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Original Article

Measurement of oral bacterial counts in dogs by dielectrophoretic impedance

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ABSTRACT — The purpose of this study was to determine the suitability for measurement of oral bacterial counts (OBC) in dogs using a new device that operates on the principle of dielectrophoretic impedance. Using this device, bacterial counts were successfully measured in swabs collected from the mouths of 5 non-anesthetized beagles. We tried to take samplings from 6 sites in each dog's mouth and stable counts obtained at an interval of 2 weeks showed no significant difference in any of the 6 sites over time. However, since the counts showed significant differences depending upon the number of times the swab was rubbed on the sampling site, and the time from feeding affects oral bacterial counts, special attention is needed on these 2 issues. The new device allows rapid measurement of oral bacterial counts in dogs under appropriate conditions. The simplicity of this method may make it useful in studies on agents affecting OBC in dogs.

Key words: Oral bacterial count, Dog, Bacterial counter, Dielectrophoretic impedance

INTRODUCTION

The mouths of humans and dogs contain numerous bacteria that are known to cause oral infections. For example, periodontal disease, which arises in, and destroys, periodontal tissue, is a common oral infection in both species (Eke *et al*., 2012; Kortegaard *et al*., 2008; Hirai *et al*., 2013). There is also strong evidence that a focus of infection in the oral cavity can cause diseases in distant organs (Gorrel, 2008; Tonetti and Van Dyke, 2013). To determine the influence of substances affecting oral hygiene, a simple method for measuring changes in the numbers of oral bacteria is desirable. Currently, available methods of measuring oral bacterial counts include culture, turbidimetry, fluorescence staining, and real-time polymerase chain reaction (PCR) testing (Ishikawa *et al*., 2008; Kepner and Pratt, 1994; Lyons *et al*., 2000), but all these methods are cumbersome and require specialist skills; thus, their use is largely restricted to research purposes.

A device for measuring bacterial counts on the basis of dielectrophoretic impedance measurement (DEPIM) has recently been developed (Kikutani *et al*., 2012). Measurements obtained using this device may provide an index for evaluating the oral hygiene of household pets. However, the effective use of this device requires specifications be drawn up to ensure sample collection under consistent conditions. In this study, we investigated sampling sites, sampling methods, and sampling times in order to determine optimal sampling conditions for the measurement of oral bacterial counts in dogs by using a Bacterial Counter® (DU-AA01NP-H, Panasonic Healthcare Co., Ltd., Tokyo, Japan).

MATERIALS AND METHODS

Animals

In this study, we used 5 beagles (male, 3-6 years old) with no obvious oral or systemic disorders that had not been administered antibiotics during the previous 6 months. These dogs were kept in cages at room temperature (21 ± 2°C) and 55 ± 5% humidity, with a 12-hr light/dark cycle. They were fed twice daily, once in the morn-

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ing and once in the evening (CLEA Dog Diet CD-5M®; CLEA Japan, Inc., Tokyo, Japan), and freely provided tap water to drink from a container. All experimental protocols were approved by the Azabu University Animal Experiment Committee.

Oral bacterial counts

Bacterial counts were measured using a Bacterial Counter® according to the manufacturer's instructions. The procedure can be summarized as follows: (1) The sampling site was rubbed 1, 3, or 5 times with a special sterile swab. (2) The swab was stuck onto the center of a disposable cap filled with sample solution, and the cap was placed into the main unit of the device. (3) A sensor chip was inserted into the main unit and the lid was closed to begin measurements.

Statistical analysis

Statistical analyses were performed using the computational software package Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). Results are expressed as mean ± standard deviation values. Non-paired and paired Student's t-tests were used for statistical analysis, with the level of significance set at < 5%.

RESULTS

First, we compared samples taken at 6 sites: the back of the tongue, buccal oral mucosa, maxillary canine (104/204), mandibular canine (304/404), fourth maxillary premolar (108/208), and first mandibular postmolar (309/409). Sampling was performed again at the same 6 sites 2 weeks later to investigate day-to-day variation.

Bacterial counts, expressed as colony-forming units (cfu)/mL, were measured successfully from samples obtained from all 6 sites in the dogs' mouths. A comparison of bacterial counts from different sampling sites showed no significant differences at any of the 6 sites. Therefore, there was no significant day-to-day variation and we could detect the bacterial distribution in each dog's mouth around the teeth stably. Another point to note was the bacterial counts in the samples from the back of the tongue and the cheeks were almost the same, but were lower than the counts in samples taken from 4 other tooth-surface sites (Fig. 1).

