



Feline Study Puts PCR to the Test

by Matt Barron

When Dr. Belle Nibblett set out to investigate the presence of parasitic bacteria in cat populations, the usual research path from test to results became a winding road that led the graduate student back to the test itself. Nibblett, a resident in small animal medicine at the Western College of Veterinary Medicine (WCVN), initially wanted to compare the presence of hemotropic *Mycoplasma* in cats housed at the Saskatoon SPCA with cats treated at WCVN's Small Animal Clinic. Formerly known as *Haemobartonella* and *Eperythrozoon* species, the renamed hemotropic mycoplasmas (or hemoplasmas) are small, epicellular parasites of cat red blood cells that cause a range of clinical signs culminating in life-threatening anemia.

Nibblett suspected that the blood-bound bacteria would be a bigger problem with the SPCA's feline population since feral cats would be at higher risk of infection through transmission from fleas and ticks as well as from contact with other cats. She had good reason to think the presence of the parasite would be high: a retrospective study of WCVN's feline cases between 1995 and 2006 found that 14 per cent of anemic cats were infected with hemotropic *Mycoplasma* species.

In a healthy cat, hemotropic *Mycoplasma* infection may remain subclinical. But if a healthy cat's infected blood is accidentally donated to an unhealthy cat, severe anemia and even death could result. That situation, points out Nibblett, is something the WCVN wants to prevent.

***Mycoplasma* tricky to test**

For various reasons, hemotropic *Mycoplasma* can't simply be grown on a *petri* dish and identified like most bacteria. Nibblett's study would have been impossible without recent advances in PCR (polymerase chain reaction) technology — a DNA-based technique whose sensitivity is thought to be unparalleled.

Yet, after two months of collecting and analyzing blood samples from 50 cats, Nibblett found that only one of the SPCA cats and none of the WCVN cats had tested positive for the bacteria. "It was absolutely a surprise because the initial screening had shown such very high numbers," she says.

Nibblett and her graduate supervisor, Dr. Elisabeth Snead, began suspecting a problem with the sensitivity of the PCR test. After all, the researchers sought very small numbers of bacteria, not the millions typically grown in a *petri* dish.

The focus of Nibblett's study shifted to include the PCR test itself: was there anything wrong with the test? This proved to be an important question. After all, PCR technology isn't just used by researchers: veterinarians also rely on PCR testing when they need to confirm particular diagnoses.

Above: Dr. Belle Nibblett examines a female patient while a clinical team works in WCVN's new anesthesia induction area.

Photos: Michael Raine

Nibblett decided to resubmit the same blood samples to the laboratory and see if the chosen lab could produce the same answer twice using its PCR technology. In each case, the lab's results were consistent. This seemed to rule out the possibility that problems with the PCR test caused the low numbers in her study, or the possibility that the test wasn't sensitive enough.

Nibblett says changing rates of infection over the past few years is a possible explanation for the fewer number of subclinically-infected cats in her study. In any case, after increasing the number of cats to be screened for hemotropic *Mycoplasma* infection to 115, the graduate student found that 12 per cent of SPCA cats were infected with hemotropic *Mycoplasma* while four per cent of the client-owned cats were infected.

Can you see the difference?

While the PCR assay was exonerated in her study's test results, Nibblett still had questions about the PCR test in general: would she get the same answer if she sent the same blood sample to two different laboratories? Curiously, she received a different answer on multiple samples. For instance, one laboratory detected two *Mycoplasma* species in the blood sample while the other lab detected only one species.

"Myself, as a doctor, I've given up on the idea that tests are black and white," Nibblett says. "There's often a fair bit of grey area within the answer of diagnostic testing. We want to accept it (the diagnostic test) as being perfect, but it's not. So we have to become aware of its limitations — such as this study that shows you can't get the same results from every lab."

PCR testing is very sensitive and useful if it's understood, points out Nibblett. But as her study showed, there was a 10 per cent discrepancy rate between results from PCR testing at two different laboratories. This isn't necessarily due to a

problem in how the laboratories perform the test: it's actually because the test is slightly different at various laboratories. If veterinarians are unaware of the test's limitations, there could be problems for their patients as well as for veterinary research.

"That difference might be significant if your cat tests negative (for a disease), but it's really positive. They won't get the antibiotics they need," explains Nibblett. She adds that it's far better to send samples to the same laboratory to maintain consistency in the results.

While it's desirable for laboratory tests to be standardized by the International Standards Organization (ISO), PCR testing still hasn't gone through that process. Nibblett hopes that this study will draw attention to the test's limitations among veterinary researchers and practitioners.

As for the circuitous development of her research study, Nibblett would have liked to find more conclusive results on the risks of *Mycoplasma* infection in cats and possibly a better way of treating the disease.

At this point, no research team has published a consistently effective antibiotic therapy for *Mycoplasma* infection in cats.

The lack of "positive" cats in her study prevented Nibblett from looking into effective antibiotic protocols, "but that's par for the course in doing research," acknowledges Nibblett. **V**



Roaming Males More at Risk

If your kitty is a mixed-breed male that roams outdoors and likes to get in the occasional brouhaha with his rivals, he's at a higher risk of developing hemotropic *Mycoplasma*-induced anemia.

Those risk factor indicators stem from a retrospective study that was conducted by Dr. Belle Nibblett and her WCVM collaborators: Drs. Elisabeth Snead, Cheryl Waldner, Sue Taylor and Marion Jackson.

During her research work, Nibblett reviewed the medical records of 170 feline patients at the WCVM Veterinary Teaching Hospital between 1995 and 2006. All of the selected patients had a primary diagnosis of anemia while records showed that 23 of the cats had clinical disease caused by hemotropic *Mycoplasma* (HM) infection.

Based on those numbers, the rate of HM infection among anemic cats was 14 per cent. Cats with HM infection were less likely to be purebred and male cats were almost three times more likely to be infected than females. Access to the outdoors was present in 95 per cent of the infected cats. As well, close to 20 per cent of the HM-infected cats had been in a cat fight within a six-week period before coming to the veterinary teaching hospital.

Clinical signs included lethargy, weakness or anorexia in 74 per cent of the HM-infected anemia cases. Veterinarians classified the anemia as regenerative in almost 60 per cent of cases.

In addition, veterinarians recorded the retroviral status of 19 of the 23 patients: 10 per cent of those cases tested positive for feline immunodeficiency virus and 15 per cent tested positive for feline leukemia virus. Half of the HM-induced anemia cases received blood transfusions as part of their therapy.

What was the prognosis for the HM-infected cats? WCVM researchers defined a good outcome as survival one year after the cat was brought to the hospital. Overall survival of HM-infected cats was 63 per cent, and all of the cats that had no concurrent illness recovered and survived. Factors that reduced a cat's chances of surviving included positive retroviral status, concurrent illness and the presence of a non-regenerative anemia.

In June 2007, Nibblett had the chance to share findings from this study with her peers during the annual American College of Veterinary Internal Medicine (ACVIM) Forum in Seattle, Washington. Besides giving a poster presentation, Nibblett's research was part of the Forum's research abstract program.