The importance of the canine normal bacterial microbiome vis a vis the innate immune system is becoming more and more apparent, both in terms of cutaneous homeostasis and atopic dermatitis, both in humans and dogs. The cutaneous microbiome is also important in the etiopathogenesis of, and protection against, canine pyoderma. Although the distribution and, to some extent, the dynamics of the microbiome of the dog's skin are understood, there is little quantitative data on the density of the normal flora, merely its constituents. Allaker et al reported a cup scrub method, expressing the mean density of bacteria on the ventral abdomen of ten healthy dogs as 2.02 log colony forming units (CFU) cm⁻² (circa 104.71 CFU cm⁻²). They also reported that coagulase-negative Staphylococci were more likely found on the hairs of the shoulder and rump than on the skin of the ventral abdomen.

The constituents of the canine normal microbiome, using 16s rRNA next-generation sequencing, have been published. The constituents have been reported to vary strongly between individuals, with the presence of cohabiting family members, diet, and both topical and systemic treatment. There is, however, a strong, species-dependent, core microbiome, which has been discussed elsewhere. Using a 50% relative species prevalence, in a temporal study, as an indicator of being a core member of

**OBJECTIVE**

To report the density, and the major constituents, of the bacteria on the skin surface of healthy dogs and to assess if scraping the skin before sampling was necessary.

**ANIMALS**

20 healthy dogs were recruited for the study, with informed consent in all cases.

**METHODS**

Flocked swabs were used to sample the skin surface and to sample the skin surface after superficial scraping with a blunted spatula. Both samples were taken within a brass guide of 3.5 cm⁻² area. Next-generation 16S rRNA sequencing was used to identify and quantify components of the bacterial microbiome.

**RESULTS**

The median density of the bacterial microbiome on the ventral abdomen of 20 healthy dogs was approximately 1.1 X 10⁵ cm⁻² (IQR 1.22 X 10⁴, 1.6 X 10⁵ cm⁻²). Sphingomonas species were isolated on 17 of the 20 dogs and Corynebacterium kroppstedtii from 15.

**CLINICAL RELEVANCE**

This is the first study to report the density of the canine skin microbiome. Superficial scraping of the skin before swabbing does not affect the result of sampling the microbiome in healthy dogs. These results will increase our understanding of the biology of canine skin.

**Keywords:** dog, skin, biome, quantification, sphingomonas
the cutaneous bacterial community, it was proposed that *Propionibacterium acnes* (Syn Cutibacterium acnes), *Haemophilus* species, and *Corynebacterium* species be so considered. The authors pointed out that other researchers found different results. For example, Rodrigues Hoffmann et al., found *Ralstonia* species, *Moraxella* species, and *Porphyromonas* species as the most abundant core members. Thus, highlighting the necessity of recognizing the potential for marked individual variation.

Studies assessing the microbiome of the dog’s skin have traditionally used a cotton swab, although cup-scrub methods were developed, and used, to allow quantitative results. Cup-scrubbing of the skin was originally envisaged to yield samples for culture-based methodology. Although cup-scrubbing has been used in microbiome studies, it might be considered impractical for clinically based studies.

Using 16S rRNA next generations sequencing technology the breadth and complexity of the canine cutaneous microflora has become apparent. It has been estimated that only about 1% of all microorganisms comprising the skin microbiome are, in fact, culturable.

A comparison of swab types for collection and recovery of organisms from the skin found flocked swabs superior to those made of cotton. In humans, approximately 85% of skin bacteria are located within the upper 6 layers of the stratum corneum. Recently, Duclos and colleagues published a technique, whereby a blunted spatula scraped away the first few layers of epidermis, wherein most of the flora lies, before sampling. Using light microscopy it was concluded that more bacteria were collected using this technique, than by, for example, simply using a swab, tape, or impression smears. Comparative studies from human skin showed that a swab was calculated to collect circa 10,000 bacteria cm$^{-2}$, a scrape method would yield circa 50,000 cm$^{-2}$, and a biopsy sample of 1,000,000 cm$^{-2}$. These studies comparing swab, scrape, and biopsy, and between swabbing and tape stripping concluded that all methods were comparable with regard to sampling the diversity of the skin microbiota, but were not comparable with regard to yield of cultivable organisms. The density of the microbiome on human skin has been stated as between 1.1 X 10$^5$ and 4.4 X 10$^6$ cm$^{-2}$, using a swab and culture. Ihreke and Schwartzman, using a swab and culture and contact plates, calculated the cutaneous bacterial count bacteria on healthy canine skin as circa 350 cm$^{-2}$. These numbers for the density of the bacterial microbiome on the canine skin are considered an understatement, particularly when compared with human studies, in which 16S rRNA next-generation technology was used.

