Infectious Disease Diagnostic Assays

Michael R. Lappin, DVM, PhD, DACVIM (Internal Medicine)

Dogs and cats are commonly exposed to infectious disease agents. The following article is an update on the most common infectious disease assays used in small animal practice. In some situations, results of pathogen-specific molecular diagnostic assays can be used to assist the practicing veterinarian in the management of patients with infectious diseases. However, with some infectious disease agents, the assays are positive in healthy and ill animals and can be falsely negative and so the predictive values of the assays vary.
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Infectious disease agents capable of inducing clinical illness are common in dogs and cats around the world. A combination of signalment, history, and physical examination findings are used to develop a list of differential diagnoses, ranking the most likely infectious agents involved. For example, young, unvaccinated puppies with bloody diarrhea and neutropenia are generally infected by canine parvovirus. Kittens with conjunctivitis and a dendritic ulcer are usually infected by feline herpesvirus 1 (FHV-1). Results of a complete blood cell count, serum biochemical panel, urinalysis, radiographs, or ultrasonography can also suggest infectious diseases. For example, an outdoor dog with acute onset of clinical illness with renal azotemia, pyuria but no bacteriuria, and normal kidneys on radiographs likely has leptospirosis. After the tentative diagnosis is made, the clinician and owner decide whether to treat empirically or perform further diagnostic tests to confirm the diagnosis. When possible, gathering information via diagnostic test results is preferred, so that treatment, prevention, prognosis, and zoonotic issues can be addressed optimally.

There are 2 major groups of infectious disease assays: assays that confirm the continued presence of the organism (cytology, culture, fecal examination techniques, antigen assays, immunohistochemical stains, molecular diagnostic assays), and antibody assays. Documenting that an infectious agent is still present is generally the best way to make a definitive diagnosis. However, certain organism demonstra-

tion techniques have low sensitivity, are expensive, are invasive, are not adequately validated, or require specialized equipment. Antibody detection assays are commonly used to aid in the diagnosis of specific infectious diseases in these situations. However, when used alone, antibody detection is generally inferior to organism demonstration for several reasons:

- antibodies can persist long after an infectious agent has been eliminated;
- positive antibody assay results do not confirm that the clinical disease was induced by the infectious agent;
- results of serum antibody assays can be negative in peracute infections if humoral immune responses have not had time to develop; and
- some immune-compromised animals may not be able to mount a humoral immune response.

In some situations, the use of both organism demonstration techniques and antibody assays concurrently (ie, ehrlichiosis in dogs) may give the most useful information using a single clinical sample.

Sensitivity is the ability of an assay to detect a positive sample, and specificity is the ability of an assay to detect a negative sample. Positive predictive value (PPV) is the ability of an assay result to predict presence of disease; negative predictive value (NPV) is the ability of an assay result to predict absence of disease. Sensitivity, specificity, PPV, and NPV vary with each test and infectious agent and must be factored into the interpretation of infectious disease assay results.[1]

Organism Demonstration Assays

Cytology

There are cytologic findings associated with many bacterial, rickettsial, fungal, and parasitic organisms of dogs and cats that may lead to a presumptive or definitive diagnosis. Cytologic evaluation of exudates, bone marrow aspirates, blood smears, synovial fluid, gastric brushings, duodenal secretions, urine, prostatic washings, airway washings, fecal

smears, tissue imprints, skin scrapings, and aspiration biopsies is inexpensive and is considered a primary diagnostic procedure for many clinical infectious diseases. Stains routinely used for the diagnosis of infectious agents in small animal practice include Wright's-Giemsa, Diff-Quik, acid-fast, and Gram stains. The laboratory should be contacted for specific specimen handling information based on the suspected organism.

Cytological findings can be used to achieve a definitive diagnosis for some infectious agents. For example, identification of *Cryptococcus* spp organisms on cytological evaluation of a lymph node aspirate from a cat with rhinitis confirms the diagnosis. Detection of a hemoplasma on cytological examination of a thin blood smear from an anemic cat or dog confirms the diagnosis. Polymerase chain reaction (PCR) assays can then be used to determine whether the organism is *Mycoplasma hemofelis*, *M. hemocanis*, or another one of the agents in the genus.

Some infectious agents can be present in small numbers, so failure to document an organism cytologically does not totally exclude the diagnosis (low NPV). *Ehrlichia* spp infections are examples of common infectious agents rarely documented cytologically. These agents generally require results of serology and PCR to confirm exposure or current infection. Some organisms like respiratory *Mycoplasma* spp are rarely documented cytologically because of their small size, whereas other organisms require special stains for optimal visualization. For example, *Mycobacterium* spp are not readily stained by rapid stains used in small animal practices, and if macrophages or neutrophils are detected, acid-fast staining is indicated to assess for the organisms within the cytoplasm of infected cells. Bacterial culture of all samples with increased numbers of neutrophils or macrophages should always be considered.

Culture Techniques

Bacteria, fungi, viruses, and some protozoans can be cultured. In general, a positive culture can be used to establish a definitive diagnosis, particularly if there is clinical illness and concurrent evidence of inflammation. If the organism is easy to grow (primarily bacteria), culture is preferred over cytology or molecular diagnostic assays because culture may be combined with antimicrobial susceptibility testing to determine optimal antibiotic therapy. Successful culture is dependent on the collection of optimal materials without contamination and on transportation of materials in the most appropriate culture medium to the laboratory as quickly as possible to minimize organism death and nonpathogen overgrowth.

