12-9-2015

A Comparison of Gastrointestinal Bacterial Population Between Indoor Cats and Outdoor Cats

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Abstract

This study used fecal samples from four indoor cats and four indoor-outdoor cats in order to identify the microbial communities in the gut of cats. This information was then used to compare the microbial phyla between both groups for any differences. Total microbial DNA was isolated from each fecal sample, and the 16S rRNA gene was sequenced using Illumina MiSeq high throughput method. The sequences were identified using the bioinformatics program mothur. The results show that indoor cats had a more diverse microbial community as compared to outdoor cats. Indoor cat samples had 26% more microbial species, and eight more phyla compared to outdoor cat samples. The predominant phyla present in both indoor and outdoor cats were *Firmicutes, Bacteroidetes, Actinobacteria,* and *Proteobacteria*. Indoor cats also contained a significant number of *Fusobacteria*. After analyzing a dendrogram, it was found that indoor cat microbial populations were more related to each other compared to outdoor cat bacterial populations, whereas outdoor cat microbial populations were quite diverse from each other.

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The American Humane Association acknowledges that there are two types of cats: indoor cats or outdoor cats. Indoor cats are defined as cats that have not been allowed outdoors since being adopted or rescued, or have not been allowed outdoors at all during their lifetime. Outdoor cats are mainly feral cats or stray cats, who live outdoors for the majority of their lifetime. However, it should be noted that many cats are indoor-outdoor cats, a halfway point between the two categories. Indoor-outdoor cats are defined as cats that have a permanent home indoors, but are allowed to freely roam the outdoors of their own accord.

Shelter operators and veterinarians are of the opinion that cats should be kept indoors throughout their lifetime. The reasons for this include physical harm to the cat from wild animals and vehicles, the possible loss of the pet, or the possibility of the cat being infected with a viral illness such as Feline Leukemia Virus or rabies (Little, 2011). Cats are also a primary reason for bird population fluctuations due to their predatory behavior (Lepczyk et al., 2004). Despite these reasons, many owners still allow their cats to roam outdoors, and this exposes them to all the dangers listed above.

Since humans interact so closely with their pets, they are at risk for infection from the many zoonotic diseases that can be passed from cats to humans (Day et al., 2012). Therefore, it is important to know about the many dangerous microorganisms that inhabit cats so as to further educate pet owners about potential risks. Table 1 lists different bacterial agents and their corresponding diseases. These diseases can be transferred through inhaling or ingesting feces, urine, saliva, or a break in the skin after being scratched or bitten by a cat.

Table 1: A list of various bacterial species found in cats and the corresponding zoonotic diseases they cause in both humans and cats.

Bacterial Species	**Associated Diseases**
Bacillus anthracis	Anthrax
Bartonella species	Cat-scratch disease, endocarditis
Bordetella bronchiseptica	Pertussis
Borrelia burgdorferi	Lyme disease
Campylobacter jejuni	Gastroenteritis, Guillaine-Barre syndrome
Chlamydophila felis	Respiratory problems and inflammation of conjunctiva in cats
Corynebacterium diphtheria	Diphtheria
Coxiella burnetii	Q fever
Francisella tularensis	Tularemia
Group A Streptococcus	Strep throat, impetigo
Helicobacter species	Stomach ulcers
Listeria monocytogenes	Listeriosis
Leptospira species	Leptospirosis
Mycoplasma haemofelis	Feline Infectious Anemia
Rickettsia felis	Flea-borne spotted fever in cats
Salmonella species	Salmonella infection
Yersinia species	Plague, yersiniosis

Identifying the differences in the number of different microbial species present in indoor cats and outdoor cats could also help to create a healthier lifestyle for cats. In humans, a higher diversity of gut microbes are correlated with improvements in health. Certain bacterial species, such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum,* have been linked to lower rates of obesity and better mental health. Although there have been no studies linking these benefits to cats, this information could be a benchmark for gauging feline health in the future.

