COMPRISON OF THE DISTRIBUTION OF ORAL CAVITY BACTERIA IN VARIOUS DOG POPULATIONS

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ABSTRACT

Dental diseases are common in dogs. Oral diseases which are chronic bacterial infections found in dogs, resulting as tooth decay, gingivitis, periodontal diseases and malodor. Salivary bacteria and the formation of dental biofilms on teeth are predominant factors in the etiology of those diseases. In this study we compared the distribution of supragingival oral bacteria of dogs from different habitats: municipal shelter dogs, Israel Defense Forces (IDF) and client owned dogs. Salivary samples from each dog were examined for total supragingival bacteria, lactobacilli and mutans streptococci bacteria. Our results show that the IDF dogs had significantly lower number of total oral supragingival bacteria as well as mutans streptococci bacteria compared to dogs from the municipal shelter and/or client owned dogs. Dogs from the municipal shelter had a significantly higher number of lactobacilli than both IDF and client owned dogs. A significantly higher mean count of total supragingival bacteria was found in mixed breed dogs compared to pure breed dogs. A comparison of the influence of the commercial dried dog food vs. the commercial dried dog food and human food leftovers on the mean general bacteria count and mutans streptococci revealed significantly lower counts in dogs that were fed only commercial dried food as opposed to dogs that ate dried food and human food leftovers. This study showed that the quantity of caries causing bacteria and the quantity of total supragingival bacteria are related to the environment in which the dogs were kept.

Keywords: dogs, populations, supragingival bacteria, lactobacilli, mutans streptococci

INTRODUCTION

Comprehensive microbial oral research on humans has strongly indicated the relationship between certain types of oral bacteria and dental diseases such as caries, gingivitis and periodontal diseases (1). As in humans, the outcomes of these oral diseases may result in oral-facial pain, dental calculus, halitosis, caries, gingivitis, periodontal diseases, eating disorders and eventually also loss of teeth in canines (2-5).

The prevalence of dental diseases in dogs increases up to more than 80% by 5 years of age (6-13). No conclusive data as far as the distribution of caries in dogs is available. The reports on incidence of caries in dogs vary between 5%-70% (14-15). The process of dental caries starts with the accumulation of supragingival, aerobic bacteria on teeth surfaces. This biofilm leads to caries and may be accompanied with halitosis (7,10-11,13,16-22).

The presence of mutans streptococci and lactobacilli in the oral cavity is directly associated with oral diseases like caries. These two types of bacteria are important etiological substances of the dental biofilm as well as the free bacteria in saliva (16,23-30). The mutans streptococci group includes several types of serotypes of the streptococci found in human and in various animals. Members of this group adhere well to the teeth surfaces, create biofilm, and secrete lactic acid as a result of carbohydrate fermentation from the diet. Lactobacilli also generate lactic acid and are also highly resistant to acid. The organic acids generated by oral bacteria cause demineralization of tooth enamel resulting in tooth cavities.

The aim of this study was to investigate the effect of environmental conditions including the type of diet in three different dog populations, in order to better understand the microbiology of dental caries in dogs. Improvement of oral health is an important factor in quality of life of canines.

MTHERIALS AND METHODS

Population

Dogs from three different habitats were chosen for this study. Ninety four generally healthy dogs participated in this trial. The dogs were not treated with any kind of tooth paste or given chewed bones or other kinds of oral hygiene treatment. None of the dogs had systemic disease. They also were not given any medications at

least 7 days before saliva samples were taken.
The first group consisted of 30 dogs from a municipal dog shelter located in Lod. The distribution of the breeds in this group was as follow: 15 mixed breed dogs, 5 Labrador Retriever, 4 German Shepherd (GSD), 2 Siberian Husky, 1 Samoyed, 1 Visla, 1 Amstaff American Staffordshire and 1 Miniature Pincher. In this group since no accurate age history was available, age was excluded from the analysis. Twenty three females and 7 males were examined in this group. The daily diet of these dogs was commercial dried food and human food leftovers.
The second group consisted of 31 dogs from the IDF (Israel Defense Forces), located in the Tel-Aviv district. All dogs were pure breed: 19 Belgian Shepherd (Malinois), 5 GSD, 4 Labrador Retriever and 3 Jack Russell. The age of these dogs was between 1 and 7 years. Nine females and 22 males were examined in this group. These dogs were raised in the same conditions and ate the same commercial dried food.
The third group was comprised of client owned dogs, which were examined at the Veterinary Teaching Hospital of the Hebrew University of Jerusalem, located in Rishon Lezion. This group was comprised of 33 dogs: 18 mixed breed, 2 Golden Retrievers, 2 GSD, 1 Visla, 1 Amstaff American Staffordshire,1 Siberian Husky, 1 Newfoundland, 1 Labrador Retriever, 1 Dog De Bordeaux, 1 Akita, 1 Rottweiler, 1 German Pointer, 1 Boxer and 1 Border Collie. Twenty five females and 8 males were examined in this group. The daily diet of these dogs was commercial dried food with or without human food leftovers.

