

When Dr. Janet Hill's cat was suffering from mysterious bowel problems, her veterinarian offered up a slew of possible diagnoses: pathogens, problematic food, even parasites. Hill, a microbial ecologist with an extensive understanding of the intestine, asked many questions.

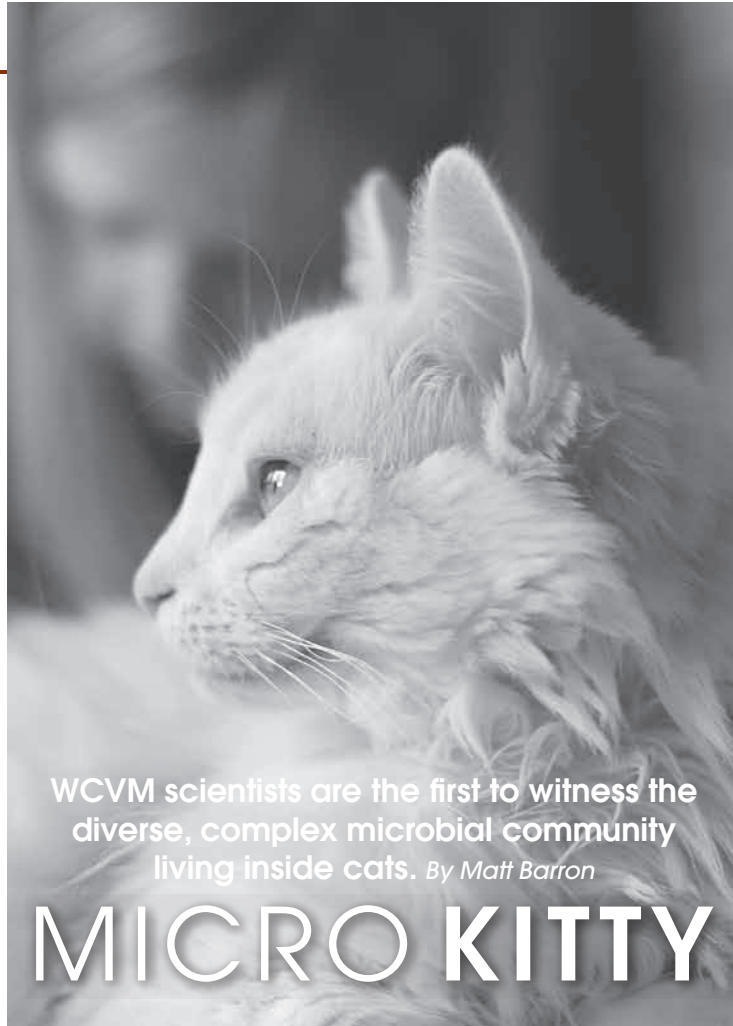
"I'm the nightmare client," jokes Hill, an assistant professor in the Western College of Veterinary Medicine's Department of Veterinary Microbiology.

Hill's cat was eventually diagnosed with inflammatory bowel disease; along with diarrhea, it's one of the more common ailments afflicting cats today. In fact, intestinal health issues are one of the main reasons behind veterinary visits for cat owners.

However, despite its significance to cats' health and nutrition, scientists know little about what constitutes a healthy, microbial community in the feline intestine. Without this knowledge, treatment for intestinal ailments will always be second best.

Hill and her research colleagues at the veterinary college are hoping to change that reality by exploring the complex, largely unknown microbial world of the cat intestine. As Hill explains, the lack of awareness stems from the ineffectiveness of the testing technique of choice: Petri dish cultures. These techniques can only detect about 10 per cent of the trillions of microorganisms thriving in the gut.

Hill, along with members of her research team, have begun to discover the other 90 per cent — a microbial community so vast and complicated that some microbiologists call it a separate organ. Based on what scientists



WCVM scientists are the first to witness the diverse, complex microbial community living inside cats. *By Matt Barron*

## MICRO KITTY

have learned in human medicine, some of these microorganisms can digest indigestible substances while others even play a role in immunity by outnumbering — and outcompeting for food and space — other disease-causing microorganisms.

"It's quite a new field," Hill says. "Even in human medicine, where you would expect the most depth, we have a very poor understanding of how this system works because it's incredibly complex. You're talking about hundreds, maybe thousands of species, living there."

