Atlanta, Georgia (CDC), reported isolating 36 cultures of Group IIj, 17 from human lesions resulting from bites or scratches of dogs and cats (19). There is as yet no evidence that Group IIj causes disease in the dog. Its

role may be that of a secondary invader. Without guanine-cytosine percentages and base homologies one can only speculate on generic classification at this time. Considering the characteristics described in Table 1 and its non-fermentative nature, Group IIj appears to resemble the genera *Moraxella* (specifically *M. phenylpyruvica*) and *Brucella* (specifically *B. canis*) most closely.

CDC reported isolating 85 strains of Group EF-4; 66 of these were recovered from humans and, of these 66, 32 were from humans who were bitten by dogs or cats (19). Group EF-4 strains have not been incriminated as a cause of disease in the dog. On the basis of the preceding characteristics described in Table 1, Group EF-4 appears to resemble most closely the genera *Pasteurella* and *Actinobacillus*.

Group M-5 resembles bacteria of the genus *Moraxella* (19) on the basis of biochemical reactions (Table 1) and cellular and colonial characteristics. Of 41 cultures studied at CDC, 25 were recovered from infected wounds caused by dog bites. There is yet no evidence that suggests M-5 is associated with disease in the dog.

Caryophanon was described as a gram-positive, motile, large rod or filament, and was originally isolated from cow dung (8). *Caryophanon* was also described as a gram-negative organism (15) which concurs with the results of this study. Further studies described this organism in water, intestines of arthropods and vertebrates, and in decomposing organic material (11). *Caryophanon* has been seen not infrequently in Wright-stained smears from oral mucosa of dogs (3). An attempt was made to recover *Caryophanon* from several dogs that yielded it the

first time. The organism was not reisolated. It may be that *Caryophanon* is unable to establish permanent residence in the gingiva and is only a part of the transient flora.

The organisms classified as *Pasteurella*-like differed from *P. multocida* in their negative indol and occasionally acid from maltose reaction. Smith (16) reported that dog strains of *P. multocida* frequently possessed such special characteristics as acid production from maltose but not xylose and mannitol, low pathogenicity for mice, saline and acid sensitivity, and absence of capsules. Other *Pasteurella*-like organisms recovered from humans bitten by dogs or cats were described as producing some gas from glucose (20).

Actinomyces viscosus was implicated as the causative agent of periodontal disease with subgingival plaque in hamsters (9). Experimentally induced infections were produced in mice from hamster strains (9). Although pathogenicity for man is yet undetermined, *A. viscosus* has been isolated from the human oral cavity (1). In this study, *A. viscosus* was found in the gingiva of 5 dogs with clean teeth and healthy gums. *Actinomyces viscosus* has been reported as the cause of 6 cases of actinomycosis in dogs (4).

Although there was considerable variation in the oral flora among individuals, a number of organisms were recovered with fair consistency. These included streptococci, staphylococci, and *Mycoplasma,* 3 gram-negative bacteria associated with dog bites in humans (Groups IIj and EF-4, M-5), and occasionally such potential pathogens as *Pasteurella* and *Actinomyces*. *Caryophanon, Neisseria, Acinetobacter, Corynebacterium,* and *Bacillus* were sporadically isolated from the canine gingiva.