Microbial Sampling
Salivary samples were obtained from the buccal side of the mandibular and maxillar pre-molar teeth of each dog. The bacterial samples were plated immediately on selective media and transferred for incubation within 1 to 2 hours.
Two types of oral bacteria were enumerated using two selective agar media as follows: ROG (Rogosa) for lactobacilli (31) and MSB (Mitis Salivarious, Bacitracin) for mutans streptococci (32). Total supragingival bacteria were enumerated using BHI (Brain-Heart Infusion) agar media.
The samples were incubated in an environment enriched with 5% CO_2, for 24 hours at 37°C for the total supragingival bacteria and 72 hours for the mutans streptococci and lactobacilli. After incubation, bacteria growth was measured visually as the colony forming units (CFU) using plate viewer (New Brunswick Scientific, New Brunswick, NJ, USA).
The estimation of bacterial growth was performed in a semi-quantitative method using a modified commercial scale between 1-7 (CRT bacteria, Ivoclar-vivadent, Liechtenstein) (30). A value of 1 was recorded for the lowest CFU ranking and 7 as the highest CFU ranking based on a scale provided in the CRT kit (Ivoclar-vivadent, Liechtenstein).
All samples were taken by the same examiner using the same technique and procedure. Each sample was examined by the same viewer.

Statistical analysis
All tests applied to the bacterial variables were non-parametric as these variables were semi-quantitative. The comparison of the three dog populations was carried out using the Kruskal-Wallis non-parametric ANOVA test. The non-parametric Mann-Whitney test was applied for comparing pairs of groups, with the Bonferroni correction of the significance level for multiple pair wise comparisons. The Spearman rank correlation coefficient was calculated in order to assess the association between two variables. All tests applied were two-tailed, and a p-value of $\leq 5\%$ was considered statistically significant. Each result is a mean of the four samples taken from each dog.

RESULTS
The three tested dog populations were compared as far as total supragingival bacterial counts**.** A statistically significant difference ($p<0.001$) was found between the three groups in the total number of oral supragingival bacteria. The lowest total bacteria ranking was found in the IDF dogs (median 4.25), the median ranking of client owned dogs was 5.75 while the municipal dogs showed the highest ranking of 6.25. The IDF dogs had a significantly lower number of total oral supragingival bacteria in comparison to dogs from the municipal shelter and/or client owned dogs [Significant according to the Bonferroni correction (Fig 1.).
Lactobacilli levels from dogs of the three populations, showed a statistically significant difference among the three groups ($p<0.001$). This difference was due to the fact that both IDF and client owned dogs had a median ranking of 0 while the median of the municipal dogs was 0.875. Dogs from the municipal shelter had a significantly higher number of lactobacilli than both IDF and client owned dogs [Significant according to the Bonferroni correction (Fig 2.).
Similar results were obtained for the mutans streptococci bacteria. In this case as well, a statistically significant difference was found among the three groups ($p =0.016$), median ranking was 0, 0 and 0.125 in the IDF, client owned dogs and municipal shelter groups, respectively. There was a lower count in dogs belonging to the IDF in comparison to dogs from the municipal kennel and/or client owned dogs [Significant according to the Bonferroni correction (Fig 3.).
The comparison between mixed breed dogs and pure breed dogs for total supragingival bacteria indicated a

statistically significant higher count in mixed breed dogs than in pure breed dogs [p =0.004, median is 6.000, and 4.875 in the mixed breed dogs and pure breed dogs groups, respectively (Fig 4.).

No statistical differences were found between the mean number of lactobacilli and mutans streptococci bacteria in mixed breed dogs vs. pure breed dogs.

A comparison of the influence of the commercial dried dog food vs. the commercial dried dog food and human food leftovers on the mean general bacteria count revealed significantly lower counts in dogs that ate only commercial dried food as opposed to dogs that ate commercial dried food and human leftover food [p =0.002, median 4.5 and 6.0 in the commercial dried dog food and the commercial dried dog food and human food leftovers groups, respectively (Fig 5.).

A significant difference between dogs that ate commercial dried food and dogs that ate commercial dried food and leftovers was noticed also in the mean count of mutans streptococci (p = 0.006, median 0, and 0.125 in the commercial dried dog food and the commercial dried dog food and human food leftovers groups, respectively), but no significant difference was shown in the mean count of lactobacilli.

No correlation was found between prevalence of any bacteria and the gender or age of the dogs examined.