Culture results of body systems with normal bacterial and fungal flora, including the skin, ears, mouth, nasal cavity, trachea, feces, and vagina, are the most difficult to interpret. Positive-culture results coupled with cytological evidence of inflammation suggest the organism is inducing disease. Culture of a single agent, particularly if the organism is relatively resistant to antimicrobials, is more consistent with a disease-inducing infection than if multiple, antibiotic-susceptible bacteria are cultured.

For some organisms, culture is difficult or has never been accomplished. For example, the hemoplasmas of dogs and cats (previously *Hemobartonella felis* and *H. canis*) can be detected on the surface of red blood cells but have never been successfully cultured. For other organisms, culture takes several weeks to become positive (ie, *Bartonella* spp), or the techniques are cumbersome and not so widely available (ie, *Ehrlichia* spp). In these situations, molecular diagnostic assays may be the optimal way to prove current infection.

Fecal Examination

Examination of feces can be used to identify bacteria, fungi, and parasites that can be associated with diseases of the gastrointestinal (Table 1) and respiratory (Table 2) tracts. The techniques used most frequently include direct, saline solution, and stained smears, fecal flotation, and the Baermann technique. The American Association of Feline Practitioners (www.catvets.com) recommends performing at least a fecal flotation, fecal or rectal cytology, and direct smear on feces from cats with potentially infectious diarrhea.[2] These assays can easily be performed in the veterinary clinic. The Companion Animal Parasite Council is an excellent source of information concerning these assays and the parasites that infest dog and cats (www.capcvet.org).

Immunologic Techniques

Infectious agents or their antigens can be detected in body fluids, feces, cells, or tissues with immunologic techniques. In general, polyclonal or monoclonal antibodies against the agent in question are used in a variety of different test methodologies, including direct fluorescent antibody assay with cells or tissue, agglutination assays, and enzyme-linked immunosorbent assay (ELISA). The sensitivity and specificity will vary among tests but are generally high for most assays. The NPV of most assays is high if performed on appropriate samples (ie, pretreatment). Positive results with these tests generally confirm infection; however, the PPV for disease varies by the agent and the assay. For example, many normal dogs and cats are positive for *Giardia* antigen in feces, and so a positive assay result in an animal with diarrhea does not prove disease causation.

In the United States, commercially available antigen assays used most frequently for the detection of antigens in serum or plasma include *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Dirofilaria immitis*, and feline leukemia virus (FeLV). The *Cryptococcus neoformans* latex agglutination procedure can also be performed on aqueous humor, vitreous humor, and cerebrospinal fluid (CSF). *Cryptosporidium parvum* antigen, *Giardia* spp antigen, parvovirus antigen, and *Clostridium perfringens* and *C. difficile* enterotoxin assays are available for use with feces. Parvovirus assays detect both canine and feline parvovirus antigen and may be positive transiently after administration of modified live vaccines.[3,4] Most *Giardia* antigen tests marketed for use with human

Table 1. Demonstration Techniques for Canine and Feline Gastrointestinal Parasites

Organism	Form in Stool	Species Infected	Optimal Fecal Examination Technique
Cestodes			
Dipylidium caninum	Egg	B	Identification of adult
Echinococcus granulosa	Egg	D	Zinc sulfate centrifugation; other flotations
Echinococcus multilocularis	Egg	B	Zinc sulfate centrifugation; other flotations
Taenia spp	Egg	B	Identification of adult
Protozoans			
Balantidium coli	Trophozoite	B	Direct or saline smear
	Cyst	D	Zinc sulfate centrifugation; other flotations
Cryptosporidium parvum	Oocyst	B	Acid-fast or monoclonal antibody stain
Entamoeba histolytica	Trophozoite	B	Direct or saline solution smear
	Cyst	D	Zinc sulfate centrifugation; other flotations
Giardia spp	Trophozoite	B	Direct or saline solution smear
	Cyst	B	Zinc sulfate centrifugation; other flotations
Cystoisospora spp	Oocyst	B	Sugar or zinc sulfate centrifugation
Tritrichomonas foetus	Trophozoite	B	Direct or saline solution smear
	Cyst	D	Zinc sulfate centrifugation; other flotations
Toxoplasma gondii	Oocyst	B	Sugar or zinc sulfate centrifugation
Flukes			
Eurytrema procyonis	Egg	C	Fecal sedimentation
Nanophyetus salmincola	Egg	D	Fecal sedimentation
Platynosonum fastosum	Egg	C	Fecal sedimentation
Helminths			
Ancylostoma spp	Egg	B	Zinc sulfate centrifugation; other flotations
Ollulanus tricuspis	Egg	C	Zinc sulfate centrifugation; other flotations
Physaloptera spp	Egg	B	Zinc sulfate centrifugation; other flotations
Spirocerca lupi	Egg	D	Zinc sulfate centrifugation; other flotations
Strongyloides stercoralis	Larvae	B	Baermann technique
Toxocara spp	Egg	B	Zinc sulfate centrifugation; other flotations
Toxascaris spp	Egg	B	Zinc sulfate centrifugation; other flotations
Trichuris vulpis	Egg	D	Zinc sulfate centrifugation; other flotations
Uncinaria stenocephala	Egg	B	Zinc sulfate centrifugation; other flotations