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APPENDIX

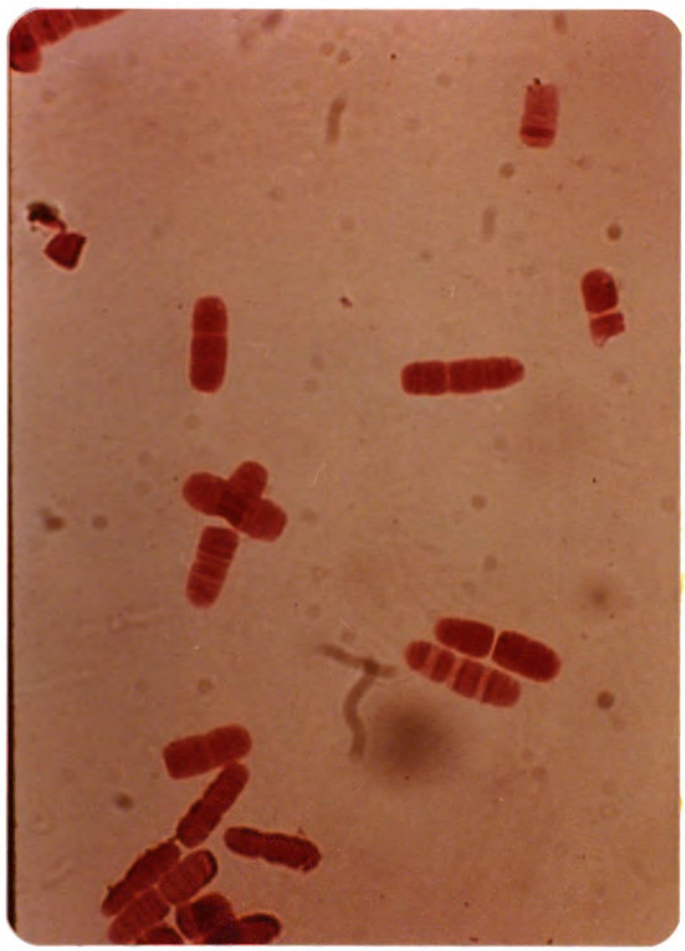

Figure 1. Gram stain of *Caryophanon*.

Table A-1. A summary of the identification data used in studying the gingival flora of 50 dogs

Code	Age	Sex	Breed	Code	Age	Sex	Breed
1	4 yrs.	F	German Shorhaired Pointer	19	5 wks.	M	Mongrel
2	2 yrs.	M	Mongrel	20	5 wks.	M	Mongrel
3	20 mo.	F	St. Bernard	21	6 wks.	M	Mongrel
4	5 yrs.	F	Boxer	22	9 wks.	M	Miniature Schnauzer
5	11 mo.	M	Labrador	23	3 yrs.	F	Mongrel
6	7 mo.	F	Sheltie	24	12 yrs.	M	Mongrel
7	5 mo.	F	Mongrel	25	6 mo.	F	Mongrel
8	6 yrs.	M	Welsh Corgi	26	11 wks.	F	Great Dane
9	18 mo.	M	Irish Setter	27	3 yrs.	M	Mongrel
10	2 yrs.	M	Cockapoo	28	3 mo.	M	English Setter
11	7 wks.	F	German Shepherd	29	3 mo.	F	Collie
12	1 yr.	F	Mongrel	30	2 mo.	F	Dachshund
13	12 yrs.	F	Beagle	31	2 mo.	M	Boston Terrier
14	4 yrs.	F	Mongrel	32	3 mo.	M	Beagle
15	3 yrs.	M	Mongrel	33	4 mo.	F	Norwegian Elkhound
16	4 wks.	M	Mongrel	34	3 mo.	F	Chow-Chow
17	4 wks.	M	Mongrel	35	2 mo.	F	Siberian Husky
18	3 mo.	F	Maltese	36	3 mo.	F	Collie

Table A-1 (continued)

Code	Age	Sex	Breed	Code	Age	Sex	Breed
37	2 mo.	F	American Eskimo	44	2 yrs.	M	German Shepherd
38	3 mo.	M	Pomeranian	45	4 yrs.	M	Mongrel
39	3 mo.	M	Collie	46	7 mo.	F	Mongrel
40	3 mo.	M	Irish Setter	47	4 yrs.	M	St. Bernard
41	3 mo.	M	Cocker Spaniel	48	7 mo.	M	Mongrel
42	8 mo.	M	Mongrel	49	5 yrs.	M	German Shepherd
43	9 mo.	F	Mongrel	50	6 mo.	F	Sheltie

Table A-2. Identifiable aerobic microorganisms isolated from gingival scrapings of 50 dogs

