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# Infectious Diseases

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## FUNGAL AND RICKETTSIAL DISEASES

Jennifer Stokes

### FUNGAL DISEASES

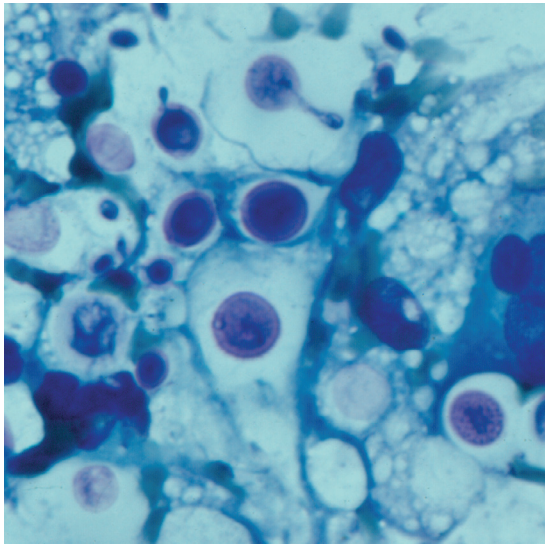
Systemic fungal infections are rarely documented in cats. Approximately 7 per 10,000 of the total population of animals presenting to veterinary teaching hospitals in North America are diagnosed with a systemic mycosis.<sup>10</sup> In most cases, dogs are the more susceptible species; the exception is cryptococcosis, which is 5 to 6 times more likely to be diagnosed in cats.<sup>42</sup> Cryptococcosis, histoplasmosis, coccidioidomycosis, blastomycosis, and sporotrichosis will be discussed separately, and recommendations for treatment will be described at the end of the section.

#### Cryptococcosis

Of the organisms causing systemic mycosis in cats, *Cryptococcus* is most commonly diagnosed. In the largest retrospective study evaluating deep mycotic infections in 571 cats, 46.1% of the infections were due to *Cryptococcus*.<sup>10</sup> The organism is round to ovoid in

shape, thin walled with diameter of 2.5 to 8  $\mu\text{m}$  (Figure 33-1).<sup>17,20</sup> In tissues, it is surrounded by a heteropolysaccharide capsule that varies in thickness depending on the strain and environment. The capsule provides resistance to desiccation and virulence.<sup>20</sup> *Cryptococcus* multiplies asexually with narrow-based budding. The infectious particle, the basidiospore, is adapted to be dispersed by air and has properties that allow it to adhere to and penetrate respiratory epithelium and cause infection.<sup>20</sup>

Typically, cryptococcosis in people and domestic animals is caused by one of two species: *Cryptococcus neoformans* (var. *neoformans* or var. *grubii*) or *C. gattii*.<sup>20</sup> Globally, *C. neoformans* var. *grubii* is the most common isolate associated with disease in people and animals. *C. neoformans* causes almost all cases in the United States and Europe.<sup>20,31</sup> Within the United States, the highest incidence of cryptococcosis in cats is reported in California, Florida, Virginia, and Iowa.<sup>10</sup> *C. gattii* most commonly causes disease in tropical and subtropical areas, including Australia, Papua New Guinea, Southeast Asia, and Central Africa; it has also been documented to cause infection in the temperate climate of the Pacific northwest, including Vancouver, Canada.<sup>37</sup> *C. albidus* was confirmed to cause one case of systemic disease in a cat in Japan but has not been recognized as a significant pathogen in cats.<sup>31</sup>

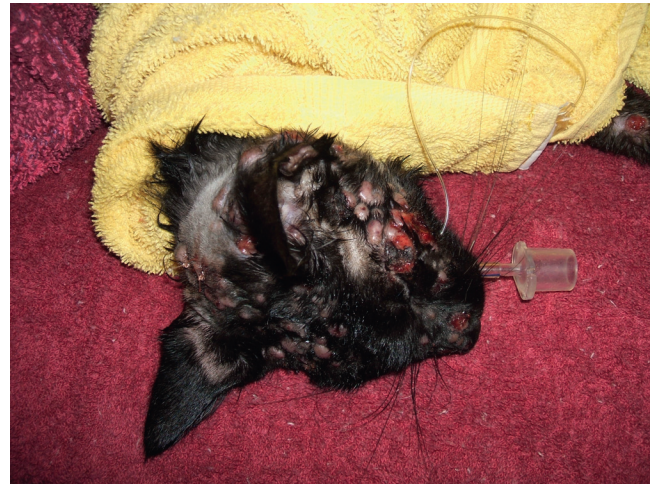


**FIGURE 33-1** Cytologic diagnosis of *Cryptococcus* showing encapsulated yeast forms in a Diff Quik-stained smear. (Courtesy Richard Malik. [Figure 61-2, B in Greene CE, editor: Infectious diseases of the dog and cat, ed 3, St Louis, 2006, Elsevier.]

*Cryptococcus* can be isolated from a variety of substances, depending on the geographic location of the organism as well as the species.<sup>42</sup> *C. neoformans* is consistently found in pigeon feces and soil enriched by avian feces and less often in milk, fermenting fruit juices, air, dust, wasp nests, grass, and insects.<sup>20,42</sup> *C. gattii* is found in hollows of *Eucalyptus* and fig trees in Australia and some fir trees in western Canada.<sup>20,42</sup> Risk factors for *C. gattii* infection in pets in Vancouver include proximity to logging sites or other areas of commercial soil disruption and owners hiking or visiting a botanical garden.<sup>14</sup> It is viable in feces for up to 2 years in moist environments.<sup>20</sup> Ultraviolet light and dry conditions can decrease viability.

The exact mode of transmission is unknown, but most likely occurs by inhalation of yeast cells or basidiospores.<sup>13,20</sup> Once inhaled, *Cryptococcus* lodges in the nasal passages and causes mycotic rhinitis; lower respiratory infection is uncommon because most organisms are larger than the alveolar diameter of 2  $\mu\text{m}$ .<sup>17</sup> Some strains are particularly virulent and will destroy adjacent facial bones and spread locally.<sup>20</sup> Rarely, cryptococcosis occurs secondary to a penetrating skin wound, causing localized infection.<sup>37</sup>

Clinical signs depend upon location of infection. Infection usually involves the nasal cavity, skin, subcutis, central nervous system (CNS), and regional lymph nodes. Dissemination has been documented (Figure 33-2). As mycotic rhinitis of the rostral nasal cavity occurs most often, sneezing, wheezing, and unilateral or bilateral nasal discharge are common presenting complaints. Respiratory signs have been reported in 26% to 83% of cats with cryptococcosis.<sup>12,39</sup> In another study, 63% of 263 cats had nasal discharge, and 12.5% had



**FIGURE 33-2** Disseminated *Cryptococcus* infection. (Courtesy Richard Malik.)



**FIGURE 33-3** Cryptococcal osteomyelitis may lead to facial deformity. (Courtesy Richard Malik.)

cough or dyspnea.<sup>10</sup> Thickening and inflammation of nasal mucosa or nasal granulomas may be visible. Osteomyelitis may occur, leading to facial deformity, including broadening of the nose or swelling of adjacent tissue (Figure 33-3). If the nasopharynx is affected, clinical signs may be absent until the infection spreads through cribriform plate and causes meningitis.<sup>38</sup> Alternatively, cats may present dyspneic or with stertorous breathing because of obstruction by fungal granulomas. Lymph node involvement was present in 39% of 263 cats.<sup>10</sup> Mycotic pneumonia and hilar lymphadenomegaly because of *Cryptococcus* is rare in cats.

Cutaneous nodules were documented in 41% of 263 cats with cryptococcosis (Figures 33-4 and 33-5).<sup>10</sup> Oral lesions occasionally occur in cats with *Cryptococcus* infection and may appear as diffuse ulceration of the oral mucosa of the tongue, gingiva, or palate, or as proliferative lesions.<sup>43</sup> Central nervous system involvement also occurs, either because of erosion of nasal infection through the cribriform plate or possibly by



**FIGURE 33-4** Cutaneous *Cryptococcus* nodule on the nasal planum. (Courtesy Jessica Baron.)



**FIGURE 33-5** Cutaneous *Cryptococcus* nodule on the inside of the pinna. (Courtesy Paige May.)

hematogenous spread. Neurologic signs occur in 8% to 26% of cats with cryptococcosis and may manifest as blindness, pupil changes, ataxia, depression, and temperament changes.<sup>10,12,41</sup>

Cryptococcosis has been diagnosed in cats less than 1 month of age and in those greater than 15 years of age. The mean age at diagnosis is about 6 years, with 58% of cats being between 2 to 7 years of age.<sup>10,17</sup> Some, but not all studies, have shown breed predisposition, with Abyssinian, Siamese, Birman, and Ragdoll cats being over-represented compared with domestic shorthairs.<sup>10,17,42</sup> Indoor as well as outdoor cats are susceptible to infection. A gender predisposition for cryptococcosis in male cats is inconsistently reported. Cryptococcosis occurs most commonly in immunocompromised people, but most studies of the infection in cats do not show an association with retrovirus infection or other causes of immunosuppression.<sup>20</sup>

### BOX 33-1

#### Methods for Diagnosis of Cryptococcosis in Cats

1. Fungal culture
2. Cytology
  - a. Nasal swab or wash
  - b. Biopsy or aspiration samples
  - c. Vitreous or subretinal fluid
3. Serology: antigen latex agglutination
  - a. Serum
  - b. Cerebrospinal fluid
  - c. Vitreous fluid

A definitive diagnosis of cryptococcosis requires culturing the organism from infected tissue (Box 33-1). Both *C. neoformans* and *C. gattii* can be cultured from the nasal cavity of asymptomatic patients. Culture of most systemic fungal infections is laborious and poses a zoonotic hazard to laboratory staff. Culture of *Cryptococcus* on Abouraud dextrose agar may take up to 6 weeks to be evident.<sup>17</sup> In most cases, a presumptive diagnosis is made by cytologic evaluation. The organism may be detected from nasal swab samples, nasal washing, and nasal tissue biopsy imprint or from aspiration of other infected tissues. Rhinoscopy or advanced imaging may aid in diagnosis. If ocular involvement is present without evidence of disease elsewhere, vitreous or subretinal fluid may be aspirated for cytologic evaluation. Quik-Dip (Mercedes Medical, Sarasota, Fla.), Wright Giemsa, or new methylene blue stains enhance visualization of the thickly encapsulated, broad-based budding yeast cells. If cryptococcosis cannot be confirmed cytologically or histopathologically, then serology can be performed. The antigen latex agglutination test is highly specific and sensitive in detecting *Cryptococcus* capsular antigen in dogs. It can be performed on serum, cerebral spinal fluid, or vitreous fluid. The specificity and sensitivity has not been described in cats, but infected cats can have extremely high titers (>1:65,536).<sup>37</sup> Serial serologic testing can be used to assess response to treatment, and a favorable prognosis often accompanies a decrease in titer.

Overall, the prognosis for cats with cryptococcosis is good, if the disease is not severe, there is no CNS involvement, and treatment is of appropriate duration. Animals presenting with or progressing to CNS disease are 4 times more likely to die than those without CNS signs.<sup>12</sup> In one retrospective study of 59 cats from Sydney, Australia with cryptococcosis, 76% were successfully treated.<sup>41</sup> Determining the ideal duration for treatment can be difficult. It is typically recommended to treat for at least 1 month past clinical resolution, and sometimes therapy is needed for 9 months or longer. If fungal

granulomas are present in the nasal cavity or nasopharynx, debulking the abnormal tissue may aid in treatment. Itraconazole is the drug most commonly used for treatment of feline cryptococcosis, but other azoles as well as amphotericin B have been used successfully.<sup>37</sup>

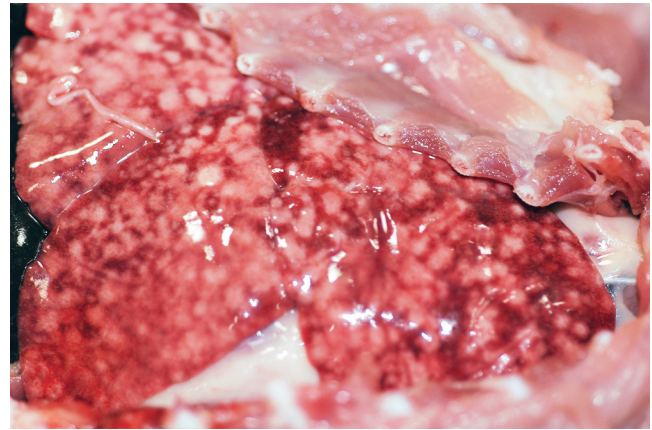
There is no evidence that cryptococcosis is either contagious or zoonotic, but pets may act as sentinels for people.

