

## Serodiagnostic Aids and Management Practice for Feline Retrovirus and Coronavirus Infections

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Virus particles were first visualized by Jarrett et al.<sup>33</sup> in 1964 in feline lymphosarcoma (LSA) tissue, and in feline infectious peritonitis (FIP) lesions by Zook et al.<sup>58</sup> in 1968. Since these early reports, much has been learned about both of these viral agents. The feline leukemia virus (FeLV) is a horizontally and vertically transmitted retrovirus and is the causative agent of the most important fatal infectious disease complex of the American domestic cats.<sup>43</sup> The FIP virus (FIPV) is a horizontally transmitted coronavirus that is the inciting agent of a lethal, immunologically mediated disease characterized by fibrinous serositis and disseminated perivascularitis.<sup>7</sup> Safe and efficacious vaccines are still unavailable for both of these virus infections. Thus, until such vaccines are developed, control of these infections must be based on accurate identification and isolation of diseased animals and asymptomatic, chronic carriers.

### FELINE RETROVIRUS INFECTIONS

Replication-competent retroviruses carry with them a unique and fascinating enzyme called *reverse transcriptase*.<sup>26</sup> This enzyme is capable of generating a deoxyribonucleic acid (DNA) copy of the retroviral genome that is then inserted into the chromosomal DNA of the infected cell. This alien intruder, known as a *provirus* or *proviral DNA*, then is replicated whenever host-cell division takes place and can serve as a template for the intracellular production of new virus particles. The new virions are assembled in the cytoplasm of the cell and are released at the cell membrane, where they acquire a lipid envelope and glycoprotein surface receptors

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(peplomers). A cell infected with a retrovirus is infected essentially for the duration of its existence, as are all of its daughter cells.<sup>26</sup> *Because a version of their genetic material becomes a part of the total genetic information of the cells they infect, retroviruses are among the most intimate parasites known in nature.*

### Feline Retroviruses

Cats are susceptible to natural infection with at least three members of the Retroviridae family: FeLV, FeSV (the feline sarcoma virus), and a virus known as RD-114. Of these, only FeLV is a truly *exogenous* agent—that is, infection is spread from cat to cat as a contagion. FeSVs, replication-defective mutants of FeLV, apparently arise within individual cats by recombination when a small piece of chromosomal DNA is erroneously incorporated into an FeLV provirus. The resulting FeSV is unable to replicate without “assistance” from replication-competent FeLV (“helper” virus) because a portion of its genetic information is lost during recombination. In nature, FeSV recombinational events apparently occur infrequently, and the recombinant viruses are probably not contagious *per se*.<sup>17</sup> The virus known as RD-114 is a true *endogenous* virus. That is, multiple RD-114 proviruses are present within the chromosomal DNA of all cells of all domestic cats and are transmitted vertically through the germline. However, production of virus is usually repressed, so that the agent is not contagious. The RD-114 retrovirus does not appear to be related to FeLV or FeSV and has not been proven to cause any recognized disease in cats.<sup>16</sup>

### Immunogenic Significance of Major FeLV Structural Components

Individual particles of FeLV consist of two distinct morphologic components: a dense inner core, or *nucleoid*, and an outer envelope containing an immunogenically important 70-kilodalton glycoprotein, *gp70*. This glycoprotein is the principal antigen present in the viral peplomers, which are responsible for attachment of the virus to cells during infection. Virus-neutralizing antibody (VNA) directed against *gp70* is an essential component of a successful immunologic response to FeLV, and the presence of this antibody in serum is an indication of past FeLV exposure.<sup>9</sup> Most persistently viremic cats produce little or no VNA. Most healthy cats in the general feline population also have little or no VNA, but only because they have not been exposed to an infective dose of FeLV. By contrast, about 40 to 50 per cent of healthy, exposed cats have protective levels of VNA. Such cats are generally believed to be resistant to subsequent FeLV infection, and most will not become persistently viremic.<sup>9, 16, 30</sup>

A 27-kilodalton protein moiety, *p27*, is a structural component of the inner viral core and is the major FeLV group-specific antigen.<sup>16</sup> It can be found in great abundance in the cytoplasm of infected leukocytes and platelets, and in soluble form in plasma and serum of viremic cats. This protein provides the major antigenic basis for both the indirect immunofluorescence assay (IFA) and the enzyme-linked immunosorbent assay (ELISA) for FeLV. The significance of the immunologic response to *p27* itself is at present uncertain, because antibody directed against it is not protective and prevents neither viremia nor FeLV-related disease.<sup>10</sup>