CODE	Group IIj	Group EF-4	*M. phenylpyruvica*	*M. nonliquefaciens*	M-5	M-4	*M*-"species"	*E. aerogenes*	*Escherichia coli*	*Acinetobacter calcoaceticus*	*P. multocida*	*Pasteurella*-"like"	*B. catarrhalis*	*Neisseria*-"species"	*Staphylococcus aureus*	*Staph. epidermidis*	"Other" *Micrococcaceae*	α-hemolytic streptococci	β-hemolytic streptococci	γ-hemolytic streptococci	*Corynebacterium*"sp."	*Actinomyces viscosus*	*Actinomyces* "species"	*Bacillus*	*Caryophanon*	*Mycoplasma*
1	X	X		X														X			X					X
2	X						X						X					X								X
3					X				X	X[a]		X					X	X			X					X
4					X						X					X	X	X			X	X				X
5		X														X		X								X
6	X	X							X									X								X
7																X		X				X				X
8														X			X	X								X
9	X	X												X				X			X				X	X
10	X	X		X							X						X	X			X	X				X

Table A-2 (continued)

CODE	11	12	13	14	15	16	17	18	19	20	21
Group II]	x	x	x	x	x						
Group EF-4		x		x							
M. phenylpyruvica				x				x			
M. nonliquefaciens											
M-5		x	x		x						x
M-4											
M-"species"											
E. aerogenes											
Escherichia coli	x					x	x		x	x	
Acinetobacter calcoaceticus		x[a]		x[a]	x[a]						
P. multocida											
Pasteurella-"like"	x										
B. catarrhalis											
Neisseria-"species"			x								
Staphylococcus aureus	x							x			x
Staph. epidermidis		x				x	x		x	x	x
"Other" *Micrococcaceae*				x		x			x		x
α-hemolytic streptococci	x	x			x		x		x		
β-hemolytic streptococci	x										
γ-hemolytic streptococci			x								
Corynebacterium"sp.					x					x	
Actinomyces viscosus											
Actinomyces "species"											
Bacillus			x		x	x	x				
Caryophanon					x			x			
Mycoplasma	x	x	x	x	x			x			

Table A-2 (continued)

CODE	22	23	24	25	26	27	28	29	30	31	32
Group IIj		x	x	x	x						x
Group EF-4		x					x			x	
M. phenylpyruvica						x					
M. nonliquefaciens											
M-5			x	x							
M-4						x					
M-"species"											
E. aerogenes											
Escherichia coli	x	x									
Acinetobacter calcoaceticus											
P. multocida		x									
Pasteurella-"like"					x					x	x
B. catarrhalis						x					
Neisseria-"species"				x	x						
Staphylococcus aureus	x									x	
Staph. epidermidis								x	x		
"Other" *Micrococcaceae*						x					
α-hemolytic streptococci	x	x			x	x	x	x	x	x	
β-hemolytic streptococci			x	x							
γ-hemolytic streptococci							x	x			
Corynebacterium"sp."		x				x					x
Actinomyces viscosus			x	x							
Actinomyces "species"				x		x					
Bacillus											
Caryophanon								x		x	
Mycoplasma		x	x	x	x	x	x	x	x	x	x

Table A-2 (continued)

CODE	33	34	35	36	37	38	39	40	41	42	43
Group IIj	X						X			X	X
Group EF-4					X		X			X	
M. phenylpyruvica											
M. nonliquefaciens											
M-5											
M-4											
M-"species"											X
E. aerogenes											
Escherichia coli											
Acinetobacter calcoaceticus											
P. multocida											
Pasteurella-"like"											
B. catarrhalis					X						
Neisseria-"species"											
Staphylococcus aureus		X			X						
Staph. epidermidis			X	X					X		
"Other" *Micrococcaceae*											
α-hemolytic streptococci	X	X	X	X	X	X	X	X		X	
β-hemolytic streptococci										X	
γ-hemolytic streptococci											
Corynebacterium"sp."											X
Actinomyces viscosus											
Actinomyces "species"							X				
Bacillus											
Caryophanon		X		X		X		X	X		
Mycoplasma	X	X	X	X	X	X	X	X	X	NT	NT

Table A-2 (continued)

