

Do Viruses Cause VAFS?

When Dr. Beverly Kidney returned to WCVN in 1997 to begin her PhD research on *vaccine-associated feline sarcomas* (VAFS), she was already familiar with the deadly disease.

For 10 years, the veterinary pathologist had worked in a busy diagnostic laboratory in Langley, B.C., where she had often peered through her microscope lens at the spindle cells of this tumour in feline tissue samples.

The experience was invaluable as she began working with Dr. Marion Jackson to design her three-year research program on potential viral causes of VAFS – a disease that has caused anxiety among cat owners and veterinarians for the past decade.

“Having background knowledge about VAFS – being familiar with its appearance in a histological setting and understanding what’s already known about it – allowed me to go directly into research mode,” says Kidney.

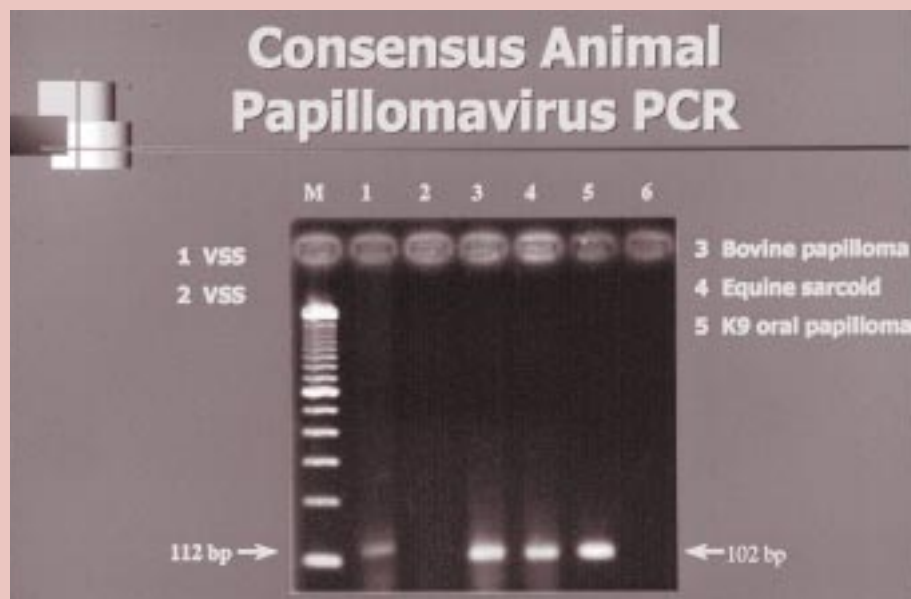
VAFS develops in a very small number of cats (one to 3.6 cats out of every 10,000), but what makes the disease so alarming is its association with vaccine administration. Since 1991, veterinarians have found a higher than expected number of sarcomas forming at common vaccine injection sites in cats. In 1996, several national veterinary organizations formed the Vaccine-Associated Feline Sarcoma Task Force to address the health issue through targeted research at North American universities.

Other researchers have considered the possible link between VAFS and viruses. A few years ago, Jackson and Dr. John Ellis confirmed that the *exogenous* form of feline leukemia (FeLV) wasn’t present in the vaccine-associated sarcomas. But since no further investigative work had been done on the *endogenous* form of FeLV in the tumours nor on several other viruses that potentially cause cancer, it was the ideal place for Kidney to start.

Retro Thinking

With funding from Saskatchewan’s Health Services Utilization and Research Commission, Kidney set out to determine whether she could detect viral DNA in 100 tissue samples – 50 from VAFS clinical cases and another 50 from non-VAFS cases.

Initially, Kidney focused on three retroviruses: feline immunodeficiency virus (FIV), feline foamy virus (FeFV), and the endogenous form of FeLV.



PCR TESTING: During her investigation of vaccine-associated feline sarcomas, Dr. Beverly Kidney analyzed hundreds of PCR test results like the one above. This particular slide shows the results of PCR products obtained after Kidney used consensus animal papillomavirus primers.

What is a retrovirus?

Retroviruses are a group of RNA-containing viruses that can produce DNA by using their RNA as a template (a process called *reverse transcription*) and incorporating the new DNA into genome of infected cells.

What’s a polymerase chain reaction (PCR) assay? What’s a consensus primer?

A PCR assay is a sensitive, molecular technique that’s used to identify and amplify a specific DNA segment within a cell. Researchers use a machine called a thermal cycler to heat and cool a solution that includes the DNA sample, the enzyme called DNA polymerase, and two *consensus primers* – short segments of DNA designed to bind with the desired DNA segment.

The heat cycle breaks the bonds between DNA strands. As the solution cools, the primers bind to the separated strands while DNA polymerase rapidly builds a new strand by joining the free nucleotide bases to the primers. By repeating this process, researchers can selectively replicate the DNA segment found between the two primers.