Significant updates in guidelines for the treatment of canine pyoderma have been suggested, and these highlight the urgent need to reduce antibacterial usage. Patently, a knowledge of the density of the cutaneous flora would be helpful when developing antimicrobial treatments, both systemic and topical, for it would allow a quantitative measurement of the effect on the cutaneous microbiome, in addition to a clinical assessment.

This study was undertaken to assess the density of the canine normal bacterial microbiome on the ventral abdomen of normal dogs, and its major constituents, using next-generation DNA sequencing, and to assess if a superficial scrape before swabbing affected the results.

**Methods**

The methods used in the study were comprehensively described in the accompanying paper describing the mycobiome of the 20 healthy dogs. The only difference relates to the primer sequence, and the absolute microbial quantification methodology, which targeted the 16S rRNA V1–V3 region for bacteriome analysis as previously described.

**Statistical Analysis**

The statistical analysis was performed using Stata version 15.1. The outcome variable was the number of bacteria cm$^{-2}$ obtained from the swabs of the ventral abdomen. An examination of the distribution of the measurements suggested that these were positively skewed, with smaller measurements and a small number of large measurements; the $P$ value was 0.05.

As the 2 swab samples came from the same dog, this gives rise to paired data. Due to the distribution of the outcome values, the Wilcoxon matched pairs test was used for the analysis.

**Results**

There were 9 males and 11 females, with a mean age of 4.9 years (range = 9 months to 12 years). There were 20 A samples and 20 B samples.

There were 151 species of bacteria identified. Seventeen species were found on 3 or more dogs and these are listed, ranked (Table 1). The median bacterial count on the A samples was 5.2 X 10$^2$ cm$^{-2}$ (IQR 18422, 162841), giving a total count of 1.07 X 10$^5$ cm$^{-2}$). The 2 most prevalent species were *Propionibacterium acnes* and *Corynebacterium kroppstedtii*, and *Nocardiooides* spp (Table 1). Sixteen species of *Sphingomonas* spp were found on 17 of the dogs, with a median count of 460 cm$^{-2}$ (IQR 158, 1806). The 2 most prevalent species were *Sphingomonas aerulata* and *Sphingomonas* spp which were found on 11 and 10 dogs, respectively (Table 1). Nine dogs carried 4 or 5 species and, where present, in only 3 of the 17 dogs was a single species isolated.

*C kroppstedtii* was found on 15 of the 20 dogs with a median count of 650 per cm$^{-2}$.
Table 1—Summary of the 17 species of bacteria found on 3 or more of the 20 normal dogs, their prevalence (P) and abundance (A). (IQR 317.5, 3475). Nocardioides spp was found on 12 with a median of 680 (IQR 247.5, 1300). Rothia kristinae (aka Propionibacterium acnes) were found on 6 dogs, respectively (Table 1).

