Boffin Access

Open Access Journal | Volume: 2.2

International Journal of Veterinary and Animal Medicine

ISSN 2517-7362

Bacterial Microbiome of Nasal Swabs from Healthy Dogs and their Owners: Bacterial Diversity, Intra- and Interspecies Interface

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Abstract

The nasal cavity microbiome plays important roles in the overall health of the host. There is still a gap of information on the microbial diversity and interface in the nasal cavity of pets and their owners. The objective of this study was to explore the nasal inhabitant microbiome and their interface in apparently healthy dogs and their owners applying the 16S tag-encoded FLX 16s Rdna amplicon pyrosequencing (bTEFAP). The nasal swab samples were collected form healthy dogs (n=5) and their corresponding owners (n=5). Nasal genomic DNA was extracted and used to create 16S rRNA gene amplicons, which were subjected and assessed to 454 pyrosequencing. In both dogs and human nasal swabs the predominant nasal bacterial phyla were *Firmicutes, Proteobacteria* followed by *Bacteroidetes, Actinobacteria, Tenericutes* and *Synergistetes*. Phylum *Fusobacteria* was identified only in human nasal swabs. A total of 18 classes were identified of which *Gammaproteobacteria* and *Bacilli* were dominantly found in most nasal swab samples. Out of 59 Genera, a total of 171 Operational Taxonomic Units were detected which was dominated by *Stapylococcus.* In dogs nasal swab samples, the abundant species were *Staphylococcus felis*, *Staphylococcus cohnii* and *Staphylococcus intermedius*. The dogs and the owners shared from 9 to 29% similar bacterial species. The pyrosequencing molecular approach applied in this study allowed us to identify and assess the relative abundance of nasal microbiome, intra- and interspecies interface in dogs and human nasal samples. Understanding of the nasal microbiome in across apparently normal different species will enable insight into microbial diversity, interface, involvement, and the possible pathogenic impact of the bacteria.

Keywords

Nasal cavity; Microbiome; Dog; Human; Pyrosequencing

Introduction

Traditionally, microbiology was almost entirely culture-dependent; it was necessary to grow microbes in the laboratory in order to identify and further study and characterize the organism. Since non-culture protocols have been established as methods to identify and characterize the bacterial communities, the microbiome detection, understanding of interactions between bacteria and their host has considerably improved [1-3]. Earlier studies utilizing culture-based microbial detection have now been replaced in research setting with detection and identification of microbes using nucleic acid-based methods, which appear to be less biased and more sensitive than traditional culture [4,5]. In recent years, numerous studies have been described that explored rich and complex bacterial communities present in the paranasal sinuses of healthy adults, and documented a surprising preponderance of anaerobic organisms [6,7].

In contrast to targeted molecular methods such as real-time PCR, global molecular approaches such as DNA sequencing offer attractive strategies for the identification of unknown pathogens from clinical specimens. Uncultured bacterial pathogens have previously been identified directly by DNA sequencing and have highlighted the importance of sequence-based identification [8].

It is estimated that over 60% families in the Western world own a pet. The majority of these households keep a dog [9]. Many studies showed that the health benefits of pet ownership is not only limited to provide comfort and companionship, but they have also found that the pets have positive impacts on nearly all life stages including decreasing

Article Information

DOI: 10.31021/ijvam.20192120
Article Type: Research Article
Journal Type: Open Access
Volume: 2 **Issue:** 2
Manuscript ID: IJVAM-2-120
Publisher: Boffin Access Limited

Received Date: 08 June 2019
Accepted Date: 04 July 2019
Published Date: 08 July 2019

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Citation: Yehualaeshet T, Edmonds-Wiggins G, Jones C, Graham M, Dillard K, et al. Bacterial microbiome of nasal swabs from healthy dogs and their owners: bacterial diversity, intra- and interspecies interface. Int J Vet Anim Med. 2019 Jul;2(2):120

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stresses and promoting relaxation [9,10]. Evidence has shown that owning a pet can increase the activity of pet owners and consequently reduced serum cholesterol, low triglyceride levels, and fewer cardiovascular events [11,12].