CODE	Group IIj	Group EF-4	*M. phenylpyruvica*	*M. nonliquefaciens*	M-5	M-4	*M-"species"*	*E. aerogenes*	*Escherichia coli*	*Acinetobacter calcoaceticus*	*P. multocida*	*Pasteurella-"like"*	*B. catarrhalis*	*Neisseria-"species"*	*Staphylococcus aureus*	*Staph. epidermidis*	"Other" *Micrococcaceae*	α-hemolytic streptococci	β-hemolytic streptococci	γ-hemolytic streptococci	*Corynebacterium* "sp."	*Actinomyces viscosus*	*Actinomyces* "species"	*Bacillus*	*Caryophanon*	*Mycoplasma*
44					X			X	X	X[q]								X								NT
45							X				X	X						X						X		NT
46		X															X	X								NT
47							X				X				X				X		X					NT
48		X					X									X		X								NT
49	X															X				X						NT
50							X		X		X				X											NT

X^a = *Acinetobacter calcoaceticus* var *lwoffi*.

X^b = *Acinetobacter calcoaceticus* var *anitratus*.

NT = not tested.

Table A-3. Frequency of isolation of aerobic gram-negative bacteria recovered in gingival scrapings with respect to gender

	FEMALES (23)			MALES (27)		
	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
Group IIj	10		43.5%	9		33.3%
Group EF-4	7		30.4%	8		29.6%
Moraxella	10		43.5%	10*		37.0%
M. phenylpyruvica		2	(20.0%)		1	(9.1%)
M. nonliquefaciens		1	(10.0%)		1	(9.1%)
M-5		5	(50.0%)		4	(36.4%)
M-4		0	(0.0%)		1	(9.1%)
M-"species"		2	(20.0%)		4	(36.4%)
Enterobacter aerogenes	0		0.0%	1		3.7%
Escherichia coli	5		21.7%	6		22.2%
Acinetobacter calcoaceticus	3		13.0%	2		7.4%
var *lwoffi*		3	(100.0%)		1	(50.0%)
var *anitratus*		0	(0.0%)		1	(50.0%)

Table A-3 (continued)

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	FEMALES (23)			*MALES (27)*		
Pasteurella	6		26.1%	5**		18.5%
Pasteurella multocida		3	(50.0%)		3	(50.0%)
Pasteurella-like		3	(50.0%)		3	(50.0%)
Neisseria	6		26.1%	4		14.8%
*Branhamella catarrhalis****		3	(50.0%)		2	(50.0%)
Neisseria "species"		3	(50.0%)		2	(50.0%)
Caryophanon	4		17.4%	6		22.2%

*One male dog had both *M. phenylpyruvica* and M-4. Therefore, the total species isolates do not add up to 10.

**One male dog had both *Pasteurella multocida* and a *Pasteurella*-like organism. Therefore, the total species isolates do not add up to 5.

***Formerly *Neisseria catarrhalis*

Table A-4. Frequency of isolation of aerobic gram-positive bacteria recovered in gingival scrapings with respect to gender

	FEMALES (23)			MALES (27)		
	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
Micrococcaceae	15		65.2%	15[c]		55.6%
Staph. epidermidis		7	(46.6%)		9	(47.4%)
Staph. aureus		4	(26.7%)		4	(21.0%)
Other species		4	(26.7%)		6	(31.6%)
Streptococci[a]	19		82.6%	22		81.5%
α-hemolytic streptococci		17	(80.9%)		19	(79.1%)
β-hemolytic streptococci		2	(9.5%)		3	(12.5%)
γ-hemolytic streptococci		2	(9.5%)		2	(8.3%)
Corynebacterium	5		21.7%	8		29.6%
Actinomycetes[b]	3		13.0%	4		14.8%
Actinomyces viscosus		3	(75.0%)		2	(50.0%)
Actinomyces "species"		1	(25.0%)		2	(50.0%)

Table A-4 (continued)

	FEMALES (23)			MALES (27)		
	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
Bacillus	1		4.3%	5		18.5%
OTHER MYCOPLASMA	20/20		100.0%	15/21		71.4%

[a]Several dogs had more than one species of *Streptococcus*. Therefore, the total species isolates do not add up to 19 in the females or 22 in the males.

[b]One female dog had *Actinomyces viscosus* and an *Actinomyces*-like organism. Therefore, the total species isolates do not add up to 3.

[c]Several dogs had more than one organism of this type. Therefore, the total species isolates do not add up to 15.