This study seeks to identify and compare the microbiome of gastrointestinal bacteria in indoor cats and outdoor cats. The hypothesis is that indoor cats will have a more diverse gut

microbiome compared to outdoor cats, because they are exposed to a wider variety of environments and diets.

Methods

A fecal sample was collected from a total of 8 felines of various breeds: 4 of them were fully indoor cats while the other 4 were indoor-outdoor or fully outdoor cats. The fecal samples were then isolated using the PowerSoil DNA Isolation Kit (MO BIO Laboratories). This kit works to separate debris and humic acids from bacterial cells, helps in cell lysis, prevents degradation of DNA, and cleans the DNA for sequencing. Only the center of the fecal sample was used in isolation, to avoid contamination by other microbes on the outer surface of the fecal sample. The protocol was followed exactly as given by the kit, with the exception of the steps for the addition of solution C1. Solution C1 was added to the sample and this mixture was heated at 65°C for 10 minutes in a thermomixer, before being vortexed for another 10 minutes.

After isolation, the DNA was quantified using a Qubit fluorometer (ThermoFisher Scientific). The Qubit fluorometer contains fluorescent dyes that are initially not very fluorescent but become intensely fluorescent upon binding to DNA, RNA, or proteins. The fluorometer is very sensitive and can detect very low amounts of fluorescence, and displays this information in a simple graph. This is a failsafe to ensure that the isolation techniques worked, and that there will be enough DNA for sequencing.

After quantification, the isolated DNA was sent to the RTSF Genomics Core at Michigan State University (MSU). At MSU, the DNA underwent polymerase chain reaction (PCR) of the 16S rRNA gene to increase the concentration of DNA available for sequencing. The sequencing was carried out using the MiSeq System (Illumina). During sequencing, contigs were created by

hybridizing sample DNA to oligos on a flow cell surface. Hybridization allows for the formation of single stranded regions of DNA which can then be paired with the corresponding nucleotide to form double stranded DNA. Each nucleotide releases a specific fluorescent signal upon binding, and the readings of these signals allow us to determine the exact nucleotide sequence of the complimentary DNA strand, as well as the original DNA strand.

The contigs generated from MiSeq were analyzed using the bioinformatics program mothur. This program eliminated bad sequence pairings, chimeric DNA, and duplicates of DNA sequences to narrow down the number of sequences that needed to be analyzed. mothur also joined the contigs of sequenced DNA to give complete readings of the 16S rRNA region of bacterial DNA. This information was used to cluster the identified bacteria according to their domain, kingdom, phylum, class, order, family, and genus. The results were tabulated and graphed, and a dendrogram was made to show the relationships between species.

Results

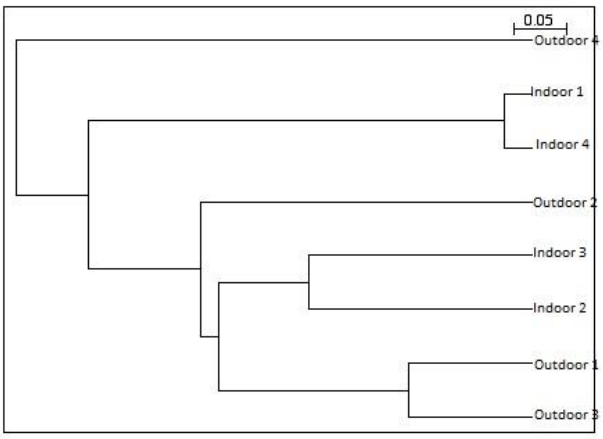

Figure 1: A dendrogram showing the relationships between bacterial populations in each sample. Indoor cats are more related to each other compared to outdoor cats.

The microbial communities in indoor cats are very closely related to each other. However, there seems to be less relatedness among outdoor cat bacterial populations. Figure 1 shows a dendrogram of the different samples in relation to each other. Indoor cat 1 (sample 1) is most closely related to indoor cat 4 (sample 4) and indoor cat 2 (sample 2) is most closely related to indoor cat 3 (sample 3). Outdoor cat 1 (sample 5) is

most closely related to outdoor cat 3 (sample 7) while outdoor cat 2 (sample 6) and outdoor cat 4 (sample 8) are not closely related to any other sample.