<table>
<thead>
<tr>
<th>Species of bacteria</th>
<th>P</th>
<th>A, mean (IQR)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphingomonas spp</td>
<td>17</td>
<td>460 (158, 1806)</td>
<td>Member of core flora?</td>
</tr>
<tr>
<td>Corynebacterium kroppstedtii</td>
<td>15</td>
<td>650 (317.5, 3475)</td>
<td>Member of core flora?</td>
</tr>
<tr>
<td>Nocardioides spp</td>
<td>12</td>
<td>680 (247.5, 1300)</td>
<td>Environmentia</td>
</tr>
<tr>
<td>Sphingomonas aerilata</td>
<td>11</td>
<td>330 (145, 588)</td>
<td>Member of core flora?</td>
</tr>
<tr>
<td>Sphingomonas aurantica-faeni</td>
<td>8</td>
<td>280 (205, 655)</td>
<td>Member of core flora?</td>
</tr>
<tr>
<td>Rothia kristinae</td>
<td>7</td>
<td>570 (190, 420)</td>
<td>Normal flora, man</td>
</tr>
<tr>
<td>Cutibacterium acnes</td>
<td>6</td>
<td>3450 (2275, 3825)</td>
<td>Normal flora dog</td>
</tr>
<tr>
<td>Conchiformibius steedae</td>
<td>6</td>
<td>310 (225, 3500)</td>
<td>Dog oral cavity</td>
</tr>
<tr>
<td>Porphyromonas spp</td>
<td>5</td>
<td>280 (190, 420)</td>
<td>Member of core flora?</td>
</tr>
<tr>
<td>Knoella spp</td>
<td>5</td>
<td>1150 (820, 2375)</td>
<td>Environmentia</td>
</tr>
<tr>
<td>Marmoricola spp</td>
<td>5</td>
<td>535 (245, 1725)</td>
<td>Member of core flora?</td>
</tr>
<tr>
<td>Sphingomonas glacialis</td>
<td>5</td>
<td>280 (205, 655)</td>
<td>Member of core flora?</td>
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<tr>
<td>Bergeyella zoohelcum</td>
<td>4</td>
<td>310 (93.5, 1750)</td>
<td>Dog oral cavity</td>
</tr>
<tr>
<td>Staphylococcus pseudintermedius</td>
<td>4</td>
<td>290 (267.5, 320)</td>
<td>Normal flora dog</td>
</tr>
<tr>
<td>Sphingomonas humi-swanesi</td>
<td>4</td>
<td>230 (210, 245)</td>
<td>Member of core flora?</td>
</tr>
<tr>
<td>Rothia sp</td>
<td>3</td>
<td>210 (110, 755)</td>
<td>Normal flora, man</td>
</tr>
<tr>
<td>Streptococcus canis</td>
<td>3</td>
<td>2000 (660, 4800)</td>
<td>Dog oral cavity</td>
</tr>
</tbody>
</table>

All are to median count cm⁻² with IQR.

Discussion

The major aim of the study was to describe the bacterial cell density, and the major constituents, of the bacterial microbiome on the skin of the ventral abdomen of healthy dogs. A secondary aim was to investigate if a spatula scrape before swabbing affected the results.

Knowledge of the density of the cutaneous microbiome, and the species of bacteria, will contribute to our understanding of canine skin biology. In addition, it could provide an objective method of assessing the efficacy of a putative systemic or topical antibacterial treatment for skin infection. Currently, such assessment is made on clinical score, by counting bacteria per high power field with a light microscope or by regular biomark assay utilizing Next Generation Sequencing using 16S rRNA. By using a sampling guide, of known areas, clinical changes may be correlated with quantitative microbiological changes.

Dysbiosis is a feature of canine atopic dermatitis and superficial pyoderma. Further studies evaluating the usefulness of Next Generation Sequencing for assessing the efficacy of antibacterial treatment for skin infection are needed, and using quantitative data might increase our understanding of these dysbiotic changes.

The total bacterial count was circa 1.1 x 10⁶ cm⁻², of a similar range to that reported on human skin, of between 1.1 X 10⁵ and 4.4 X 10⁶ cm⁻². The ventral abdomen of the dog is relatively hairless and dry, and, presumably, a similar habitat to human skin, although it is much closer to the ground, and, thus, perhaps more exposed to contaminants.

There was no significant difference between the A and B samples suggesting that, while the scrape technique might be of value when investigating diseased skin, it is not superior to a swab on healthy skin. It is possible that the lack of difference between the A and B samples might represent species differences. For example, a study into the yield of bacteria from human skin with swab, scrape and biopsy did find a difference and it is possible that our scrapes were too superficial to show any difference. However, our results might suggest that, in the healthy dog, flocked swabbing is sufficient. This suggestion is supported by Garcia-Fonticoba et al. whose study demonstrated that there were no viable bacteria in the deeper layers of the skin. If our conclusion is corroborated in future studies, it will greatly simplify sampling.