Table A-5. The occurrence of aerobic microorganisms isolated from 6 sporting dogs

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	GRAM-NEGATIVE			*GRAM-POSITIVE*		
Group IIj	2		33.3%	2		33.3% *Micrococcaceae*
					2	(100.0%) *S. epidermidis*
Group EF-4	4		66.7%			
				5		83.3% streptococci[a]
Moraxella	1		16.7%		5	(83.3%) α-hemo. strep.
M. phenylpyruvica		1	(100.0%)		1	(16.7%) γ-hemo. strep.
Neisseria	1		16.7%	2		33.3% *Corynebacterium*
Neisseria "species"		1	(100.0%)			
Caryophanon	3		50.0%			
OTHER						
MYCOPLASMA	6/6		100.0%			

[a]One animal had more than one species of *Streptococcus*. Therefore, the total species isolates do not add up to 5.

Table A-6. The occurrence of aerobic microorganisms isolated from 4 hounds

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	GRAM-NEGATIVE			*GRAM-POSITIVE*		
Group IIj	2		50.0%	1		25.0%) *Micrococcaceae*
					1	(100.0%) *S. epidermidis*
Moraxella	1		25.0%			
M-5		1	(100.0%)	3		75.0% streptococci
					2	(66.7%) α-hemo. strep.
Neisseria	1		25.0%		1	(33.3%) γ-hemo. strep.
Neisseria "species"		1	(100.0%)			
				1		25.0% *Corynebacterium*
Pasteurella	1		25.0%			
Pasteurella-like		1	(100.0%)	1		25.0% *Bacillus*
OTHER						
MYCOPLASMA	4/4		100.0%			

Table A-7. The occurrence of aerobic microorganisms isolated from 15 working dogs

	GRAM-NEGATIVE			GRAM-POSITIVE		
	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
Group IIj	5		33.3%	11		73.3% *Micrococcaceae*[a]
					4	(33.3%) *S. aureus*
Group EF-4	3		20.0%		5	(41.6%) *S. epidermidis*
					3	(25.0%) other species
Moraxella	5		33.3%			
M-5		3	(60.0%)	14		93.3% streptococci[b]
M-"species"		2	(40.0%)		12	(75.0%) α-hemo. strep.
					3	(18.7%) β-hemo. strep.
Escherichia coli	5		33.3%		1	(6.2%) γ-hemo. strep.
Enterobacter aerogenes	1		6.6%	3		20.0% *Corynebacterium*
Pasteurella	5		33.3%	2		13.3% Actinomycetes
Pasteurella multocida		3	(60.0%)		1	(50.0%) *A.*-"species"
Pasteurella-like		2	(40.0%)		1	(50.0%) *A. viscosus*
Acinetobacter calcoaceticus	2		13.3%			
var *lwoffi*		1	(50.0%)			
var *anitratus*		1	(50.0%)			

Table A-7 (continued)

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	GRAM-NEGATIVE			*GRAM-POSITIVE*		
Neisseria	4		26.6%			
Neisseria "species"		2	(50.0%)			
*Branhamella catarrhalis**		2	(50.0%)			
Caryophanon	2		13.3%			
OTHER						
MYCOPLASMA	11/11		100.0%			

[a]One animal had two species in this group. Therefore, the total species isolates do not add up to 11.

[b]Several animals had more than one species of *Streptococcus*. Therefore, the total species isolates do not add up to 14.

*Formerly *Neisseria catarrhalis*.

Table A-8. The occurrence of aerobic microorganisms isolated from terriers (1), toys (2), and non-sporting dogs (2)

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	GRAM-NEGATIVE			*GRAM-POSITIVE*		
			TOYS (2)			
Moraxella	1		50.0%	1		50.0% *Micrococcaceae*
M. phenylpyruvica		1	(100.0%)		1	(100.0%) *Staph. aureus*
Caryophanon	2		100.0%	1		50.0% streptococci
					1	(100.0%) α-hemo. strep.
OTHER						
MYCOPLASMA	2		100.0%			
			NON-SPORTING DOGS (2)			
Group EF-4	1		50.0%	2		100.0% *Micrococcaceae*
					2	(100.0%) *Staph. aureus*
Pasteurella	1		50.0%			
Pasteurella-like		1	(100.0%)	2		100.0% streptococci
					2	(100.0%) α-hemo. strep.