Figure 2 shows a graph of the phyla present in each fecal sample. The results show that *Firmicutes* are the most abundant phylum in every sample of both indoor cats and outdoor cats. The three other phyla that were highly represented include *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*. Indoor cats also contained a significant number of *Fusobacteria*. The microbiome of the indoor cats contained 19 different phyla of bacteria compared to 11 phyla in the outdoor cats.

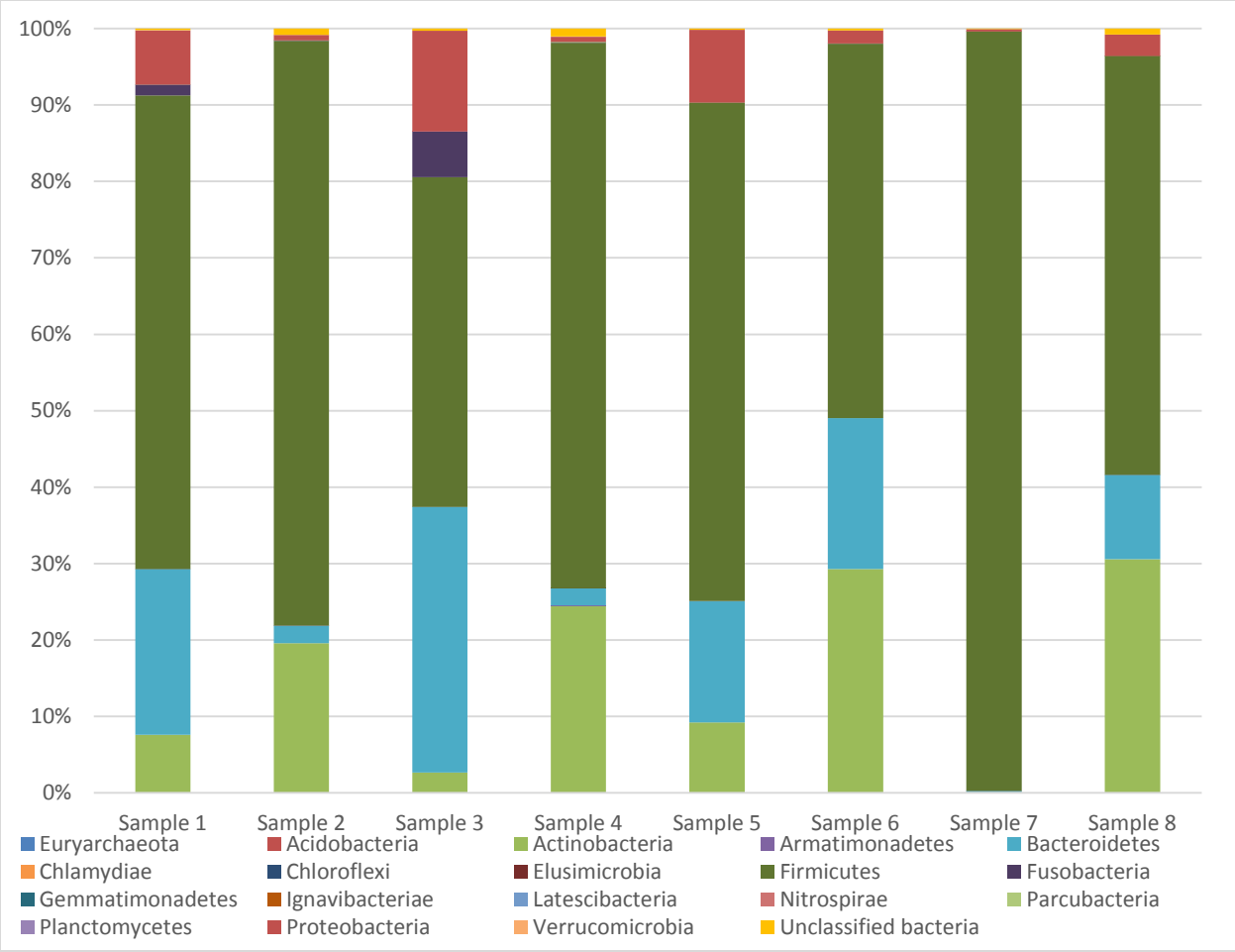

Figure 2: A cumulative graph of all bacterial phyla present in the gut microbiome of four indoor cats and four outdoor cats.

Comparing the most abundant genus in each phylum of bacteria for indoor and outdoor cats showed many differences. For *Firmicutes*, *Catenibacterium* was most abundant in indoor cats while *Lactobacillus* was most abundant in outdoor cats. For *Bacteroidetes*, *Prevotella* was most abundant in both indoor and outdoor cats. For *Actinobacteria*, *Collinsella* was most abundant in indoor cats while *Bifidobacterium* was most abundant in outdoor cats. For *Proteobacteria*, *Sutterella* was most abundant in indoor cats while *Anaerobiospirillum* was most