Sphingomonads are Gram-negative bacilli, typically found in the environment, and they are regarded as opportunistic pathogens. Of the 12 studies reporting 16S rRNA analysis of the canine biomes, all but 1 found Sphingomonas spp, although sometimes in low numbers. Two of did not report any taxa recovered in low numbers, but they did recover the species in their studies (personal communications). One study found Sphingomonas spp on the same dogs at both sampling times, 30 days apart, although the species recovered were not reported.

Figure 1—A graphical portrayal of the relative prevalence of bacterial species on the ventral abdomen of 30 healthy dogs.
C. kroppenstedtii was recovered from 15 of the dogs. C. kroppenstedtii appears to be an opportunistic pathogen in humans and has been found almost exclusively in female patients, and mainly from breast abscesses and cases of granulomatous mastitis. Older et al. noted an increased abundance of untyped corynebacteria in their study, which compared the microbiome of atopic and healthy dogs. They suggested that species-level analysis might be worthwhile, a recommendation supported by our finding that 75% of our healthy dogs were carrying C. kroppenstedtii.

Corynebacterium spp, Porphyromonas species, Cutibacterium acnes (syn Propionibacterium acnes), and, perhaps, Haemophilus species, have been proposed as core members of the canine skin bacterial flora. P. acnes has been reported from dogs previously, where it was found on 63.6% of dogs, a higher prevalence than in this study.

Nocardioides species and Knoellia species are environmental bacteria, presumably contaminants. In contrast, S canis, Conchiformis steedae, Bergeyella zoohelcum, and Staphylococcus pseudintermedius are members of the dog's oral microbiome. These members of the canine oral microbiome may be present on the ventral abdomen of healthy dogs by simple transference to the skin during grooming, as is thought to be the case, for example, of S pseudintermedius in the dog. B. zoohelcum has been isolated from the skin of people in prolonged contact with therapy dogs, with no history of being bitten and it has not been reported previously on the skin of healthy dogs. Notwithstanding, it might explain why B. zoohelcum can be found on people in contact with therapy dogs.

R. kristinae (syn K. kristinae) is a gram-positive coccus. It is found on human skin and is considered part of the normal flora, although it is an occasional pathogen. Song et al. reported that cohabiting humans and dogs may share components of their cutaneous biome and our finding of R. kristinae on dogs and the finding of B. zoohelcum on humans might be examples of this.

Our data, and that reported in other studies, of the canine microbiome suggest that the canine cutaneous flora is very broad and very variable. Torres et al. suggested that a core community of cutaneous bacteria might be identified across a broad group of animals. Identifying a core member of the microbiome is difficult as individual dogs have their own microbiome, which is similar across all body sites. We suggest that Sphingomonas species might be considered candidates as part of the core gram-negative bacteria flora on normal dogs not just on the basis of its prevalence in the various studies but also because Leverett et al. found Sphingomonas spp on the same dogs, 30 days apart.

We have also presented data to suggest that the status of B. zoohelcum and C. kroppenstedtii, on the dog's skin be reassessed in the light of their zoonotic potential.

There are 2 major limitations to a study of this type, the single site sampling and the lack of a temporal aspect, both of which can be addressed in future studies, now that a method has been described.

While we cannot rule out contamination from the home environment, we took steps to minimize DNA contamination at the time a sampling by minimizing transit time in the waiting room and using disinfection products on the consulting-room table anticipated to denature any residual DNA. Cusco et al. was the only one of the recent papers, which used 16s RNA new technology tools to investigate the canine biome, to perform an environmental control. It could be argued that the steps described above and the 2-center study, using both surface swabs and postscrape samples, mitigate against environmental contamination.

In conclusion, we have found that the density of the bacterial microbiome on the ventral abdomen of 30 healthy dogs was approximately 1.1 X 10^9 cm^-2. We conclude that scraping the skin before sampling is not necessary on healthy dogs.

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None reported.

**Disclosures**

The authors have nothing to declare, No AI-assisted technologies were used in the generation of this manuscript.

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