Abbreviations: D, dog; C, cat; B, dog and cat.

feces and the test labeled for use with dog or cat feces detect the *Giardia* assemblages that infect dogs or cats.[5] Occasionally, samples will be antigen-positive but cyst-negative on fecal flotation. In this situation, it is unknown whether the antigen test is falsely positive or the fecal flotation is falsely negative. None of the currently available *C. parvum* antigen tests marketed for use with human feces consistently detects *C. felis* or *C. canis* and so should not be used with feces from dogs and cats. However, a direct fluorescent antibody assay for human cryptosporidiosis and giardiasis detects dog and cat strains and is available at most commercial laboratories. *Clostridium* spp enterotoxins can be detected in the

Table 2. Demonstration Techniques for Common Canine and Feline Respiratory Tract Parasites

Organism	Form in Stool	Species Infected	Optimal Fecal Examination Technique
Aelurostrongylus abstrusus (lungworm)	Larva	C	Baermann technique
Andersonstrongylus milksi (lungworm)	Larva	D	Baermann technique
Eucoleus aerophila	Egg	D	Zinc sulate or other flotation
Crenosoma vulpis (lungworm)	Egg	D	Zinc sulfate or other flotation
Eucoleus bohemi (nasal worm)	Egg	D	Zinc sulfate or other flotation
Filaroides hirthi (lungworm)	Larva	D	Baermann technique
Oslerus osleri (tracheal nodular worm)	Egg or larva	D	Zinc sulfate or other flotation and Baermann technique
Paragonimus kellicotti (lung fluke)	Egg	B	Fecal sedimentation
Pneumonyssoides caninum (nasal mite)	None	D	None, visualization of adults

Abbreviations: D, dog; C, cat; B, dog and cat.

stool of healthy dogs and dogs with diarrhea, and so the PPV is <100%.[6]

Immunocytochemical and immunohistochemical techniques are commercially available for the documentation of a variety of infectious disease agents. These procedures are particularly valuable for the detection of viral diseases, detection of agents present in small numbers, and differentiation among agents with similar morphologic features. Tissues collected from animals with suspected infectious diseases can be evaluated by several different techniques. For example, focal feline infectious peritonitis granulomatous disease can be documented by immunohistochemical staining.[7] Tissue samples should be aseptically placed in appropriate transport media for culture procedures or inoculated into laboratory animals, if indicated, before further handling. Gently blotting the cut edge of the tissue on a paper towel to remove excess blood and then lightly touching the tissue multiple times to a microscope slide make tissue impressions for cytological or immunocytological examination. Tissue specimens can then be frozen, placed into 10% buffered formalin solution, or placed into glutaraldehyde-containing solutions. Frozen specimens are generally superior for immunohistochemical staining and PCR. Routine histopathologic evaluation is performed on formalin-fixed tissues. Special stains can be used to maximize the identification of some infectious agents. The clinician should alert the histopathology laboratory to the infectious agents most suspected to allow for appropriate stain selection. Glutaraldehyde-containing fixatives are superior to other fixatives for electron microscopic examination of tissues, and this technique can be more sensitive than other procedures for demonstration of viral particles.

Molecular Diagnostic Assays

Assays to amplify DNA and RNA of infectious agents are among the newest organism demonstration techniques now commonly available to veterinary practitioners. PCR assays are used to amplify small quantities of DNA to detectable levels. By use of a reverse transcriptase (RT) step, RNA is converted to DNA, therefore the technique can also be used to detect RNA (RT-PCR). In general, PCR is more sensitive than cytologic or histopathologic techniques and is comparable with culture and laboratory animal inoculation. PCR assays are of great benefit for documentation of infections, particularly if the organism in question is difficult to culture (eg, respiratory *Mycoplasma* spp) or cannot be cultured (eg, hemoplasmas). Specificity can be very high, depending on the primers used in the reaction. For example, primers can be designed to detect one genus but not others. Primers can also be designed to identify only one species. For example, a PCR assay can be developed to detect all *Ehrlichia* spp or just one species, such as *E. canis*.

Because of the inherent sensitivity of the reaction, PCR can give false-positive results if sample contamination occurs during collection or at the laboratory performing the procedure. False-negative results can occur if the sample is handled inappropriately; this is of particular importance for detection of some RNA viruses by RT-PCR. Results may also be affected by treatment, and it is therefore best to collect samples before administration of antimicrobial agents. Another potential problem is that minimal standardization exists among commercial laboratories offering PCR techniques, and results may therefore vary between laboratories. In addition, minimal external quality control exists and so the veterinary practitioner must trust that the laboratory providing the assay is using appropriate controls.

