



To titer

or to revaccinate

Measuring antibody titers is becoming common in practice—but remains confusing, even controversial

By Katie Burns

When and why has it become more common for veterinarians to measure antibody titers when deciding whether to revaccinate cats and dogs?

An antibody titer is a measure of the concentration of antibodies in the blood, as determined by a test involving repeatedly diluting a blood sample and exposing those dilutions to an antigen. The shorthand is to refer to all measurements of antibody concentration as titers.

Dr. Richard Ford, emeritus professor of internal medicine at the North Carolina State University College of Veterinary Medicine, said factors inside and outside the profession are driving the increase in antibody titer testing.

In the late 1990s and early 2000s, the American Association of Feline Practitioners, AVMA, and American Animal Hospital Association released guidelines on vaccination suggesting that core vaccines have a longer duration of immunity than one year. The AAFP and AAHA guidelines recommended vaccinating every three years. Dr. Ford said many practices began measuring titers “to provide evidence to themselves that in fact the vaccinations are lasting longer than one year.”

Part of the impetus for the guidelines was concerns about the potential

adverse effects of vaccines. Recently, Dr. Ford said, concerns about adverse effects of vaccines in children have spilled over into veterinary medicine.

The technology for measuring antibodies also has improved, he said. There are now affordable point-of-care test kits that provide useful information within 20 to 25 minutes. Dr. Ford said, “Two things loom, and I think every veterinarian should be aware of this. One, what is the indication for using the test? And, two, how do you interpret the test results?”

For all forms of antibody testing, it remains confusing and even controversial whether the results are a good measure of immunity.

According to the AAHA guidelines, antibody testing is useful for monitoring immunity to certain viruses in dogs. The AAFP guidelines recommend defined revaccination intervals for cats. Guidelines from the World Small Animal Veterinary Association favor antibody testing for determining duration of immunity of core vaccines in dogs. But the current AVMA “Vaccination Principles” sound a cautionary note.

“I get the impression that some veterinarians are just not doing any vaccinations until the titer falls into the negative threshold level, and then they boost. So that might mean some dogs or cats will go on for multiple years without needing a vaccination. Some develop excellent levels of antibody that are sustained virtually for their lives following the initial vaccination series. And others don’t seem to sustain them that well.”

*Dr. Richard Ford,
emeritus professor
of internal medicine,
North Carolina State
University College
of Veterinary Medicine*

Principles of titers

According to the AVMA principles: “When serological titers are used to help determine the vaccination/protection status of an animal, veterinarians should make sure these data have been clinically correlated to host-animal protection studies for the specific diseases and species being tested. For most common vaccine antigens, the correlation between serological response to vaccination, long-term serostatus, and protection in the host animal has not been adequately established. The lack of these data often precludes practitioner’s ability to make well-informed vaccination decisions based on serostatus alone.”

Dr. Laurel Gershwin, a professor who teaches immunology at the University of California-Davis School of Veterinary Medicine, represents immunology on the AVMA Council on Biologic and Therapeutic Agents, which has oversight of the “Vaccination Principles.” The principles don’t make recommendations on specific vaccines or specific vaccination intervals, but Dr. Gershwin said there are good data that immunity from core vaccines in cats and dogs should last for three years.

“For those clients that are reticent about not having a distemper vaccine every single year, for example, those are a great indication to go ahead and do a titer,” she said. “Having said that, when we measure antibody, antibody is only part of the equation.”

A cat or dog could respond to a vaccine with a strong cell-mediated immune response, which is difficult to measure outside a research setting. She said, “Even if you have a titer that is less than what is considered acceptable, that doesn’t necessarily mean that the pet would get sick if he or she were challenged with just the street virus, walking down the road and sniffing noses with a dog that was infected, for example. But, obviously, one would want to boost those.”

Dr. Gershwin said the gold standard

in titer testing involves sending a blood sample to a laboratory to, say, perform a virus neutralization assay to measure antibodies to canine distemper virus or parvovirus or to perform a hemagglutination inhibition test to measure antibodies to parvovirus.

