THE FELINE SKIN MICROBIOME: THE MICROORGANISMS INHABITING THE SKIN OF HEALTHY AND ALLERGIC CATS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

The Feline Skin Microbiome: The Microorganisms Inhabiting The Skin of Healthy And Allergic Cats. (May 2015)

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The skin in inhabited by a multitude of microorganisms. In order to further understand how disease and the microbiome are related, we propose to set a standard for what the commensal bacterial microbiome is on the skin of cats. To describe the cutaneous bacterial microbiome of cats and its relationship with disease, the skin surfaces on various regions of 10 normal cats and 10 allergic cats were sampled. Genomic DNA was extracted from skin swabs and sequenced using primers that target the V4 region of the 16S rRNA in bacteria.

The sequences revealed that there is a significant difference in species diversity and richness between haired and non-haired/mucosal sites. No significant difference in alpha or beta diversity was seen between cats or between body sites, other than between nostril and each site. There is a significant difference in the species richness and diversity between allergic and healthy cats, but not in microbiome composition.

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NOMENCLATURE

ANOSIM	Analysis of similarities
DSH	Domestic Short Hair
DMH	Domestic Medium Hair
DLH	Domestic Long Hair
QIIME	Quantitative Insights Into Microbial Ecology

CHAPTER I

INTRODUCTION

The body is colonized by a variety of microorganisms. These microorganisms can be helpful, by educating the immune system, and competing and inhibiting growth of pathogenic microorganisms, or can be pathogenic, resulting in disease in affected tissues, causing damage to the host. The populations present in and on the body vary with the different locations. They differ between individuals due to intrinsic and extrinsic factors, such as immune status and environment. An imbalance in the microbiome can result in dysbioses of these microorganisms causing a variety of problems. Understanding the interactions of these microbial populations residing on the skin is very important to learn how these can cause skin diseases. By studying the microbiome of skin in healthy individuals, a standard is set for what is normal, and these parameters can be further used to understand how these microorganisms may be causing infections or disease (1,2).

Several studies have been performed to describe the microbiome in various organs in humans, including the skin (4). In the skin, it was found that moist areas are primarily colonized by *Staphylococcus* and *Corynebacterium,* whereas in dry sites there was more diversity in organisms present (1). Studies have also compared the degree of diversity among different skin regions. Statistical analysis indicates that the vaginal region had the lowest diversity and oral had the most diversity (4).

In the field of Veterinary Medicine, there have been several studies about the gastrointestinal tract microbiome (3,5). The microbiome differs throughout the gastrointestinal system with Fusobacteria, Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes being the dominant bacteria present in most areas with varying relative frequencies (5,8). In addition to studies evaluating the gastrointestinal microbiome, there have been studies on the oral microbiome of cats and dogs. In the oral microbiome of healthy cats it was found that most bacteria belong to the phyla Proteobacteria, Bacteroidetes, and Firmicutes (6). In dogs, it was found that 39.2% of the sequences were represented by bacteria from the genus *Porphyromonas* (7).

However, very few studies have been conducted on the skin microbiome of animals. Recently, one study was published to further our understanding of the canine skin microbiome. In dogs, it was found that the bacteria present on the skin varies greatly between different regions with the majority of the bacteria present in all areas belonging to the phylum Proteobacteria, and family *Oxalobacteriaceae*. The study showed that species richness was higher in regions of haired skin when compared with mucosal surfaces (2).

The skin of individuals with skin lesions, such as atopic dermatitis (AD), is colonized by a different microbiome than individuals who are not affected. It is still uncertain if the changes in microbiome are due to cellular and molecular alterations in the skin surfaces of affected individuals or if a change in the microbiome results in skin lesions. Regardless, there is a significant relationship between alterations in the microbiome and disease (2).

We hypothesize that the analysis of the data will reveal a significant difference in the bacterial microbiome in each cutaneous area studied. Furthermore, we expect to see variation in diversity and different bacterial taxa present in healthy cats compared to allergic cats.