## Histoplasmosis

The second most common cause of systemic mycosis in cats is due to *Histoplasma*; 16.7% of 571 cats diagnosed with systemic fungal disease had histoplasmosis in one study.<sup>10</sup> *Histoplasma* has a global distribution. Pathogenic species include *H. capsulatum*, *H. duboisii*, and *H. farcininosum*. *H. capsulatum* causes infection in the continental United States, and *H. duboisii* is the causative agent in Africa. The organism thrives in warm (22° C to 29° C) and moist environments, particularly in temperate and subtropical areas.<sup>22</sup> *H. capsulatum* is most commonly isolated from moist, nitrogen-rich soil containing bird or bat feces.<sup>20,32</sup> In the environment, the organism exists as a mycelial form and within a host as a yeast.

*H. capsulatum* is most commonly diagnosed in North and South America, India, and southeastern Asia, although it has been documented in every continent with the exception of Antarctica.<sup>22</sup> *H. capsulatum* is endemic in the U.S. Midwest, South and areas along the Ohio, Mississippi, and Missouri rivers. It is sporadically reported elsewhere, including California, Ontario, Canada, and Australia.<sup>7</sup> Although reported in 31 states within the United States, the highest incidence is in Oklahoma, Texas, Virginia, and Louisiana.<sup>10,22</sup>

The life cycle of *H. capsulatum* is similar to other dimorphic fungi. The mycelial stage living in soil is resistant to environmental damage. It sporulates at temperatures around 22° C, and the spores are known as microconidia or macroconidia.<sup>22</sup> Lower respiratory infection most likely occurs because of inhalation of infective microconidia. Some cases of histoplasmosis are isolated to the gastrointestinal (GI) system, but an oral route of infection has not been confirmed.<sup>22</sup> At body temperature (37° C), the inhaled organism transforms into the yeast phase within the lungs, is phagocytized, and replicates intracellularly. Infection may be limited to the lower respiratory system and regional lymph nodes, or yeast-laden macrophages may disseminate the organism via lymphatics or hematogenously. In most patients, cell-mediated immunity is effective in controlling the infection; however, if a large number of organisms are inhaled or if immune compromise exists, severe disease may occur.<sup>22</sup> In patients with an effective immune system, dormant infections may be reactivated because of immunocompromise.



**FIGURE 33-6** Histoplasmosis pneumonia in a cat. (Courtesy Eric Snook.)

Unlike other systemic fungal infections, histoplasmosis occurs equally in dogs and cats.<sup>32</sup> Histoplasmosis has been diagnosed in cats less than 8 weeks of age to more than 15 years, and it occurs most commonly in cats less than 4 years of age.<sup>10,32</sup> The mean age at time of diagnosis is 3.9 years.<sup>7</sup> Both outdoor and exclusively indoor cats are at risk of infection.<sup>10,30</sup> No consistent gender bias is reported. Breed predilection is not consistently described, but Persian cats were predisposed in one report.<sup>10</sup> Although one retrospective study found that concurrent infection with feline leukemia virus (FeLV) was present in 15% of 96 cats with histoplasmosis, in most reports co-infection with feline retroviruses is uncommon.<sup>10,29,30,32</sup>

Although infection with *H. capsulatum* may be asymptomatic and self-limiting, dissemination is common in cats and may occur in up to 95% of cases, despite a lack of systemic clinical signs.<sup>10</sup> The organs most commonly affected include the lungs (Figure 33-6), GI tract, lymph nodes, spleen, liver, bone marrow, eyes, and adrenal glands. The incubation period is approximately 12 to 16 days in people and dogs and is likely the same for cats.<sup>10,22</sup> Clinical signs are often present for 2 to 3 months prior to presentation.<sup>29</sup> In 96 cats with histoplasmosis, the most common clinical signs, which occurred in 67% of infected cats, were nonspecific and included lethargy, weakness, and fever.<sup>10</sup> Respiratory signs were present in 39% of the cats and ocular in 24%. When pulmonary involvement is present, clinical signs may include abnormal lung sounds, tachypnea, or dyspnea. Ocular abnormalities included blepharitis, conjunctivitis, anterior uveitis, chorioretinitis, optic neuritis, and retinal detachment.<sup>10,22</sup> Lymphadenopathy and hepatosplenomegaly can occur with dissemination. If present, bone marrow involvement may lead to cytopathies.<sup>32</sup> Uncommon sites for infection include skin, bone, CNS, and oral cavity.<sup>33,53</sup> Specific GI signs occur less commonly in cats than in dogs. Oral infection may manifest as ulcerated tissue or proliferative lesions on the gingiva or palate.<sup>33</sup>

Routine laboratory tests are often abnormal in cats with histoplasmosis, but findings are not pathognomonic for infection. Anemia, thrombocytopenia, leukopenia, or pancytopenia may be present. The most common hematologic abnormality is normocytic, normochromic, nonregenerative anemia, which may be due to chronic inflammation, bone marrow involvement, and GI blood loss. Neutrophilic leukocytosis with monocytosis can also be diagnosed, or the leukogram may be normal. The organism can be seen in phagocytic cells on blood smears, and in a study of 56 cases, it was evident in 20% of the cats with histoplasmosis.<sup>10</sup> Other abnormalities reported include thrombocytopenia and severe pancytopenia.<sup>22</sup> Biochemical abnormalities have included hypoalbuminemia, hyperglobulinemia, elevated liver enzyme activity, hyperbilirubinemia, and hypercalcemia.<sup>32</sup> In one report, radiographic abnormalities were present in over 87% of cats with histoplasmosis, and the most common finding was a diffuse or nodular interstitial pattern in the lungs.<sup>10</sup> Hilar lymphadenomegaly was rare. Abdominal fluid, hepatomegaly, or splenomegaly may be present.

Definitive diagnosis of histoplasmosis requires identification of the organism cytologically or histologically. *Histoplasma* is usually present in clusters within cells of the mononuclear phagocyte system in the infected organs. When stained with Wright or Giemsa, *H. capsulatum* appears as a small (2 to 4  $\mu\text{m}$ ) round body with a basophilic center and lighter halo caused by shrinkage of the yeast during the staining process.<sup>22</sup> Diff-Quik can also be used to stain cytologic preparations. In cats, the organism is most commonly found by fine-needle aspirate cytology of infected organs, including lung, lymph node, dermal lesions, spleen, liver, or bone marrow. Organisms may be seen on evaluation of fluid collected by endotracheal wash, bronchoalveolar lavage, thoracocentesis, or cerebrospinal tap. Histopathologic abnormalities include granulomatous inflammation, but *H. capsulatum* may be difficult to see with routine staining. If fungal disease is suspected then special stains, such as periodic acid–Schiff (PAS), Gomoris methenamine silver, or Gidley fungal stain, should be requested. Immunostaining has been used to diagnose histoplasmosis in skin biopsy samples.<sup>22</sup>

Diagnosis of histoplasmosis by culture of the organism is rarely performed because of the zoonotic risk for laboratory personnel; it can also take up to 4 weeks for results to be available. Serologic testing is unreliable and false negatives are common in animals with clinical disease, and false-positive results may occur in previously infected patients with residual antibodies.<sup>22</sup> MiraVista Diagnostics (Indianapolis, Ind., [www.miravistalabs.com](http://www.miravistalabs.com)) has developed a test that can detect *H. capsulatum* antigens in urine, serum, or cerebrospinal fluid (CSF); this has been used to diagnose histoplasmosis in people. Sensitivity is increased if both

urine and serum is tested. The test can be used to monitor response to treatment; titers decrease with effective therapy and increase with disease relapse. In people, cross reactivity occurs with blastomycosis, coccidioidomycosis, and penicilliosis. At the author's institution, the urine antigen test has been used for monitoring purposes in dogs confirmed to have histoplasmosis, based on cytologic diagnosis. The test is likely applicable in cats, but more research needs to be performed to determine sensitivity and specificity.

Although histoplasmosis may be self-limiting when isolated to the lungs, treatment is recommended to avoid dissemination. As with most systemic fungal diseases diagnosed in cats, itraconazole is the medication of choice. Prognosis varies depending on the extent of disease. Of 56 cats in which the outcome was known in one study, 68% died or were euthanized.<sup>10</sup>

Prevention consists of avoiding exposure to soils likely to harbor *H. capsulatum*, including those contaminated by bird or bat feces. Histoplasmosis is not zoonotic.

### Coccidioidomycosis

Coccidioidomycosis was diagnosed in 9.2% of cats with systemic fungal disease in one study.<sup>10</sup> *Coccidioides* is a dimorphic fungus that grows in soil as a mycelium. Mycelia germinate to form thick-walled, barrel-shaped, rectangular, multinucleate arthroconidia that are 2 to 4  $\mu\text{m}$  wide and 3 to 10  $\mu\text{m}$  in length.<sup>24</sup> Mycelium can persist in soil indefinitely and arthroconidia are environmentally resistant. When soil containing *Coccidioides* is disturbed, arthroconidia are released and dispersed.<sup>24</sup> They can germinate and produce new hyphae or serve as a source of infection.

*Coccidioides* is found in a specific ecologic location, known as the Lower Sonoran life zone.<sup>24</sup> This area includes the southwestern United States, Mexico, and Central and South America.<sup>21</sup> It is also known as valley fever. These regions have sandy, alkaline soil, and high temperatures, with the summer mean greater than 26.6° C and the winter mean 4° C to 12° C.<sup>24</sup> In addition, elevation and annual rainfall are low. During prolonged periods of dry, hot weather, *Coccidioides* survives below the soil as deep as 20 cm.<sup>24</sup> After rainfall, the organism replicates near the soil surface and releases large numbers of infective arthroconidia that disseminate.<sup>24</sup>

Infection is most common when the soil is dry and is disturbed, such as by dust storms, earthquakes, or crop harvesting, or following the rainy season.<sup>21</sup> *C. immitis* is the species found in California in the San Joaquin Valley, while *C. posadasii* is found in all other endemic areas.<sup>24</sup> Disease is most common in California, Arizona, and southwestern Texas and less commonly diagnosed in New Mexico, Nevada, and Utah. Infections outside of endemic areas are sporadic. In such cases, the individual

may have traveled to an endemic area and then had activation of dormant organisms years later.<sup>24</sup> In people, most infections are asymptomatic, with only 40% of people developing clinical signs.<sup>24</sup> This may be true in other species.

Coccidioidomycosis occurs primarily following inhalation of infective arthrospores, and less than 10 organisms can cause infection.<sup>21</sup> Uncommonly, infection will occur following inoculation of the organism into the skin. There is one case report of a veterinary assistant developing coccidioidomycosis after being bitten by an infected cat.<sup>16</sup> Rarely, there have been suspect cases of dogs becoming infected after contacting fomites contaminated with arthrospores.<sup>21</sup>

Once inhaled and spread to the alveoli, arthroconidia convert to spherules because of the higher temperature and increased carbon dioxide level.<sup>21,24</sup> Once the spherule matures, it eventually ruptures, releasing up to 300 endospores.<sup>24</sup> After inhalation, it takes approximately 3 days for the endospores to form, but clinical signs usually do not occur for 2 weeks.<sup>21</sup> It is thought that endospores are able to disseminate through blood and lymphatics to distant sites, and cats with disseminated disease may have no infection in the respiratory system.<sup>49</sup> When skin inoculation occurs, infection may be limited to the dermis or subcutis.