Although PCR assays can be one of the most sensitive for documentation of infections, positive test results do not always prove that the infection is resulting in clinical illness (low PPV). For example, because the technique detects DNA of both live and dead organisms, positive test results may be achieved even if the infection has been controlled. Interpretation of test results for a single animal can be difficult if the targeted organism commonly infects the background population of healthy animals. For example, "*Candidatus* Mycoplasma haemominutum" DNA can be amplified from approximately 15% of all cats, whether anemic or healthy.[8,9] Thus, although PCR is the most sensitive way to document infection by this agent, the PPV is actually very low. In addition, currently available PCR assays cannot discriminate between vaccine and field strains for some agents. For example, currently available PCR assays for FHV-1 also amplify modified live vaccine strains, so a positive result does not even indicate presence of a pathogenic strain.[10] Real-time PCR can be used to determine the amount of microbial DNA in a sample. It is possible that the DNA load will correlate to the presence of disease for some agents. Based on these findings, it is very important that small animal practitioners carefully assess the predictive values of currently available PCR assays as well as the expertise and reliability of the laboratory that will be performing the assays. New PCR assays are being developed frequently, but may not be required or optimal except for many canine and feline infectious disease agents. The clinical use of commonly available PCR assays will be discussed throughout this article.

Antibody Detection
Serum

A variety of different methods exist for detecting serum antibodies against infectious agents, including complement fixation, hemagglutination inhibition, serum neutralization, agglutination, agar gel immunodiffusion, indirect fluorescent antibody (IFA), ELISA, and Western blot assays. Complement fixation, hemagglutination inhibition, serum neutralization, and agglutination assays generally detect all antibody classes in a serum sample. Western blot immunoassay, IFA, and ELISA can be adapted to detect specific immunoglobulin (Ig) M, IgG, or IgA responses.

Comparison of IgM, IgA, and IgG antibody responses against an infectious agent can be used to attempt to demonstrate recent or active infection. In general, IgM is the first

antibody produced after antigenic exposure, with antibody class shift to IgG occurring in days to weeks. Serum and mucosal IgA immune responses have also been studied for some infectious agents, including *Toxoplasma gondii*, feline coronaviruses, and *Helicobacter felis.*

Timing of antibody testing is important. In general, serum antibody tests in puppies and kittens cannot be interpreted as specific responses until at least 8 to 12 weeks of age because of the presence of antibodies from the dam passed to the puppy or kitten in the colostrum. Most infectious agents can induce disease within 3 to 10 days after initial exposure; with the use of many assays, serum IgG antibodies are usually not detected until 1 to 2 weeks after initial exposure. Based on these facts, falsely negative serum antibody tests during acute disease can be common in small animal practice. If specific serum antibody testing is initially negative in an animal with acute disease, repeat antibody testing should be performed in 2 to 3 weeks to assess for seroconversion. Documentation of increasing antibody titers is consistent with recent or active infection. It is preferable to assess both the acute and conva-lescent sera in the same assay on the same day to avoid interassay variation.

Diagnostic use of some serologic tests are also limited be-cause of the presence of antibodies induced by vaccination. Examples include feline coronaviruses, some *Borrelia burg-dorferi* assays, FHV-1, parvoviruses, calicivirus, and canine distemper virus.

The clinician should interpret positive results in serum an-tibody tests only as evidence of present or prior infection by the agent in question. Recent or active infection is suggested by the presence of IgM, an increasing antibody titer over 2 to 3 weeks, or seroconversion (negative antibody result on the first test and positive antibody result on convalescent testing). However, detection of recent infection based on antibody testing does not always prove disease because of the agent in question. Conversely, failure to document recent or active infection based on serologic testing does not exclude a diag-nosis of clinical disease. For example, many cats with toxo-plasmosis develop clinical signs of disease after serum anti-body titers have reached their plateau. The magnitude of antibody titer does not always correlate with active or clini-cal disease; for example, many cats with clinical toxoplasmo-sis have IgM and IgG titers that are at the low end of the titer scale. Conversely, many healthy cats have IgG titers greater than 1:16,384 years after infection with *Toxoplasma gondii.*

Body Fluids

Some infectious agents induce disease of the eyes or central nervous system (CNS). Documentation of agent-specific an-tibodies in aqueous humor, vitreous humor, or CSF can be used to support the diagnosis of infection of these tissues. Quantification of ocular and CSF antibodies is difficult to interpret if serum antibodies and inflammatory disease are present; serum antibodies leak into ocular fluids and CSF in the face of inflammation. Detection of local production of antibodies within the eye or CNS has been used to aid in a

number of infectious agents including canine distemper virus infection, feline toxoplasmosis, and feline bartonellosis.[11,12]

Conclusions

In summary, there are organism demonstration techniques or antibody assays available for many infectious disease agents in pets with variable predictive values. Results of infectious agent assays should not be interpreted alone, but should be combined with other parameters to document a clinical in-fectious disease, including:
- appropriate signalment, history, and physical examination findings;
- clinical signs referable to the agent;
- positive cytology, culture, antigen assay, PCR assay or se-rologic evidence of exposure to the agent;
- exclusion of other causes of the clinical syndrome; and
- response to treatment.

The infectious disease agent in question is the likely cause of the clinical syndrome when these criteria are met. How-ever, it is possible that the clinical syndrome may have spon-taneously resolved during the treatment period. In addition, many antimicrobial drugs have other nonspecific effects. For example, doxycycline has antiinflammatory properties, and so some apparent clinical responses may not have been from the antibiotic activity.[13]

The purpose of the remaining part of the article is to use several common infectious disease agents to emphasize im-portant points concerning use of molecular diagnostic assays in practice.