Titer testing is a good idea for some cats and dogs, Dr. Gershwin believes. These include animals that have a history of or genetic predisposition to poor responsiveness to a vaccine, that have an allergic reaction to or other adverse effect from a vaccine, or that have immunosuppression.

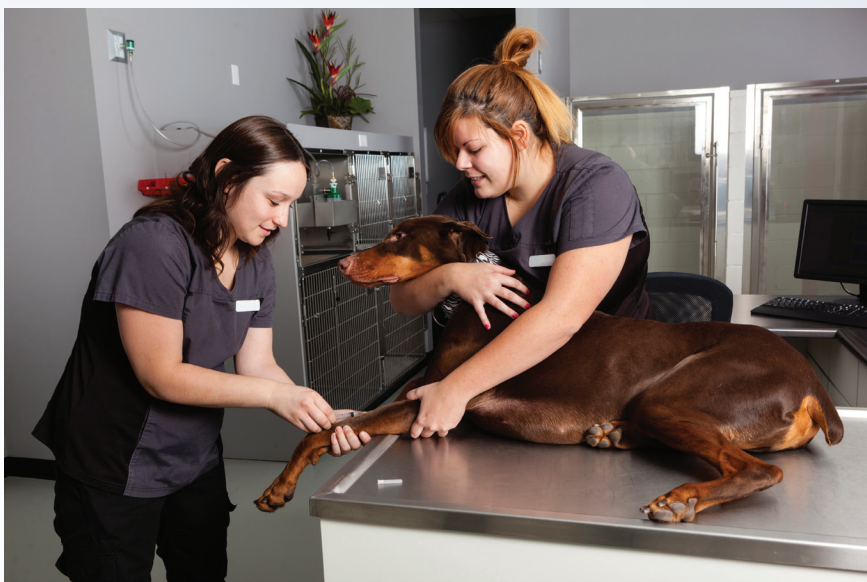
Titers in practice

The AAHA Canine Vaccination Guidelines state that “despite the confusion and controversy surrounding antibody testing,” the testing is useful for monitoring immunity to canine distemper virus, canine parvovirus type 2, canine adenovirus type 1, and rabies virus. The guidelines note, “Some clients are now having titers performed for CDV and CPV-2 in lieu of revaccinating.”

Dr. Ford of North Carolina State University, an author of the AAHA and AAEP guidelines on vaccination, said point-of-care test kits that correspond well to gold-standard titer testing are available for canine distemper virus, canine parvovirus, and canine adenovirus as well as the parvovirus that causes feline panleukopenia.

The test kits have a variety of applications, he said. Animal shelters could do titers during animal intake or during an outbreak to help control disease without euthanasia. A veterinarian might finish the vaccination series for a puppy, then test whether the dog has been immunized before starting puppy socialization or doggy day care.

More clients are asking whether booster vaccines are necessary, Dr. Ford said, and the cost has come down for titer testing. He said, “I get the impression that some veterinarians are just not doing any vac-



cinations until the titer falls into the negative threshold level, and then they boost. So that might mean some dogs or cats will go on for multiple years without needing a vaccination. Some develop excellent levels of antibody that are sustained virtually for their lives following the initial vaccination series. And others don't seem to sustain them that well."

Dr. Ford uses the acronym PIE to help with interpretation of titer tests. Depending on the nature of the disease, a positive result can imply protection, infection, or exposure. A positive result on a titer test for Lyme disease or leptospirosis implies infection. A positive result on a titer test for ehrlichiosis implies exposure.

For titer tests as with all tests, Dr. Ford added, practitioners should consider data on the likelihood of false-positive and false-negative results.

AAFP recommendations

The AAFP Feline Vaccination Advisory Panel Report states, "Because antibody titers may not reliably correlate with, or predict, the degree of protection or susceptibility for an individual cat, the Advisory Panel recommends employing defined revaccination intervals rather than measuring antibody titers to assure protection."

According to the report, most cats that have a positive result on a titer test for feline panleukopenia are immune to the disease. Titers for feline herpesvirus-1 and feline calicivirus "may not necessarily correlate well with protective immunity and should not be used to predict protection in the future." Titers for feline leukemia virus and feline immunodeficiency virus "do not correlate with immunity and should not be used to determine the need for vaccination."