Table A-8 (continued)

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	GRAM-NEGATIVE			*GRAM-POSITIVE*		
Neisseria	1		50.0%			
*B. catarrhalis**		1	(100.0%)			
Caryophanon	2		100.0%			
OTHER						
MYCOPLASMA	2		100.0%			
		TERRIERS (1)				
Escherichia coli	1		100.0%	1		100.0% *Micrococcaceae*
					1	(100.0%) *Staph. aureus*
				1		100.0% streptococci
					1	(100.0%) α-hemo. strep.

*Formerly *Neisseria catarrhalis*.

Table A-9. The occurrence of aerobic microorganisms isolated from 20 mongrels

	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	GRAM-NEGATIVE		
Group IIj	10		50.0%
Group EF-4	7		35.0%
Moraxella	12		60.0%
M. phenylpyruvica		1	(8.3%)
M. nonliquefaciens		1	(8.3%)
M-5		5	(41.7%)
M-4		1	(8.3%)
M.-"species"		4	(33.3%)
Escherichia coli	5		25.0%
Acinetobacter calcoaceticus var *lwoffi*	3		15.0%
		3	(100.0%)
Pasteurella[a]	3		15.0%
Pasteurella multocida		3	(75.0%)
Pasteurella-like		1	(25.0%)

	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	GRAM-POSITIVE		
	12		60.0% *Micrococcaceae*[b]
		1	(6.2%) *Staph. aureus*
		8	(50.0%) *S. epidermidis*
		4	(43.7%) other species
	15		93.7% streptococci[c]
		13	(81.2%) α-hemo. strep.
		3	(18.7%) β-hemo. strep.
	7		35.0% *Corynebacterium*
	4		20.0% Actinomycetes[d]
		4	(80.0%) *A. viscosus*
		1	(20.0%) *A.*-"species"
	5		25.0% *Bacillus*

Table A-9 (continued)

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
	GRAM-NEGATIVE			*GRAM-POSITIVE*		
Neisseria	3		15.0%			
Neisseria "species"		1	(33.3%)			
*B. catarrhalis**		2	(66.7%)			
Caryophanon	1		5.0%			
OTHER						
MYCOPLASMA	1		5.0%			

[a]One animal had a *P. multocida* and a *Pasteurella*-like organism. Therefore, the total species isolates do not add up to 3.

[b]Several animals had more than one species. Therefore, the total species isolates do not add up to 12.

[c]One animal had an alpha- and a beta-hemolytic *Streptococcus*. Therefore, the total species isolates do not add up to 15.

*Formerly *Neisseria catarrhalis*.

Table A-10. Isolation of aerobic gingival bacteria as related to the ages of male and female dogs

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
AGE		*<1 year*			*1-3 years*			*4-6 years*			*>6 years*	
TOTAL NUMBER OF DOGS		*32*			*9*			*7*			*2*	
GRAM-NEGATIVE ORGANISMS												
Group IIj	8		25.0%	6		66.7%	3		42.9%	2		100.0%
Group EF-4	9		28.1%	4		44.4%	2		28.6%	0		0.0%
Moraxella[a]	6		18.7%	7		77.8%	5		71.4%	2		100.0%
M-5		2	(33.3%)		4	(50.0%)		1	(20.0%)		2	(100.0%)
M.-"species"		4	(66.7%)		4	(50.0%)		4	(80.0%)		0	(0.0%)
Escherichia coli	8		25.0%	3		33.3%	0		0.0%	0		0.0%
Acinetobacter calcoaceticus	0		0.0%	4		44.4%	1		14.3%	0		0.0%
Pastuerella[a]	5		15.6%	3		33.3%	4		57.1%	0		0.0%
Neisseria	5		15.6%	3		33.3%	1		14.3%	1		50.0%
Caryophanon	8		25.0%	2		22.2%	0		0.0%	0		0.0%
Enterobacter	0		0.0%	1		11.1%	0		0.0%	0		0.0%