The objectives of this study are:

1) to describe the various bacteria present on different skin surfaces of healthy cats;
2) to identify significant differences between the skin microbiome of healthy and allergic cats

The data presented here will allow us to better understand the composition and diversity of bacterial species between different regions and types of skin (haired, non-haired/mucosal surfaces), and between healthy and allergic cats.

CHAPTER II

METHODS

Participants

Healthy Cats

Ten healthy cats participated in this study with their ages ranging from 2 years old to 17 years old. There were 6 spayed females (3 DSH, 1 DMH, and 2 DLH) and 4 castrated males (2 DSH, 1 DMH, and 1 DLH). All of these cats lived with other animals. Seven of the cats were kept indoors, two spent time both inside and outside, and one was kept solely. All cats were evaluated by a board certified dermatologist, and none of these cats had skin lesions, history of pruritus or any history of cutaneous disease in the past 6 months. These patients were not treated with antibiotics, anti-inflammatory or immunosuppressive drugs for at least 6 months prior to sample collection.

Table 1. Physical and environmental characteristics of healthy cats enrolled in this study.

Cat	Skin	Sex	Breed	Age	Environment
1	Healthy	CM	DLH	5	Indoor
2	Healthy	SF	DSH	2	Indoor
3	Healthy	CM	DSH	13	Indoor
4	Healthy	CM	DSH	7	Outdoor
5	Healthy	SF	DMH	4.5	Indoor
6	Healthy	SF	DSH	7	Indoor
7	Healthy	SF	DSH	9.5	Indoor
8	Healthy	SF	DLH	13	Indoor
9	Healthy	SF	DLH	15+	Outdoor
10	Healthy	CM	DMH	6	Indoor

Allergic Cats

Ten cats with allergic skin disease were enrolled in this study. Their ages ranged from 5 to 11 years old. There were 5 spayed females (4 DSH and 1 Siamese), 1 intact female (DSH), 3 castrated males (2 DSH and 1 Siamese), and 1 intact male (Persian). All but two of these cats

lived or had contact with other animals. Six of the cats were kept indoors and the other four spent time both inside and outside. All allergic cats were evaluated by a board certified dermatologist. Allergic cats in this study were defined as those that showed manifestations of pruritus to include any of the common cutaneous reaction patterns in cats (self-induced alopecia/fur mowing, miliary dermatitis, flea allergic dermatitis, eosinophilic skin lesions, and/or cervicofacial (pruritic) dermatitis) and where other parasitic and infectious causes of pruritus have been ruled out. For most patients, the clinical diagnosis of allergy was made when other causes of pruritus were ruled out or deemed highly unlikely. Seven patients had confirmed negative cytological evaluation, skin scrapings and/or anti-mite treatment trials.

Table 2. Physical and environmental characteristics of allergic cats enrolled in this study.

Cat	Skin	Sex	Breed	Age	Environment
12	Allergic	CM	DSH	9	Indoor
13	Allergic	CM	Siamese	8	Indoor
14	Allergic	CM	DSH	11	Indoor
15	Allergic	SF	Siamese	10	Indoor
16	Allergic	F	DSH	5	Outdoor
17	Allergic	SF	DSH	9	Indoor
18	Allergic	M	Persian	9	Indoor
19	Allergic	SF	DSH	11	Indoor
20	Allergic	SF	DSH	7	Indoor
21	Allergic	SF	DSH	8	Indoor

Sample Collection

Both the 10 healthy and 10 allergic cats were swabbed at 5 sites. The 5 sites included the axilla, groin, interdigital, lumbar, and nostril.

Three Isohelix buccal swabs (Cell Projects Ltd., Kent, UK) were used per skin site. Two swabs were added into a MoBio PowerBead tube containing 750 µl of buffer (MoBio Laboratories, Carlsbad, CA) and the other swab was stored in a 2 ml collection tube without any reagents and immediately stored at 4^{o}C, followed by storage at -80^{o}C. The swabs on the PowerBead tubes were stored for no longer than 30 days at 4^{o}C until extractions were performed.