### Tiny organisms make it big

Last year, the Companion Animal Health Fund (CAHF) provided financial support for a first step by Hill's research team: a study to produce a comprehensive, sequence-based description of the bacterial community of the feline intestine. Hill and her PhD student, Dr. Atul Desai, conducted the investigation in collaboration with Dr. Anthony Carr, a specialist of small animal internal medicine at WCVM.

While the ability to study this new universe is attributed to new technology, the sample source of bacteria is familiar to everyone: stool samples. As part of the study, researchers collected fecal samples from cats that live indoors as well as others that live outdoors.

After team members extracted DNA from individual samples, a gene sequence common to all existing microorganisms was sequenced.

# INSIDE SCOOP ON BACTERIA

If you count all the human cells in your body and multiply that number by 10, you would calculate the mind-tweaking quantity of bacteria that call your body home.

Cats have roughly the same number, with a good portion of microorganisms inhabiting that mysterious part of the cat that even scientists take for granted: the intestine. Dr. Janet Hill's research at the Western College of Veterinary Medicine has shown that this vast microbial universe has surprising consequences for not only the cats themselves but also for humans.

One reason Hill has taken interest in the cat intestine is that this complex environment can serve as a reservoir for disease agents such as *Campylobacter* bacteria. Infections by pathogenic bacteria in humans remain the major cause of gastrointestinal disease in Canada.

While *Campylobacter* infections have proven a major burden to the health care system, most research on the bacteria focuses on those species that

cause disease in livestock. Pet owners, however, might be surprised to learn that cats and dogs can actually host such human pathogens as *Campylobacter*.

"But we humans can transmit it to cats, too," Hill says. "That's the thought, anyway. If *Campylobacter* bacteria can live in either cats or humans, it's not necessarily guaranteed that they won't move in the other direction."

Hill wants to know what role *Campylobacter* and other families of microorganisms play in human diseases so future prevention and treatment

One reason that Dr. Janet Hill has taken interest in the cat intestine is that this complex environment can serve as a reservoir for disease agents such as *Campylobacter* bacteria.

*Chaperonin-60 (cpn60)* is employed as a “universal target” for identifying microorganisms in sequence-based microbial ecology studies. “I use the analogy of looking at a pinky fingerprint,” Hill says. “If you walked into a crowded room of people, you could take a fingerprint of all the people and find out who they are.”

The result of their work is the largest study to date of the complex microbial community found in feline feces. The team developed two *libraries* (electronic databases) of cloned *cpn60* gene fragments that represent the microbial diversity present in the outdoor and indoor cats’ fecal samples.

When Desai compared the intestinal libraries of cats living outdoors to those living indoors, he found a difference. “Indoor cat libraries were more diverse as compared to outdoor cat libraries,” explains the graduate student.

Specifically, the indoor cat library had three times as many different sequences identified than the outdoor cat library. The indoor cat library was dominated by sequences identical to *Bifidobacterium spp.*, while the outdoor library was largely composed of *Lactobacillus*-like sequences.

In May 2008, second-year veterinary student Kristyna Musil took the study further by investigating the extent of animal-to-animal variation in the cats’ microbial populations (see “Student Scientist” on page 4). Musil’s research showed that besides differences between pools of samples, there are also enormous variations between individual cats living in either environment.

Diversity was exactly what the research team was hoping to find: “Our overall objective was to create a collection of sequences that represents the universe of possible organisms found in cats’ feces. By including both indoor and outdoor cats in our study, I think we’ve been successful in maximizing the diversity of species that we could observe,” says Hill.

### Next phase of exploration

Major differences exist between the microbial communities living within the intestines of cats, humans and other species. That means scientists can’t just apply what they know about people to cats and hope that it leads to better treatments for feline ailments such as flatulence, stool odour and inflammatory bowel syndrome.

strategies can be created. With funding from the Saskatchewan Health Research Foundation, she’s developing methods of detecting and identifying a broad diversity of species, including *Campylobacter*.

Hill wants to know the prevalence of these bacteria, and how the identified and unknown species of *Campylobacter* cause gastrointestinal disease.

After all, the cat intestine itself is still a relative unknown, and she’s looking at many of these species of microorganisms for the first time. Using DNA sequencing, Hill is detecting a wealth of microorganisms that have been largely overlooked by other scientists.