As is typical of fungal disease, cell-mediated immunity is much more effective in resolving infection with *Coccidioides* than is humoral immunity. Antibody formation typically occurs but is more useful as a diagnostic aid than in fighting the infection. Although people who recover from coccidioidomycosis are considered immune to reinfection, recurrence in dogs and cats is common. It is not known if this is due to premature discontinuation of treatment or to a lack of long-term immunity to *Coccidioides*.<sup>21</sup>

Clinical disease varies from subclinical to fatal; it is not known why some infected animals have self-limiting disease and others die despite treatment. Evaluation of necropsy reports from 1995 to 2005 from the Arizona Veterinary Diagnostic Laboratory found that in fatal coccidioidomycosis cases, 25% of the animals involved were cats. It is unclear if this finding is due to more severe disease in cats or if coccidioidomycosis is underdiagnosed antemortem in cats. Infection is most commonly reported in middle-aged cats, with no breed predisposition. There is also no correlation between coccidioidomycosis and feline retrovirus infection.<sup>21</sup>

Specific clinical manifestations depend on the site of infection and are extremely varied, making early diagnosis a challenge. *Coccidioides* appears to be able to infect most tissues. Fever is commonly present at diagnosis, as well as nonspecific signs, including lethargy, anorexia, and weight loss. Respiratory signs are uncommonly recognized, and lung and bone involvement occurs less frequently in cats than in dogs. Ocular lesions, including

chorioretinitis and anterior uveitis, occur with similar frequency among cats and dogs.<sup>24</sup> In cats, approximately 50% of cases are disseminated.<sup>21</sup> Subclinical infection may occur in up to 70% of dogs; it is unknown if this is true in cats.<sup>21</sup>

Coughing is uncommon, but 25% of cats may present dyspneic. The CNS can be infected, and granulomatous mass lesions in the brain are more common than fungal meningitis. Ocular signs may include anterior uveitis, subretinal granulomas, retinal detachment, and blindness. There is also a report of cats that presented for periocular swellings with systemic signs, including weight loss, unkempt hair coat, and lethargy. Clinical ophthalmologic abnormalities were bilateral in each cat and included hyperemia, conjunctival masses, fluid-filled periorbital swellings, granulomatous chorioretinitis, nonhematogenous retinal detachments, and anterior uveitis.<sup>51</sup> Cats were diagnosed with coccidioidomycosis using a combination of clinical findings, serology, and, in two cases, visualization of *Coccidioides* spherules by either aspiration cytology or biopsy. Active anterior uveitis and periocular swelling resolved with treatment. Chorioretinal granulomas, although persistent, significantly decreased in size.<sup>51</sup>

Infection is often limited to the lungs and perihilar lymph nodes, although dissemination of the organism through the blood and lymphatics can occur.<sup>21</sup> When dissemination occurs, the skin is the most frequent site of infection. In 15 cats with coccidioidomycosis that underwent post-mortem evaluation, all had multiorgan involvement.<sup>21</sup> Nonhealing cutaneous lesions, including abscesses, dermatitis, chronic draining tracts, and ulcerations are the most common clinical manifestations.<sup>21</sup> Cutaneous and subcutaneous lesions were reported in 56% of cats in one series of 48 cases.<sup>49</sup>

In infected cats, laboratory abnormalities have included nonregenerative anemia, neutrophilic leukocytosis with left shift, monocytosis, eosinophilia, hypoalbuminemia, and hyperglobulinemia. Sensitivity and specificity of serology in cats is unknown. In the report of 48 cats with coccidioidomycosis, of the 39 cats undergoing testing, all were seropositive at some point during their illness.

Cytologic confirmation may be made by evaluation of aspirates of affected lymph nodes, skin lesions, or lungs. The organism can be seen on unstained slides and appears as a large (10 to 80  $\mu\text{m}$ ) round, double-walled structure containing endospores.<sup>24</sup> Multiple biopsy samples may need to be collected and evaluated in order to see the organism histologically. Although spherules can be detected with routine hematoxylin and eosin stain (H&E), they are easier to see when PAS or Grocott-Gomori methenamine silver stain is used. Commercial laboratories that practice biosafety precautions can isolate *Coccidioides* on culture. The mycelia that grow on culture media are highly

infectious. Serologic testing using tube precipitin and complement fixation techniques were useful in 48 cats with coccidioidomycosis.<sup>24</sup>

Itraconazole is typically used for treatment of feline coccidioidomycosis. Of 53 cats diagnosed with coccidioidomycosis, 67% survived with treatment.<sup>10</sup>

## Blastomycosis

*Blastomyces dermatitidis* is the saprophytic dimorphic fungus that causes blastomycosis. It exists in a mycelial form and reproduces sexually, producing infective spores. At body temperature, the spores transform into yeast and replicate asexually. Budding yeasts are 5 to 20 µm in diameter and have a thick, refractile, double-contoured cell wall.<sup>36</sup> Dogs and people are the species infected most commonly, but blastomycosis has been reported in other animals, including bats, horses, sea lions, wolves, ferrets, and nondomestic cats.<sup>19,36</sup> It is rarely reported in domestic cats.

The most likely reservoir for *Blastomyces* is soil. Because normal soil organisms destroy most *Blastomyces* organisms, specific environmental conditions are needed for *Blastomyces* to survive. *Blastomyces* thrives in a sandy, acidic soil near water. It has also been isolated from decaying wood and vegetation, animal excrement, a beaver dam, and animal waste.<sup>19,36</sup> Even in endemic areas, blastomycosis occurs in geographically restricted foci. Living near water is a risk factor for blastomycosis in dogs. In people, disturbing soil is associated with infection, and precipitation may facilitate the release of infective spores. Most people and dogs are likely exposed to *Blastomyces* on their own property, because there has been documentation of repeated cases occurring at the same location despite occupation by multiple families.<sup>3</sup> The source of infection in cats is unclear. Many reported cases of feline blastomycosis have occurred in strictly indoor cats. One study analyzed 60 environmental samples obtained from four homes in which blastomycosis was diagnosed in cats, and all were negative for *B. dermatitidis*.<sup>3,19</sup>

During a 10-year period, eight cases of blastomycosis were diagnosed in cats at the veterinary teaching hospital (VTH) in Illinois, while during a span of 11 years, 5 cats were diagnosed with blastomycosis at the VTH in Tennessee.<sup>3,19</sup> The prevalence of canine blastomycosis in Tennessee between 1979 and 1989 was 1.2%, compared with less than 0.1% in cats.<sup>4</sup> Of 571 cats diagnosed with systemic mycosis, 41 (7.1%) were infected with *B. dermatitidis*.<sup>10</sup> *B. dermatitidis* mainly causes disease in the United States and Canada, but is endemic in Africa and India and has been documented in Europe and South America.<sup>6</sup> Within North America, endemic areas include the Mississippi, Missouri, and Ohio river valleys; the mid-Atlantic states; and the Canadian provinces of Manitoba, Ontario, and Quebec.<sup>36</sup> Blastomycosis has also

been diagnosed in New York, Wyoming, South Dakota, Colorado, and Saskatchewan.<sup>6,27,36</sup> In a retrospective review of 41 cats diagnosed with blastomycosis, most cases occurred in Oklahoma, Tennessee, and Wisconsin.<sup>10</sup> Risk factors and the epidemiology of blastomycosis are unknown in cats because of its rarity.

Infection occurs most commonly by inhalation of infective spores, which establish infection within the lungs. Direct inoculation of the organism through skin puncture may occur. Blastomycosis is thought to disseminate through lymphatics and hematogenously. There is great variation in host response to infection, because dogs are 10 times more likely to develop blastomycosis than people; blastomycosis is rarer in cats. No seasonality has been reported in infected cats.

There are so few cases described in the literature that it is not possible to determine predisposing factors. Many reports provide conflicting data. Most cases described in the literature were diagnosed at necropsy.<sup>19</sup> Males may be slightly predisposed; one study reported that 69% of 36 infected cats were male.<sup>3</sup> Breeds described as being predisposed include Siamese, Abyssinian, and the Havana Brown, but this is not supported in all reports.<sup>10,19</sup> Affected cats have ranged in age from 6 months to 18 years.<sup>4</sup> The typical age of infected cats varies among publications: In three studies, 75% were less than 4 years of age, 42% were less than 4 years, and 87% of cats were greater than 7 years.<sup>19</sup> Duration of clinical signs prior to diagnosis has ranged from 3 days to 7 months.<sup>10,19</sup> A history of immunosuppression is rarely present; 10% of 41 cats with blastomycosis were FeLV positive, none were FIV positive, and one was positive for feline infectious peritonitis (FIP).<sup>10</sup>

Based on thoracic radiographs and necropsy data, infection in cats occurs most commonly in the lung, even though respiratory signs may be absent (Figure 33-7).<sup>19</sup> *B. dermatitidis* has been documented in lymph node, kidney, eye, CNS, skin, GI tract, pleura, peritoneum,

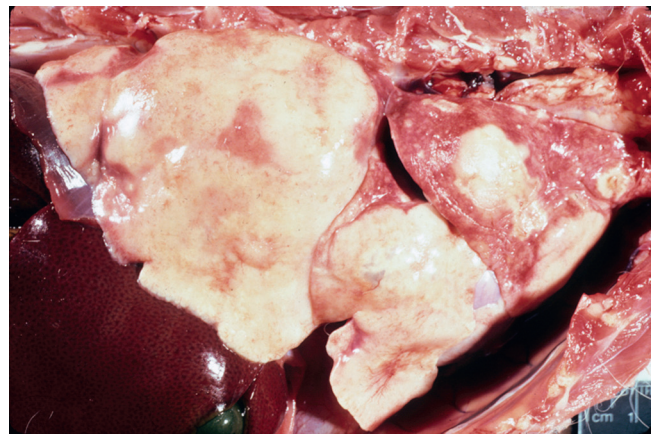


FIGURE 33-7 Pulmonary blastomycosis in a cat. (Courtesy Jennifer Stokes.)



heart, liver spleen, trachea, and adrenal glands.<sup>19,36</sup> Infection is frequently disseminated. Clinical signs vary depending on the site of infection. The most commonly reported clinical signs in cats with blastomycosis vary among publications, but dyspnea, lethargy, weight loss, and fever are frequently present.<sup>19</sup> Other reported respiratory signs have included cough, tachypnea, sneezing, and increased bronchovesicular signs.<sup>19</sup> Central nervous system, ocular, and dermatologic involvement may manifest clinically as well. Ocular changes described include retinal granulomas and detachment, chemosis, corneal edema, and uveitis.<sup>6,7,19</sup> Skin lesions may be draining tracts or nonulcerated dermal masses and range from a few millimeters to a few centimeters in diameter.<sup>19</sup>

Diagnosis of blastomycosis can be difficult in cats, particularly because there are no pathognomonic clinical manifestations. Hematologic and biochemical changes are neither specific nor consistent among infected cats but may include anemia, leukopenia or leukocytosis, monocytosis, hyperglobulinemia, hypoalbuminemia, and hypercalcemia.<sup>19</sup> Radiographic changes may include poorly defined soft tissue opacities with nodules or masses or alveolar lung consolidation and pleural effusion.<sup>10,19</sup>

Definitive diagnosis is made by cytologic or histologic identification of *B. dermatitidis*. Pyogranulomatous inflammation is commonly seen associated with large numbers of broad-based budding yeasts. Diagnosis has occurred by cytologic exam of fine-needle aspirate samples of infected skin, draining tracts, lymph nodes, and lung. Bronchoalveolar lavage can also be performed to diagnose pulmonary blastomycosis.<sup>19</sup> In dogs, the sensitivity and specificity of the agar-gel immunodiffusion test (AGID) was reported as 91% and 96%, respectively. The usefulness of AGID testing for blastomycosis in cats is unknown. Of three cats with blastomycosis tested using AGID, one was positive.<sup>10</sup> MiraVista Diagnostics offers an antigen test for *B. dermatitidis* that has been validated in dogs and has the greatest sensitivity when urine is tested.<sup>50</sup> It is unknown if this test is sensitive or specific in cats.

Currently, treatment with itraconazole is recommended.<sup>6</sup> The prognosis is guarded to poor. Of four cats treated for blastomycosis, three died within 12 days of diagnosis.<sup>3</sup>

### Sporotrichosis

Sporotrichosis is a mycotic disease of humans and many animal species caused by the dimorphic fungus *Sporothrix schenckii*, which is endemic worldwide. Zoonotic transmission of *S. schenckii* between cats and people has been documented and is considered an emerging zoonosis.<sup>11,45,48,54,55</sup> *S. schenckii* survives in the environment, typically in decaying vegetation, and people and animals

are infected by wound contamination or penetrating foreign bodies. The organism becomes pathogenic because of its dimorphic abilities. After entering the skin through a puncture, bite, or scratch, the fungus converts to a yeast phase. The organism has also been isolated from the nails and oral cavity of cats and presumably can be inoculated into bites, scratches, or puncture wounds.<sup>47,48</sup> Three clinical syndromes of sporotrichosis are known in cats:

1. Localized cutaneous
2. Lymphocutaneous
3. Multifocal disseminated

The localized and lymphocutaneous forms are the most common. Cutaneous lesions are most commonly found on the face, nasal planum (Figure 33-8), tail base, and legs and may be solitary or multiple. Lesions appear after an incubation period of about 1 month and first appear as draining puncture wounds mimicking bacterial fight-wound abscesses or cellulitis. Treatment with antibiotics does not result in resolution. The lesions may then become ulcerated and form large, crusted areas. The localized form may progress to the lymphocutaneous form, especially if not treated. In the lymphocutaneous form, cutaneous nodules may progress to draining ulcers of the skin, subcutis, and lymph nodes. The disseminated form is primarily found in the liver and lungs, but involvement of other organs has been documented.