Suppression of normal protective immunologic responses is one of the most important consequences of persistent FeLV infection. Both the humoral and cellular arms of the immune system are affected by the virus.<sup>22, 45, 54</sup> A major cause of FeLV-induced immunosuppression appears to be a specific FeLV structural protein, *p15(E)*, that is associated with the viral envelope.<sup>39</sup> *Both intact as well as disrupted (inactivated) virus particles retain immunosuppressive capabilities.*<sup>22</sup> The significance of FeLV-induced immunosuppression is especially apparent when one considers the array of secondary diseases associated with FeLV infection.<sup>16</sup> In addition, immunosuppression mediated by *p15(E)*, even from "inactivated" virus, is a major consideration in the design of a safe and efficacious FeLV vaccine.

### Transmission of FeLV

All persistently viremic cats are excretors of infectious FeLV and probably remain so for the rest of their lives.<sup>16</sup> They thus serve as a reservoir of infection for healthy, uninfected, susceptible cats with which they come into contact. Many cats that ultimately develop immunity experience an initial transient viremia lasting from 1 to 2 days or up to several months, during which time they can excrete infectious FeLV from the oropharynx.<sup>30, 31</sup>

Excretion of FeLV in persistently viremic cats occurs primarily by way of salivary secretions, although virus may also be present in respiratory secretions, milk, feces, and urine.<sup>16, 19</sup> Thus, the social grooming habits of cats, biting, sneezing, and the urban practice of sharing litter boxes and feeding bowls probably represent the major modes of spread of FeLV among pet cats. In addition, in utero transfer of virus across the placenta and excretion in colostrum are also known to occur, so that kittens may become infected either through an infected queen or by close contact with other persistently viremic cats.<sup>18, 32</sup> *Prolonged close contact (days to weeks) between cats is usually required for effective transmission of FeLV.* Thus, asocial cats that participate little in "group activities" appear to be less readily infected than more "outgoing" cats.<sup>14</sup> Virus can also be spread in blood transfusions from viremic cats and possibly also by hematophagous arthropods. The time period between initial exposure to an infective dose of FeLV and the development of either persistent viremia or immunity is quite variable and may be dependent in part upon the route of virus transmission.<sup>14</sup>

In common with a number of other enveloped ribonucleic acid (RNA) viruses, FeLV is extremely unstable once outside the cat and is rapidly inactivated by warm, dry environmental conditions and by most common household detergents and disinfectants. Infectivity of virus in saliva left to dry at room temperature has been shown to decline to inconsequential levels within 3 to 4 hours.<sup>12</sup> However, infectivity of FeLV in liquid suspension at room temperature may persist for several days and for even longer periods at refrigerator temperatures.<sup>12</sup>

### Serodiagnostic Aids for FeLV Infection

Currently, there are three basic serodiagnostic procedures for determination of the FeLV and FeLV-immune status of an animal: (1) detection

of viral antigens; (2) detection of VNA; and (3) detection of antibody to the feline oncornavirus-associated cell membrane antigen (FOCMA).

**Detection of Viral Antigens.** The p27 core protein provides the major antigenic basis for both the IFA and ELISA FeLV antigen detection tests. A positive test for FeLV by IFA implies that a cat is excreting FeLV and is a potential health hazard to uninfected cats, especially kittens and cats on immunosuppressive drug therapy. Approximately 97 per cent of cats with a positive IFA test will remain positive for life.<sup>16</sup> A negative IFA test indicates that no detectable infected blood cells are present. It does not exclude the possibility that a cat is incubating FeLV at the time of testing, nor does it imply that a cat has developed immunity to FeLV. A positive test by ELISA indicates the presence of circulating FeLV p27 in the blood fraction (serum, plasma, whole blood) tested. Most, *but not all*, cats positive by ELISA are actively excreting FeLV. A negative ELISA test indicates that no detectable FeLV p27 is present, but, as with the IFA test, does not exclude the possibility of virus incubation and is not an indication of immunity to FeLV.