Culture-based methods were used previously and much lower number of bacterial microbes were identified in the canine and/or human nasal cavity [13-15]. The nares environment is colonized by highly diverse microorganisms that are distinct from other regions of the integument [14]. Pyrosequencing has been used to explore microbes from several organ systems, including nasal cavity [16,17], gastrointestinal tract [18], fecal [19] skin [20], oral cavity [19,21], and vagina [22]. The technique generates similar data to Sanger sequencing and is rapid, reproducible, high throughput, user-friendly, and cost-effective [23].

Since pyrosequencing has been established as a method to identify and characterize the microbiome bacteria [17,24], the identification, typing and understanding of microbial interactions between bacteria and their environments has noticeably advanced [3,23,25,26]. The objective of this study was to explore the intra- and interspecies diversity of nasal microbiome by applying the bacterial 16S tag-encoded FLX-titanium amplicon pyrosequencing (bTEFAP) technique to characterize its host diversity in dogs and the human owner.

Materials and Methods

Sample sources and collection

Prior to initiating the experiments, necessary paper work for animal and human care study procedures was approved by The Human Participants Review Committee (HPRC) that serves as the Institutional Review Board (IRB), Tuskegee University. Five apparently healthy dogs and their respective owners were selected and the dog owners were oriented about the objectives of the sampling and the bioethical certification. Once the owner agreed, surface nasal swab samples were collected from the dogs and the owners. Sterile cotton-tipped swabs (Thermo Fisher Scientific, Waltham, MA) were used to collect the nasal swabs. While swabbing the nasal cavity, appropriate care was taken not to damage the nasal epithelial lining and cause bleeding. After swabbing, the swab sticks were immediately placed back into the tube containing sterile solution and the samples were transported in a refrigerated container to the laboratory and frozen at -80°C untilnucleic acid extraction,

DNA Extraction and Pyrosequencing

Total nucleic acid (DNA) was extracted from the nasal swab samples using PrepMan DNA Sample Preparation kit (Applied Biosystems, Foster City, CA) as described by the manufacturer. Once the DNA was eluted, it was quantified using NanoDrop spectrophotometer (NanoDrop ND-1000, Nano-Drop Technologies, Wilmington, DE). The transcriptase synthesis was performed using cDNA Synthesis Kit (Agilent Technologies, Santa Clara, CA) as described previously [27]. The cDNA was submitted for pyrosequencing testing laboratory (Research and Testing Laboratory, Lubbock, TX). Bacterial tag-encoded FLX 16s rDNA amplicon pyrosequencing (bTEFAP) approach was performed as described earlier [28]. To determine the identity of the bacteria in the remaining sequences, they were first queried using BLASTn against a database of high-quality bacterial 16S rRNA gene sequences derived from NCBI [29].

Data analysis

Raw data from bTEFAP were screened and trimmed based upon quality scores and binned into individual sample collections. The compiled data was analyzed to determine the relative percentages of bacteria for each individual sample. The relative abundance of each species and genus were expressed as a percentage of the total number of isolates. Data was compiled and relative percentages of each bacterial identification were plotted accordingly using Microsoft® Office Excel.

Results and Discussion

By using pyrosequencing molecular approach, the nasal microbiomes of dogs (n=5) and the owners (n=5) were collected, examined and analyzed. The results showed that the nasal cavities of dogs and their owners were inhabited by a highly species-rich bacterial community, and suggest the intra-and interspecies interface similarities and differences between the nasal microbiome of healthy dogs and their owners. The tag-encoded pyrosequencing approach reported here allowed us to detect and identify bacteria that otherwise might be fastidious, obligate intracellular, or noncultivable. In addition to the survey, the pyrosequencing dataset also allowed assessment of the relative abundance of the taxonomic levels of bacteria inhabiting the nasal cavity.

Nasal microbiome from dogs

Bacteria identified in dog nasal swabs by bTEFAP comprised of 8 phyla, 15 classes, 21 orders, 26 Families, 44 Genera, and 118 species. Predominant bacterial phyla present in dogs nasal cavity were *Firmicutes*, followed by *Proteobacteria* and *Bacillus* (Graph 1). Class *Cytophagia, Deltaproteobacteria, Sphingobacteria,* and *Synergistia* were recovered only in dog samples, not from human samples (Figure 1).