DNA Extraction and Sequencing

Genomic DNA was extracted from skin swabs using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories) using the manufacturer's protocol. Extracted DNA was sequenced at MR DNA lab in Shallowater, TX, on an Illumina miSeq instrument. The 16s rRNA gene was sequenced using primers forward 28F: GAGTTTGATCNTGGCTCAG and reverse 519R: GTNTTACNGCGGCKGCTG.

Data Analysis

The raw sequences received were processed using QIIME to perform quality filtering, definition of OTUs (sequences with 97% similarity), and removal of chimeras, as previously described (2). Processed sequences were then classified by comparing it to the Greengenes database. Alpha diversity was calculated to determine species richness and diversity in each sample. Beta diversity was calculated to measure similarity between samples (9). The ANOSIM function of PRIMER 7 (PRIMER-E Ltd., Luton, UK) was used with the UniFrac distance matrix to compare different samples to see if there was any difference in bacteria present ($p \geq 0.001$ considered significant). A Kruskal-Wallis test was performed using JMP11 (SAS, Marlow, Buckinghamshire) since the data was not normally distributed.

CHAPTER III

RESULTS & DISCUSSION

Skin Microbiome of Healthy Cats

Species Richness and Diversity Between Sites Sampled

Alpha diversity calculations indicated that there is a difference in the number of observed species found in each body site. The three of the indices used to calculate alpha diversity (chao1, shannon, and observed species) had similar results, with samples from interdigital area having the most and nostril having the least number of observed species (Figure 1). The nostril in particular was significantly different than the other regions (p-value < 0.015), except for the lumbar area according to observed species. Statistical analysis also revealed that according to chao1 and observed species calculations, interdigital and lumbar skin are also significantly different (p-value < 0.05).

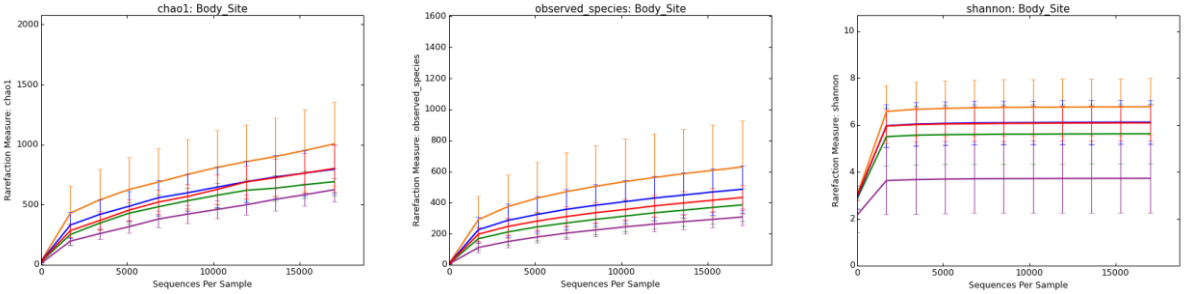

Figure 1. Alpha diversity (chao1, observed species, and Shannon) plots comparing axilla (red), groin (blue), interdigital (orange), lumbar (green), and nostril (purple).

It was found that haired skin (interdigital, lumbar, groin, and axilla) had a higher number of observed species than mucosal sites (p-value < 0.001), like the nostril (Figure 2).