It is thus important that positive FeLV tests be repeated within 2 to 3 months in order to determine whether the viremia is transient or persistent. Virtually all cats positive by IFA will be persistently viremic.<sup>16</sup> *Both transiently and persistently viremic cats can excrete infectious FeLV for the duration of the viremia.*<sup>30, 31</sup>

In the last several years, comparative studies of FeLV antigen detection tests have identified some cats that remain positive by ELISA but negative by IFA or by virus isolation for many months.<sup>23, 32, 36, 37</sup> As many as 30 per cent of cats positive by ELISA may be negative by one or both of the other methods.<sup>32</sup> Tests performed 1 to 10 months after initial testing have shown that the FeLV status of most of these cats remains unchanged.<sup>32, 36, 37</sup> The cellular source of the FeLV antigen causing the persistently positive ELISA results has not yet been definitely identified. The most recently published studies indicate that persistently ELISA-positive, IFA-negative cats, unlike their persistently IFA-positive counterparts, do not give birth to infected kittens and do not appear to be excreting FeLV.<sup>30, 32</sup> Tentatively, then, the following cautious recommendation may be made: Until further research should prove otherwise, cats remaining persistently ELISA-positive and IFA-negative for a period of at least 3 months may be considered free of *infectious* FeLV.<sup>30, 32, 37</sup> However, studies have not progressed for a long enough period to determine whether these cats are still at risk to develop one or more of the FeLV-related diseases.

**Detection of VNA.** Cats with protective levels of VNA have resisted generalized FeLV infection and in most cases are protected against subsequent development of persistent viremia. However, because a DNA copy of the genetic information of FeLV stably integrates into the host cell chromosomal DNA during viral infection and replication, *latent FeLV proviral infection resulting in malignant transformation at some time in the future cannot be excluded in cats with VNA.* Indeed, previously exposed FeLV-negative cats treated with corticosteroids can experience a reactivation of their infection, presumably owing to the continued presence of

integrated FeLV proviral DNA<sup>46, 49</sup> (see the section on the problem of latency).

**Detection of FOCMA Antibody.** A certain percentage of cats exposed to FeLV will develop antibody against FOCMA, a tumor-specific antigen found on the surface of FeLV-infected cells that have undergone malignant transformation.<sup>9, 16</sup> This antigen appears to be structurally similar to the gp70 of FeLV subgroup C.<sup>52</sup> Antibody titers to FOCMA tend to be higher in cats that have resisted generalized FeLV infection and in persistently viremic but healthy cats, and lower (often nonexistent) in cats with FeLV-induced malignancy. In general, the higher the FOCMA antibody titer, the greater the probability that a cat is protected against the oncogenic effects of FeLV.<sup>16</sup> However, recent research has suggested that a more complex situation may exist, with a constellation of specific antibodies comprising the anti-FOCMA immune response.<sup>15, 52</sup>

### Control of FeLV Infections

Elimination of FeLV from an infected household can be achieved by implementation of an FeLV test-and-removal program using the IFA test.<sup>16</sup> This program has been highly effective in removing infectious FeLV from multiple-cat households. In a survey of 45 households from which 159 FeLV-positive cats were removed, 561 of 564 (99.5 per cent) FeLV-negative cats remained negative upon subsequent retesting.<sup>18</sup> Infected multiple-cat households in which FeLV test-and-removal has not been implemented have experienced infection rates over 40 times greater than those experienced by households in which the program has been successfully introduced.<sup>18</sup>

**FeLV Test-and-Removal.**<sup>1, 16, 43</sup> All cats in the household should be tested by IFA, regardless of age or condition. All cats found positive should be removed, and the household premises cleaned with a commercial detergent or disinfectant. All litter boxes and food and water bowls should be thoroughly cleaned or replaced. Cats that initially tested negative should be tested several times over a period of 8 to 12 months, in the event they were infected just before the first test, prior to onset of detectable viremia, or are cycling in their level of detectable viremia. The time period between exposure and viremia is extremely variable, and an infected cat that tested negative initially may be positive when tested again later. During the testing period, no new cats should be allowed to enter the household. If any FeLV-positive cats are found on subsequent testing, they should be removed and another period of quarantine and testing imposed. All cats in the household should test negative for FeLV in two tests at least 3 months apart for the household to be considered free of infectious FeLV.<sup>16</sup>

All new cats entering an FeLV-negative household should be tested prior to entry. Those that test negative should be quarantined in separate quarters for 3 to 5 months and retested negative one to two times before being allowed to intermix with the established FeLV-negative household population. New cats should, of course, be obtained only from FeLV-free environments. Routine yearly or twice-yearly testing for FeLV is suggested for cats in multiple-cat households owing to the variable incubation period.