Use of Molecular Diagnostic Assays for Common Infectious Disease Agents in Small Animal Practice

Blood-borne Agents

The new names for *Hemobartonella felis* are *Mycoplasma hemofelis*, "*Candidatus* Mycoplasma haemominutum," and "*Candidatus* M. turicensis." The new names for *H. canis* are *M. hemocanis* and "*Candidatus* M. hematoparvum." In cats, *M. hemofelis* is apparently more pathogenic than "*Candida-tus* M. haemominutum" and "*Candidatus* M. turicensis." However, fever and anemia have been documented in all 3 species. In dogs, the hemoplasmas are generally only associ-ated with disease if the dog is immunosuppressed. Diagnosis of hemoplasma infections in dogs and cats is based on dem-onstration of the organism on the surface of erythrocytes on examination of a thin blood film or PCR assay. Organism numbers fluctuate, and therefore blood film examination can be falsely negative >50% of the time.[8] The organism may be difficult to find cytologically, particularly in the chronic phase. Thus, PCR assays are the tests of choice because of sensitivity, particularly if the cytological examination is neg-ative. Both conventional and quantitative PCR assays are available and have similar sensitivity, specificity, and predic-tive values. Unfortunately, DNA copy numbers do not cor-

relate to the presence of disease for the hemoplasmas. However, quantitative PCR assays can be used to monitor DNA copy numbers during antimicrobial drug research studies. Samples should be obtained for PCR assays before antibiotics. Treatment with doxycycline or a fluoroquinolone may be indicated if any of the agents are detected by PCR in cats or dogs with fever or anemia. Additionally, the American College of Veterinary Internal Medicine recommends screening blood donor dogs or cats by hemoplasma PCR assays.[14] There is minimal clinical utility after hemoplasma PCR assay results because most animals do not become negative with treatment.

Dogs in the United States are known to be infected by *Ehrlichia canis*, *E. ewingii*, *E. chaffeensis*, *Anaplasma phagocytophilum*, *A. platys*, and *Neorickettsia risticii*.[15] Each of these agents can result in fever and cytopenias, and the clinical syndrome is collectively known as ehrlichiosis. The primary agent in the United States is *E. canis* because of the distribution of *Rhipicephalus sanguineus*, whereas the prevalence of the other agents varies geographically. Cats may be infected by *E. canis*-like organisms as well as by *A. phagocytophilum*.[16,17] Little is known about the other agents in these genera in regards to cats; because the organisms are in different genera, serological cross reactivity is variable. Thus, although the clinical syndromes can be similar, there is no single serological test to document all the infections, and, other than the commercially available kit, there is no standardization among laboratories offering serology. In addition, some cats with *E. canis*-like infection do not seroconvert.[16] Dogs and cats with ehrlichiosis can be clinically ill before seroconversion. Cytological assessment of blood smears is usually negative, and culture is expensive, has poor sensitivity, and is not readily available. Thus, PCR assays for *Ehrlichia* spp, *Anaplasma* spp, and perhaps *N. risticii* (atypical ehrlichiosis in dogs) should be performed on blood of dogs and cats with acute fever or cytopenias before treatment. PCR assays can be designed to amplify each individual organism and can be offered in panels. Alternately, primers are available to amplify all of the organisms in a single reaction, and subsequent sequencing can be used to determine the infective species. The predictive value of these PCR assays is currently unknown. However, it seems prudent to treat a clinically ill, PCR-positive dog or cat. Dogs with ehrlichiosis can be followed by PCR to prove cure; however, infection can reoccur, and so tick control should be maintained.[15] Minimal information concerning the clinical use of repeated PCR testing of cats with these infections is available. Blood donors should be screened for *Ehrlichia/Anaplasma/Neorickettsia* and, if positive, excluded from the program even if treated because of the difficulty in sterilizing the blood.[14]

Some dogs become clinically ill after exposure to *Rickettsia rickettsii*, and there are an additional 4 spotted fever group organisms in the United States that induce cross-reacting antibodies. Therefore, antibodies against *R. rickettsii* do not prove Rocky Mountain spotted fever. Cats can be infected by *R. felis* and have demonstrated antibodies against *R. rickettsii*. We assayed 92 pairs of cat blood and flea extracts from Alabama,

Maryland, and Texas in a recent study in our laboratory; we used PCR assays that amplify a region of the citrate synthase gene and the outer membrane protein B gene.[18] Of the 92 pairs, 62 of 92 (67.4%) flea extracts and none of the cat blood samples were positive for *R. felis* DNA. In another study, we identified *R. felis* and *R. rickettsii* antibody prevalence rates in cats with fever to be 5.6% and 6.6%, respectively; however, neither organism was amplified from blood.[19] These results proved that cats were sometimes exposed, but further data are required to determine the significance of disease associations. *Rickettsia* spp DNA has been amplified from healthy dogs, but the infective species are currently unknown.[20] The predictive values of *Rickettsia* spp PCR assays in clinical syndromes in dogs and cats have not been established.