She has also been characterizing the complex intestinal communities of other species including chickens, pigs and humans. While many of these species share common species of microorganisms, each has its own distinctive species of bacteria — many of which have yet to be identified.

But as scientists gain a better understanding of feline-specific intestinal bacterial populations, they can help food manufacturers design better diets to combat these illnesses — and even improve overall health. However, disturbing the community has repercussions: immune defenses can fail, and disease can result.

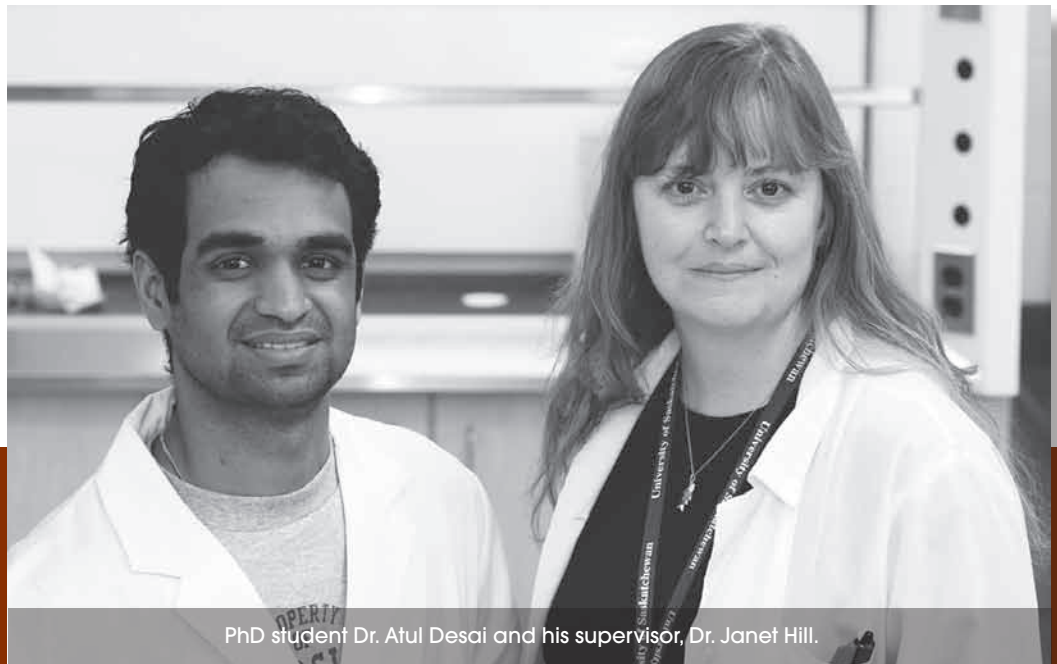
What especially excites Hill is that her lab has been the first to witness a universe on a microcosmic scale with the great resolution of DNA techniques. And it’s only the beginning. As Hill describes, trillions of bacteria exist in the feline intestine and range from the most common species to the rarest of organisms.

Many of these species have yet to be discovered, and yet may be implicated in certain diseases, or contribute to some aspect of cat health. Only by identifying these species and their functions can proper treatments — similar to the development of pro- and prebiotic treatments for humans — be developed.

The rarest organisms — those found in tiny numbers — are difficult to discover since Hill’s instruments aren’t sensitive enough to detect them. However, she plans to use an even newer form of technology called *next generation sequencing* technology, whose resolution is strong enough for the task.

“With the current technology we’re using, we know we’re seeing what is potentially the tip of the iceberg,” says Hill. ▽

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PhD student Dr. Atul Desai and his supervisor, Dr. Janet Hill.

Hill offers a huge electronic database called *cpnDB* (<http://cpndb.cbr.nrc.ca/>) that stores the DNA sequences from well-characterized reference strains of bacteria. The online *cpnDB* collection provides a high quality reference data set to which scientists can compare sequences from unknown organisms.

Ultimately, a deeper insight of microbial communities could contribute to better treatments of diseases caused by these microorganisms. It could also be the basis for proactive measures such as boosting the immune responses of animals and humans to prevent disease.

Visit Dr. Hill’s web site (<http://homepage.usask.ca/~jeh369/>) to learn more about her team’s research.