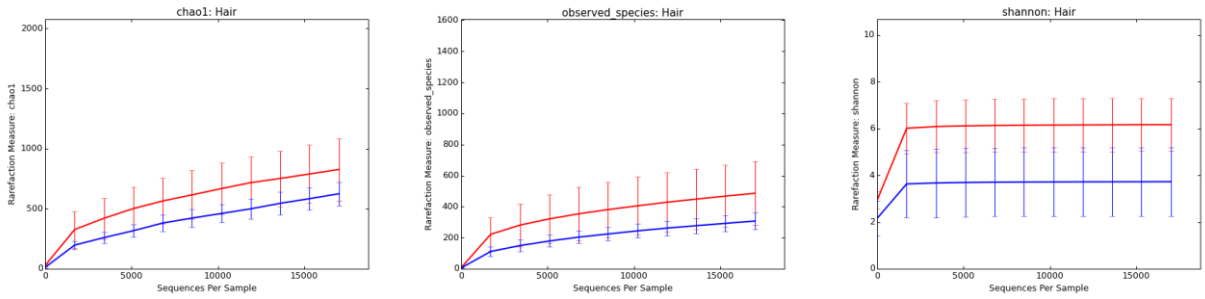

Figure 2. Alpha diversity (chao1, observed species, and Shannon) plots comparing haired (red) and mucosal (blue) surfaces.

Beta diversity calculations showed that there is a difference in bacteria present between mucosal and haired sites (Figure 3). Using the ANOSIM function and a p-value of 0.001, there is a significant difference in microbiome composition between haired and mucosal areas.

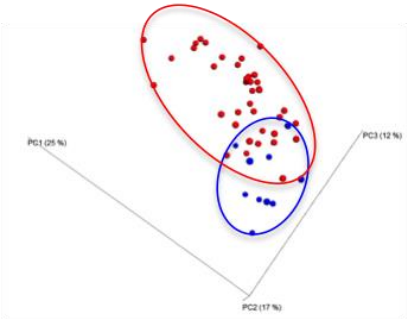

Figure 3. Beta diversity (Unifrac weighted) plot comparing haired (red) and mucosal (blue) surfaces.

Common taxa

The most abundant phylum in the different regions of healthy cats was found to be Proteobacteria, followed by Firmicutes and Bacteroidetes (Figure 4). The nostril had the highest relative abundances of Proteobacteria, with the most abundant family being *Moraxellaceae*. In the other sites the most abundant family was *Pasteurellaceae.* The most predominant family of the Firmicutes phyla in all sites was *Staphylococcaceae*, with highest relative abundances of

Staphylococcaceae seen in the interdigital region. The most abundant family in the phyla Bacteroidetes was found to be *Porphyromonadaceae*, with most of it being in the nostril.