Blood culture, PCR assay on blood, and serologic testing can be used to assess dogs or cats for *Bartonella* spp infection.[21] *Bartonella vinsonii* and *B. henselae* infections are most common in dogs, whereas *B. henselae* and *B. clarridgeiae* are most common in cats. There are several other less common species that infect dogs and cats, and not all of the species induce cross-reacting antibodies. Thus, as for the *Ehrlichia/Anaplasma/Neorickettsia* group, PCR assays that amplify multiple *Bartonella* species can be superior to serology if collected before treatment. However, a combination of culture and PCR may be required to detect some infections in dogs.[22] Serologic testing can be used to determine whether an individual dog or cat has been exposed; however, both seropositive and seronegative animals can be bacteremic, limiting the diagnostic use of serologic testing. Thus, testing healthy cats or dogs for *Bartonella* species infection is not currently recommended and should be reserved for those with suspected clinical bartonellosis. However, because *Bartonella* spp infection is so common in healthy animals, even positive-culture or PCR results do not prove clinical bartonellosis. For example, although we detected *Bartonella* spp DNA in more cats with fever than pair-matched cats without fever, the healthy cats were still commonly positive.[23] Treatment usually does not permanently eliminate infection, and so there appears to be minimum clinical benefit to repeating PCR assays after successful treatment.

Cytauxzoon felis in clinically affected cats is usually easily identified on cytological examination of blood smears or splenic aspirates. Serologic testing is not commercially available. PCR can be used to amplify organismal DNA from blood or splenic aspirates from cytologically negative cats.[24] Nonpathogenic *C. felis* strains can be detected in the blood of healthy cats, and therefore the PPV of PCR assays is less than 100%. However, some strains of the organism are extremely pathogenic, and so it seems prudent to institute treatment in clinically ill, positive cats. Whether there is clinical benefit to repeating PCR assays after successful treatment is unknown.

Babesia canis and *B. gibsoni* infections occur in dogs of the United States; there are no species that infect cats in this country. Serological assays are available for both organisms, but positive test results do not prove anemia from *Babesia* spp infection. PCR assays are now commercially available for

both organisms. These assays should be considered in high-risk dogs with hemolytic anemia-like greyhounds, pit bull terriers, or dogs exposed to pit bull terriers.[25] The predictive values of *Babesia* spp PCR assays are unknown, but it seems prudent to institute treatment in clinically ill, positive dogs. It is unknown whether there are clinical benefits to repeating PCR assays after successful treatment.

Antibodies against feline immunodeficiency virus (FIV) are detected in serum in clinical practice most frequently by ELISA. Comparisons between different tests have shown the results of most assays are comparable.[26] It is believed that infection is incurable once established, thus presence of serum antibodies in unvaccinated cats indicates persistent infection. Clinical signs can occur before seroconversion in some cats, and some infected cats never seroconvert, thus false-negative reactions can occur. Results of virus isolation or RT-PCR on blood are positive in some antibody-negative cats. Virus isolation or RT-PCR on blood can also be performed to confirm infection. However, FIV is not present in the blood in high levels, and so false-negative results are common.[27] Thus, the assay is not very accurate for distinguishing a vaccinated cat from a naturally exposed cat.

Most cats with FeLV infection are antigenemic, and therefore molecular diagnostic assays are not usually necessary in clinical practice. However, the use of newer, sensitive RT-PCR assays have been used to accurately characterize the stages of infection, although these assays do not have widespread commercial availability.[28]

RNA of both feline infectious peritonitis (FIP) virus and feline enteric coronavirus (FECV) can be amplified from the blood and feces of cats, and so positive test results do not always correlate with the development of FIP. Amplification of the mRNA of the M gene by RT-PCR has had mixed results in 2 studies performed to date. A study demonstrated that 13 of 26 apparently normal cats were positive for FECV mRNA in blood, suggesting that the PPV of this assay for the diagnosis of FIP was low.[29]

Gastrointestinal Infectious Agents

The primary bacteria associated with gastrointestinal tract disease in cats and dogs include *Salmonella* spp, *Campylobacter* spp, *Clostridium* spp, and *Helicobacter* spp. The optimal diagnostic test for *Salmonella* spp and *Campylobacter* spp is culture with antimicrobial susceptibility testing rather than PCR. Although little information is available in cats, it appears in dogs that it is optimal to perform a combination of both PCR and enterotoxin assays on feces from those suspected to have *C. perfringens* or *C. difficile* infections.[30] However, some healthy dogs can also be positive in all assays, and therefore the PPV is not 100%. *Helicobacter* spp infection is suspected on cytological or histopathological detection of gastric spiral organisms and urease-positive test results on tissue biopsies. PCR assays can be used to document the presence of *Helicobacter* spp DNA in tissues. However, *Helicobacter* spp can be detected in the tissues of many healthy dogs and cats, and therefore the PPV

of assays for this organism group is low.[31] Treatment usually does not permanently eliminate gastrointestinal bacterial infections, resulting in minimum clinical benefit to repeating PCR assays after successful treatment.