At a species level, ten *Staphylococcus species* (*S. caprae, S. epidermidis, S. felis, S. haemolyticus, S. intermedius, S. lugdunensis, S. pasteuri, S. saprophyticus, S. schleiferi,* and *S. warneri*) and five *Streptococcus species* were identified (*S. equinus, S. minor, S. pluranimalium, S. sanguinis,* and *S. suis*). The Genus Bacillus recorded at the species level were *B. aryabhattai, B. megaterium, B. pumilus, B. simplex, B. stratosphericus and B. subtilis*. These include some lists of bacteria colonizing the nasal cavity, which are not frequently and previously mentioned.

Nasal microbiome from human owners

A total of seven bacterial phyla, 13 classes, 21 orders, 24 families, 42 genera and 114 species were recovered (Graph 1). At class level, member of *Firmi*+K26:P32*cutes* Gammaproteobacteria, followed by *Bacillales* commonly colonize the nasal cavity (Figure 1).

Nine *Staphylococcus spp* recorded were *S. aureus, S. caprae, S. epidermidis, S. felis, S. haemolyticus, S. intermedius, S. schleiferi, S. simiae,* and *S. warneri*. Within the genus *Streptococcus, S. parasanguinis* species was detected from human nasal swab samples. In general, solated bacterial genera with more than one species were *Staphylococcus* (13), *Bacillus* (6), *Streptococcus* (6), *Neisseria* (4), *Moraxella* (3), *Clostridium* (2), *Enterococcus* (2), *Pasteurella* (2), *Pseudomonas* (2), *Rothia* (2), and *Serratia* (2).

Intra- and Inter species interface of the nasal microbes

Generally, it is anticipated that different bacterial species colonize the dogs and their owner's nasal cavity. Three of the dogs and the corresponding owners (Group 1, 2 and 4) shared 24.4% to 28.3% of the recorded bacterial isolates. Group 3 and 5 shared 9.7% and 9.3% of the common bacteria, respectively (Table 1). In both dogs and human, the predominant nasal bacterial phyla included *Firmicutes* and *Proteobacteria* (Graph 1).

A total of seven phyla were detected in both human and dogs out of which *Firmicutes* and *Proteobacteria*, respectively, were predominant bacterial phyla present in both host nasal swabs. The phylum *Fusobacteria* was found only in dog's nasal sample (Graph 1). The dendogram plot (Figure 2) shows the clustering of human and dog's nasal sample.

Seven *Staphylococcus species* (*S. caprae, S. epidermidis, S. felis, S. haemolyticus, S. intermedius, S. schleiferi* and *S. warneri*) were found in both dogs and human samples. *Staphylococcus aureus* and *S. simiae*, were recorded only in human nasal swabs whereas *Staphylococcus cohnii, S. lugdunensis, S. pasteuri* and *S. saprophyticus* were identified only from dog nasal swabs. From genus *Streptococcus,* only *S. parasanguinis* was identified in human nasal swabs, the rest four species (*S. equinus, S. minor, S. pluranimalium* and *S. sanguinis*) were found only in dog nasal Swabs.

Scientific researches revealed links between the environment and their health [30]. Pets and human share same environments thus

	Sample source	Number of bacterial spp recorded	Common isolates (in percentage)
Group 1	HNS-1	66	
	DNS-1	47	
	HNS-1/DNS-1*	32	28.3
Group 2	HNS-2	58	
	DNS-2	30	
	HNS-2/DNS-2*	25	28.4
Group 3	HNS-3	12	
	DNS-3	19	
	HNS-3/DNS-3*	3	9.7
Group 4	HNS-4	14	
	DNS-4	27	
	HNS-4/DNS-4*	10	24.4
Group 5	HNS-5	19	
	DNS-5	24	
	HNS-5/DNS-5*	4	9.3

HNS: Human nasal sample; DNS: Dog nasal sample; HNS-1/DNS-1*: Bacterial species found in both human and dog nasal swabs in percentage