Outbreaks of sporotrichosis are thought to be rare. In a large series of 347 cats with naturally acquired sporotrichosis in an epidemic in Rio de Janeiro, the median age was 2 years, and cats of male gender predominated.<sup>46</sup> Most cats were infected through fight wounds, and multiple skin lesions were common. Most lesions were on the head. The skin lesions were varied and included small crusted lesions, subcutaneous nodules that progressed to draining lesions and ulcers, extensive



**FIGURE 33-8** Sporotrichosis nodule on the nasal planum of a cat. (Courtesy Vic Menrath.)

exudative ulcers, and extensive zones of necrosis that exposed muscle and bone. More than 25% of cats had lymphangitis and regional lymphadenitis. The most common extracutaneous signs were respiratory signs, such as sneezing and dyspnea. Subclinical infections were also documented.

It does not appear that infection with FeLV or feline immunodeficiency virus (FIV) is a predisposing factor for sporotrichosis in cats.<sup>46,54</sup> Concurrent infection with FIV does not affect clinical outcome.<sup>46</sup>

Diagnosis of sporotrichosis in cats is most often by cytologic examination of exudates and aspirates from abscesses or nodules or impression smears from skin lesions. Smears stained with a Romanowsky-type stain typically contain large numbers of yeastlike organisms that are often cigar shaped but may appear as round budding shapes. Histopathology is not a reliable method of diagnosis; in two published case series, the organism was not present in more than 1 of 3 affected cats.<sup>9,46</sup> Failure to find the organism in biopsy specimens may be due to sampling early in infection or individual variation in immune response. Definitive diagnosis is by fungal culture of exudate from deep within a draining tract and/or macerated tissue samples.

The drug of choice for sporotrichosis in cats is oral itraconazole. Ketoconazole and sodium iodide have also been reported as effective treatments, but the rate of adverse effects is high.<sup>46,54</sup> Successful treatment of localized disease with a combination of oral itraconazole and intralesional amphotericin B has been described.<sup>25</sup> Secondary bacterial infections should be treated according to culture and sensitivity results for 4 to 8 weeks. Antifungal treatment should be continued for 1 month past resolution of clinical signs to prevent recurrence. Treatment may be required for months to over 1 year; therefore client compliance may be an obstacle to achieving cure even though the prognosis is good. People handling cats suspected or confirmed with sporotrichosis should wear gloves as well as wash their hands and arms with a disinfectant scrub.

## Antifungal Therapy

The best therapy for management of systemic fungal disease in cats is ultimately dependent on the individual patient. Preexisting medical conditions, site of fungal infection, and cost of therapy are factors to consider when choosing treatment (Table 33-1).

Amphotericin B is fungicidal and causes cell death by binding to ergosterol in the fungal cell membrane and disrupting membrane stability. It has a broad spectrum of efficacy against many fungal species and was initially the treatment of choice for systemic mycosis in people and animals. It has been proven to eliminate fungal meningitis.<sup>37</sup> Its potential for nephrotoxicity limits the total dose that may safely be administered to a patient, and its use in patients with compromised renal function is not recommended. Newer formulations are safer but more expensive. The three types of newer formulations of amphotericin B include a lipid mixture (Abelcet), a colloidal suspension (Amphotec), and a liposome-encapsulated form (AmBisome). The lipid complex is the least expensive and has been used the most in veterinary medicine.<sup>26</sup> It is 8 to 10 times less nephrotoxic than the original amphotericin B, when administered to healthy dogs.<sup>26</sup> The new formulations are taken up rapidly by the reticuloendothelial system, leading to the high drug levels in infected organs, including the liver, spleen, and lungs.<sup>26</sup> A higher cumulative dose of the new formulations may be administered without increasing risk of drug uptake by the kidneys and nephrotoxicity. The lipid-complexed amphotericin B has been used successfully in veterinary patients for treatment of cryptococcal meningitis, histoplasmosis, coccidioidomycosis, blastomycosis, and other systemic mycoses.

Indications for use of lipid complexed amphotericin B include cryptococcosis with CNS involvement, in mycotic infections that are severe or progressive and in cats that cannot tolerate oral administration of antifungal agents. An appropriate dose of Abelcet in cats is 1 mg/kg intravenously spanning 2 hours. Therapy is

**TABLE 33-1** Drugs for Treatment of Systemic Fungal Infections in Cats

Drug	Dose	Comments
Amphotericin B	Original formulation: 0.5 mg/kg, IV, 3 times/week Lipid mixture (Abelcet): 1 mg/kg, IV for 2 hours, 3 times/ week	Cumulative dose in cats should not exceed 4 to 6 mg/kg for the original formulation, and 12 mg/kg for the lipid mixture
Flucytosine (Ancobon)	50 mg/kg, PO, q8h	Used in combination with amphotericin B
Itraconazole (Sporanox)	10 mg/kg, PO, q24h	Administer capsules with food; administer liquid on empty stomach
Fluconazole (Diflucan)	30-50 mg/cat PO q12h 75 mg/cat PO q12-24h	

Note: Duration of treatment is difficult to determine but should be at least 1 month past clinical resolution.

administered 3 times weekly for an accumulative dose of 12 mg/kg.<sup>26</sup> If the original formulation of amphotericin B is used, a dose of 0.5 mg/kg IV 3 times weekly has been recommended.<sup>37</sup> Monitoring for changes in renal function, such as creatinine, blood urea nitrogen (BUN), and glucosuria, is indicated. Amphotericin B may also be effective as a fungicide by causing immunomodulation and activating macrophage uptake and killing of fungal organisms.<sup>26</sup> Amphotericin B combined with flucytosine may provide the greatest efficacy when treating cats with disseminated disease and/or CNS involvement. This combination is considered by some as the treatment of choice for feline cryptococcosis.<sup>37</sup>

Flucytosine is rarely used as sole therapy, but is combined with other antifungals to increase efficacy. It is synergistic when combined with amphotericin B and penetrates the blood–brain barrier. It has been associated with drug reactions in dogs, and its use may be limited to the first 10 to 14 days of treatment.<sup>37</sup>

Azole antifungals inhibit ergosterol biosynthesis, interfering with fungal membrane function.<sup>26</sup> One benefit of azole drugs is that they allow for treatment of patients without hospitalization. Itraconazole is considered the drug of choice for treatment of most cases of systemic mycoses that are not immediately life threatening in cats.<sup>26,37</sup> It does not easily cross the blood–brain, blood–eye, or blood–prostate barriers. Although it does not penetrate these organs well, it has been used successfully to treat fungal meningitis in cats. Such success may be due to a decrease in the blood–brain barrier associated with inflammation. It is more effective than ketoconazole and has fewer side effects. Side effects can include GI upset, hepatic disease with elevations in alanine aminotransferase activity, and rarely, cutaneous lesions resulting from vasculitis. The capsule formulation of itraconazole should be administered with food to increase absorption, while the liquid formulation should be given after a fast. It should not be administered with antacids. Itraconazole is typically dosed at 10 mg/kg PO q24h for treatment of cryptococcosis, histoplasmosis, coccidioidomycosis, and blastomycosis.<sup>6,22,37</sup>

Fluconazole is effective in treatment of systemic mycoses, particularly when there is involvement of the CNS, eye, or urinary system. It may be the most effective antifungal for treatment of feline cryptococcosis.<sup>37</sup> It has also been used in cats that cannot tolerate itraconazole or in which itraconazole is ineffective. Published doses include 30 to 50 mg/cat PO q12h and 75 mg/cat PO q12-24h.<sup>37</sup> Ketoconazole is not considered a drug of choice for management of feline fungal disease but has been used successfully in treatment of *C. gattii*. It has a higher rate of side effects and is less efficacious than itraconazole.

Newer azoles are on the market and are being used in veterinary medicine. Voriconazole is a derivative of fluconazole but has greater potency and a broader

spectrum of activity. It is highly bioavailable when administered orally and also comes in an IV formulation.<sup>26</sup> Posaconazole is an itraconazole analog. It has been used successfully in animal models for the treatment of systemic histoplasmosis and coccidioidomycosis, as well as cryptococcal meningitis.<sup>26</sup> There is little published data describing use of these newer azoles in cats.

In summary, itraconazole can be considered the first choice for treatment of systemic fungal disease in cats. Cats with severe, progressive, or immediately life-threatening disease may need amphotericin B therapy. When cats are suspected to have CNS involvement, flucytosine combined with amphotericin B may be the best treatment. Patients should be monitored regularly for drug toxicity and side effects. The ideal duration of treatment is unknown, but treatment for at least 1 month past clinical resolution is recommended; if the patient's owner can afford to treat for an additional month or two, that is recommended in order to decrease risk of recurrence. A decrease in antibody titers is often associated with effective treatment but does not always indicate cure. In the future, with newer diagnostic tests available, including the fungal antigen tests offered by MiraVista Diagnostics, we may be able to serially monitor antigen levels in infected cats and determine an appropriate time to discontinue antifungal therapy.

## RICKETTSIAL DISEASES

*Rickettsia* are obligate intracellular gram-negative bacteria that are transmitted by an arthropod vector, typically a tick. Their pathogenicity in people and dogs is well understood; in cats little is known currently. *Ehrlichia* organisms primarily infect leukocytes, while *Anaplasma* species typically infect erythrocytes, endothelial cells, platelets, as well as leukocytes. Reclassification of several rickettsial organisms within the families Rickettsiaceae and Anaplasmataceae (order Rickettsiales) occurred in 2001.<sup>40</sup> The genera *Ehrlichia* was moved from the family Rickettsiaceae to the family Anaplasmataceae, while the genera *Rickettsia* remains in the family Rickettsiaceae.<sup>40</sup> Within the genera *Ehrlichia*, *E. phagocytophila*, *E. equi*, and *E. platys* were moved to the genus *Anaplasma*, while *E. risticii* and *E. sennetsu* now belong to the genus *Neorickettsia*.<sup>40</sup>

Specific information about ehrlichiosis, anaplasmosis, and *Rickettsia felis* will be described subsequently. Cats appear less susceptible than dogs to common vector-borne diseases, including ehrlichiosis and anaplasmosis. There are several reports of ill cats presenting with clinical signs that are similar to the ones caused by rickettsial disease in dogs. Presumptive diagnosis of clinical rickettsial disease in cats has been based on appropriate clinical signs combined with the presence of morulae

(intracellular clusters of organisms), positive serology, positive polymerase chain reaction (PCR) analysis, and/or response to treatment with doxycycline.

Confirmation of rickettsial organisms as the causative agent of disease in cats is difficult. *Rickettsia* are difficult to culture, and morulae are infrequently present.<sup>35</sup> In addition, the presence of morulae that are *E. canis*-like, for example, does not confirm infection with the specific *Ehrlichia* species because the morulae may belong to another species or genera. Serology has been used to diagnose rickettsial infections, but there are limitations. Serologic techniques among diagnostic laboratories are not standardized. Because there may be yet undiscovered rickettsial species targeting cats, serology results may be negative despite clinical disease. There is serologic cross reactivity among some rickettsial organisms, making diagnosis of infection with a specific species difficult.

The use of molecular techniques including real-time PCR may increase detection of rickettsial pathogens in cats. PCR is sensitive and specific, particularly in the early phase of disease prior to antibody formation. It can be used to detect rickettsial DNA in blood, body fluids, bone marrow, and tissue samples. However, positive PCR results do not confirm an infection in the absence of clinical disease. Although PCR is highly sensitive, false-negative results can occur. If a rickettsial organism is harbored within a tissue, then PCR of blood samples would likely be negative. In addition, in infections with intermittent or brief bacteremia, negative results from testing of blood cannot rule out infection.

At this point, it is recommended that both PCR and serology be used to diagnose suspect rickettsial infections in cats. In addition, measurement of convalescent titers and serial examination of blood or tissue samples by PCR are likely to increase diagnostic efficiency. It is also important to recognize that one arthropod host may transmit multiple pathogens, leading to co-infection. This may explain the variation in clinical signs and response to therapy of cats suspected of having rickettsial disease. It is recommended that samples be screened for multiple organisms simultaneously in areas in which vectors for rickettsial disease are endemic.

Although the most effective therapy for treatment of feline rickettsial diseases is unknown, the American College of Veterinary Internal Medicine recommends that suspect ehrlichial cases be managed with doxycycline at a dose of 10 mg/kg/day for 28 days.<sup>35</sup> Treatment of other rickettsial infections with doxycycline is also appropriate.