“When a retrovirus infects a cell, it integrates into the host’s cell DNA, and there’s always the chance that it could cause a mutation in the DNA at the site. And any time we have mutations in DNA, that could potentially lead to a tumour,” explains Kidney. A retrovirus could also alter expression of certain cellular genes, resulting in increased or decreased levels which could contribute to tumour development.

For the first two viruses, Kidney used *polymerase chain reaction* (PCR) assays to look for the DNA sequences of FIV or FeFV within the tumour cells. But the sensitive, molecular technique didn’t find any complementary sequences in vaccine-associated sarcoma tissue samples.

For the endogenous form of FeLV, Kidney used a two-step process called *reverse transcriptase PCR* that allows researchers to extract messenger RNA

rather than DNA from the samples: “We’re trying to find out whether the endogenous virus is activated. If it is, activation would make the virus produce RNA.”

Many different species have endogenous retroviruses “buried” in their DNA. Although parts of these retroviruses are transmitted into germ cells of the host, they don’t produce infectious virus because they’re incomplete sequences. “But if they’re somehow activated – for example, by inflammation associated with vaccination – they can cause problems,” says Kidney.

That wasn’t the case with VAFS: Kidney found that the amount of RNA for endogenous FeLV was no greater in the vaccine-associated tumours than it was in the non-vaccine-associated sarcomas.

Other Potential Viruses

Kidney expanded her investigation to three DNA viruses: the *papillomavirus*, *polyomavirus* and the *herpesvirus* which are all recognized as potential causes of cancer in human beings and other species.

“Our thinking with these viruses was could they have contaminated a vaccine, or could there be contamination which is introduced under the cat’s skin when vaccination takes place?” explains Kidney, who received funding from the VAFS Task Force for the second phase of her research. “Another speculation was whether latent forms of the viruses in the tissue are reactivated by the vaccine.”

For all three viruses, Kidney tried to detect the presence of particular viral antigens or proteins using immunohistochemistry testing, but no traces of the virus were found.

Then, Kidney used PCR assays to detect viral sequences in the DNA extracted from the VAFS tissues. While other researchers have developed *consensus primers* to detect polyomavirus and herpesvirus, Kidney took on the enormous task of developing new primers for identifying any type of animal papillomavirus. She also used primer sequences previously established by other researchers to evaluate the VAFS tissues for the presence of bovine polyomavirus, bovine papillomavirus and feline herpesvirus.

“We came up with negative findings – all of these viruses were not involved in the pathogenesis of VAFS. That rules out the possibility that latent viruses are involved in the vaccine-associated sarcomas,” says Kidney.

Although she didn’t find a specific cause of VAFS, her work has narrowed the focus for future research – including her own. In particular, Kidney wants to investigate aspects of cell cycle control:

Career Path Takes Some Curves

Dr. Beverly Kidney’s career path has taken a couple of constructive detours since she received her undergraduate degree from WCVM in 1979.

After practising veterinary medicine in Regina and Calgary for several years, she returned to WCVM in 1984 for a two-year, non-thesis Master’s program in clinical pathology. “I always enjoyed the lab portion of veterinary medicine, so that’s when I decided to work on a diagnostically-oriented pathology degree,” says Kidney, who completed her Master of Veterinary Science degree in 1986.

She spent another year at WCVM as an assistant professor, then accepted a full-time job with Central Laboratory for Veterinarians in Langley, B.C. Kidney’s new job was filled with variety since the private laboratory worked on diagnostic cases from all over B.C. and Alberta — including samples from Vancouver Aquarium’s beluga and killer whales.

But, after 10 years of B.C. living, Kidney wanted to be closer to her parents who still live in her hometown of Tisdale, Sask. She was also interested in learning about and applying new molecular techniques in veterinary pathology.

“Once I decided to come back, I made up my mind to pursue my PhD, then work toward a faculty position at WCVM,” says Kidney. She fulfilled both goals earlier this year, defending her PhD thesis in March then becoming an associate professor in WCVM’s veterinary pathology department on June 1.

“Obviously, what I did isn’t how most people work toward a PhD,” says Kidney, who has no regrets about her career choices. “But learning new concepts is so much easier when you can draw on past experiences.”



PET-OWNING PATHOLOGIST: Dr. Beverly Kidney holds Gina whose litter mate, George, also lives with Kidney and her husband in Saskatoon. The couple also own Goldie, a Labrador-cross.

“We need to determine particular patterns that occur in the sarcomas’ cell-cycling process and find a common thread. But it’s challenging because in most cancers, more than one factor is involved.”

While researchers continue to search for the causes of VAFS, new research on the duration of immunity for particular vaccines is helping to ensure that veterinarians aren’t over-vaccinating cats, and vaccine manufacturers are developing

non-adjuvanted vaccines that will minimize inflammation.

“These kinds of preventive actions will probably have the most influence on reducing VAFS cases,” says Kidney. “It’s a balancing act for practitioners because no one wants to cause disease in patients. But on the other hand, we can’t forget how vital vaccinations are for preventing serious disease and infection in pets.” 🐾