Laboratory Diagnosis of Feline Viral Respiratory Disease

By: Roger Maes, DVM, PhD; Annabel Wise, DVM, PhD; Ingeborg Langohr, DVM, PhD, Dipl ACVP; Matti Kiupel, DVM, PhD, Dipl ACVP

The two most important viral agents inducing upper respiratory disease in cats are feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV). Excellent reviews on both of these diseases have been published recently.1, 2, 3, and 4

FHV-1 causes both acute and chronic disease. Acute disease is characterized by fever, rhinotracheitis and keratoconjunctivitis. Chronic FHV-1 infections are associated with chronic conjunctivitis, sinusitis and keratitis (Figure 1). Ocular diseases in cats are most frequently associated with FHV-1 infection.5 Acute FCV infections present with fever, upper respiratory signs and oral ulceration (Figure 2) in most cases. Chronic stomatitis or gingivitis can also be caused by FCV.

Laboratory diagnosis of acute FHV-1 and FCV infections is accomplished by virus isolation or PCR on extracts of nasal, conjunctival and oropharyngeal swabs. Blood is not a very useful specimen for detection of FHV-1, since viremia is limited. Use of synthetic swabs (Dacron, nylon) and submission of the swabs in 0.5-1 mL of sterile saline are preferred. Samples collected with commercial swabs containing liquid transport medium are very appropriate, but the commercial swabs containing semi-solid (Amies) transport medium are not suitable for virological testing. Although the transport conditions for swabs to be tested for the presence of FHV-1 by PCR have been reported to be less critical,6 our overall recommendation is to submit samples on ice packs by overnight mail, to maximize sample quality.

We currently use real-time PCR as the default method for the detection of FHV-1 DNA and FCV RNA, but we also offer virus isolation for both if needed. An advantage of virus isolation is that infectious virus is being detected, but sample quality is more critical and the turnaround time is longer. The diagnosis can also be made on formalin-fixed biopsy samples, using immunohistochemistry for FHV-1 and FCV.

FIGURE 1: Large corneal ulcer accompanied by marked chemosis in a cat with feline herpesviral keratoconjunctivitis.

Positive PCR results have to be interpreted in the overall context of the disease process. A good example of this for FHV-1 is given in a publication by Vogtlun et al.7 For FHV-1 detection in ocular swabs from experimentally inoculated cats, either VI or PCR was comparable in sensitivity between days 1 and 19 post-exposure. PCR signals were still strong between days 19-24, but virus was seldom isolated.

FIGURE 2: Feline calicivirus-induced ulcer in the tongue of a tiger cub. (Photo by Dr. Thomas Mallaney)

Some of the samples collected at 80 days post-inoculation were still positive by PCR. The take-home message from these data is that finding FHV-1 DNA by PCR does not always imply presence of infectious virus. Another implication is that providing information about the strength of a positive PCR result is more meaningful than just reporting a positive or negative result. In the case of FCV, shedding of infectious virus continues well beyond the clinical phase in many cats. The detection of FCV or FCV RNA does not by definition imply an etiological role.

Other factors to be kept in mind when interpreting laboratory results are recent vaccination with a modified live vaccine and, in the case of FHV-1, reactivation of latent virulent or, potentially, vaccine virus. Recent vaccination with modified live vaccines can temporarily lead to false positive results. Both FHV-1 field virus and modified live FHV-1 vaccine viruses establish latency, primarily in trigeminal ganglion neurons. Asymptomatic reactivation of FHV-1 occurs fairly easily in cats and could lead to a positive PCR result that is unrelated to the disease process under investigation.

For additional information on these and other viral diseases, please contact the laboratory at 517.353.1683.

References
A Picture is Worth a Thousand Words

By: Rebecca Smedley, DVM, MS, Dipl ACVP

Assessment of surgical margins on large, oddly-shaped tissue specimens is a challenging task for the histology technician, pathologist, and submitting veterinarian. It is often difficult to communicate results of a margin evaluation to a submitting veterinarian in a written report, or even by phone. With certain tumor cases, it is important for the clinician to know to which margin the tumor extends in order to better direct additional resection.

Recommended guidelines for submitting tissues for full margin evaluation can be found on our website at: www.animalhealth.msu.edu/Sections/Surgical_Pathology. Under the heading Topics of Interest in Surgical Pathology, there are three PDF documents entitled: “Guidelines for Special Tissue Submissions,” “Evaluation of Surgical Margins,” and “Standard Trimming Method.” Even with these guidelines, however, margin interpretation and communication about margin assessment can still be difficult.

DCPAH’s Surgical Pathology service can now digitally photograph tissues submitted for full margin evaluation before they are trimmed. The histology technician who trims the tissues can then “draw” on the photos to indicate where each section of tissue is located. This is especially helpful when large numbers of sections are needed to adequately assess all lateral and deep margins. Knowing how the tissues are sectioned helps the pathologist assess the true surgical margins. In order to assist the submitting veterinarian to understand the results of a margin evaluation, the photographs are now attached to the case in WebView. If photos were taken, a link will be available in WebView when the case number is entered. For example, see Figures 1 and 2.

