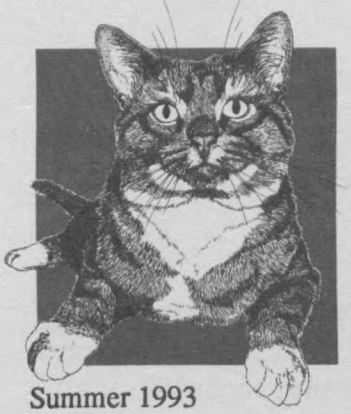

Feline Health Topics

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Feline Immunodeficiency Virus Tests and their Interpretation

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Serologic tests can be a valuable aid in the diagnosis and management of feline immunodeficiency virus (FIV) infection and related diseases. The proper use and interpretation of the tests are critical. Veterinarians must understand not only the types of tests available and their formats, but also the basics of FIV pathogenesis in order to interpret and act upon the results of these serologic tests.

FIV Pathogenesis and Prevalence

Feline immunodeficiency virus is a lentivirus associated with an immunodeficiency disease in domestic cats.[1-4] The disease, known as feline immunodeficiency syndrome (FIS) or, more commonly, feline AIDS, is characterized by fever, lymphadenopathy, leukopenia, anemia, anorexia and cachexia, chronic secondary infections, and neurologic disorders. Like human AIDS, FIS is caused by infection or damage of lymphocytes with subsequent impairment of immune system function.

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Feline immunodeficiency virus has been demonstrated to be efficiently transmitted through bite wounds, with virus being shed in the saliva of infected cats,[2-4] possibly in the form of infected leukocytes. The seroepidemiologic data support the theory that bites are a major mode of transmission. Serologic surveys of multiple-cat households with at least one FIV-positive cat indicate that transmission through casual contact is, at best, inefficient under natural conditions.[6] Placental or colostral transmission for FIV from a queen to her offspring occurs infrequently.[3]

Serologic tests for FIV indicate that the virus is distributed widely throughout North America. Estimated prevalence of FIV infection in the United States is 1.5% to 3% in the healthy cat population and 9% to 15% in cats exhibiting signs of clinical illness.[4-6] Antibodies to FIV have also been detected in cats from Japan, the United Kingdom, the Netherlands, France, Australia, New Zealánd, and Taiwan.[7-17] Retrospective serologic surveys conducted in the United States, Great Britain, and Japan indicate that FIV infection has been well established in the domestic cat population for more than 20 years.[9,18,19] In addition, infection with FIV or a closely related lentivirus has been detected by serologic methods in several species of nondomestic felids, both in captive and in free-ranging populations.[20-23]

Male cats are approximately three times as likely to be infected with FIV as female cats. Free-roaming cats are much more likely to be infected than cats

housed strictly indoors; very few purebred cats housed in catteries are infected with FIV.[4,10,11] The prevalence of FIV infection increases with age, with a mean age of about 5 years at the time of diagnosis.[7,24] The higher prevalence of infection in male cats has been attributed to an increased likelihood of territorial aggression, resulting in a disproportionate number of bite wounds, in male cats.[4] Likewise, free-roaming cats have a much greater chance of aggressive contact with other cats than do indoor cats, and older cats have had more opportunity for such contact to occur.

When cats are experimentally infected with FIV, neutralizing antibodies appear three to four weeks after infection; however, virus can be isolated from infected cats in spite of high neutralizing antibody titers.[25] Antibodies to the envelope glycoprotein (gp120) and the major core proteins develop first,[3,26,27] with antibodies to the transmembrane protein (gp40) and the *pol* gene products generally appearing 4 to 8 weeks after infection.[27] Antibody levels peak after several weeks and remain elevated for months to years following infection.[3,26,27] Although most cats develop high antibody levels within a few weeks after FIV infection, several antibody-negative, virus-positive cats have been identified.[28] Some cats may have undetectable or very weak antibody levels for weeks to months before finally seroconverting.[3] In addition, FIV antibody levels may drop precipitously in some cats during the terminal stages of disease.[29]