Persistently viremic cats should never be used for breeding purposes, because infected queens will transmit the virus to their offspring.

Certain modifications of the test-and-removal program may be made for households in which both FeLV-negative and FeLV-positive cats are kept.<sup>1</sup> The positive cats in these households should be isolated from contact with *all* other cats. This will not only prevent the spread of infectious FeLV but will also decrease exposure of immunosuppressed, viremic cats to other infectious agents to which they may have an enhanced susceptibility. No new cats should be introduced at any time, and the FeLV-positive cats should not be allowed to breed. Separate litter boxes and feeding bowls should be provided for positive and negative cats, and cleanliness and personal hygiene should be maintained at all times.

### The Problem of Latency

The persistence of the integrated provirus in infected cells and in their descendants is an important aspect of the replication cycle of retroviruses.<sup>26</sup> Cells so infected frequently persist in the face of an active immunologic response against the infecting retrovirus, a phenomenon well-recognized in ovine progressive pneumonia, equine infectious anemia, and bovine leukemia virus infection. Only recently, however, has the full significance of latent proviral infection of cats with FeLV begun to be appreciated.

As outlined earlier in this article, many cats naturally exposed to FeLV experience an initial, transient viremia lasting for a short but variable period of time, during which an active immunologic response to the virus develops that results in the disappearance of virus from the bloodstream and apparent recovery from infection. Thus, even in multiple-cat households and catteries where FeLV is endemic, apparently only a minority of exposed cats (estimated to be about 30 per cent) fail to overcome the infection and instead proceed to develop persistent viremia.<sup>16</sup> However, recent studies have shown that many recovered cats develop a persistent infection of myelomonocytic precursor cells in the bone marrow and of certain nodal T lymphocytes.<sup>38, 49</sup> Thus, "*FeLV-negative*" cats are not necessarily free of FeLV. Healthy FeLV-negative cats that lack both VNA and FOCMA antibody have probably never been exposed to an infective dose of FeLV and thus are truly free of the virus. VNA and/or FOCMA antibody in healthy FeLV-negative cats, however, are the telltale "footprints" of previous FeLV exposure and, in light of the new data, probably indicate, in many cases, the existence of latent FeLV proviral infection. In addition, some cats with FeLV-negative LSA have been shown to harbor latent FeLV infections in the bone marrow.<sup>49</sup>

Cats latently infected with FeLV are not viremic and, thus, do not shed infectious FeLV into their environment. However, administration of corticosteroids can reactivate latent infections, *with re-emergence of FeLV into the bloodstream (and reversion to FeLV-positive status)*<sup>46, 49</sup> Blood smears from such cats usually contain FeLV-infected cells as determined by IFA, but plasma samples may be negative by ELISA, suggesting that most extracellular virus is complexed with antibody and, thus, not available for ELISA detection.<sup>46</sup> Because corticosteroid release from the adrenal glands is a natural physiologic response of animals to stress, it seems

reasonable to suspect that certain stressful situations in the day-to-day existence of modern cats, such as overcrowding, movement to new quarters, territorial conflicts, pregnancy and lactation, improper nutrition, and perhaps intercurrent diseases, may serve to reactivate latent FeLV infections in nature. This might explain occasionally observed instances when a cat with a long history of negative FeLV tests in an FeLV-negative, closed cattery suddenly becomes FeLV-positive. The virus may not have come from the outside; it may have been present in the cat all along.

Further research is currently in progress on this important problem. Hopefully, additional information about latent FeLV infections will become available over the next several years, especially regarding the potential of cats with reactivated infections to excrete FeLV, and the long-term risks (if any) of latently infected cats to develop one or more of the FeLV-related diseases.