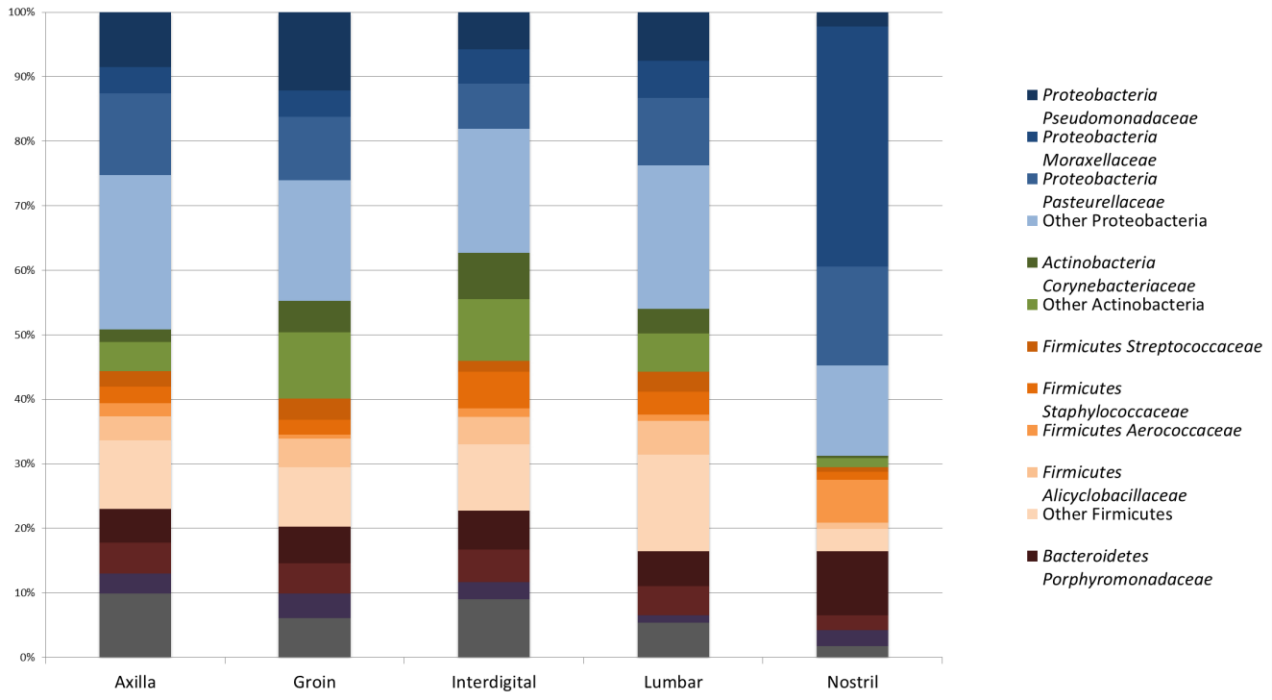

Figure 4. Graphs showing makeup of microbiome by phyla and family.

Skin Microbiome of Healthy vs. Allergic Cats

Species Richness and Diversity

Alpha diversity plots indicate that there is a significant difference in the diversity and richness of the microbiome of allergic and healthy cats (p-value < 0.0001). Healthy cats have a larger number of observed species and more evenness and abundances of species, as based on the Shannon, observed species, and chao1 diversity analyses. The diversity and richness varied significantly between healthy and allergic cats in every individual site, except for the nostril in chao1 and observed species calculations (p-value < 0.03).

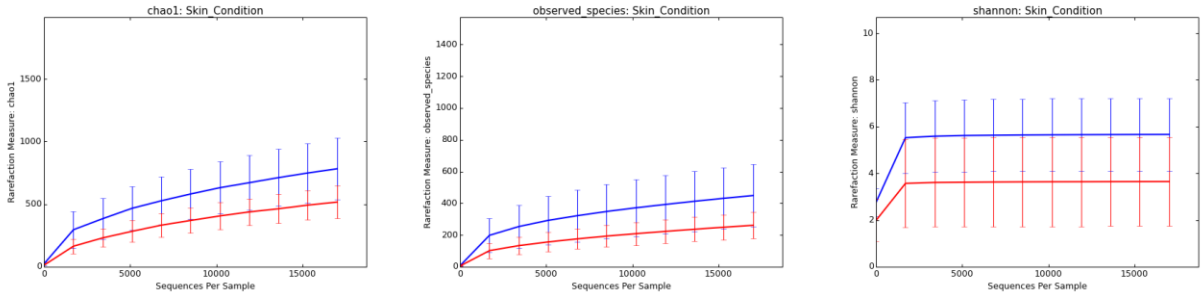

Figure 5. Alpha diversity (chao1, observed species, and Shannon) plots comparing allergic (red) and healthy (blue) skin.

Plots generated by beta diversity calculations show that there is clustering between samples from allergic dogs and separate clustering for samples from healthy dogs (Figure 6). ANOSIM revealed there is not a significant difference in microbiome composition between healthy and allergic cats.

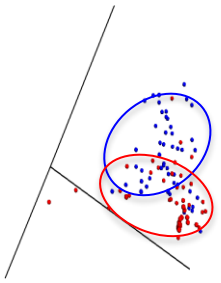

Figure 6. Beta diversity (Unifrac weighted) plot showing clustering of healthy (blue) and allergic (red) samples.

Common taxa

The composition of the microbiome of healthy and allergic cats differs significantly ($p < 0.0001$). The most abundant phyla in all regions of both healthy and allergic cats was found to be Proteobacteria. Allergic cats had significantly more Proteobacteria, except in the nostril ($p<0.0036$). The skin of healthy cats is inhabited by more Firmicutes than the skin of allergic cats ($p<0.0001$). The sites that were the most significantly different between healthy and allergic cats were the axilla and groin (Figure 7).