Clinical Signs

The clinical signs associated with FIV infection in domestic cats are diverse due to the immunosuppressive nature of the disease.[1,7] Diseases associated with FIV infection cannot be distinguished clinically from feline leukemia virus (FeLV)-associated immunodeficiencies. Among the most common clinical findings in FIV-infected cats are gingivitis, stomatitis, and periodontitis. Chronic, nonresponsive or recurrent infections of the external ear and skin also are commonly seen. Chronic upper respiratory tract disease occurs in about 30% of FIV-positive cats, and persistent diarrhea due to chronic enteritis occurs in 10% to 20% of infected cats.[4,7] Pyrexia is a frequent finding in the later stages of disease, and severe wasting occurs in some cats. Abortion, infertility and other reproductive failures have been reported in infected queens;[1] however, reproductive disorders have not been linked statistically with FIV infections. Some FIV-infected cats have experienced seizures, behavioral abnormalities and other neurologic disorders, usually in the terminal stages of disease.[1,28,30]

Infections with FIV can be differentiated from feline leukemia virus (FeLV) infections only by appropriate laboratory tests. In most circumstances, concurrent testing for both retroviruses is indicated. Certainly, cats with signs of immunosuppression

Feline Health Topics

A publication for veterinary professionals

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and cats entering multiple-cat facilities should be tested for both FIV and FeLV.

Diagnostic Tests for FIV

The diagnosis of FIV infection usually depends on the detection of FIV-specific antibodies in serum, plasma or whole blood. Tests to detect antibodies to FIV in saliva are also being developed. The presence of FIV antibodies correlates well with persistent FIV infection.[4] One common exception to the "antibody-positive, therefore virus-positive" scenario is the young kitten which is seropositive due to the presence of maternal antibody. Thus, FIV tests in kittens require careful interpretation.

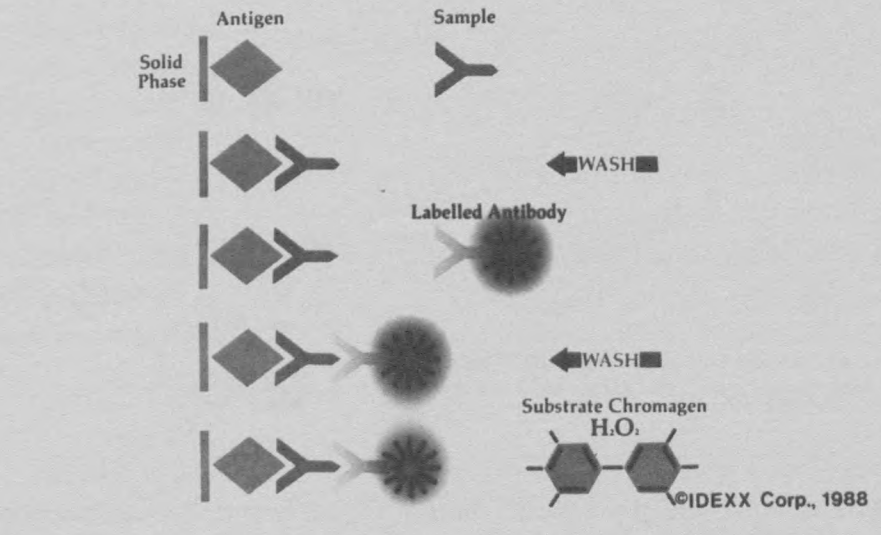

Figure 1. *Schematic of an Indirect Antibody ELISA format. FIV antigens are adhered to a solid phase (well, membrane, etc.), and a sample of whole blood, serum or plasma containing FIV- specific antibodies is added. After washing to remove nonspecific antibodies, a second antibody (against cat IgG) labeled with an enzyme is reacted with the FIV antigen-antibody complex. A color change occurs after the addition of the substrate/chromagen solution.*

Three basic techniques are employed in FIV antibody tests—the enzyme-linked immunosorbent assay (ELISA), the indirect immunofluorescence antibody assay (IFA), and the western blot or immunoblot assay.