Table A-10 (continued)

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
AGE		*<1 year*			*1-3 years*			*4-6 years*			*>6 years*	
TOTAL NUMBER OF DOGS		*32*			*9*			*7*			*2*	
GRAM-POSITIVE ORGANISMS												
Micrococcaceae[a]	21		65.6%	4		44.4%	5		71.4%	0		0.0%
S. aureus		8	(32.0%)		0	(0.0%)		1	(16.7%)		0	(0.0%)
S. epidermidis		13	(52.0%)		1	(25.0%)		2	(33.3%)		0	(0.0%)
Other species		4	(16.0%)		3	(75.0%)		3	(50.0%)		0	(0.0%)
Streptococci[a]	24		75.0%	9		100.0%	6		85.7%	2		100.0%
α-hemo. strep.		22	(81.5%)		9	(100.0%)		4	(66.7%)		1	(33.3%)
β-hemo. strep.		3	(11.1%)		0	(0.0%)		1	(16.7%)		1	(33.3%)
γ-hemo. strep.		2	(7.4%)		0	(0.0%)		1	(16.7%)		1	(33.3%)
Corynebacterium	3		9.3%	6		66.7%	4		57.1%	0		0.0%
Actinomyces	4		12.5%	2		22.2%	1		14.3%	0		0.0%
Bacillus	3		9.3%	1		11.1%	1		14.3%	1		50.0%

Table A-10 (continued)

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
AGE		*<1 year*			*1-3 years*			*4-6 years*			*>6 years*	
TOTAL NUMBER OF DOGS		*32*			*9*			*7*			*2*	
OTHER												
MYCOPLASMA	21/27		77.8%	8/8		100.0%	4/4		100.0%	2/2		100.0%

[a]Several animals had more than one species.

Table A-11. Isolation of aerobic gingival bacteria as related to the ages of male dogs

	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence
AGE			*<1 year*			*1-3 years*			*4-6 years*			*>6 years*
TOTAL NUMBER OF DOGS			*16*			*6*			*4*			*1*
GRAM-NEGATIVE ORGANISMS												
Group IIj	3		19.7%	4		66.7%	1		25.0%	1		100.0%
Group EF-4	6		37.5%	2		33.3%	0		0.0%	0		0.0%
Moraxella	2		12.5%	5[a]		83.3%	2		50.0%	1		100.0%
M-5		1	(50.0%)		2	(33.3%)		0	(0.0%)		1	(100.0%)
M.-"species"		1	(50.0%)		4	(66.7%)		2	(100.0%)		0	(0.0%)
Escherichia coli	5		31.2%	1		16.6%	0		0.0%	0		0.0%
Acinetobacter calcoaceticus	0		0.0%	2		33.3%	0		0.0%	0		0.0%
Pasteurella	2		12.5%	1		16.6%	3[a]		75.0%	0		0.0%
Neisseria	0		0.0%	3		50.0%	1		25.0%	0		0.0%
Caryophanon	4		25.0%	2		33.3%	0		0.0%	0		0.0%
Enterobacter	0		0.0%	1		16.6%	0		0.0%	0		0.0%

Table A-11 (continued)

	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence
AGE	*1 year*			*1-3 years*			*4-6 years*			*6 years*		
TOTAL NUMBER OF DOGS	*16*			*6*			*4*			*1*		
GRAM-POSITIVE ORGANISMS												
Micrococcaceae	10[a]		62.5%	2		33.3%	3		75.0%	0		0.0%
S. aureus		3	(21.4%)		0	(0.0%)		1	(33.3%)		0	(0.0%)
S. epidermidis		8	(57.1%)		0	(0.0%)		1	(33.3%)		0	(0.0%)
Other species		3	(21.4%)		2	(100.0%)		1	(33.3%)		0	(0.0%)
Streptococci	11		68.7%	6		100.0%	4		25.0%	1		100.0%
α-hemo. strep.		11	(84.6%)		6	(100.0%)		2	(50.0%)		0	(0.0%)
β-hemo. strep.		1	(7.7%)		0	(0.0%)		1	(25.0%)		1	(100.0%)
γ-hemo. strep.		1	(7.7%)		0	(0.0%)		1	(25.0%)		0	(0.0%)
Corynebacterium	2		12.5%	4		66.7%	2		50.0%	0		0.0%
Actinomyces	1		6.3%	2		33.3%	0		0.0%	1		100.0%
Bacillus	3		19.7%	1		16.6%	1		25.0%	0		0.0%