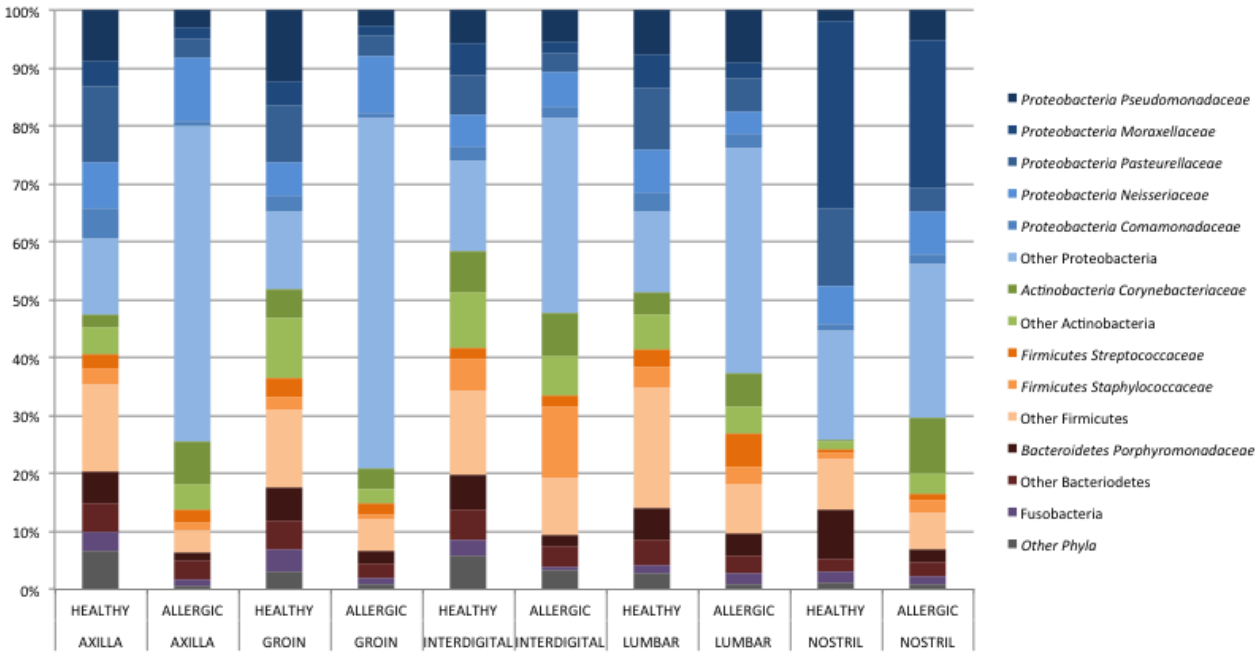

Figure 7. Graphs showing make up of microbiome by phyla and family.

This study's results are similar to that of a similar study done with dogs (2). Unlike in the dogs, where *Oxalobacteriaceae* was the most abundant family, *Pasteurellaceae* was the most abundant bacterial family found in most of the samples from cats. In both cats and dogs, the most abundant phylum was Proteobacteria. In the cats, Proteobacteria made up more than 50% of the bacteria found in most samples, but in dogs, although Proteobacteria was the most prevalent phylum found, it accounted for less than 50% of the bacteria found in most samples.

CHAPTER IV

CONCLUSIONS

It has been demonstrated that the skin microbiome of cats is very diverse and varies between different body sites and between skin conditions (healthy and allergi). Haired sites are much more diverse and even than mucosal sites. The microbiome of allergic cats is less diverse and less rich than that of healthy cats. A significant difference was seen in diversity between healthy and allergic cats. No significant difference was found between healthy and allergic cats in overall microbiome composition, however significant differences were found in abundance of certain phyla and families. More research will need to be done to see how this relates to disease and what other factors might play a part in the microbiome-disease relationship. Along with bacteria, the presence of other microorganisms will need to be considered.

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