Table 1: Bacterial species recorded from dogs and their corresponding owners

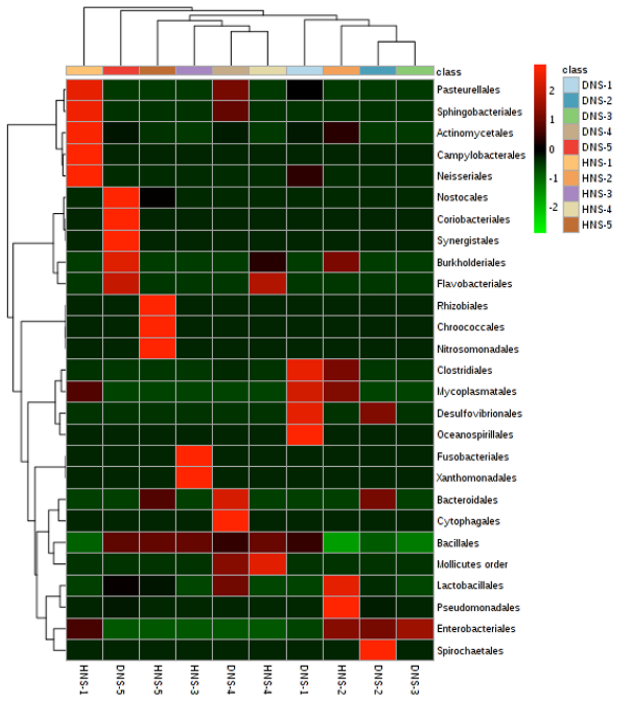


Figure 1: Heat map depicting bacterial class diversity and relative abundance in nasal swabs from dogs (DNS-1 DNS-2 DNS-3 DNS-4 DNS-5) and human (HNS-1 HNS-2 HNS-3 HNS-4 HNS-5)

are exposed to many of the same pollutants as human and vice versa. Previous studies have been published using culture, PCR and DNA sequence-based identification to investigate bacterial populations in the nasal cavity of dogs and other pets [31-33]. Establishing the baseline data for the microbiome in apparently healthy hosts

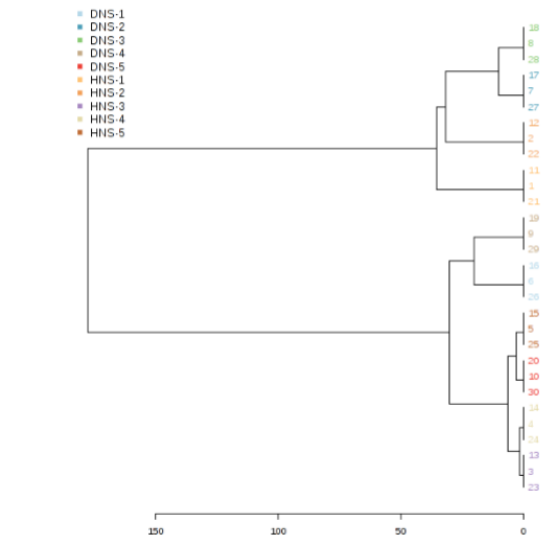
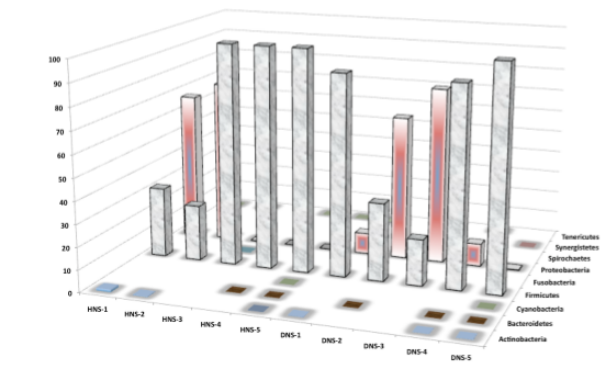


Figure 2: Dendrogram representation of hierarchical clustering. Legend: Nasal swabs from dogs (DNS-1 DNS-2 DNS-3 DNS-4 DNS-5) and Nasal swabs from human (HNS1 HNS-2 HNS-3 HNS-4 HNS-5)



Graph 1: Summary of bacterial Phyla (expressed as percentage) from dogs and human nasal swabs identified by DNA pyrosequencing (HNS= Human nasal swab; DNS=Dog nasal swab)

may support to identify the role of bacterial communities in the pathophysiology of disease and further to explore the bacterial ecosystem in apparently healthy and disease situation.