The most common enteric protozoans in dogs and cats include *Cryptosporidium* spp, *Giardia* spp, *Isospora* spp, and *Tritrichomonas fetus*. Although infection is common, it is unusual to find *C. felis* or *C. canis* after fecal flotation in cats because of the small size of the oocysts. Acid-fast staining of a thin fecal smear is cumbersome and insensitive, and antigen assays that are titrated for use with human feces are inaccurate when used with canine or feline feces. Immunofluorescent antibody staining of a thin fecal smear with a commercially available kit for the detection of *C. parvum* and *G. lamblia* in people appears to detect most *Cryptosporidium* spp and *Giardia* spp in dog and cat feces. This assay is commonly combined with fecal flotation by the author for the initial screening of cats with small bowel diarrhea and dogs with unexplained small bowel diarrhea that persists after routine therapeutic trials. PCR assays have been shown to be more sensitive than IFA for detection of *C. felis* in cats, but little is known about sensitivity in the dog.[32] However, because of the inherent sensitivity, PCR assays are likely to detect a number of subclinical carriers, and so the PPV will be less than 100%. *Cryptosporidium felis* and *C. canis* are not considered to be significant zoonotic agents, therefore positive PCR assay results are only potentially significant when detected in feces of dogs or cats with diarrhea.[33] *Cryptosporidium* spp PCR assays are indicated in IFA-negative dogs or cats with unexplained small bowel diarrhea and when the genotype of *Cryptosporidium* is to be determined. Treatment usually does not permanently eliminate infection, and so there appears to be minimum clinical benefit to repeating PCR assays after successful treatment.

The diagnosis of *Giardia* spp infection is easy to achieve in dogs or cats with small bowel diarrhea with the combination of fecal flotation techniques and wet mount examination. Fecal antigen assays and the *Giardia* IFA are also accurate. Fecal PCR assays can be inaccurate because of the presence of PCR inhibitors and should only be used when the genotype of *Giardia* is to be determined.[34] Treatment usually does not permanently eliminate *Giardia* infection, and reinfection can occur within days. Thus, there appears to be no clinical benefit in testing healthy animals or repeating PCR assays after resolution of diarrhea.

Some kittens and puppies with large bowel diarrhea are infected by *Toxoplasma fetus* (rare). Most clinically affected animals have cytological evidence of infection on wet mount examination. In addition, the organism is readily cultured from feces. However, PCR assays can be more sensitive than culture, and results return more quickly. Subclinical carriers are common, and therefore amplification of *T. fetus* DNA from feces does not always prove that the agent is the cause of the clinical disease (poor PPV).[35] Treatment may not eliminate infection, and reinfection is common. Thus, there appears to be little clinical benefit in testing healthy animals or repeating PCR assays after resolution of diarrhea.

The primary viral agents associated with gastrointestinal disease in cats include feline coronaviruses, feline panleukopenia virus, FeLV, and FIV. In dogs, canine coronavirus, canine distemper virus, and canine parvoviruses are most common. FeLV and FIV assays are generally performed on blood, not feces. Coronaviruses in dog and cat feces can be documented by electron microscopy, virus isolation, or molecular assays. RT-PCR can be used to detect FECV and FIPV RNA in feces of cats and canine coronavirus in feces of dogs. However, positive results do not prove feline infectious peritonitis in cats but merely the presence of a coronavirus.[36] In addition, RT-PCR assay results cannot distinguish a cat or dog that is merely colonized with a coronavirus from one with diarrhea induced by a coronavirus. Enteric coronaviruses are rarely a problem in puppies and kittens with good husbandry and do not cause diarrhea in adults.[6] Thus, there appears to be no reason to routinely perform coronavirus RT-PCR assay on the feces from a dog or cat. Feline panleukopenia virus and canine parvoviruses can be detected in dog and cat feces by electron microscopy, virus isolation, canine parvovirus antigen assays, and molecular diagnostic assays. There appears to be no reason to routinely perform parvovirus PCR assays on canine and feline feces because fecal antigen assays are widely available, easy to use, and detect current strains of canine and feline parvoviruses.[4] Other organisms like astroviruses and reoviruses may infect some animals, but these agents have rarely been associated with disease. Thus, whether specific diagnostic assays for these agents are needed is unknown.

Respiratory Infectious Agents

Of the bacteria that have been associated with respiratory disease in dogs and cats, PCR assays are now routinely available for *Bordetella bronchiseptica*, *Mycoplasma* spp, *Streptococcus zooepidemicus* and *Chlamydophila felis*. Although *B. bronchiseptica* is a well-defined primary pathogen in dogs, the organism can be isolated from many clinically normal cats.[37] Thus, the PPV of culture and PCR assay are low in cats. *Bordetella bronchiseptica* is easily grown, and culture is superior to PCR for this agent because antimicrobial susceptibility testing can be performed on isolates. The organism is not usually eliminated by treatment, and therefore posttherapeutic follow-up culture or PCR assay has minimal benefits. *Chlamydophila felis* is a common differential diagnosis for cats with clinical evidence of conjunctivitis and rhinitis, although it is not a common cause of lower airway disease. The organism is difficult to culture, and so detection of microbial DNA by PCR assays can be useful clinically. However, not all PCR-positive cats are clinically ill, and so the PPV can be low. PCR assays results can be used to prove a cattery has been cleared of the infection after treatment.[38] *Mycoplasma* spp organisms are normal commensal organisms of the mucous membranes of multiple species including dogs and cats. *Mycoplasma felis* has been associated primarily with conjunctivitis in cats but is suspected as a primary cause of rhinitis in cats as well. There are multiple *Mycoplasma* spp of dogs and

cats, and the pathogenic potential for most is unknown. If other primary diseases are present, even nonpathogenic *Mycoplasma* spp may be associated with the disease process.[39,40] *Mycoplasma* spp culture can be difficult, and antimicrobial susceptibility is not provided by most laboratories. Therefore, *Mycoplasma* spp PCR assays have at least moderate clinical utility. Genus-specific primers should probably be used because the pathogenic potential of all *Mycoplasma* spp is not known. However, the PPV of the assays is likely to be low because *Mycoplasma* spp are common flora. The organism is not usually eliminated by treatment, and so posttherapeutic follow-up culture or PCR assay has minimal benefit.