abundant in outdoor cats. In addition, since indoor cats had a large number of *Fusobacteria*, this was also analyzed and it was found that an unclassified genus of order *Fusobacteriaceae* was most abundant. Figure 3A shows the breakdown of bacterial phyla in each sample of indoor cats, and Figure 3B does the same for each sample of outdoor cats.

Discussion

On the whole, indoor cats had a more diverse gut microbiome compared to outdoor cats, having 8 more phyla of bacteria represented in their feces. These results go against the initial hypothesis, which stated that outdoor cats would have a more diverse gut microbiome. This phenomenon could be due to the close contact between humans and indoor cats. Just as cats can influence human gastrointestinal microbiomes, perhaps the opposite holds true as well. Humans are also capable of bringing in a diverse array of bacteria from the outside environment, either on their skin, their shoes, plants, and other household items.

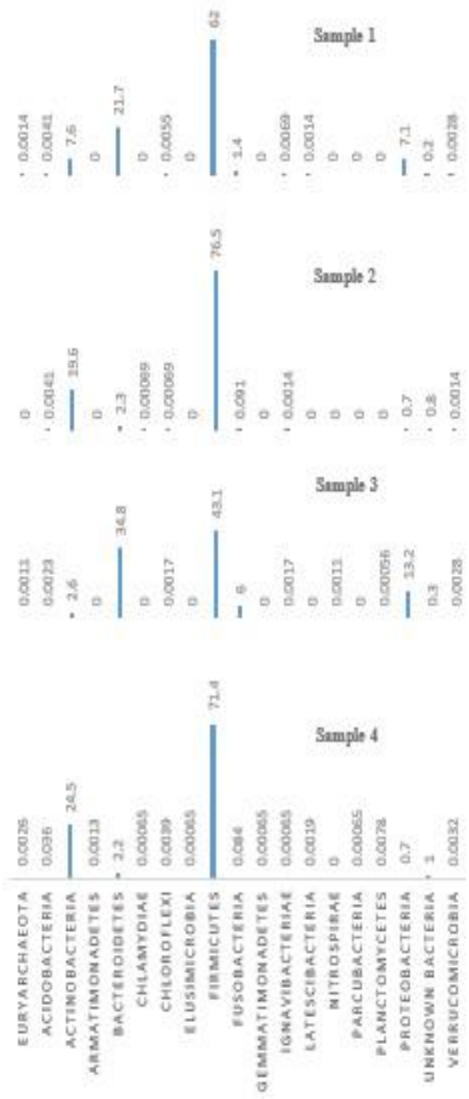

Figure 3 A: The percentages of each phylum present in each sample of indoor cats. Firmicutes are the most abundant phyla, followed by Bacteroidetes, Actinobacteria, and Proteobacteria.