Further in-depth comparison of the bacterial counts in samples obtained from different tooth surfaces (that is the distribution of bacteria in each dog's mouth) showed that bacterial counts were higher in samples taken from the molars than in those from the canines, and the individual variation in bacterial counts at each of the 6 sites tended to be low. Although there were some individual differences

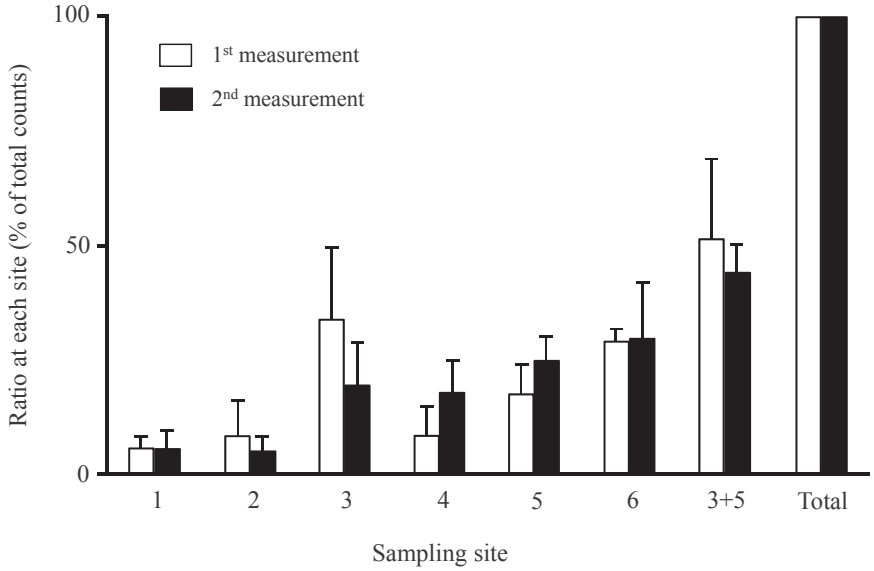

Fig. 1. Bacterial counts at different oral sampling sites. Results are expressed as the mean ± standard deviation. Measurements were performed twice at an interval of 2 weeks. 1: Back of the tongue, 2: Buccal oral mucosa, 3: Maxillary canine, 4: Mandibular canine, 5: 4th maxillary premolar, 6: 1st mandibular postmolar, 3 + 5: Maxillary canine + 4th maxillary premolar, Total: All of sites 1-6. (There were no significant differences between the 1st and 2nd measurements at individual 6 sites.)

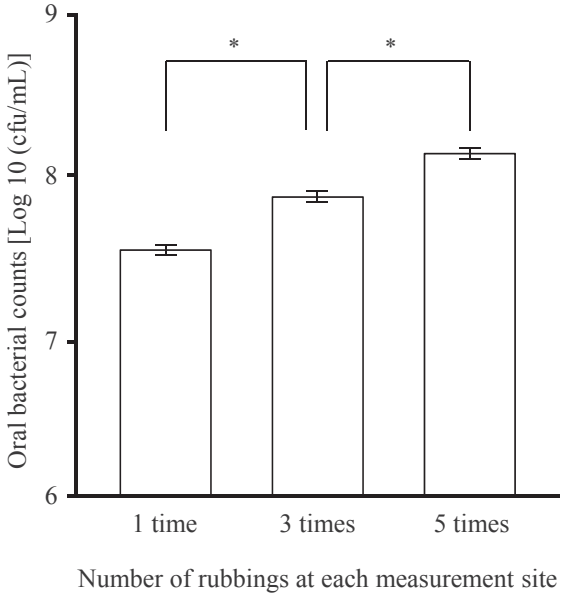

Fig. 2. Effect of the number of times the swab was rubbed on the sample site. Results are expressed as the mean ± standard deviation (*$p < 0.05$).

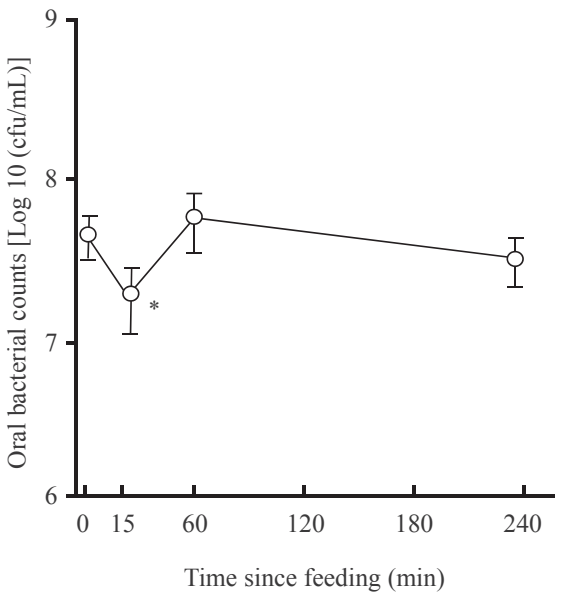

Fig. 3. Effect of feeding on oral bacterial counts in dogs. Results are expressed as the mean ± standard deviation (*$p < 0.05$ vs. Time 0). Food was given at time 0 (10 a.m.).

between total bacterial counts from all 6 sites, all counts ranged from 0.99×10^8 cfu/mL to 2.10×10^8 cfu/mL. There was no major difference between these values and bacterial counts at individual sites or total bacterial counts re-measured 2 weeks later (1st measurement: $1.68 \pm 0.48 \times 10^8$ cfu/mL; 2nd measurement: $1.30 \pm 0.40 \times 10^8$ cfu/mL).