## Ehrlichiosis

Vectors for *E. canis* include the ticks *Rhipicephalus sanguineus* and *Dermacentor variabilis*, and clinical ehrlichiosis in dogs has been well recognized and understood for

decades.<sup>40</sup> Although the first evidence of naturally transmitted ehrlichiosis occurring in cats was described in 1986, our understanding of the disease in cats and which *Ehrlichia* species are infective to cats is incomplete.<sup>35</sup> Evidence for feline ehrlichiosis includes cytologic identification of *E. canis*-like morulae on blood smears, positive *E. canis* serology, and PCR evidence of ehrlichial organism DNA in blood.<sup>35</sup>

Feline ehrlichiosis has been recognized globally, because blood from five cats in North America and France was positive for DNA most consistent with *E. canis*.<sup>35</sup> In addition, *Ehrlichia*-like morulae have been detected in peripheral leukocytes of cats in the United States, Kenya, France, Brazil, Sweden, and Thailand.<sup>5,35</sup> Serology has been used as a diagnostic tool for evaluation of feline ehrlichiosis; however, a limitation is that seropositivity does not equate with active infection. There is a lack of standardization in available methodologies, and variable serologic cross reactivity occurs among species of *Ehrlichia*, *Neorickettsia*, and *Anaplasma*.<sup>35</sup> Some cats with presumed ehrlichiosis test negative for *E. canis* antibodies but positive for *N. risticii*. Antibodies for *N. risticii* and *Ehrlichia* have been detected cats from Maryland, Virginia, California, and Colorado.

The pathogenesis of feline ehrlichiosis is thought to be similar to that of ehrlichiosis in dogs.<sup>35</sup> Clinical disease has been described in 55 cats with probable *E. canis*-morulae in mononuclear cells, *E. canis*-like DNA in blood, or seropositivity for *E. canis* +/- *N. risticii*.<sup>35</sup> Affected cats ranged from 1 to 14 years of age with no gender predisposition; most cats were domestic shorthairs.<sup>5,35</sup> Some cats had a history of tick infestation. Clinical signs included fever, anorexia, lethargy, weight loss, pallor, splenomegaly, lymphadenopathy, and anemia.<sup>35</sup> Clinicopathologic abnormalities included anemia (both regenerative and nonregenerative), hyperglobulinemia, hypoalbuminemia, and positive antinuclear antibody titers. Both leukocytosis and leukopenia were documented.<sup>35</sup> Some cats had radiographic evidence of interstitial lung disease.<sup>35</sup> Concurrent infection with *Mycoplasma haemofelis*, *M. haemominutum*, *Cryptococcus neoformans*, feline immunodeficiency virus, or feline leukemia virus were documented.<sup>35</sup>

Cats with suspect ehrlichiosis have been treated with doxycycline, tetracycline, or imidocarb.<sup>35</sup> In three cats, clinical resolution occurred with doxycycline therapy: 5 mg/kg PO q12h for 21 days. Five cats seropositive for *N. risticii* initially had clinical relapse after doxycycline therapy, but clinical resolution occurred after treatment with a higher dose: 10 mg/kg PO q12h for 21 days. Imidocarb dosed at 5 mg/kg IM administered as two injections 14 days apart was successful in treating two cats in Kenya.

The modes of transmission of feline ehrlichiosis are unknown, although vector transmission and spread through blood transfusion have been

documented. Prevention may be managed by minimizing a cat's exposure to vectors, administering monthly flea and tick preventatives, as well as by screening potential blood donors for rickettsial species.

Although people, dogs, and cats may develop ehrlichiosis, there is no evidence that the disease can be transmitted directly from cats to other species.

## Anaplasmosis

*Anaplasma phagocytophilum* is the causative agent of anaplasmosis in dogs and people, and there is evidence that cats can develop the disease after experimental inoculation as well as natural transmission. *Ixodes* tick species are vectors for transmission of *A. phagocytophilum* to dogs and are likely vectors for cats.<sup>2,34</sup> At this point, it is not known if other modes of transmission, such as the ingestion of or contact with *A. phagocytophilum*-infected rodents, occurs in cats.<sup>34</sup> In initial research studies, cats inoculated with *A. phagocytophilum* were found to have morulae in eosinophils but were asymptomatic.<sup>34</sup> In a subsequent study, when cats with and without FIV infection were inoculated, they developed clinical disease.<sup>34</sup>

Other evidence for the susceptibility of cats to anaplasmosis includes the detection of *A. phagocytophilum* DNA in the blood of naturally infected cats in Sweden, Denmark, Ireland, and the United States. Additionally *A. phagocytophilum*-like morulae have been detected in neutrophils of infected cats in Brazil, Kenya, and Italy.<sup>34</sup> *A. phagocytophilum* morulae have been confirmed in the neutrophils of Swedish cats.<sup>34</sup> Prevalence of *A. phagocytophilum* antibodies in 416 cats from six states in the United States was 4.3%, but blood samples were PCR negative for DNA from *Anaplasma* and *Ehrlichia* species.<sup>2</sup> In Florida, 553 cats were tested for *A. phagocytophilum* by PCR and all were negative.<sup>2</sup> At this time, it is not known if the prevalence of anaplasmosis is rare in cats or underdiagnosed because of limitations of current diagnostic tests.

The pathogenesis of feline anaplasmosis is likely similar to that in other species. The clinical manifestations of anaplasmosis in six cats diagnosed with infection, based on PCR documentation of *A. phagocytophilum* DNA with or without serologic evidence have been described.<sup>34</sup> Cats were 9 to 14 months of age, and both castrated males and spayed females were infected.<sup>34</sup> Cases occurred in Massachusetts, Connecticut, and Sweden.<sup>34</sup> Clinical abnormalities were most often mild and included fever, lethargy, anorexia, tachypnea, and the presence of *Ixodes* tick.<sup>34</sup>

Clinicopathologic abnormalities included thrombocytopenia, neutrophilia with left shift, lymphopenia, and mild hyperglycemia. All cats were FIV and FeLV negative. Morulae were detected in only one cat; 24% of its neutrophils were affected. Of the three cats in which *A. phagocytophilum* serology was performed at

presentation, two were seronegative and the third had a titer of greater than 1:640. Subsequently, the seronegative cats seroconverted, illustrating that negative serology at the time of initial clinical illness does not rule out anaplasmosis in cats. Titers for *A. phagocytophilum* increased, decreased, or fluctuated over time, so use of serology to confirm resolution of infection is not recommended. With treatment, five of the six cats became PCR negative within 15 to 139 days after diagnosis.<sup>34</sup> All cats were seronegative for *E. canis*.<sup>34</sup> Clinical disease in these 6 cats was milder than anaplasmosis in dogs; data from one study of cats experimentally co-infected with FIV and *A. phagocytophilum* suggests that immunocompromised cats may have more severe clinical disease.<sup>15</sup>

Although initial microbial therapy varied among the six affected cats, all were ultimately treated with tetracycline or doxycycline for 20 to 28 days. The dose of doxycycline administered was 5 to 10 mg/kg PO q12h and 22 mg/kg PO q8h for tetracycline. All cats had clinical improvement within 48 hours after administration of tetracycline or doxycycline.

## Rickettsia felis

The cat flea, *Ctenocephalides felis* is a reservoir and vector for *R. felis*, which is widely disseminated within tissues of the cat flea.<sup>1,44</sup> Naturally infected *C. felis* fleas have been found worldwide, although prevalence of infection based on detection of *R. felis* DNA using PCR varies.<sup>8,28,44,52</sup> In Italy, prevalence of *R. felis* in 320 cat fleas from 117 animals was 11.9%, while the prevalence was 9% in Germany.<sup>8,18</sup> In one study in the United States, the prevalence of *R. felis* DNA in 226 cat fleas from 103 animals was 9%, while in another study 67% of cat fleas collected from cats from Alabama, Maryland, and Texas were positive for *R. felis*.<sup>1,28</sup> *R. felis*-infected fleas have also been found in California, Florida, Georgia, Louisiana, New York, North Carolina, Oklahoma, and Tennessee. *Rickettsia felis* DNA has been found in two research cats exposed to fleas infected with *R. felis*.<sup>28</sup> Most cats exposed to *R. felis*-infected fleas do not develop antibodies. This data suggest that *R. felis* may not cause clinical disease in cats, bacteremia may be brief or intermittent, or the organism is harbored in tissues so that blood samples tested by PCR are negative.

Cats may be potential reservoirs for *Rickettsia felis* and a source of infection in people. The pathogenicity of *Rickettsia felis* in cats is poorly understood. Cats experimentally infected remained asymptomatic but seroconverted between 2 to 4 months.<sup>23</sup>

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## VIRAL DISEASES

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Viral infections of cats are common, especially in the young. Many of the viral agents affecting cats can cause serious, even lethal disease. Several cause lifelong infections and affected cats are important sources in multicat settings. Most of the agents are very contagious, spreading easily from cat to cat. Additionally, some such as feline parvovirus and calicivirus, are quite hardy and may persist in the environment for weeks or months. Identification of the infecting agent is critical in these multicat settings in order to aid control and prevention. Vaccines to protect against several of these agents have been developed, some of which are considered core vaccine components. Like viral diseases in other species, very few antiviral chemotherapeutics are available for treatment. However, the repertoire of efficacious drugs is increasing as more research is performed. This chapter describes the most common viral agents of concern in cats.

### FELINE HERPESVIRUS-1

Feline herpesvirus-1 (FHV-1) is the agent of viral rhinotracheitis and is a common respiratory pathogen of cats. An Alphaherpesvirinae subfamily member of the Herpesviridae family, the virus is a double-stranded DNA virus with an icosahedral protein capsid and a lipid envelope containing several viral glycoproteins. As a DNA virus, the mutation rate of herpesviruses is relatively low; thus antigenic variation among FHV-1 strains is not a major concern. The lipid membrane encasing the virion is derived from the infected cell, and contributes to the virus' ability to survive desiccation, making it an efficient respiratory pathogen. However, it also contributes to the virus' lability in the environment; it survives up to 18 hours in a damp environment (less in dry conditions) after shedding onto inanimate objects and is unstable as an aerosol.<sup>87</sup> In addition, it is easily inactivated by any detergent or soap.

### Transmission and Pathogenesis

Most cats become infected with feline herpesvirus as kittens. Direct contact with an infected cat is the most efficient mode of transmission, but spread by aerosolized droplets over short distances or by indirect contact with contaminated objects is also important. Unlike herpesviruses of other animal species, feline herpesvirus primarily targets epithelia of the upper respiratory tract and conjunctiva and only rarely spreads beyond these tissues to cause systemic disease. Virus replication in these cells results in cell death (cytolysis) and loss. This may manifest as ulceration, necrosis, and inflammation in the oronasal and pharyngeal tissue. In the conjunctiva, epithelial necrosis may also occur, with serosanguinous to purulent discharge, which may be profuse. In severe cases, erosion to the bone may occur in the nasal cavity from rhinitis, and the resultant distortion of bone and cartilage may lead to chronic rhinosinusitis (cats known as "snufflers").

In a manner similar to all herpesviruses, FHV-1 enters a latent state in innervating sensory nerves after acute infection. In cats, this most commonly occurs in the trigeminal ganglion, and is estimated to occur in about 80% of infections.<sup>88</sup> From this latent state, the virus can be reactivated, especially during stressful episodes, leading to replication in the epithelia, virus shedding, and in a minority of cats, disease. Termed recrudescence, it can be stimulated by any stressor, including trauma, concurrent disease, parturition, boarding, or changes in social hierarchy. Recrudescence episodes are often asymptomatic, and may be an important mechanism of maintaining the virus in a population. As new, immunologically naïve kittens are introduced, whether by birth (e.g., breeding cattery) or intake (e.g., shelter setting), asymptomatic shedders may expose them to the virus.