Table A-11 (continued)

AGE	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence	No. of positive canine samples	No. of positive samples isolated	Percent of incidence
	<1 year			1-3 years			4-6 years			>6 years		
TOTAL NUMBER OF DOGS	*16*			*6*			*4*			*1*		
OTHER												
MYCOPLASMA	8/14		57.1%	5/5		100.0%	1/1		100.0%	1/1		100.0%

[a]Several animals had more than one species.

Table A-12. Isolation of aerobic gingival bacteria as related to the ages of female dogs

	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
AGE	*<1 year*			*1-3 years*			*4-6 years*			*>6 years*		
TOTAL NUMBER OF DOGS	*16*			*3*			*3*			*1*		
GRAM-NEGATIVE ORGANISMS												
Group IIj	5		31.2%	2		66.7%	2		66.7%	1		100.0%
Group EF-4	3		19.7%	2		66.7%	2		66.7%	0		0.0%
Moraxella	4		25.0%	2		66.7%	3		100.0%	1		100.0%
M-5		1	(25.0%)		2	(100.0%)		1	(33.3%)		1	(100.0%)
Others		3	(75.0%)		0	(0.0%)		2	(66.7%)		0	(0.0%)
Escherichia coli	3		19.7%	2		66.7%	0		0.0%	0		0.0%
Acinetobacter calcoaceticus	0		0.0%	2		66.7%	1		33.3%	0		0.0%
Pasteurella	3		19.7%	2		66.7%	1		33.3%	0		0.0%
Neisseria	5		31.2%	0		0.0%	0		0.0%	1		100.0%
Caryophanon	4		25.0%	0		0.0%	0		0.0%	0		0.0%
Enterobacter	0		0.0%	0		0.0%	0		0.0%	0		0.0%

Table A-12 (continued)

AGE TOTAL NUMBER OF DOGS	No. of positive canine species	No. of positive species isolated	Percent of incidence	No. of positive canine species	No. of positive species isolated	Percent of incidence	No. of positive canine species	No. of positive species isolated	Percent of incidence	No. of positive canine species	No. of positive species isolated	Percent of incidence
	<1 year 16			1-3 years 3			4-6 years 3			>6 years 1		
GRAM-POSITIVE ORGANISMS												
Micrococcaceae	11		62.5%	2		66.7%	2[a]		66.7%	0		0.0%
S. aureus		5	(45.4%)		0	(0.0%)		0	(0.0%)		0	(0.0%)
S. epidermidis		5	(45.4%)		1	(50.0%)		1	(33.3%)		0	(0.0%)
Other species		1	(9.1%)		1	(50.0%)		2	(66.7%)		0	(0.0%)
Streptococci	13[a]		81.2%	3		100.0%	2		66.7%	1		100.0%
α-hemo. strep.		11	(78.6%)		3	(100.0%)		2	(100.0%)		0	(0.0%)
β-hemo. strep.		2	(14.3%)		0	(0.0%)		0	(0.0%)		0	(0.0%)
γ-hemo. strep.		1	(7.1%)		0	(0.0%)		0	(0.0%)		1	(100.0%)
Corynebacterium	1		6.2%	2		66.7%	2		66.7%	0		0.0%
Actinomyces	3[a]		19.7%	0		0.0%	1		33.3%	0		0.0%
Bacillus	0		0.0%	0		0.0%	0		0.0%	1		100.0%

Table A-12 (continued)

AGE	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence	No. of positive canine samples	No. of positive species isolated	Percent of incidence
TOTAL NUMBER OF DOGS	*<1 year* 16			*1-3 years* 3			*4-6 years* 3			*>6 years* 1		
OTHER												
MYCOPLASMA	13/13		100.0%	3/3		100.0%	3/3		100.0%	1/1		100.0%

[a]Several animals had more than one species.