### FELINE CORONAVIRUS INFECTIONS

Coronaviruses are a large and widely distributed group of RNA viruses and are important causes of upper respiratory and enteric disease, hepatitis, vasculitis, serositis, and encephalomyelitis in several species of birds and mammals.<sup>51</sup> FIPV, canine coronavirus (CCV), transmissible gastroenteritis virus (TGEV) of swine, and human respiratory coronaviruses of the 229E group comprise an antigenic cluster of closely related viruses within the Coronaviridae family.<sup>44</sup> In fact, the major structural polypeptides of FIPV, TGEV, and CCV are so antigenically similar that some regard these three viruses as host-range variants rather than as individual viral species.<sup>24</sup>

#### Feline Coronaviruses

Cats are susceptible to natural infection not only with FIPV but also with certain enteric coronaviruses that may or may not be variants of FIPV (or vice versa).<sup>20, 40, 42</sup> These feline enteric coronaviruses (FECVs) can produce a range of effects from asymptomatic infection of the gastrointestinal tract to severe enteritis, in either kittens or adult cats. The nature of the relationship between FECVs and FIPV is perhaps illuminated by the observation that certain FIPV strains are capable of producing either FIP or enteritis, or both.<sup>20, 21</sup> Enteritis can also be produced in newborn piglets by oral exposure to virulent FIPV.<sup>57</sup> It is thus quite possible that FECVs and FIPV may represent pathogenetic (rather than host-range) variants of a single coronavirus type—variants possessing, however, a broad spectrum of virulence from asymptomatic infection, to enteritis, to lethal disseminated FIP. Spectra of such breadth and character are not without precedent in virology. For example, different strains of murine hepatitis coronavirus can produce different disease conditions in different strains of mice, including hepatitis, serositis, enteritis, vasculitis, encephalomyelitis, or asymptomatic infection.<sup>51</sup> In cats, variants of feline calicivirus produce a wide range of effects from asymptomatic infection to severe pneumonia, and possibly enteritis.<sup>13, 25</sup> It is perhaps also pertinent to recall that the human gastroin-

testinal tract is the normal habitat for a number of viruses with significant pathogenic potential, such as hepatitis A virus and poliovirus.

Recent reports indicate that at least two other coronaviruses in the FIPV antigenic cluster can infect cats under experimental conditions: TGEV, which produces an asymptomatic infection and is excreted in feces for as long as 3 weeks after exposure,<sup>47</sup> and CCV, which also produces an asymptomatic infection and is excreted from the oropharynx for at least 1 week.<sup>6, 53</sup> At present, the frequency of infection of cats in nature with these two coronaviruses is not known.

### **Immunogenic Significance of Major FIPV Structural Components**

Individual coronavirus particles are characterized morphologically by a fringe of large, radiating surface peplomers resembling the rays or corona of the sun. As is the case with FeLV, the peplomers are responsible for attachment of the virus to cells during infection and for the induction of VNA. The significance of VNA titers to coronavirus in cats, either healthy cats or those with FIP, has not been satisfactorily determined. The presence of this antibody is not necessarily an indication of protective immunity, because many cats with FIP are VNA-positive.<sup>4, 5, 8, 48</sup> Moreover, because of the especially close antigenic relationship between FIPV and FECVs, TGEV, and CCV, commonly utilized assays for VNA (or for other types of coronavirus antibody) cannot yet identify with certainty the exact coronavirus against which the antibody was raised (see the section on coronavirus antibody testing in cats).

Cats with FIP also produce antibody against the two other major structural components of FIPV: the inner viral core and the outer envelope, in which the protruding peplomers are embedded.<sup>24, 29</sup> As with VNA, there is as yet no clear consensus on the significance of the antibody response to these antigens. However, recent studies have demonstrated distinct structural differences among the envelope polypeptides of FIPV, TGEV, and CCV—differences that potentially could assist in serologic identification of the coronavirus inciting the antibody response in a given serum sample.<sup>24</sup>

### **Transmission of FIPV**

The route by which FIPV is spread in nature is still unknown. However, it is most likely that initial infection results from ingestion and/or inhalation of the virus.<sup>7</sup> Virus is probably excreted into the environment by a number of routes—in oral and respiratory secretions, feces, and possibly urine. Close contact between cats is usually required for effective transmission of FIPV, although the possibility of virus transmission via excreta and by other indirect methods (on clothing, bedding, feeding bowls, and so on) also exists. The potential for transmission by hematophagous arthropods is unknown. Transmission of FIPV across the placenta to the developing fetus, although suggested by several reports,<sup>11, 41</sup> has not yet been documented.