### Clinical Signs

The typical presentation of FHV infection is that of upper respiratory tract disease (see also Chapter 30): sneezing, nasal and/or ocular discharge, fever, depression, and decreased appetite following an incubation period of 2 to 6 days.<sup>13</sup> Conjunctivitis is not uncommon, and can progress to severe hyperemia and chemosis, with mucopurulent ocular discharge (see also Chapter 29). Infection may lead to corneal ulceration because of the viral damage of the corneal epithelium. In fact, FHV-1 is believed to be the most common cause of feline ocular disease, and corneal ulceration in a cat should be assumed to be a consequence of FHV-1 infection until proven otherwise.<sup>110</sup> This may manifest as a typical dendritic ulcer or may progress to involve the stroma, leading to a desmetocele.<sup>110</sup> Occasionally, cats may manifest with stromal keratitis; this uncommon manifestation is a consequence of the immune response to herpesvirus



**FIGURE 33-9** Herpesvirus may cause an ulcerative dermatitis that may be multifocal, often involving the face or planum nasale.

antigen rather than direct destruction by the virus itself. The corneal stroma becomes infiltrated with mononuclear white blood cells, primarily lymphocytes, which may lead to blindness.<sup>110</sup>

Less common manifestations of FHV-1 are ulcerative dermatitis and stomatitis. Ulcerative dermatitis may be multifocal, often involving the face or planum nasale, but may involve other areas of the skin (Figure 33-9). Affected cats may not have concurrent or historical evidence of respiratory infection.<sup>161</sup> Because lesions may involve eosinophils in addition to neutrophils, and intranuclear viral inclusions may not always be found, misdiagnosis as eosinophilic granuloma complex is a concern.<sup>161</sup> Cases of stomatitis are also relatively uncommon and may involve the soft palate and tongue.<sup>109,161</sup> An association with chronic gingivostomatitis has not been found.<sup>238</sup>

## Diagnosis

Diagnostic testing for FHV-1 infection primarily involves virus detection, because most cats are seropositive from either natural exposure or vaccination. Prevalence rates for seropositive status may be as high as 97%.<sup>180</sup> In addition, studies have shown that the magnitude of the FHV-1-specific antibody levels does not necessarily correlate with presence of either acute or chronic FHV-1 infection.<sup>180</sup>

Diagnosis of the classic presentation of upper respiratory tract disease in kittenhood is relatively straightforward. Methods for viral detection include virus isolation, viral antigen detection, and detection of viral genetic material. With ocular involvement, conjunctival and/or corneal swabs, scrapings, or brushings are collected for testing. In addition, pharyngeal and/or nasal swabs should be collected from cats with upper respiratory tract disease.<sup>299</sup>

Virus isolation is the gold standard because it identifies actively replicating virus, but may have a turnaround time of several days to a week. Samples should be shipped chilled and preferably overnight to the testing laboratory. Virus isolation may be falsely negative with chronic herpesviral-induced disease. This is due to the presence of locally produced neutralizing antibodies on the mucosal surface, preventing viral replication in cell culture. In addition, virus may be isolated from clinically normal cats.<sup>277</sup> Viral antigen detection using immunofluorescence is fast and inexpensive; however, sensitivity is relatively low, especially in chronic infections. This testing is done on corneal, conjunctival, or oropharyngeal scrapings, and samples must be collected prior to fluorescein administration to avoid test interference.

Genetic detection using polymerase chain reaction (PCR) has become the most commonly used assay for virus detection. This assay, done on similar samples as those described above, amplifies viral genetic material through repeated rounds of DNA synthesis. The amplified viral material is generally identified using a probe (e.g., TaqMan real-time PCR). This technology has very high sensitivity and specificity, and does not require viable virus, unlike virus isolation. The exquisite sensitivity of PCR is a double-edged sword, however, because it may detect subclinical, recrudescing, and even latent infections; thus positive results must be interpreted carefully.<sup>110,284,291</sup> Studies have shown that, using PCR, FHV-1 may be detected in many clinically normal cats,<sup>178,276,291</sup> as well as in normal corneas.<sup>277</sup> In addition, it appears possible that PCR assays may detect vaccine virus as well as field strains.<sup>178</sup> Therefore an increase in test sensitivity does not necessarily equate with diagnostic sensitivity. Genetic detection by PCR may also be used to identify virus in skin lesions of ulcerative dermatitis resulting from FHV-1.<sup>161</sup> In addition, histopathology may identify viral inclusions, and immunohistochemistry can be used for viral detection in biopsy samples.<sup>109,161</sup>

## Treatment

Advancements have been made in the treatment of FHV infection in cats, and in fact, this is one agent for which specific antiviral medications are available. Although none are approved for veterinary use in the United States, some success has been achieved with their use. It is critical to remember, however, that human antiviral medications should not be used unless safety and efficacy have been proven in cats because some have proven to be highly toxic, even fatal to cats. Topical antivirals used in cases of FHV-1 ocular disease include trifluridine, vidaribine, and idoxuridine. These drugs are virostatic, and must be given often; thus owner compliance may be a challenge. Typically, recommendations are to apply these as many times as possible throughout the



day, usually every 4 to 6 hours being the maximum. Recently, topical instillation of a 0.5% cidofovir solution every 12 hours led to clinical improvement and decreased viral shedding in experimental FHV-1 infection.<sup>80</sup> Its usefulness in natural infection is being evaluated. The advantage to this medication is its less frequent administration.

Systemic nucleoside analogs developed for human herpesvirus infections have shown some efficacy against feline herpesvirus, at least in vitro. Toxic side effects have been reported with some, such as acyclovir, but others, such as ganciclovir, may prove to be useful clinically. Famciclovir has been shown to be effective for FHV-1-associated ocular disease, rhinosinusitis, and dermatitis in at least one study.<sup>184</sup> Clinical trials are required to optimize the dose and schedule.

Interferon (IFN; both human IFN-alpha and recombinant feline IFN-omega) has been used with some success, and has been shown to be efficacious in vitro.<sup>267</sup> To date, recombinant feline interferon omega (rFeIFN) is not available in North America. Effectiveness of rFeIFN in vivo for dermatitis associated with FHV-1 has been shown in at least one case report.<sup>105</sup>

L-lysine given orally inhibits herpesviral protein synthesis and restricts virus replication by antagonizing the growth promoting effect of arginine. In vitro, FHV-1 replication was significantly reduced when lysine was present in the growth medium.<sup>179</sup> It is optimal when used early in infection, or as a means to prevent recrudescence during stress, where it has been shown to reduce viral shedding in latently infected cats.<sup>181</sup> However, studies evaluating its usefulness in preventing upper respiratory tract disease (URTD) in multicat settings, such as shelters, have shown no positive effect from daily lysine supplementation.<sup>243</sup> In fact, one study actually found increased viral shedding and severity of signs of URTD in shelter cats fed lysine supplements.<sup>62</sup> In another study evaluating the effectiveness of dietary lysine supplementation in shelter cats with enzootic upper respiratory disease, mean disease scores were higher for cats fed the lysine-supplemented diet.<sup>183</sup> However, food intake (and therefore lysine intake) decreased when lysine was added to the diet. In addition, cats in the study were group housed and group fed so that individual lysine intake could not be monitored. Despite this, oral administration as a bolus (250 to 500 mg/cat/day) for acute infections and as a supplement for prophylaxis in cats with recurrent signs has been recommended.<sup>182</sup> Lysine administration to cats appears to be safe, though the effects of long-term administration on plasma arginine concentrations are not known.<sup>183</sup> In one study, plasma arginine concentrations declined in lysine-supplemented cats during a 52-day monitoring period, leading the authors to recommend monitoring of plasma arginine in cats receiving long-term lysine supplementation.<sup>183</sup>

In experiments, bovine lactoferrin has been shown to inhibit virus attachment and entry, and may eventually be available as an antiviral treatment for FHV-1.<sup>17</sup> An immune-enhancing probiotic, *Enterococcus faecium* SF68, used as a dietary supplement has been shown to reduce evidence of clinical disease associated with chronic infection.<sup>160</sup> Although the study size was small (12 cats), the findings warrant further clinical evaluation. At least one study has shown improvement in adult cats with chronic rhinitis by administration of liposomal complexes containing interleukin-2 (IL-2) DNA as an immunotherapeutic.<sup>298</sup> Another approach under investigation is the use of ribonucleic acid interference to inhibit FHV-1 replication.<sup>305</sup>

## Prevention and Control

Protection following recovery is not long-lived, and reinfections may occur. Antigenic variation is not a significant problem with feline herpesvirus; thus the antigenic coverage of available vaccines is adequate. Vaccines do not prevent infection or production of the carrier state. They do offer protection from disease, however, and FHV-1 is considered a core component of feline vaccines.<sup>50,249,284</sup> The nonadjuvanted modified live vaccines that contain FHV-1 in combination with other agents have been shown to be both efficacious and safe when administered as directed. In multicat situations where FHV-1 infection is endemic, intranasal vaccination may be used in kittens for early protection from clinical disease and decreased viral shedding.<sup>159</sup> In addition, response to intranasal vaccination is not affected by the presence of maternal antibody. More information on FHV-1 vaccination is found in Chapter 8.

## FELINE CALICIVIRUS

Feline calicivirus (FCV) is a highly contagious respiratory pathogen of cats. In addition to the classic respiratory disease, FCV is associated with several other disease syndromes, including polyarthritis, gingivostomatitis, and systemic vasculitis. The virus is classified as a *Vesivirus* in the family *Caliciviridae*. It is a small nonenveloped virus, making it very hardy in the environment, and it is easily spread by fomites, including pet owners and hospital staff.<sup>133</sup> The viral genome is single-stranded RNA, giving it a significant mutation rate, much higher than that of FHV-1. This may lead to changes in antigenicity (many strains that vary antigenically exist) as well as virulence.

The gene encoding the capsomer protein, the major structural protein, has variable regions that distinguish strains of FCV. These regions also contain important immunologic epitopes; thus antigenic variability among strains is common, and has an impact on vaccine



**FIGURE 33-10** The most common lesion associated with feline calicivirus infection is oral ulceration, commonly on the margins of the tongue.

efficacy. For most vaccines, there is sufficient antigenic overlap to allow cross protection to heterologous strains following immunization with one strain of FCV, but protection against all field strains may not be equal. Genetic variability may also have an impact on disease phenotype, but does not segregate with antigenicity; that is, differences in disease manifestations do not correlate with differences in antigenicity. This, too, has an impact on vaccine design and development.

### Transmission and Pathogenesis

FCV is shed in secretions from the oropharynx, conjunctiva, and nose. Transmission is most efficient by direct cat-to-cat contact and by fomites. Aerosol transmission is less important, because sneezed macrodroplets do not travel far (less than 4 feet). A major source of infection is asymptomatic carrier cats that shed virus continuously. Unlike FHV-1, FCV shedding is not influenced by stress. There is a high prevalence of FCV in healthy cats (up to 24%, depending on the assay). Carrier cats may shed for months to years (even lifelong),<sup>302</sup> although one study showed that 50% of infected cats ceased shedding within 75 days.<sup>86</sup> Long-term analysis of FCV shedding patterns in five naturally infected colonies revealed three distinct patterns of shedding in individuals: cats that shed virus consistently, cats that shed virus intermittently, and cats that never shed virus.<sup>45</sup> Re-infection after recovery is possible.

Feline calicivirus primarily targets epithelia of the upper respiratory tract, oral cavity, and conjunctiva. Unlike FHV-1, it is not associated with corneal infection and ulceration. The most common lesion associated with FCV infection is oral ulceration. In general, clinical signs begin as vesicular lesions in the mouth, and are commonly seen on margins of the tongue (Figure 33-10).<sup>239</sup>



**FIGURE 33-11** Epithelial necrosis associated with virulent systemic calicivirus infection occurs in skin as well as mucous membranes, leading to ulceration that often involves the ears (A), face (B), and paws. (Courtesy Dr. Patricia Pesavento, University of California, Davis, Calif.)

As the overlying epithelia necroses, the lesions ulcerate and become inflamed. Feline calicivirus may also target alveolar epithelia of the lower respiratory tract. Some strains appear to be quite pneumotropic, leading to severe interstitial pneumonia.

Infection with FCV also produces a transient viremia, leading to widespread distribution of the virus.<sup>239</sup> In most cases, this dissemination does not manifest clinically. Uncommonly, disease beyond the respiratory tract may occur. Lameness associated with acute synovitis may occur, and although the precise mechanism of disease remains unclear, viral antigen associated with joint macrophages has been identified.<sup>239</sup>

Rarely, a virulent systemic (VS-FCV) manifestation may occur, and may appear as an outbreak within a population.<sup>132,223,247,260</sup> This syndrome involves widespread vasculitis and multiorgan failure and has occurred in vaccinated animals.<sup>223</sup> Epithelial infection and necrosis occurs in skin as well as mucous membranes, leading to ulceration that often involves the ears, face, and paws (Figures 33-11).<sup>239</sup> The mortality rate of this syndrome is quite high.

The underlying pathogenesis of this virulent clinical manifestation appears to involve viral mutations leading to hypervirulence, though the precise mutation remains unknown. In each documented outbreak where data is available, the virulent strain seems to have appeared spontaneously by mutation from caliciviruses already present in the group. Each isolate has been genetically unique. VS-FCV isolates are not members of a single clade.<sup>212</sup> Instead, these mutant viruses are emerging from several different lineages intermixed with other field strain FCVs. In addition, the emergence of these variants seems to involve host and environmental conditions as well. Thus far, no common mutation has been identified; however, at least one report describes point mutations leading to an additional glycosylation site in the capsomer protein of some hemorrhagic isolates.<sup>2</sup> Interestingly, most of the outbreaks associated with this form of FCV have arisen in shelter or rescue situations. One theory is that in these settings, FCV infection may be endemic in the population; in these situations, rapidly replicating virus that can attain high titers in a relatively short period of time is selected for because of the immunity of the endemically infected population.<sup>239</sup> When introduced into a population, this rapidly replicating, “hot” variant may lead to systemic dissemination and disease.

Host parameters have also been speculated to play a role in VS-FCV cases. In particular, immunopathologic mechanisms may contribute to the disease production.<sup>74</sup> Local modulation of cytokine levels have been found associated with lesions, and may contribute to the vasculitis and increased vascular permeability seen.