The basic principle of all the ELISAs is detection of FIV antibodies in body fluids evidenced by a visible color change; however, the tests vary somewhat in format (microwell, barrel or probe). The tests employ whole virus antigens (IDEXX tests) or a synthetic FIV-specific peptide (Synbiotics tests) as the substrate for FIV antibody detection (Figures 1 and 2).

The IFA uses FIV-infected cells as a substrate which binds to FIV-specific antibodies in serum or plasma. The antibodies are then labeled with an anti-cat IgG antibody conjugated to a fluorescent dye and detected by examination with a fluorescent microscope.

In the immunoblot technique, FIV-specific proteins from purified virions or virus-infected cell lysates are separated according to size by electrophoresis. The proteins are then transferred or "blotted" to a nitrocellulose membrane. FIV-specific antibodies in serum or plasma will bind to the individual viral proteins on the membrane. These antibodies are then labeled with an anti-cat IgG antibody, usually conjugated to an enzyme (such as horseradish peroxidase). This complex is detected by development with a substrate/chromagen solution (giving a color change at the site of the specific FIV protein) or by another system such as chemiluminescent autoradiography.

Testing Errors

Commercial ELISA tests (from IDEXX Corp. and Synbiotics) are available for use in veterinary clinics, hospitals, and diagnostic laboratories; some laboratories also offer IFA or immunoblot (western blot) assays for FIV antibodies.[27,31-33] In general, FIV ELISAs have similar sensitivites to the IFA and immunoblot procedures. However, false-positives

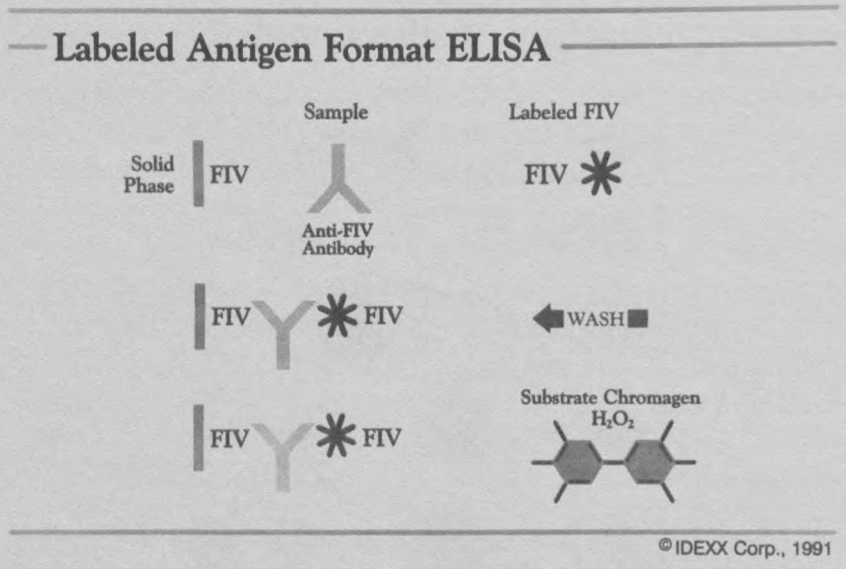

Figure 2. *Schematic of a Labeled Antigen ELISA format. FIV antigens are adhered to a solid phase (well, membrane, etc.), and a sample of whole blood, serum, plasma, or saliva containing FIV-specific antibodies is added. Additional FIV antigens labeled with an enzyme are reacted with the FIV antigen-antibody complexes. The bivalent anti-FIV antibody is then "sandwiched" by the FIV antigens. After washing, a substrate/chromagen solution is added, and a color change occurs if FIV-specific antibodies are present.*

(decreased specificities) with ELISAs may occur due to operator error (especially inadequate washing) or nonspecific reactivity with cell culture components. The likelihood of false-positive results increases when testing cats at low risk for FIV infection;[34] therefore, positive ELISA tests for FIV antibody should be confirmed by IFA or immunoblot, especially when the cat is asymptomatic or at low risk for infection, or when euthanasia of an FIV-positive cat may be considered. The presence of maternally-derived FIV antibodies in kittens less than 4 to 5 months of age results in positive tests with any of the antibody assays, even though the kittens are usually virus negative. Causes of false-negative FIV tests include insufficient levels of antibody in the sample tested (early infection or poor immune response), inadequate sensitivity of the assay, and operator error (incorrect sample preparation or interpretation of results).[32,35]