Shila Nordone, who holds a doctorate in immunology and was an author on the report, said, "We have yet to determine the role of cell-mediated immunity in protection of the cat long term against specific diseases post-immunization. Antibody titers do correlate with protection but are by no means indicative of absolute protection."

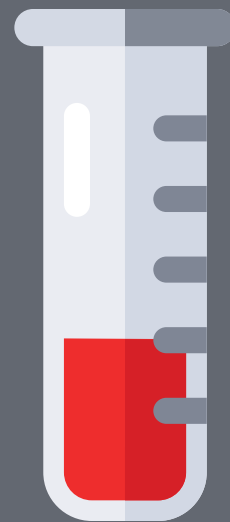
Vaccine formulations vary quite a bit, Dr. Nordone said. The AAFP recommends boosting when protection is likely to begin waning, about halfway through the expected duration of immunity.


Dr. Nordone said vaccines for feline panleukopenia are highly effective, inducing sterilizing immunity, which means the virus is blocked from replicating in the cat. She said, "FCV and FHV vaccines do not induce sterilizing

What exactly is an antibody titer?

In brief: An antibody titer is a measurement of the concentration of antibodies in the blood, as determined by a test involving repeatedly diluting a blood sample and exposing those dilutions to an antigen.

Dr. Laurel Gershwin, who teaches immunology at the University of California-Davis, added: "The last dilution of the patient's serum that produces the positive endpoint—this will differ depending upon the type of assay—is used to determine the titer. Hence, the more you can dilute the serum and get the appropriate endpoint reaction, the more antibody there is." 📌





immunity but rather minimize clinical symptoms of disease. Similarly, FIV and FeLV vaccines don't induce sterilizing immunity against the pathogens."

She concluded, "My bias, as an immunologist, is to avoid overimmunization and follow recommendations in order to maintain full protection."

Favoring titers

The Canine Vaccination Guidelines within the WSAVA Guidelines for the Vaccination of Dogs and Cats state that, while antibody testing still can be relatively expensive, "The principles of 'evidence-based veterinary medicine' suggest that testing for antibody status (for either puppies or adult dogs) should be better practice than simply administering a vaccine booster on the basis that this would be 'safe and cost less.'"

Some dogs maintain antibodies for their entire lives to canine distemper, canine parvovirus, and canine adenovirus, said Dr. Ronald D. Schultz, professor of immunology and founding chair of the Department

of Pathobiological Sciences at the University of Wisconsin-Madison School of Veterinary Medicine and an author of the WSAVA and AAHA guidelines.

In his controlled studies, he has found that dogs maintain immunity to CDV, CPV-2, and CAV seven to nine years after vaccination, as proved by protection against virulent challenge. He said, "The presence of active antibody response to these viruses is a clear indication of protection. There is no confusion on this point."

Dr. Schultz noted that the canine distemper, canine and feline parvovirus, and canine adenovirus core vaccines are all modified-live virus vaccines. In general, he said, modified-live virus vaccines provide longer-term immunity than killed virus vaccines do.

"We can use titers to know whether or not the animal does need to be revaccinated, or we can simply go on a schedule and just revaccinate," he said. While few practitioners would have thought of doing titers 10 years ago, he sees more and more titer test-

ing today. He said, "Dr. Laurie Larson in my laboratory is running thousands of gold-standard titer tests per year, and the push is coming from owners who wish to avoid unnecessary booster vaccination."

Dr. Schultz emphasized, "This is something that the owners just need to recognize: how important those core vaccines are. Every puppy and every kitten should receive them. Antibody testing assures us that the dog or cat is actually protected by the vaccine, or not, and is a very useful tool for every small animal practitioner. This is the only practical method to be certain the animal has developed an immune response to a given core vaccine."

Dr. Gershwin of the AVMA Council on Biologic and Therapeutic Agents concluded, "Pet owners need to work with their veterinarians to determine the best schedule of immunizations for their pet. This will be dependent upon individual situations, such as allergic reactivity and factors such as being on an immunosuppressive drug." 🐾

Exploring a role for titers in rabies vaccination

By Katie Burns

Most states and many municipalities have laws requiring rabies vaccination for dogs and often cats, and these laws do not allow titer testing for rabies antibodies as an alternative to booster vaccination.