The most common viruses associated with feline respiratory disease are feline calicivirus (FCV) and feline herpesvirus 1 (FHV-1).[40] Both viruses are extremely common in cats, particularly those from crowded environments like pet stores, catteries, and shelters. Adenovirus 2, parainfluenza, influenza, respiratory coronavirus, canine distemper virus, and canine herpesvirus have the most potential to induce respiratory disease in dogs. There are many FCV, and mutations resulting in new strains are common.[41] This organism is a common differential diagnosis for cats with clinical evidence of rhinitis, stomatitis, and conjunctivitis. FCV is less commonly associated with polyarthritis, lower airway disease in kittens, and virulent systemic disease.[42] Virus isolation can be used to document current infection but takes at least several days for results to return and is not performed by all laboratories. Because of widespread exposure and vaccination, the PPV of serological tests is poor. RT-PCR assays can be used to amplify calicivirus RNA with quick and rapid return of results. However, these assays also amplify vaccine strains of FCV. Accordingly, FCV RNA can be amplified from samples collected from normal carrier cats as well as clinically ill cats, and therefore FCV RT-PCR assays may have poor PPV. For example, the presence of FCV RNA failed to correlate to the presence or absence of stomatitis in cats in a study from our laboratory.[43] In addition, amplification of FCV RNA cannot be used to prove virulent systemic calicivirus infection. Results of FCV RT-PCR can also be falsely negative, and so assays may have poor NPV. Treatment does not eliminate FCV infection, and so there is no benefit to follow-up culture or RT-PCR testing.

FHV-1 is a common differential diagnosis for cats with clinical evidence of rhinitis, stomatitis, conjunctivitis, keratitis, and facial dermatitis. The PPV of serological tests is poor because of widespread exposure and vaccination. FHV-1 infection can be documented by direct fluorescent staining of conjunctival scrapings, virus isolation, or PCR. FHV-1 DNA can be amplified from conjunctival cells of approximately 25% of healthy cats, and therefore the PPV of PCR assay for this agent is low.[44] Currently used PCR assays also detect vaccine strains of FHV-1, further lessening the PPV.[10] In one study in the author's laboratory, presence of FHV-1 DNA failed to correlate to the presence or absence of stomatitis in cats.[43] Quantitative PCR may ultimately prove to correlate to the presence or absence of disease but failed to correlate to presence of conjunctivitis in one study in the author's labo-

ratory.[45] The NPV of the FHV-1 PCR assays is also in question because many cats that are likely to have FHV-1–associated disease are negative. This may relate to clearance of FHV-1 DNA from tissues by the immune reaction. Tissue biopsies have greater sensitivity than conjunctival swabs. FHV-1 DNA can be amplified from aqueous humor of some cats, but whether this indicates FHV-1 associated uveitis is unknown.[46] Treatment does not eliminate FHV-1 infection, and so there is no benefit to follow-up culture or PCR testing.

The viruses in dogs most commonly associated with respiratory disease (adenovirus 2, parainfluenza, influenza, respiratory coronavirus, canine distemper virus, and canine herpesvirus) generally have an acute course of disease.[47,48] Therefore, the syndrome is often resolving before the return of diagnostic test results from virus isolation or molecular diagnostic assays. Results of molecular diagnostic assays can also be negative by the time clinical signs develop. In addition, there are no specific treatments for the viral respiratory agents of dogs, and so a definitive diagnosis is not required to optimize the treatment plan. Thus, use of these assays has low clinical utility in individual dogs. However, when respiratory outbreaks occur in populations of dogs, determining whether the primary agent is bacterial or viral may help in the planning of treatment (bacteria) or preventative programs.

Toxoplasma gondii (dogs and cats) or *Neospora caninum* (dogs) is associated with interstitial pneumonia that can progress to alveolar disease.[49] Serological tests are available for both organisms but have low PPV because of high seroprevalence rates in normal animals. Dogs and cats with active disease may have tachyzoites present on cytological examination of airway washings. However, the agents can also be obscured by the inflammatory reaction. In these situations, PCR may be used to amplify the microbial DNA.[50] PCR can also be used to differentiate *T. gondii* from *N. caninum*.

A variety of different serological assay and organism demonstration assays are available to aid in the diagnosis of the most common fungal causes of respiratory disease in dogs and cats, including *Aspergillus* spp, *Blastomyces dermatitidis*, *Coccidioides immits*, *Cryptococcus neoformans*, and *Histoplasma capsulatum*.[51] Although molecular diagnostic assays have been evaluated in limited numbers of research studies, there is minimal information concerning predictive values. Therefore, the majority of clinical cases are diagnosed by other methods at this time.

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