The pathologist can indicate in the biopsy report that neoplastic cells extend to the margin in section A3, and the clinician can view the photos and decide exactly where additional surgical resection may be needed. The DCPAH is pleased to provide enhanced client service by offering this new technology. Access to WebView can be requested online using your DCPAH account number and clinic telephone number. If assistance is required, contact the lab at 517.353.1683.

What is an MIC and Why Should I Care?

By: Carole Bolin, DVM, PhD

Culture and susceptibility is the best way to identify bacterial pathogens and guide antimicrobial therapy. Often empiric therapy must be started prior to the availability of susceptibility results. However, it is estimated that approximately 40% of the time, antibiotic therapy should be changed after receipt of susceptibility testing results.

Our preferred method of determining antimicrobial susceptibility provides a quantitative result as a Minimum Inhibitory Concentration (MIC). The MIC is the minimum concentration of the drug that will inhibit the in vitro growth of the organism. The MIC is also translated to a qualitative result of Susceptible, Intermediate, or Resistant.

The MIC is particularly valuable because it can be used to compare the relative efficacy of different drugs and to calculate doses that may need to be customized in particularly challenging infections. The drug concentration achieved at the site of infection should be at least equal to the MIC value. The approximate drug concentration that can be reasonably achieved safely in the plasma using the normal dose and route of administration is the resistant breakpoint MIC. The resistant breakpoint MIC takes into account the clinical pharmacology of the drug and is specific for the host, dose, drug and often the organism. Resistant breakpoint MICs are determined by testing organizations and are available in various references and, for some drugs, in a table provided on the DCPAH website (Resistance Breakpoints for Antimicrobials Used in Animals).

Armed with the organism MIC measured at DCPAH and the resistant breakpoint MIC, you can begin to compare the relative efficacy of various antimicrobials to treat a given infection. The MICs for various drugs must be compared, as they relate to the resistant breakpoint MIC for that drug. The resistant breakpoint MIC is divided by the measured MIC to derive an efficacy ratio—or a measure of how far the measured MIC is from the resistance breakpoint for that drug. A drug with a high efficacy ratio should be more effective than a drug with a lower efficacy ratio. Confused? An example might be helpful.

| TABLE 1: Moderate numbers of Staphylococcus pseudointermedius |
|---------------------|---------------------|---------------------|---------------------|
| Drug                | MIC                | Interp  |
| Amox/Clav           | <= 4/2             | S       |
| Ampicillin          | <= 2               | R       |
| Cefoxolin           | <= 2               | S       |
| Cephalothin         | <= 0.25            | S       |
| Clindamycin         | <= 0.5             | S       |
| Enrofloxacin        | <= 0.5             | S       |
| Erythromycin        | <= 0.5             | S       |
| Gentamicin          | <= 1               | S       |
| Marbofloxacin       | <=0.5              | S       |
| Penicillin          | >=1                | R       |
| Trimethoprim/Sufa   | <=0.5/9.5          | S       |
| Tetracycline        | >8                 | R       |

(MIC continued on page 3)
There are several methods for detection of parasites in the feces of animals. Some fecal exams detect parasite ova, oocysts, or cysts, while others detect antigens produced by the parasite. We are often asked which type of fecal exam or diagnostic assay is the most appropriate. The answer depends on the species of animal being sampled and the parasite that is suspected. Also, the choice of assay can be driven by the need to detect a variety of parasites, using procedures that are efficient and inexpensive for the client.

We offer several types of assays for detection of parasites in feces.

**Qualitative Fecal with Sucrose Solution:**
This is a good assay for detection of helminth eggs (roundworm, tapeworm and fluke eggs) and coccidia oocysts (Figure 1). Sucrose solution may distort parasitic larvae, amoeba cysts, and *Giardia* spp. cysts, making their identification more difficult.

**Qualitative Fecal with Zinc Sulfate:**
This is an alternative for sucrose solution and is useful for detection of parasitic larvae, amoeba cysts or trophozoites, and *Giardia* sp. cysts. This solution is not optimal for *Trichuris* sp. and Strongyld eggs. Using both sucrose and zinc sulfate qualitative procedures allows a broad parasite analysis for animals, especially those with diarrhea.

**Quantitative Fecal Using the Modified McMaster Method:**
This is the ideal assay for determining the number of eggs or oocysts per gram of feces. This procedure is sensitive to 25 eggs per gram. It is used frequently to help make decisions on deworming programs in horses, sheep and cattle (Figure 2). The qualitative fecal exam is more difficult and requires more expertise than a qualitative fecal exam. If a high degree of sensitivity is desired to detect very low numbers of parasite eggs or oocysts, we recommend doing both a qualitative fecal exam and a quantitative fecal exam.