The total bacterial counts from the maxillary canine and 4th maxillary premolar sites accounted for approximately 50% of the total bacterial count at all 6 sites in both measurements, and in light of the technical ease of sampling at these 2 sites, only the maxillary canine and 4th maxillary premolar sites were used for sampling for subsequent tests.

Next, we attempted to investigate the relationship between the bacterial count and the number of times the swab was rubbed on the sampling site. Two sampling sites were used, i.e., the maxillary canine and the 4th maxillary premolar, and swabs were rubbed 1, 3, and 5 times. As shown in Fig. 2, the bacterial count increased significantly depending on the number of times the swab was rubbed. Therefore, the number of times the swab is rubbed is one important condition for the measurement of bacteria in the mouth.

Next, bacterial counts were measured in samples taken from the maxillary canine and 4th maxillary premolar 30 min before and 15 min, 60 min, and 240 min after feeding with dog food (at 10 a.m.). Although the oral bacterial count 15 min after feeding was significantly lower ($p = 0.020$) than that before feeding, counts recovered to their pre-feeding levels after 60 min ($p = 0.264$) and remained the same after 240 min ($p = 0.718$) (Fig. 3). These results suggest that the sampling of bacteria needs to be done at least 60 min after feeding time.

DISCUSSION

Methods of measuring oral bacterial counts include culture, turbidimetry, and real-time PCR testing (Ishikawa *et al.*, 2008; Lyons *et al.*, 2000), but because they all require several days to yield results, their versatility is not good. The device used to measure bacterial counts in the present study employed DEPIM (Hamada *et al.*, 2011), which uses dielectrophoresis to concentrate bacteria at the electrodes and calculate their number on the basis of the changes in impedance between the electrodes. The measurable range of bacterial counts is 1.0×10^5 cfu/mL to 9.9×10^8 cfu/mL and the measurement only takes approximately 1 min. Previous studies have shown that bacterial counts measured by DEPIM are strongly correlated with those measured by culture or fluorescence antibody testing (Kikutani *et al.*, 2012). In the present study, we

focused on examining bacterial counts in dogs.

The results showed that a device based on DEPIM can be used for rapid measurement of oral bacterial counts in non-anesthetized dogs. To our knowledge, this is the first report demonstrating that a device developed for measuring human oral bacterial counts can be used in animals. The beagles used for measurements were experimental animals reared in the Azabu University Research Institute of Bioscience; they had not undergone any particular oral care and their level of oral hygiene was considered to be similar to that of most companion animals generally kept as indoor pets. The device used in this study may therefore be usable not only for dogs in experimental facilities, but also for pets brought along to regular veterinary clinics. In addition, dogs are often used in research studies of dental treatment and oral microbiology as an animal model of human disease; therefore, this study may facilitate canine studies of human clinical conditions.

Human oral bacterial counts are known to vary according to the sampling site (Kikutani *et al.*, 2012) and time of day, and so we investigated this aspect in dogs. A comparison of bacterial counts in samples taken from 6 different sites in the dogs' mouths revealed clear differences (Fig. 1). The counts were lower at the back of the tongue and the buccal oral mucosa than on the surface of the teeth, with the highest count obtained on the surface of the 1st mandibular postmolar. Obtaining samples from the 1st mandibular postmolar of non-anesthetized dogs, however, is far from easy. In addition, although bacterial counts from the maxillary canine, mandibular canine, and 4th maxillary premolar were relatively high, individual variation meant that it would be difficult to obtain an accurate estimate of oral bacterial counts for a measurement at a single site. The total bacterial counts at the maxillary canine and 4th maxillary premolar accounted for approximately 50% of the total bacterial count. At these sites, it was also easy to secure the field of view and move the swab around during sampling. The maxillary canine and 4th maxillary premolar were therefore considered to be appropriate sites for sampling in dogs' mouths.

Sampling conditions that are not susceptible to individual variation are required in order to obtain samples that accurately reflect bacterial counts. Therefore, we investigated the number of times the swab was rubbed on the site. The total bacterial count at the maxillary canine and 4th maxillary premolar increased the more times the swab was rubbed (Fig. 2). To minimize variations in sampling and make the process easier, 3 rubbings were regarded as appropriate.

Human oral bacterial counts are known to be affected by eating habits and to vary at different times of the day (Fujimasa, 1959). An investigation of the effect of morning feeding showed that oral bacterial counts in dogs slightly decreased 15 min after feeding. This may have been due to bacterial removal by friction between food boluses and the surface of the teeth, or the antibacterial components of saliva, secretion of which increases during eating (Dawes, 2008). Eating may have a lesser effect in dogs than in humans because dogs generally have fewer molars than humans and do not grind food as much with their back teeth. This results in less friction between food boluses and the surface of the teeth. The findings of the present study show that an interval of 60 min after feeding would be sufficient when measuring oral bacterial counts in dogs.

The results of the current study indicated that rapid measurement of oral bacterial counts in non-anesthetized dogs is possible by using a device based on DEPIM. The simplicity of this method may make it useful in studies on oral hygiene in dogs.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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