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Editorial

PCR Assays: Are They Really Infectious "Disease" Tests?

Clinical syndromes induced by bacteria, fungi, protozoa, and viruses are common in dogs and cats. The diagnostic differentials for a clinical case, including the infectious agent most likely involved, are usually determined after an assessment of the signalment, history, and physical examination findings. After making a tentative clinical diagnosis, the clinician generally determines whether to test or treat. Empiric treatment can be satisfactory in some cases, such as simple, first-time infections in cats and dogs without life-threatening disease, particularly if the owner cannot afford an extensive diagnostic workup. However, a definitive diagnosis is usually preferred so that treatment, prevention, prognosis, husbandry, and zoonotic issues can be addressed optimally. Many tests are available for routine use as aids in the diagnosis of infections in dogs or cats. Assays commonly used to document the presence of an infectious agent include fecal flotation, cytology, histopathology, immunohistochemistry, culture, antigen tests, and molecular diagnostic assays. Assays that detect serum antibodies are also available for many infectious agents.

Sensitivity is the ability of an assay to detect a positive sample; *specificity* is the ability of an assay to detect a negative sample. *Positive predictive value* (*PPV*) is the ability of a test result to predict the presence of disease; *negative predictive value* (*NPV*) is the ability of a test result to predict the absence of disease. Sensitivity, specificity, PPV, and NPV vary with each assay.

Many of the infectious agents encountered in practice colonize or infect a large percentage of the pet population, resulting in positive serum antibody tests. However, the agent may induce clinically evident disease in only a small number of animals in the infected group. Examples in cats include coronaviruses, *Toxoplasma gondii*, and *Bartonella* spp. Even though antibody assays with good sensitivity and specificity are available for these agents, the PPV of the assays is very low because most cats with positive antibody test results are clinically normal. Thus, serum antibody tests are often not good infectious "disease" tests.

Polymerase chain reaction (PCR) is one of the newer molecular diagnostic assays that can be used to confirm that an organism is present in a sample. PCR amplifies small quantities of DNA to detectable levels. When a reverse transcriptase step is incorporated to convert RNA to DNA, PCR can also be used to detect RNA (RT-PCR). In general, PCR can be more sensitive than cytologic or histopathologic techniques and is comparable to culture and laboratory animal inoculation. PCR assays are of great benefit for documenting infection, particularly if the organism in question is difficult to culture (e.g., *Chlamydophila felis*) or cannot be cultured (e.g., hemoplasmas). The specificity of these assays can be very high, depending on the primers used in the reaction. For example, primers can be designed to detect all species within one genus (e.g., all *Ehrlichia* spp) or a single species (e.g., *Ehrlichia canis*).

Because of the inherent sensitivity of the reaction, PCR can give false-positive results if the sample is contaminated during collection or at the laboratory conducting the assay. False-negative results can be obtained if the sample is handled inappropriately or if the patient has received previous treatment. False negatives are of particular importance when RT-PCR is used to detect some RNA viruses. Other potential problems are that little standardization exists among laboratories offering PCR assays and there is minimal external quality control.

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[a]Dr. Lappin discloses that he has received financial support from several companies, including Heska Corporation, Pfizer Animal Health, and VCA Antech.

example, because the technique amplifies DNA from dead as well as living organisms, positive results may be achieved even if the infection has been controlled. Furthermore, when the organism being tested for commonly infects the background population of healthy animals, interpretation of results for a single animal can be difficult. Up to 20% of cats are subclinical carriers of "*Candidatus* Mycoplasma haemominutum," and my laboratory recently amplified *Bartonella* spp DNA from more than 50% of apparently healthy cats housed in shelters in states with a high flea risk. Although PCR is a sensitive way to document infection by these agents, the PPV is actually very low.

For some infectious agents, the currently available PCR assays cannot discriminate between vaccine strains and field strains. For example, currently available PCR assays for feline herpesvirus-1 and feline calicivirus also amplify modified live vaccine strains, so a positive result obtained from a nasal or pharyngeal swab does not definitively indicate the presence of a pathogenic strain. Real-time PCR can be used to determine the amount of microbial DNA in a sample, and it is possible that the DNA load will correlate to the presence of disease for some agents. However, some agents, like *Bartonella henselae,* are very host adapted, and some cats and dogs have many DNA copies of the agent in their blood without detectable clinical disease.

Based on these facts, it is my opinion that most PCR assays—like antibody assays—are not infectious disease tests. Rather, they are infectious "agent" assays that give results a veterinarian can use to diagnose an infectious disease. I believe it is very important that to properly use PCR assays, small animal practitioners must carefully assess the PPV and NPV of the currently available tests and the expertise and reliability of the laboratory that will be conducting the assays.

The clinical diagnosis of an infectious disease should include the following: (1) clinical signs referable to the agent; (2) positive culture, antigen assay, or PCR assay results or serologic evidence of exposure to the agent; exclusion of other causes of the clinical syndrome; and (4) response to an appropriate treatment. When these criteria are met, the suspected infectious agent might be the cause of the clinical disease.

Advancements in veterinary medicine lead to better diagnostics all the time; however, using tests properly and incorporating them into sound clinical judgment remain the "art" of medicine. PCR is a valuable tool, not an infallible indicator.