In common with FeLV and many other enveloped viruses, FIPV is quite unstable once outside its host and is rapidly inactivated by warm, dry environmental conditions and by most common household detergents and disinfectants.<sup>7</sup>



## Diagnosis of FIP

The clinical diagnosis of FIP is made by evaluation of history and presenting signs and the results of supportive laboratory tests.<sup>7, 85</sup> Clinico-pathologic and serologic procedures important in diagnosis include analysis of thoracic and abdominal effusions (when present), hemogram, serum protein electrophoresis, clinical chemistry profiles, serum coronavirus antibody titer, and biopsy (when possible).

It is important to remember that biopsy is the only test procedure that can *definitively* diagnose FIP in the living animal. Exploratory laparotomy with organ punch biopsy of affected tissues (especially liver, spleen, omentum, and mesenteric lymph node) is the preferred method of obtaining biopsy samples (percutaneous needle biopsy *cannot* be recommended owing to the friability of diseased organs and the potential for serious hemorrhage). Similarly, complete necropsy examination with histopathologic evaluation of suitable tissues will provide a definitive diagnosis after death. *Any FIP diagnosis made in the absence of either biopsy or necropsy examination must be considered presumptive.* This is because of the large number of "FIP look-alike" diseases that can affect cats. These include LSA and other tumors (especially those involving the liver, biliary tract, kidneys, and lungs), cardiomyopathy, septic peritonitis, diaphragmatic hernia, pyothorax, chylothorax, internal abscessation, pansteatitis, toxoplasmosis, cryptococcosis and tuberculosis.

Thus, in individual cases, clinicopathologic and serologic test procedures will assist in ruling out possible diagnoses, but only biopsy or necropsy examination will definitively identify the FIP disease process. Therefore, it follows that, as described in the following section, *the diagnosis of FIP must never be made simply on the basis of a coronavirus antibody titer determination.*

## Coronavirus Antibody Testing in Cats

Laboratory test procedures for detection of coronavirus antibody in feline serum include biologic assays such as virus neutralization (VN), for detection of VNA; nonbiologic, immunohistochemical techniques such as IFA, ELISA and kinetics-based ELISA (KELA); and other nonbiologic methods such as agar gel immunodiffusion and passive hemagglutination.<sup>4</sup> Either FIPV itself or one of the other coronaviruses in the FIPV antigenic cluster (usually TGEV or CCV) can be used as the target antigen in most of these assays. The use of non-FIPV coronaviruses in antibody testing procedures has become popular in recent years because of long-standing difficulties in routinely propagating FIPV in the laboratory. In the United States, the immunohistochemical tests (especially the IFA) have gained the greatest popularity among veterinary diagnostic laboratories, in part because of their relative ease of performance and the widespread availability of the pertinent immunotechnologies.

On the basis of serosurvey data, it has been proposed that most FIPV infections in nature result only in seroconversion without progression to recognizable clinical disease.<sup>4</sup> Serum coronavirus antibody can be found not only in cats with lethal, disseminated FIP but also in many healthy cats

and in many cats with diseases other than FIP. This indicates that exposure of cats to coronavirus(es) is much more widespread than was once believed, especially in certain selected populations. In the general healthy feline population, excluding cats in catteries and multiple-cat households, approximately 10 to 40 per cent of cats will have positive coronavirus antibody titers (*Note*: "Positive" refers only to the presence of antibody, *not* to the presence of the FIP disease process). A special situation is encountered when cats are clustered together into cattery situations, in which case positive titers are either completely absent (that is, there has been no coronavirus exposure), or are present in 80 to 90 per cent of the cats within a household (indicating efficient spread of the virus once it has been introduced). The occurrence of coronavirus antibody in a cattery does not always correlate with its FIP history; for example, antibody has been detected in healthy cats in catteries that have experienced death losses to FIP as well as in catteries that have never lost a cat to FIP.