The commercial availability and convenience of the ELISA generally makes it a more useful test than the IFA or immunoblot, especially for smaller diagnostic laboratories and veterinary practices. In addition, the IFA and immunoblot are not without problems. The nonspecific reactivity observed in about 10% of feline sera with feline-origin substrate cells of the IFA presents an opportunity for a number of false-positive results in the absence of appropriate controls and careful evaluation. Even with these controls, intense nonspecific reactivity can obscure visualization of FIV-specific reactivity, leading to indeterminate or false-negative results.[31,32]

Although the immunoblot is used as the standard for testing, it is not always accurate. The FIV surface glycoprotein (gp120) is not detected in immunoblot procedures which use purified virus as the substrate, leading to false-negative results for sera con-taining antibody to this protein alone.[32] In addition, no standards have been established for preparation of antigen, immunoblot protocols or the interpretation of immunoblot results; therefore, interpretation depends upon the criteria established by the individual laboratories involved in FIV immunoblot testing.

Indeterminant or equivocal FIV test results may be obtained when antibody levels are near the limit of detection in an assay. High levels of background (nonspecific) reactivity also may result in indeterminant tests.[31,32] One limitation of the commercial ELISA tests is that the manufacturers do not acknowledge that some samples will be in this "gray area;" the tests must be interpreted as either positive or negative. To overcome this limitation, the New York State Diagnostic Laboratory has adapted a commercial microwell plate ELISA (PetChek FIV Antibody Test Kit, IDEXX Corp.) using a kinetics

ELISA interpretation which allows results to be reported as positive, negative or equivocal.[32]

Cats with indeterminant test results or discordant results should be retested in 6 to 8 weeks; most cats will have a clearly positive or negative result on retesting. Cats with a known, recent exposure (less than 3 months prior to testing) should also be retested if the initial result is negative. In addition, FIV antibody-positive kittens should be retested at 8 to 12 months of age to allow time for maternal antibodies to disappear and for active seroconversion to occur in any virus-positive kittens.

Circulating blood levels of free virus, viral antigen, and cell-associated virus are too low to be detected consistently in most FIV-infected cats.[35] Virus isolation and detection of FIV proviral DNA or messenger RNA by polymerase chain reaction (PCR) may be useful in diagnosis of FIV infection in cats with negative or indeterminant antibody tests. Virus isolation and PCR are not available as commercial assays but may be performed in some research facilities.[35]

Summary

FIV-related disease in domestic cats is similar in many respects to human immunodeficiency virus-induced AIDS; this similarity, and because veterinarians call FIV infection "feline AIDS," can be a source of intense emotional anxiety for the pet owner. Euthanasia of a positive cat may be elected by the owner based solely on a serologic diagnosis of FIV infection regardless of the cat's current health status, particularly in a cattery situation. In such cases, it becomes extremely important to know the limitations of available test procedures so appropriate follow-up testing can be performed if necessary to detect false-positive results. In general, the available tests for detection of FIV antibody perform well and are very useful as diagnostic tools. *However, despite some manufacturers' claims, no test is 100% accurate at all times and under all conditions; therefore, critical decisions about patient care should never be based solely on a single test result.* ■

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Research Briefs

Comparison of Kittens Fed Queen's Milk with those Fed Milk Replacers

Clinicians at Angell Memorial Animal Hospital in Boston, Massachusetts compared 15 two-week-old kittens fed queen's milk and milk replacers. The kittens were randomly assigned to one of three treatment group for four weeks—queen's milk, commercial kitten milk replacer (CMR) and an experimental milk replacer (EXP).

Kittens fed queen's milk suckled ad libitum, whereas CMR- and EXP-fed kittens were tube-fed every six hours. The kittens were weaned at six weeks of age and were fed a feline growth diet ad libitum for an additional four weeks. They were examined at 2, 4, 6, 8 and 10 weeks of age. Tests included an ophthalmic examination and blood sample collection for complete blood count, serum biochemical and amino acid analyses.