## Clinical Signs

Clinical presentations with FCV infection can vary from mild upper respiratory tract disease to viral pneumonia to lethal systemic disease. The typical presentation is similar to FHV infection; though the ocular discharge generally remains serous, corneal ulcers do not occur and oral ulcers are common. Typically, cats present with vesicular and ulcerative lesions of the oral cavity that may also involve the lips, nares, and even paronychia skin. Sneezing, hypersalivation, serous ocular and nasal discharge, and fever are seen. Ocular lesions include conjunctival hyperemia, chemosis, and blepharospasm.<sup>133</sup> The majority of infections are mild and self-limiting. Acute lameness with joint and muscle pain may be seen in kittens associated with either FCV vaccine strains or field virus.<sup>219</sup> Affected cats may be febrile, and about 25% have oral ulceration. Clinical signs resolve quickly, usually within 72 to 96 hours.<sup>239</sup> Feline calicivirus has also been associated with chronic lymphoplasmacytic gingivostomatitis.<sup>61</sup> However, other pathogens and host factors likely also play a role in this syndrome.<sup>239</sup>

For virulent systemic FCV (VS-FCV) infection, disease is typically more severe in adults than kittens. Clinico-pathologic abnormalities associated with VS-FCV are generally nonspecific, such as neutrophilia, hyperglobulinemia, and elevated liver enzymes. Characteristic clinical signs that have been described include subcutaneous edema, particularly of the head and limbs, ulceration of pinna and footpads, and crusting lesions of the face, ears, and limbs.<sup>133,239</sup> In addition, signs such as jaundice, dyspnea, vomiting, and diarrhea, may be observed. However, mild or subclinical infections may also occur, and asymptomatic cats are able to transmit fatal disease.<sup>133</sup> Mortality rates have been reported as high as 60%.<sup>228</sup> On necropsy, affected cats commonly have hepatocellular necrosis, interstitial pneumonia, and fluid in body cavities.<sup>133,228</sup>

Persistent infections following recovery from acute disease are not uncommon. Unlike FHV, persistent FCV infections are not latent and shedding is continuous. These asymptomatic shedders are important sources of the virus in a population and may be the source of new variants. Infected cats may continue to shed the virus throughout their lifetime, but most shed for periods of weeks to a few months.

## Diagnosis

The presence of severe oral ulcerations is an important clinical indicator of FCV infection, even in cases of VS-FCV. Confirmation of a diagnosis of FCV, as with FHV, relies primarily on detection of the virus, because the majority of cats are seropositive for FCV. Viral identification is particularly important in multicat settings. Virus isolation is the gold standard, because it detects replicating virus. Virus can be isolated from conjunctival and nasal swabs, but the highest success rate is achieved with oropharyngeal swabs. Samples should be shipped cooled (e.g., with ice packs) overnight to the testing laboratory. Antigen detection on slides made from swabs of the same sites, as for virus isolation, can also be done but is generally of lower sensitivity. As with FHV, detection of FCV nucleic acid by PCR is being used more frequently for diagnosis and is done on the same samples as for virus isolation.<sup>3</sup> The drawback of PCR testing for FCV not observed with FHV is the genetic variation of FCV, potentially leading to false-negative results. At least one report has identified a highly conserved genetic region of the virus that can be targeted for detection of the majority of field strains.<sup>3,4</sup> However, it is possible that PCR assays may also detect vaccine virus in addition to field strains.<sup>157</sup> It is recommended that samples submitted for PCR NOT be frozen; refrigeration and shipment on an ice pack is suggested.<sup>157</sup>

None of the assays described can distinguish the virus of virulent systemic disease from those causing more classic disease; this classification is currently based on

clinical presentation. In cases of suspected VS-FCV, samples from the same sites as for typical presentations should be collected. In addition, tissue samples from those animals that die should be submitted for histopathology and immunohistochemistry or PCR. These samples should include parenchymal organs and ulcerated lesions (e.g., skin, foot pads, lingual areas).

Interpretation of positive results for each of these ante mortem assays must be done in light of the fact that asymptomatic carrier states are not uncommon; thus finding the virus in an ill cat does not necessarily prove causation of the current disease.

## Treatment

Treatment of FCV infection primarily involves symptomatic and supportive care. Fluids and nutritional support (e.g., esophagostomy or gastrostomy tube feeding) are important for anorectic cats, and oxygen therapy for dyspneic cats is critical. Broad-spectrum antibiotics should be used if bacterial infection is suspected. Recombinant feline interferon has demonstrated antiviral activity in vitro, but in vivo effectiveness is unclear.<sup>209</sup> Currently, no specific antiviral medication for FCV exists. A recent study showed efficacy of virus-specific compounds in blocking FCV replication in vivo.<sup>270</sup> This technology, which is referred to as phosphorodiamidate morpholino oligomer (PMO), uses virus-specific nucleic acid sequences that bind to viral RNA, preventing translation of viral proteins. In at least one study, it was safe and reduced disease development, virus shedding, and mortality.<sup>270</sup> As this technology is developed, a commercially produced medication may become available. For cats with VS-FCV, intensive care using parameters described above are needed, and corticosteroids for the immunopathologic component may be beneficial.<sup>133,239</sup> Oral interferon-alpha has also been used in these cases, though it is not clear if it contributed to survival.<sup>133</sup>

## Prevention and Control

Vaccination is the main means of control, and, as with FHV, prevents disease but not infection nor the carrier state. Calicivirus is considered a core component of feline vaccines.<sup>50,239,249</sup> Most vaccines contain a single strain, typically strain F9 or strain 225, and these strains have been shown to be broadly cross-reactive based on neutralization studies.<sup>234</sup> Traditional calicivirus vaccines, however, do not appear to be protective against virulent systemic disease. Manufacturers are investigating the utility and inclusion of additional strains in vaccines to increase the spectrum of protection. Newer vaccine strains appear to induce neutralizing antibodies against a higher proportion of calicivirus field strains.<sup>129,235</sup> A vaccine containing a virulent systemic strain is available,

although the ability of this vaccine to protect against future outbreaks of VS-FCV disease is unknown. A bivalent vaccine containing two strains with broad cross-antigenicity based on in vitro cross-neutralization evaluation was found to provide protection against heterologous strains.<sup>235</sup> This study validated the use of cross-neutralization tests to evaluate cross-protection of vaccine strains. It is important to bear in mind that inclusion of two or more strains isolated from different disease manifestations does not necessarily insure broad protection against the varied pathogenic phenotypes. Rather, neutralization assays are critical for assessing the protective spectrum of any new vaccine. It will be difficult to achieve a vaccine that provides protection against all strains in circulation because of the antigenic variability of FCV, and continued evaluation of prevalent strains and their antigenic relatedness to vaccine strains will be critical. In a clinical setting, if vaccine breakthroughs are occurring within a cat population, boosting with a different strain of FCV may enhance the protection of the population. More information on calicivirus vaccines is found in Chapter 8.

Environmental decontamination is also important for control in multicat situations, including veterinary clinics. Because of the environmental hardiness of the virus, detergent alone will not inactivate FCV. The virus can persist for days to weeks in the environment, and disinfection requires products with oxidizing activity, such as 5% sodium hypochlorite diluted 1:32 and potassium peroxydisulfate.<sup>239</sup> Quaternary ammonium products are not effective against FCV.<sup>69</sup> Thus decontamination following examination or housing of any cat with URT infection should include cleaning with a detergent to remove organic matter, followed by disinfection.<sup>133</sup> During outbreaks of virulent disease because of FCV, stringent quarantine measures and barrier nursing are required to prevent the spread of the virus. All affected and exposed cats should be strictly isolated, and if possible, treatment away from the veterinary hospital is ideal.<sup>239</sup> Additional and more detailed control measures can be found elsewhere.<sup>133,239</sup>

## INFLUENZA VIRUS

Influenza virus is an uncommon pathogen of cats, but several occurrences have been documented. The virus known as highly pathogenic avian influenza H5N1 (HPAI H5N1) was found to infect cats in 2004 in Southeast Asia, while the human pandemic strain of 2009 (H1N1) was transmitted from a human to a cat in 2009. Because of these occurrences, it is important that veterinarians understand the virus and its pathogenesis not only for patient care but, perhaps, even more importantly for communication with the public.

Influenza viruses are members of the Orthomyxoviridae family. These viruses are enveloped viruses with a genome of single-stranded RNA. The majority of influenza viruses are classified antigenically as type A (based on internal viral proteins). Subtypes of the virus are based on antigenicity of the two viral glycoproteins embedded in the viral envelope, the hemagglutinin (H) and neuraminidase (N) proteins, and are designated by numbers (H1-16; N1-9). In addition to antigenicity, the hemagglutinin also affects virulence of the virus, and certain subtypes are associated with more pathogenic strains, notably H5 and H7. Another important characteristic of this family is the segmentation of the genome, with each virus containing seven to eight separate genetic segments encoding individual viral proteins. This allows for a unique form of viral mutation called reassortment. As with all RNA viruses, the mutation rate of the genome is quite high, and generally manifests as small point mutations, which lead to relatively minor amino acid changes in the viral proteins. Reassortment involves the exchange of entire gene segments when one or more distinct influenza viruses infect the same cell. This is often the mechanism of significant changes in antigenicity, virulence, or host/tissue tropism of influenza viruses. For example, the 2009 pandemic H1N1 influenza is a quadruple reassortant, having gene segments from four distinct influenza viruses, two mammalian and two avian in origin.<sup>24</sup> Reassortment is often referred to as “antigenic shift,” reflecting the relatively large change in the viral genome, while “antigenic drift” refers to the smaller point mutations observed from season to season within a single strain.

The natural reservoirs of influenza viruses are birds, primarily waterfowl. The most common mammalian species affected are pigs, horses, and humans; more recently, dogs have experienced infection with a variant derived from equine influenza virus H3N8. Cats are only rarely infected with influenza virus.

In 1997, outbreaks of the HPAI H5N1 occurred in poultry in Southeast Asia. Subsequent spread of H5N1 strains in birds has occurred in Europe, the Middle East, and Africa. Sporadic cases of H5N1 in humans have also occurred with relatively high mortality. In 2004, the first report of H5N1 in domestic cats in Thailand was made by the World Health Organization.<sup>287</sup> Subsequent occurrences in domestic cats, as well as tigers and leopards, have been documented in Turkey, Iraq, China, Germany, and Austria.<sup>18</sup> In the majority of cases, exposures occurred from contact with infected poultry. HPAI H5N1 has not occurred in North or South America in either birds or mammals, to date.

In 2009, a new strain of H1N1 spread to humans causing a worldwide pandemic. This virus is a reassortant that has infected a number of species, including turkeys, ferrets, and swine. Infection of a domestic cat in the United States was documented in 2009, with

infection occurring from an infected person to the pet cat in the household.<sup>274</sup>

## Transmission and Pathogenesis

Oral infection of cats, particularly with avian influenza (e.g., by consumption of infected bird carcasses) can occur. In addition, aerosol and direct contact may be a means of transmission (i.e., spread of human H1N1 to cats by contact with infected owners). Finally, indirect contact, for example, with feces from birds infected with HPAI H5N1, may occur. Infections may be subclinical or may manifest with mild to severe disease ending in death. Factors such as dose of virus, strain virulence, and host factors may have an impact on the severity of disease. Prevalence of influenza infection in cats is very low, including in areas where HPAI H5N1 occurs, and cats are not believed to be important in maintenance or transmission of influenza virus for humans.<sup>187</sup> In fact, transmission of the virus from cats to humans has never been documented.<sup>186,187</sup> It is currently not known how well HPAI H5N1 can spread from cat to cat under natural conditions, although it has been shown to occur in experimental settings.<sup>152,296</sup>

Natural and experimental infections with HPAI H5N1 lead to viral replication in the upper respiratory and gastrointestinal tracts.<sup>285</sup> Spread to the lower respiratory tract with viral replication in type II pneumocytes may occur leading to alveolar damage.<sup>286</sup> This may manifest as severe pneumonia.<sup>186,286</sup> With HPAI H5N1, viremia may also occur, leading to spread to other tissues. In fact, in addition to pneumonia, hepatic necrosis is a common finding and contributes to the pathogenesis of this virus in cats.<sup>150</sup> In addition, neurologic disease associated with a nonsuppurative encephalitis has been found in natural infection with HPAI H5N1.<sup>285</sup> Infected cats may shed the virus in respiratory secretions and feces.<sup>186</sup>

## Clinical Signs

In general, the incubation period for influenza in cats is quite short, usually 2 to 3 days. Typical signs, whether HPAI H5N1 or human pandemic H1N1, are fever, decreased appetite and activity, and respiratory signs such as dyspnea.<sup>18,285</sup> Conjunctivitis may also be observed.<sup>285</sup> With HPAI H5N1, signs of systemic spread may include icterus, hemorrhagic lesions, and neurologic signs such as seizures and ataxia.<sup>285</sup>