Most cats with histopathologically confirmed FIP have serum coronavirus antibody, often of high titer. Because cats with other illnesses may also have elevated titers (indicating coronavirus exposure), interpretation of their titers can be more challenging. The interpretation of coronavirus antibody titers in healthy cats and in cats with undiagnosed illnesses has been further complicated by recent reports that other coronaviruses (TGEV, CCV, FECVs) can also infect cats *and generate coronavirus antibody in their serum*.<sup>6, 42, 47, 53</sup> Because these viruses are all serologically cross-reactive with each other and with FIPV, and because several of them (FIPV, TGEV, CCV) are used relatively interchangeably in commercially available coronavirus antibody tests, the nonspecificity of these tests is readily apparent. The serodiagnostic potential of these tests (that is, their ability to identify cats with FIP and/or possible virus carriers/excretors) is thus limited not only by the widespread occurrence of serum coronavirus antibody in the general feline population, but also by the possibility that non-FIPV coronaviruses may be responsible for some of the seroconversions they detect. The actual distribution of antibodies in the general feline population to each of these coronaviruses is therefore unknown and will remain so until highly specific tests are developed that will be able to differentiate antibody against one coronavirus (for example, FIPV) from antibody against another antibody (for example, CCV).

These difficulties are further compounded by the plethora of test methodologies (that is, IFA, VN, ELISA, KELA) employed by different laboratories and by the absence of standardization of testing protocols. Conflicting titer results should therefore be *expected* when a serum sample is tested by different laboratories using different serologic techniques, or even by different laboratories using the *same* technique. *Titer results from a testing laboratory are best interpreted in light of specific information provided by that laboratory on the significance of titer levels generated by the individual test that it performs.*

**Effect of Recent Vaccination.** Research has revealed that antibody against bovine serum components can be found in the serum of certain cats—antibody capable of reacting with antigenically similar bovine serum components present in cell cultures used for propagating target viruses for

immunohistochemical assays such as the IFA, ELISA, and KELA.<sup>2, 3</sup> Because these components adhere tightly to both cells and virus,<sup>2, 34, 35</sup> *reactivity against them can be mistaken for a coronavirus antibody response* unless feline serum samples are tested in parallel against cell culture preparations *without* coronavirus ("negative antigen" controls).<sup>2, 3</sup> In the IFA and conventional ELISA, which are often performed without benefit of negative antigen controls, antibody to bovine serum components is a potential source of false-positive coronavirus antibody test results.<sup>2</sup> In the KELA, negative antigen controls are routinely performed for each individual serum sample tested and titer results are adjusted accordingly.<sup>2, 3</sup>

One possible explanation for the presence in feline serum of antibody reacting against bovine serum components is routine vaccination.<sup>34</sup> Cell culture vaccines prepared for use in cats (as well as vaccines for many other species) contain bovine serum components that could conceivably be a source of this noncoronavirus reactivity—reactivity that might be especially strong in serum samples drawn soon after parenteral vaccination. Both retrospective and prospective studies support this hypothesis. Using the KELA, a statistical association between recent vaccination and the presence of this noncoronavirus reactivity has been demonstrated.<sup>3</sup> However, not all cats reacted to vaccination this way, nor did all vaccines always produce this reactivity. KELA studies have shown further that this reactivity dissipates with time and that the probability of encountering it (and hence of producing an inaccurate coronavirus antibody titer) can be minimized if serum samples for elective serotesting are drawn no sooner than three to four months after the most recent parenteral vaccination.<sup>3</sup>

**General Recommendations.** The presence of serum coronavirus antibody in any cat, whether healthy or diseased, is indicative *only* of exposure to a coronavirus in the FIPV antigenic group. A positive coronavirus antibody titer, while consistent with a clinical diagnosis of FIP (recall that this type of FIP diagnosis is always presumptive), does not indicate that a cat actually has FIP, because many healthy cats and many cats with diseases other than FIP are also coronavirus antibody-positive. Neither does a positive titer indicate that a cat is protected against FIP, because most cats with FIP are also coronavirus antibody-positive. Considering that FIP occurs only sporadically in the general feline population and that most cats in FIP-problem households are coronavirus antibody-positive and yet do not contract FIP, it would appear that many cats (perhaps most cats) with coronavirus antibody are protected against the natural disease. The question remains whether it is coronavirus antibody (of some type) that actually confers this protection or whether other unknown factors are involved. Lastly, present-day coronavirus antibody tests *have absolutely no predictive value*; a positive titer in no way indicates that a cat is doomed to develop FIP at some future date.