In order to evaluate the association between the general bacteria count and each of the caries causing bacteria which were examined, the Spearman correlation coefficient was calculated. A medium positive correlation was found between BHI and ROG (Spearman's r=0.501, p<0.001) and a weak, but statistically significant, positive correlation was found between BHI and MSB (Spearman's r=0.322, p=0.006).

DISCUSSION

In this study, we compared oral microbiota of three different populations of dogs which were raised in Israel. These populations were chosen due to the variation of their environmental living conditions. The IDF dogs represent a uniform living situation in which the dogs were well treated and well fed. The second population consisted of variable client owned dogs with a varying degree of health control. The municipal dog shelter represented the lowest conditions for dog health. In this kennel some dogs were stray dogs with no accurate previous history available. The municipal shelter also suffered from financial problems, which limited its possibilities in fulfilling the health needs of the animals. Our aim was to check whether the environmental conditions in which the dog populations were raised influenced their supragingival oral microflora.

The results of this study showed indeed that there is a great influence of the diet on the dental health in dogs as reflected by significant variations in some of the supragingival bacterial species in the oral cavity. It was shown that dogs which were raised with optimal medical care and ate only commercial dried food as represented by the IDF dogs, had significantly less total supragingival bacteria as well as caries causing bacteria mutans streptococci and lactobacilli.

Another finding of the study was that in the mixed breed dogs group, the number of total oral bacteria was statistically greater than in pure breed dogs group. This trend of difference was also shown in the mean counts of lactobacilli and mutans streptococci bacteria, which was found to be higher in mixed breed dogs than in pure breed dogs. One explanation for these results might be that all the IDF dogs were pure breed. Another possibility is that an owner of a pure breed dog probably pays more attention to his dog's dental health requirements.

The type of food was found to play an important role in the potential for dental health problems of dogs. This fact is well known in human dental medicine (5,33-34). We found that the numbers of total supragingival bacteria as well as mutans streptococci bacteria were lower in dogs that were fed only commercial dried food in comparison to dogs that ate human food leftovers with commercial dried food. These results are similar to findings of other studies, which indicated the advantage of dried food by the maintenance of the periodontal tone and the fact that food when chewed dry is abrasive and scrapes off plaques (5,35-38).

Although it is well known that dental problems increase with age in dogs (6-7,9-12) this phenomena was not shown in our study. In this study no statistical differences were demonstrated in any of the parameters, which were checked regarding the age of dogs. This may be explained by the fact that most of the dogs in our study were middle aged as no puppies or old dogs were included.

Oral bacteria play a pivotal role in the etiology of dental diseases. Bacteria species found in canine are not necessarily found in human, although there are many shared types of bacteria in dogs and human. Mutans streptococci and lactobacilli which we tested are well-known as caries causing bacteria in humans and are also found in the animal kingdom including dogs (21,23-26,29-30). Indeed, about 18% of the bacteria from salivary samples of dogs were streptococci, while actinomycs species were the most abundant ones (25%). A high correlation was found when comparing the gene sequences of bacteria found in dogs' oral microbiota with sequences of human origin on GenBank (27,37). However, diversity in the distribution, percentage and serotypes of bacteria between human and animal are not surprising. Although there is abundant literature on animal studies conducted as a step before dental human studies, compared to human studies, little is known on the oral pathogens of dogs. Identifying the virulent factors is an essential step in prevention and treatment

of oral diseases. Human toothpastes and mouthwashes are two of the most popular dental medications. Several oral care products are on the market for dog owners. However, there is a need for studies focusing on dogs in order to develop specific medications which will enable better compliance,

In this study, a positive correlation was demonstrated between the total number of supragingival bacteria and the environmental conditions in which the dogs were raised.

The dogs, in which high levels of caries causing bacteria were found, are at high risk for dental disorders especially caries tartar and halitosis.

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Figures:

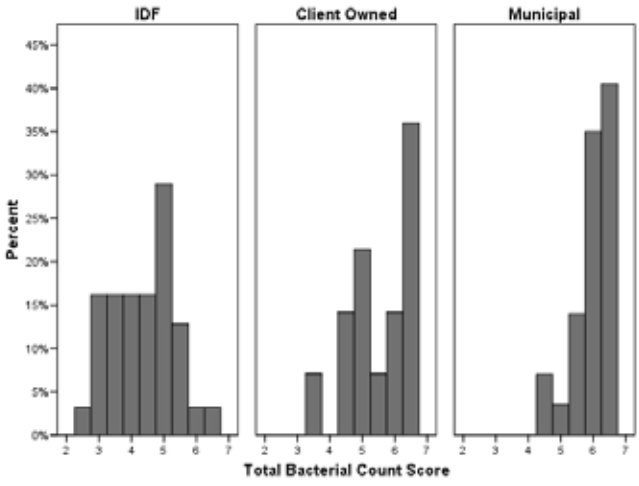

Fig 1. The mean ranking of total oral supragingival bacteria from three various dog populations