In dogs with chronic rhinitis, bacteria have been discussed as primary or secondary pathogens [33] and further studies investigating the nasal microbiome of healthy as well as diseased individuals have been reported in human [34,35] with inflammatory or neoplastic diseases of the upper airways [36] and laryngeal carcinoma compared to a healthy control group [37]. Additionally, bacterial species that commonly reside on surfaces of the human nasal passages interact with the host along a continuum from beneficial to harmful. As a consequence, the host responds along from tolerance to damage [38]. Different microbiomes for different sites in the upper airways have been described that *Actinobacteria* and *Firmicutes* accounted for the majority of nasal bacteria, with a lower prevalence of *Proteobacteria* [39,40].

Yan et al. [41], reported that the type of epithelium in healthy nasal passages has a significant impact on bacterial community diversity with no distinct patterns in bacterial composition between sites. Another study demonstrated that the nasal microbiome is distinctly different to that of the oral and buccal cavity within an

individual [34]. The self-licking habits of dogs may also be associated with possible self-contamination of oral and nasal and vice versa.

As the nares are exposed to the outdoor surroundings of humans and dogs, the microbes can be obtained from the environment. Therefore, there seems to be considerable differences between the nasal bacterial communities of different species under different circumstances. The molecular approaches have revealed new insights into this possibility and have sparked renewed interest in determining the molecular mechanisms of commensal–pathobiont interactions in the nasal microbiome [14]. The factors that shift the behavior of pathobionts from a commensal to a pathogenic state remain to be identified. However, the interplay of pathobionts with other members of the microbiome might be one such factor. *Staphylococcus pneumoniae* and *S. aureus* can be considered pathobionts (bacteria that can be benign or pathogenic). Most human interactions with *S. pneumoniae* and *S. aureus* are harmless. It is not well understood why these pathobionts colonize the nasal passages of some individuals but not others, nor are what causes a pathobiont to shift from a commensal to pathogenic state [1].

Colonization of humans with *Staphylococcus aureus* is a critical prerequisite of subsequent clinical infection of the skin, blood, lung, heart and other deep tissues. *S. aureus* persistently or intermittently colonizes the nares of, 50% of healthy adults, whereas, 50% of the general population is rarely or never colonized by this opportunistic pathogen. This suggests that microbial consortia within the nasal cavity may be important determinants of *S. aureus* colonization [14]. In at least 80% of *S. aureus* bacteremia cases in colonized subjects, the infecting strain is identical to a nasal colonizing strain detected prior to onset of bacteremia [42]. Followed longitudinally, approximately 20–30% of persons are colonized persistently with *S. aureus*, 30% are colonized intermittently, and 50% never, or rarely, are colonized and hypothesized that competition and cooperation between *S. aureus* and nares-associated microbial communities directly affects the incidence and prevalence of S. aureus colonization and subsequent infection [14].

Dogs shared most of the household environments as their owners and they are exposed to many of the same pathogens and pollutants as human. Although dogs have several positive impacts on the psychosocial and psychical health of their owners, many diseases among humans are attributed to them [19]. From public health perspective, dogs are among major reservoirs for zoonotic infections to transmit several bacterial and viral diseases to humans. Zoonotic diseases can be transmitted to human by infected saliva, aerosols, contaminated urine or feces and direct contact with the dog.

Bacterial infections including *Pasteurella, Salmonella, Brucella, Yersinia enterocolitica, Campylobacter, Capnocytophaga, Bordetella bronchiseptica, Coxiella burnetii, Leptospira, Staphylococcus intermedius* and Methicillin resistance *Staphylococcus aureus* are the most common infections transmitted to humans by dogs [43-45].

In conclusion, a complex bacterial community resides along the nasal passages in dogs and human owners. However, there is a relatively limited knowledge about the nasal microbiome including how beneficial members of the nasal microbiome might help keep healthy or pathobionts. Our results add evidence that the healthy dogs and human owner nasal microbiome are likely to enhance our understanding of the bacterial population ecosystem and get more insight to explore and compare the nasal microbiome in apparently healthy hosts. Further studies are required to understand the role and interface of the nasal microbiome in dogs and humans under various health and environmental circumstances.

Acknowledgements

The study was supported by Tuskegee University, CVM COE Grant #D34HP00001. The authors wish to thank Dr. Gopal Reddy for his technical and financial support (USDA 35-12650080) for the publication. We also appreciate the RCMI Core Facility Grant #U54MD007585.

Conflict of Interests

The authors declare that they have no competing interests.

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