Some laws specify revaccination intervals, such as three years, while others defer to vaccine labeling or the Compendium of Animal Rabies Prevention and Control. Pet owners increasingly have shown interest in titer testing as an alternative to routine booster vaccination, nevertheless, because of concerns about the potential adverse effects of vaccines.

In addition to legal limitations, titer testing has been much more expensive than administering a booster. In August 2015, the Rabies Laboratory at Kansas State University completed modifications to a titer test for rabies antibodies such that the laboratory could start offering a micro test at a reduced cost.

"It seems to make sense to us that there might be room

for titer testing for rabies" as a tool for revaccination decisions, said Rolan Davis, a reference diagnostician at the laboratory.

Susan Moore, PhD, laboratory director, said, "Pet disease status, medical treatments, and history of adverse reactions to vaccinations are examples of situations for which local or state authorities have accepted rabies titers as proof of continued immunity to rabies."

The K-State Rabies Laboratory reduced the price of a rapid fluorescent focus inhibition test for rabies antibodies partly through economy of scale, switching from 96-well plates to 384-well plates. Davis said, "We've really changed nothing about what we're doing. It's just downsizing the traditional rabies serum neutralization test."

The laboratory also switched from paper to online submission forms for the micro test to reduce data entry. Plus, the results of the micro test are not an exact titer but simply indicate whether the antibody concentration in a blood

sample is higher than 0.5 IU/mL—which is predicted to be protective on the basis of published challenge studies in dogs and cats.

In April, the K-State Rabies Laboratory did 194 of the micro tests. The laboratory also continues to offer other rabies titer tests, including a fluorescent antibody virus neutralization test for dogs and cats going to rabies-free regions.

According to the Canine Vaccination Guidelines from the American Animal Hospital Association, titer tests are useful for monitoring immunity to several viruses, including rabies virus. According to the Feline Vaccination Advisory Panel Report from the American Association of Feline Practitioners, however, “a rabies titer is only an indication of serological response to vaccination. Rabies titers are not recognized as an index of immunity.”

Dr. Richard Ford, an author of the AAHA and AAFP vaccination guidelines, emphasized that a rabies titer is not a legal index of immunity in lieu of revaccination. He said, “While immunologically speaking a titer quite likely does correspond with protective immunity, today veterinarians in practice do not have legal discretion to substitute a titer for vaccination.”

Dr. Ronald Schultz and Dr. Laurie Larson of the University of Wisconsin-Madison School of Veterinary Medicine have found that the presence of rabies antibod-

ies is highly indicative of protective immunity on the basis of challenge studies they have performed in cooperation with Dr. W. Jean Dodds and Kris Christine of the Rabies Challenge Fund and Dr. Zhen Fu of the University of Georgia College of Veterinary Medicine.

In their rabies challenge studies, Drs. Schultz and Larson found that when exposed to the rabies virus, dogs maintain immunity for up to seven years after vaccination. Although rabies vaccines are killed virus vaccines, which generally don’t provide as long of a duration of immunity as modified-live virus vaccines do, Dr. Schultz described rabies virus as an “excellent antigen.” Some rabies vaccines also have adjuvants to increase efficacy and duration of immunity.

“We’ve used virtually every test available to measure rabies antibodies, and as long as the test was antibody-positive, the dogs challenged were protected from the rabies virus challenge for as long as seven years after vaccination,” he said.

Dr. Schultz wouldn’t necessarily push for a change in laws, though. He said, “I think the requirement for a three-year revaccination cycle with rabies hopefully will get more dogs immunized. One of the goals for rabies is herd immunity because you always reduce the likelihood of disease by having the greatest number of animals immune as possible.” 🦋



The Rabies Laboratory at Kansas State University offers titer tests for rabies antibodies. Sami Pralle, research assistant, uses an automated, multichannel pipette to add stain to slides.

Photo by Tommy Theis/K-State Photo Services