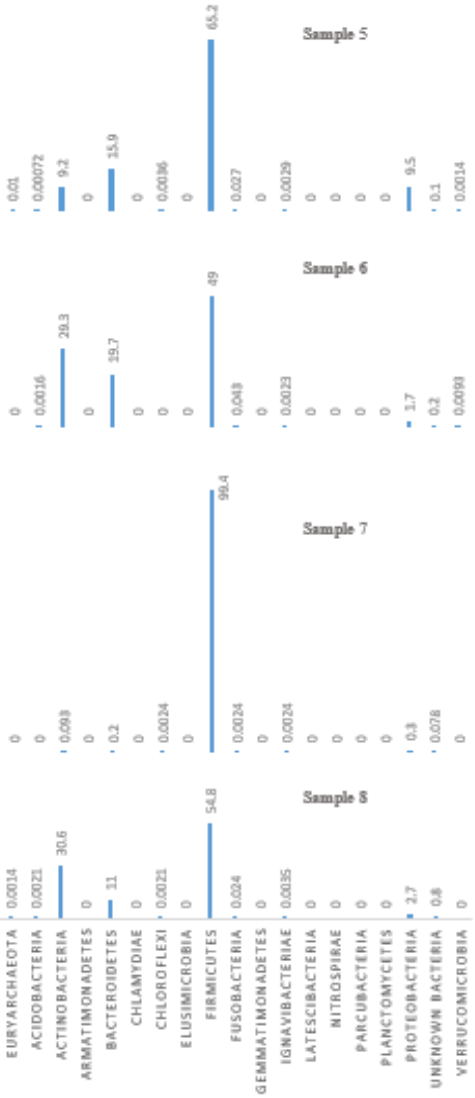

Figure 3 B: The percentages of each phylum present in each sample of outdoor cats. Firmicutes are the most abundant phyla, followed by Actinobacteria, Bacteroidetes, and Proteobacteria.

This diversity that is seen in indoor cats could be an indication that they are healthier than outdoor cats. Research has shown that indoor cats tend to live between 12-18 years, while outdoor cats live for an average of about 3 years (Loyd et al., 2013). Past studies have linked gut

diversity to longevity in the nematode *Caenorhabditis elegans* as well as in humans. Strains of *Escherichia coli* OP50 bacteria are thought to be a source of nitric oxide for *C. elegans*, a compound that plays an important role in the animal's longevity (Gusarov et al., 2013). In humans, certain species of *Lactobacillus* and *Bacteroidetes* have been shown to interfere with the inflammation pathway, reducing inflammaging and the amount of inflammatory proteins in the circulatory system (Tien et al., 2006).

The indoor cats were also exposed to a wider variety of cat food brands (Purina, Iams, Royal Canin) compared to outdoor cats. All outdoor cats had been in captivity for less than three days. However, since the outdoor cats were currently being held by animal control in Kalamazoo, they were fed the same brand of cat food (Purina) and their diets prior to being captured are unknown. Diet effects are especially significant when looking at the data for Sample 7 in Figure 2. The most prevalent genus of bacteria in the fecal sample from this cat was *Lactobacillus*, which is rarely found in cat food, but is found in yogurts and probiotics.

Catenibacterium species accounted for 13.5% of *Firmicutes* in outdoor cats. *Catenibacterium* species are known to produce short-chain fatty acids, which play a protective role in the gut (Šlapeta et al., 2015). These bacteria were also found in significantly increased amounts in dogs that were infected with *Giardia* (Šlapeta et al., 2015), a genus of protozoan parasites which cause giardiasis.

Prevotella was the most abundant genus of *Bacteroidetes* in both indoor and outdoor cats, accounting for 60.6% of *Bacteroidetes* species in indoor cats and 12% of *Bacteroidetes* species in outdoor cats. They are human gut microbes that are common in the oral cavity, and have previously been isolated from the intestines of African children (Filippo et al., 2010). They are

involved in many oral and vaginal diseases. *Prevotella* species have also been isolated from infected cat and dog bite wounds, and may be involved in the inflammation reaction (Alexander et al., 1997).