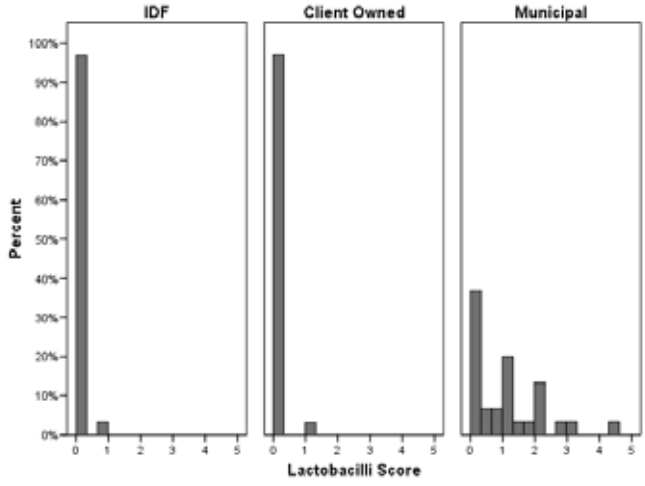

Fig 2. The mean ranking of lactobacilli from three various dog populations.

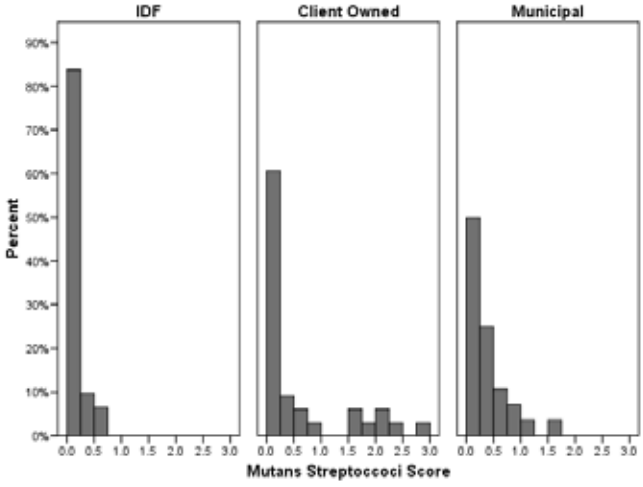

Fig 3. The mean ranking of mutans streptococci from three various dog populations.

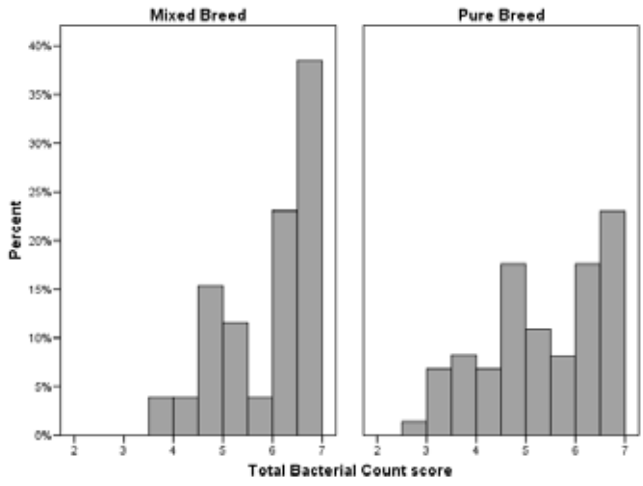

Fig 4. The mean ranking number of oral general bacteria from mixed breed dogs and pure breed dogs.

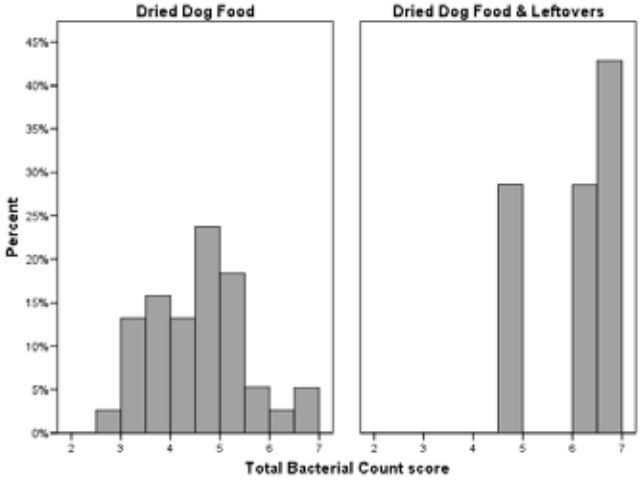

Fig 5. The mean ranking of total oral supragingival bacteria from dogs fed with commercial dried food vs. dogs fed with commercial dried food and human food leftovers