Despite all the problems with current feline coronavirus antibody testing methods, there are still situations in which determination of antibody titers can be of use to the veterinarian and to the cat owner:

1. As a screening test, to determine the presence or absence of antibody in a previously untested household, and to detect potential virus carriers (see the section

on control of FIPV infections) when introducing new cats into coronavirus antibody-negative households. *Based on our current understanding of feline coronavirus serology, screening would appear to be the major use for coronavirus antibody testing today.* Screening of cats in a household experiencing undiagnosed disease problems may be especially useful. Only about 10 to 20 per cent of the cats (a minimum number of three) in such a household need to be tested, because antibody will either be totally absent or present in 80 to 90 per cent of the animals. Although the discovery of coronavirus antibody-positive cats in such households will not diagnose the problem, *knowledge that coronavirus antibody is absent is helpful in ruling out an FIPV-group coronavirus as the culprit.*

2. As an aid (and nothing more than an aid) in the clinical diagnosis of a sick cat with signs suggestive of FIP. A coronavirus antibody titer determination should be given no more weight than any of the other routine procedures (for example, hemogram, clinical chemistry profiles, abdominal fluid cytology, radiographs, and so on) used in arriving at a clinical diagnosis. A positive titer will not diagnose FIP, but a negative titer will usually rule it out, except under certain rare circumstances that are described in the following section.

**Coronavirus Antibody-negative FIP Cases.** A very small percentage of cats with FIP (usually diagnosed at necropsy) do not have *detectable* coronavirus antibody in their serum. Several explanations for this phenomenon are possible:

1. Detectable antibody may sometimes disappear from the circulation during the terminal stages of the diseases. Thus, submission of serum from some moribund cats may result in a negative titer determination despite the presence of disseminated FIP.

2. Immune complexing is an important immunopathologic feature of FIP.<sup>27, 28, 56</sup> In certain cases, if extensive immune complexing is present at the time of testing, it is conceivable that there may be little free coronavirus antibody available to be detected. This may be the explanation, at least in part, for the absence of detectable antibody in the serum of some moribund cats.

3. The swiftness of the FIP disease process is an important factor, especially in animals without previous coronavirus exposure. Cats experiencing a rapid disease course (such as some young kittens) may display a rather slowly rising antibody response that can be more difficult to detect in the earlier stages, especially if a non-FIPV coronavirus (TGEV, CCV) is used by the laboratory for antibody detection. Although serologically cross-reactive with FIPV, these viruses nevertheless *are different* from FIPV, and thus are not as sensitive as FIPV at detecting low levels of anti-FIPV antibody.

### Control of FIPV Infections

A test-and-removal program for coronavirus antibody-positive cats similar to that utilized for FeLV infection, based upon current scientific information, *cannot be recommended.*<sup>7, 50</sup> There is no available serodiagnostic test that can specifically identify antibody-positive cats with FIP, antibody-positive cats with diseases other than FIP, or "FIP-immune" antibody-positive cats. No test is presently available that can specifically identify antibody-positive cats that are excreting FIPV into the environment or that can even identify the exact coronavirus(es) against which the antibodies in these cats were raised. Thus, *there is no known medical reason for destroying these animals.*

There is no recognized environmental reservoir of FIPV; the natural

reservoir is assumed to be infected cats. How, then, does the virus maintain itself in these animals? For how long do infected cats harbor the virus? For how long do they excrete the virus, and by what route(s)? What route is most important for effective virus transmission to other cats? Is excretion continuous or only intermittent? Is it possibly stress-related? What percentage of cats infected with FIPV actually become chronic carriers? To what extent is a coronavirus antibody-positive cat a potential disease threat to other cats with which it may come into contact? Can an infected queen infect her kittens in utero? If so, does in utero infection result in disease?

Clearly, much further research will be required before these questions and others can be satisfactorily answered. Importantly, an antigen detection test for identifying carrier animals that are excreting FIPV (and not FECVs, TGEV, or CCV), similar to those currently available for FeLV infection, is urgently needed so that rational FIPV control procedures can be devised. Until then, control must be based on isolation of cats with suspected FIP and maintenance of coronavirus antibody-negative catteries, when possible. *Euthanasia of coronavirus-antibody positive cats to achieve this latter purpose, however, cannot be justified.*

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