Collinsella species accounted for 50.9% of *Actinobacteria* in indoor cats. A previous study has found that this genus of bacteria is significantly increased in diarrheic cats (Suchodolski et al., 2015). Another study has suggested that increased numbers of these bacteria during diarrhea help to contribute to gut health (Ramadan et al., 2013).

Sutterella species accounted for 28.2% of *Proteobacteria* in indoor cats. These bacteria have been isolated from human feces, most notably from children with autism. The researchers also noted that children without autism did not have any *Sutterella* isolated from their system (Benache et al., 2012). Increased numbers of *Sutterella* species were found in cats and dogs with acute diarrhea (Honneffer et al., 2014).

Lactobacillus species accounted for 29.8% of *Firmicutes* in outdoor cats, and are used to treat gastrointestinal problems such as Crohn's disease and irritable bowel syndrome in humans. *Lactobacillus* species are often introduced into the body through probiotics and foods such as yogurts and cheeses. They have also been involved in reducing iflammaging effects by reducing the amount of circulating inflammatory proteins (Tien et al., 2006).

Bifidobacterium species accounted for 51.5% of *Actinobacteria* in outdoor cats. *Bifidobacterium* is a ubiquitous intestinal bacteria in humans and animals, and *Bifidobacterium* species are frequently used in probiotics to improve gut health in humans. It is thought that the ability to scavenge different nutrients from a variety of foods is linked to the presence of *Bifidobacterium* species in the intestines (Schell et al., 2002).

Anaerobiospirillum species accounted for 32.7% of *Proteobacteria* in outdoor cats, and are commonly found in fecal samples of healthy dogs and cats, as well as in diarrhea of humans. However, it is not found in the feces of healthy humans, and it is unknown whether the bacteria were present in high numbers because they are the cause of the diarrhea, or whether they are helping to stop the infection (Greene, 2011). Two cases were reported where *Anaerobiospirillum* species were isolated from children who experienced diarrhea, as well as their asymptomatic pet dogs, confirming that this genus of bacteria is transmissible to humans (Malnick et al., 1990).

This study had some limitations that could be improved upon. The first limitation was the small sample size of eight cats. A larger sample size will lead to more accurate results, and will provide more concrete information based on the large number of cats that are involved. The second limitation was the condition of the outdoor cats, since they were all in the care of Kalamazoo Animal Control. This would expose them to the same environment and diet, and although their stay did not exceed three days, the results would be more relevant if fecal samples were taken while the cats were still living in the wild. The third limitation was the area that was taken into account. This study used cats solely from the Michigan area (Kalamazoo, Portage, Lansing, and Bay City), but a wider area would provide information that can be generalized to other cats. Ideally, if this study were to be carried out internationally, at least two indoor cats and two indoor-outdoor cats from each state or country should be used in future studies.

Since this study only analyzed bacterial species that are present in cats, future studies could build upon this knowledge by analyzing viral species, Archaea, and eukaryotic species that affect cats and humans alike. For example, a very important eukaryotic parasite that has been extensively studied is *Toxoplasma gondii*, a protozoan that causes the disease toxoplasmosis. As for Archaea, although no pathogenic Archaea have been identified, some beneficial archaeal

species live in the gut of both humans and cats. Lastly, although the cases are rare, the rabies virus can be passed from a rabid cat to a human through a break in the skin after being bitten or scratched.

In conclusion, it is important to know and understand the intestinal microbiome of cats because many of them are kept as household pets. This puts them in close proximity to humans, and any pathogens they carry could have an adverse effect on us. Children, pregnant women, and those with weakened immune systems are especially at risk of infection. On the other hand, information about their microbiome could help us to keep our pets healthy. By analyzing the benefits that microbes confer on them, we could adjust their diets and lifestyles so as to promote health and longevity in our cats.

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