Kittens fed CMR and EXP diets had weight gain greater than that for queen's milk-fed kittens. The kittens fed CMR, however, had diarrhea throughout most of the milk-feeding trial and developed diffuse anterior and posterior lens opacification and vacuolation at the posterior Y-sutures. The lens opacities noticed in the kittens during the milk treatments resolved to a residual perinuclear halo, and a few incipient cortical opacities were observed by the end of the growth diet-feeding period. Serum arginine concentration was significantly lower in the CMR-fed kittens, but was not different during the growth diet-feeding period.

The researchers concluded that the EXP diet supported normal growth in 2- to 6-week-old kittens; CMR supported normal kitten growth rate, but resulted in diarrhea and cataract formation; and serum amino acid data indicated that low arginine concentration may have been related to the CMR-induced cataract formation.—*(Resource: Amer J Vet Res 54:901-907, 1993)*

Prevalence of Mycoplasmal and Ureaplasmal Recovery from Traceobronchial Lavages and of Mycoplasmal Recovery from Pharyngeal Swab Specimens in Cats with or Without Pulmonary-disease

(This study was done at Cornell University and partially funded by the Cornell Feline Health Center through the Veterinary College's Consolidated Research Grant Program.) Drs. Randolph, Moise, Scarlett, Shin, and Blue determined the prevalence of mycoplasmal and ureaplasmal recovery from tracheobronchial lavage specimens and the prevalence of mycoplasmal recovery from pharyngeal swab specimens from cats with (28) or without (18) pulmonary disease.

Mycoplasmas were recovered from tracheobronchial lavage specimens in 21% of cats with pulmonary disease, but in no cats without pulmonary disease. Mycoplasmal recovery from tracheobronchial lavage specimens was not significantly associated with concurrent Pasteurella spp isolation, septic inflammation, or bronchitis. Ureaplasmas were only isolated from a tracheobronchial lavage specimen in one cat with pulmonary disease and in no cats without pulmonary disease. Similar mycoplasmal recovery rates were found for pharyngeal swab specimens from cats with (39%) or without (35%) pulmonary disease.

Mycoplasmas are part of the normal pharyngeal flora in approximately a third of the feline population, but mycoplasmas are not normal inhabitants of the lower respiratory tract in cats. It is unknown whether mycoplasmas isolated from tracheobronchial lavage specimens in cats with pulmonary disease are primary pathogens or opportunistic invaders. Seemingly, ureaplasmas are seldom associated with pulmonary disease in cats, and are not normal inhabitants of the trachea and bronchi of cats.—*(Resource: Amer J Vet Res 54:897-900, 1993)* ■

Use of Human Ultralente and Beef Ultralente Insulin in Cats

Effective September 1, 1993, Eli Lilly and Company will discontinue production and distribution of its Ultralente Iletin I (extended insulin zinc suspension, USP, beef-pork) insulin. Since this is the most common long-acting insulin preparation used in the cat, the absence of this insulin will affect diabetic regulation in these animals. Acceptable long-acting insulins for the cat include Humulin U (Ultralente human insulin [rDNA origin], extended zinc suspension) supplied by Eli Lilly and Company (Indianapolis, Indiana) and Ultralente (beef) supplied by Novo Nordisk Pharmaceuticals, Inc. (Princeton, New Jersey).

Recent studies in normal cats suggest that important differences may exist between the absorption of human Ultralente and beef-pork Ultralente insulins. The human insulin appears better absorbed and, therefore, more potent than beef-pork insulin.[1] When switching to a more potent insulin (e.g., from beef-pork Ultralente to human Ultralente), consider reducing the dosage of insulin by at least 25%. With regard to beef Ultralente, insulin kinetic studies in a limited number of diabetic cats suggest that beef Ultralente is similar in potency and duration of action to human Ultralente.[2] In all cases where insulins have been switched, a glucose curve is recommended at one- to two-week intervals until regulation is achieved.—*(Resource: Feline Practice 21:2, [5], 1993)*

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