**Giardia Antigen Test:**
This is probably the best choice for detecting a *Giardia* infection in mammals. It can detect *Giardia* when other exams fail to find visible *Giardia* cysts or trophozoites. The *Giardia* Antigen Test has exceptional sensitivity and specificity; one fecal sample should be sufficient for detection of *Giardia* sp. It is important to remember that this assay should be done on fecal samples tested within 48 hours of collection. The disadvantage of the *Giardia* Antigen Test is that it is expensive, so it may not be cost-effective to perform in your clinic. The qualitative fecal using zinc sulfate can detect *Giardia* cysts, but the cysts may be shed intermittently. Hence, it is recommended that the zinc sulfate fecal exam be done on three fecal samples taken on three different days to rule out infection.

Suppose the measured MIC for an *E. coli* isolate from a dog was 1 μg/mL for enrofloxacin and 2 μg/mL for amoxicillin/clavulanate. Using the chart provided on the DCPAH website, the resistant breakpoint MICs for these drugs are determined to be >4 μg/mL for enrofloxacin (at the 5 mg/kg dose) and >32 μg/mL for amoxicillin/clavulanate. Therefore, the efficacy ratio for enrofloxacin in this particular infection would be 4 (4 divided by 1) and that for amoxicillin/clavulanate would be 16 (32 divided by 2). The drug with the higher efficacy ratio, in this case amoxicillin/clavulanate, would be predicted to be more effective in this infection. This type of calculation can be repeated to compare a whole series of drugs, and this information can be factored in with the many other considerations in choosing an antimicrobial.

Another example from a DCPAH report may be helpful. In this case (Table 1 on page 2), the data from DCPAH is shaded in light green and the analysis by the veterinarian in light blue. Assume you want to use a drug that is good for skin infections and is available for PO use, which narrows your choices somewhat. Next, to compare the relative efficacy of the drug choices, you look up the resistant breakpoint MIC and calculate the efficacy ratio as described above. In this example, there are several reasonable choices, but it appears that the fluorinated quinolones would be a better choice than amoxicillin/clavulanate.

**Note:** This information is in no way intended to supersede clinical judgment and information provided in package inserts. Recommended reference: Small Animal Clinical Pharmacology and Therapeutics by Dawne Merton Boothe, published by W.B. Saunders Co in 2003.
DCPAH Fee Increase
Scheduled for September 1st

Fees for all diagnostic services will be increased on September 1st by roughly 10%. The increase will not be applied equally to all tests. An evaluation of all services is currently being done to determine the appropriate increase. The updated schedule will be posted on the website as soon as it is finalized. Existing clients will automatically receive an updated schedule with their fall newsletter. Prices in our web catalog will continue to reflect current pricing but will be updated to the new pricing on September 1st. Please contact the lab if you have any questions concerning the new pricing.

Where Are Those Results?

Still getting your results by snail-mail? Please give us a call and have your account updated today. We have many other options available (secured web access, e-mail, or fax). We can even target your fax results to a specific part of your day: morning, afternoon, evening, and even late night!

Contact us at 517.353.1683 to make a change.

Potomac Horse Fever Season
By: Nicole Grosjean, BS, LVT and Steve Bolin, DVM, PhD

Potomac horse fever (PHF) is a seasonal disease caused by Neorickettsia risticii (formerly Ehrlichia risticii), a Gram-negative bacterium. The mode of transmission likely is ingestion of aquatic insects that carry the N. risticii bacteria. Common clinical findings include depression, anorexia, fever and diarrhea. In Michigan, PHF is seen from late spring through mid-fall. For antemortem detection of PHF, the DCPAH offers tests that detect antibody against N. risticii or DNA from N. risticii. The indirect fluorescent antibody (IFA) test measures the antibody titer raised against N. risticii from a current or previous infection, or from vaccination. The IFA test is often used on horses that have a prolonged disease course. Only 1 mL of serum is needed to determine an antibody titer. A PCR assay is often used for acute infections. Whole blood (EDTA tube) or feces can be submitted for the PCR. As part of our Equine Diarrhea Panel plus PHF (test code 60993), we often have an opportunity to test both blood and feces for PHF using PCR. Over two PHF seasons, we found 33 Equine Diarrhea Panel submissions positive for PHF. Fourteen horses were PCR positive on feces but negative on blood; 6 horses were PCR positive on blood but negative on feces; and 13 horses were PCR positive on both blood and feces.

Conclusion:
Submit both blood and feces for PHF using PCR.

Please Note...
DCPAH will be closed on Monday, July 5, 2010, in observance of Independence Day. Regular business hours will resume on Tuesday, July 6. Service hours will resume on Thursday, July 8. 2010.