## Diagnosis

Diagnosis may be established by virus detection or serology for virus-specific antibodies. For the former, virus isolation as well as genetic detection by PCR may be performed on oropharyngeal swabs or tissues post mortem.<sup>186,285</sup> In addition, immunohistochemistry for

viral antigen may be performed on tissues post mortem.<sup>285</sup>

## Treatment and Control

Treatment using human antiviral medication, such as oseltamivir (Tamiflu, Genentech, South San Francisco, Calif.) has not been clinically evaluated and is not recommended.<sup>18</sup> Supportive treatment, including oxygen therapy as needed, is generally all that is recommended. No vaccine is currently commercially available for any influenza of cats, although experimental vaccines have been designed. Control is by and large aimed at preventing exposure, including avoiding access to uncooked poultry.<sup>18,186</sup> Additional recommendations may be found elsewhere.<sup>18,186</sup>

## FELINE PANLEUKOPENIA

Feline panleukopenia is caused by feline parvovirus (FPV), and remains a significant disease of cats. In addition to FPV, the newer canine variants of canine parvovirus (CPV), specifically CPV-2a, CPV-2b, and CPV-2c reacquired the ability to replicate and cause disease in cats. All of these variants are closely related, sharing approximately 99% DNA homology. Parvoviruses are small nonenveloped viruses with a single-stranded DNA genome. A notorious property of the parvoviruses is their extreme hardiness in the environment. They are shed in feces of infected animals and may remain infectious in the environment for months, or even years, when protected by organic matter.<sup>101</sup>

Parvoviruses are unique among most DNA viruses in that they have a significant mutation rate, more similar to that of RNA viruses; thus mutations occur in circulating field virus. Feline parvovirus is in evolutionary stasis as compared with CPV.<sup>55</sup> Canine parvovirus-2 is believed to be ancestrally related to FPV. It emerged in 1978, and as it adapted to dogs, additional variants arose with relatively minor amino acid changes in the capsomer protein genes. The original CPV-2 is now believed to be extinct, and CPV-2b is the most prevalent variant in circulation. In recent years, additional variants have emerged, and differ from CPV-2b by just a few amino acid residues, with some leading to antigenic differences. The nomenclature of these variants is confusing and has led to the reporting of several distinct CPV-2c isolates. These variants have been identified in Asia, Europe, South America, and most recently, the United States.<sup>55,124,134</sup> One of these variants contains a mutation at amino acid residue 426 of the major capsid protein, an important antigenic epitope of CPV, leading to substitution of an aspartic acid residue with glutamic acid.<sup>56</sup> This mutant has been reported to be replacing CPV-2b in Italy and is present

in dogs in the United States. The disease associated with these newer variants appears to be similar to that seen with earlier strains, including vomiting, diarrhea that may be hemorrhagic, and leukopenia. The mortality rate thus far does not seem to be significantly different from that of previous isolates. Interestingly, the new canine variants that emerged from the original variant, CPV-2 (which lacked the ability to infect cats), have all reacquired the ability to infect and cause disease in cats.<sup>54,85,134</sup> This includes both CPV-2b and CPV-2c, which are currently the most prevalent variants in circulation. Thus when we discuss feline panleukopenia, it is important to bear in mind that the infecting agent may be either feline or canine in origin.

## Transmission and Pathogenesis

FPV is shed from all body secretions during active disease but is most consistently found in feces. Replication of the virus in intestinal epithelia leads to fecal shedding of the virus, which is at very high levels in the acute phase of disease ( $\geq 10^9$  TCID<sub>50</sub>/g). The period of viral shedding is usually only a few days, but recovered cats can shed virus in urine and feces for as long as 6 weeks.<sup>49</sup> Infection with FPV occurs through the oral cavity, where the virus initially replicates in local lymphoid tissue. From there, the virus disseminates via lymphatics and blood to many tissues. As discussed below, successful viral replication leading to cell lysis occurs only in those cells that are actively replicating. Destruction of intestinal crypt cells leads to blunting or complete loss of intestinal villi, while bone marrow infection leads to profound leukopenia. In addition, destruction of lymphoid tissues can contribute to the virus-induced immunodeficiency.

All parvoviruses share a tropism for cells of high mitotic index; that is, these viruses can only complete their replication cycle in cells that are rapidly dividing. With its small genomic coding capacity, much of the replication machinery for the virus must be provided by the infected cell. Parvoviruses, unlike larger DNA viruses, such as adenoviruses, have no ability to “push” cells into the cell cycle; thus cells must be actively dividing to support parvovirus replication. In kittens and adult cats, this includes cells of lymphoid tissue, blood cell precursors in the bone marrow, and intestinal crypt epithelia. In the neonate, this also includes tissues such as the cerebellum and myocardium. The virus may also target a wide variety of cells in the developing embryo or fetus, causing reproductive loss. The virus causes a lytic infection in target cells, leading to their destruction. The typical clinical presentation in kittens reflects the bone marrow and intestinal epithelia involvement. For the former, because of the shorter half-life of white blood cells compared with red blood cells, this destruction generally manifests as a severe leukopenia, though anemia

can occur. Anemia can also occur as a consequence of blood loss in the intestines.

### Clinical Signs

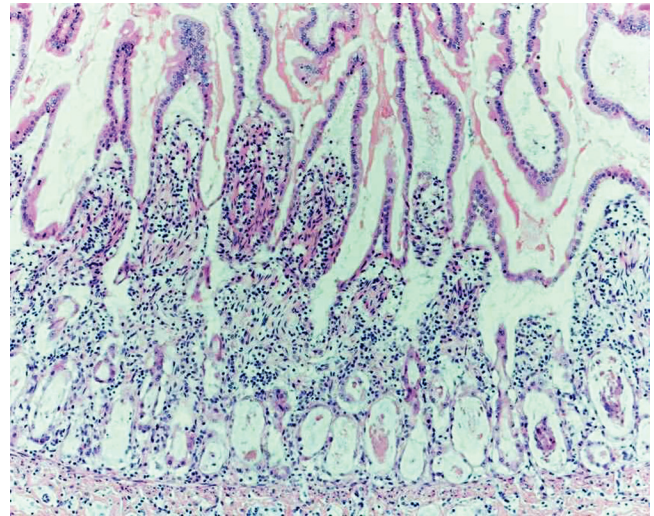
The clinical presentation of FPV infection includes profound depression, a consequence of the bone marrow depletion, anorexia, and fever. Signs referable to the intestinal infection may not be evident initially or may only include vomiting, but diarrhea that may be hemorrhagic is a hallmark sign in most infections.<sup>42,293</sup> Kittens quickly become dehydrated and may be moribund with subnormal body temperatures. The classic disease presentation is most common in kittens at the time that maternal immunity wanes and mortality is high. A study of kitten mortality in the United Kingdom revealed 25% of kitten deaths were due to FPV.<sup>37</sup>

Infection of kittens in late gestation or in the neonatal period may result in myocardial or cerebellar destruction. The latter syndrome manifests as permanent ataxia and intention tremors.<sup>293</sup> Myocardial infection has been postulated to contribute to cardiomyopathy development, but a causal association has not been proven.<sup>193</sup>

### Diagnosis

Diagnosis of feline panleukopenia is generally based on clinical presentation, the presence of severe leukopenia (often  $<2000$  cells/ $\mu\text{L}$ ), and virus detection, which is often done in-house at veterinary clinics using commercial fecal enzyme-linked immunosorbent assay (ELISA) kits. Most kits use monoclonal antibodies, specific for a single epitope of the virus, to detect the virus in fecal samples. Typically, fecal ELISA test kits designed to detect CPV-2 variants of dogs will also detect FPV.<sup>1,203</sup> Evaluation of ELISA results must be interpreted in light of vaccination history, especially in shelter situations. It has been shown that some ELISA kits may detect vaccine virus for as long as 2 weeks postvaccination.<sup>218</sup> Commercial ELISA kits currently available have shown good sensitivity and specificity for detection of virus shedding in the unvaccinated animal.

Other diagnostic options include electron microscopy to visualize the virus in fecal samples, which is typically only available at laboratories affiliated with academic institutions, and PCR for genetic detection of the virus. Electron microscopy offers the advantage of being non-specific—that is, it is an assay for any virus that may be causing enteritis, and it can detect agents such as coronavirus, rotavirus, or other viral enteric pathogens. The PCR assay is very sensitive and may detect vaccine virus or subclinical parvovirus infections; thus positive results by PCR must be interpreted in light of other relevant clinical data. Virus isolation, as well as histopathology and immunohistochemistry, can also be performed on tissues collected postmortem.<sup>156</sup> Histopathologic



**FIGURE 33-12** Feline panleukopenia virus infection in the small intestine. Photomicrograph of small intestine with radiomimetic type injury of cryptal necrosis. Crypts are dilated and lined by a reduced number of attenuated epithelial cells (H&E stain, 200 $\times$ ). (Courtesy Dr. Robert Foster, Ontario Veterinary College and Yager-Best Histovet, Guelph, Ontario, Canada.)

examination reveals crypt necrosis with villus blunting in the small intestines and cellular depletion in bone marrow and lymphoid tissues (Figure 33-12).<sup>156</sup>

### Treatment

Treatment of panleukopenia is directed at supportive care. Strict isolation and barrier nursing must be used when treating affected cats in a clinic setting. Fluids to combat dehydration, and restoration of electrolyte and acid–base balance are critical in the treatment of panleukopenia.<sup>293</sup> Colloids, plasma, or whole blood transfusion may be required in hypoproteinemic cats (protein  $<5$  g/dL). B-vitamin supplementation should be given parenterally because of decreased food intake and loss in diuresis. A platelet count and activated coagulation time should be evaluated for signs of disseminated intravascular coagulopathy (DIC). Initially, oral intake of food should be avoided to lessen vomiting and slow the bowel mitotic activity necessary for viral replication. Antiemetics may be necessary to control persistent vomiting. In addition to dehydration, a major concern is secondary bacterial septicemia resulting from the leukopenia and intestinal epithelial necrosis; thus parenteral broad-spectrum antibiotics with activity against gram-negative and anaerobic bacteria (e.g., amoxicillin/clavulanic acid with an aminoglycoside, fluoroquinolone, or cephalosporin) are an important management tool. Return to enteral nutrition is vital once vomiting ceases.<sup>293</sup> Feline recombinant interferon-omega has been shown to inhibit viral replication in vitro, and may be beneficial clinically.<sup>293</sup> Its administration has also been

recommended in pregnant queens and neonatal kittens prior to introduction to a potentially contaminated environment in order to enhance antibody production.<sup>215</sup>

The antiviral medication oseltamivir (Tamiflu) has been proposed as part of the treatment regimen for CPV infection in dogs.<sup>258</sup> This drug, designed to combat influenza virus, inhibits neuraminidase enzyme activity. Parvovirus encodes no neuraminidase function; therefore this medication has no direct effect on the virus. Proponents of its use indicate it is beneficial because of its effect on bacterial neuraminidase enzymes. The efficacy and, more importantly, the safety of this medication in cats have not been evaluated, and its use is not recommended for panleukopenia cases.

## Prevention and Control

Panleukopenia is most common in kittens and is uncommon in adults. Despite the ability of CPV-2a, CPV-2b, and CPV-2c to infect cats, FPV remains the most common cause of panleukopenia in cats.<sup>55</sup> Panleukopenia is a major concern in shelter and rescue situations where it may accumulate and survive disinfection. Thorough cleaning with a detergent to remove all organic matter, followed by disinfection with an appropriate product with oxidizing activity (e.g., 6% sodium hypochlorite, potassium peroxymonosulfate), is needed to inactivate the virus.<sup>69</sup> The virus survives disinfection with 70% alcohol and quaternary ammonium compounds.<sup>69,101</sup> Contaminated fomites and caretakers can be an important mode of transmission, and stringent precautions to prevent spread must be taken.

Passive immunization with serum from vaccinated or recovered cats is very effective, even after exposure, as long as clinical signs are not present. Serum donors should be selected with the same care as blood donors (see Chapter 25). Products containing immunoglobulins against parvovirus are available in some European countries for cats and are marketed for prophylactic and therapeutic use. Cats cannot be vaccinated with a modified live virus (MLV) product for 3 weeks after administration of immunoglobulin to avoid neutralization of vaccine virus. Repeated treatment should be avoided or anaphylactic reactions may occur.<sup>293</sup>

Immunity after recovery is likely lifelong.<sup>42</sup> Vaccination is recommended for every cat, given the severity of disease and the ability of the virus to persist in the environment.<sup>50,249,293</sup> The modified live vaccine is recommended as early as 6 weeks of age and continuing through 16 weeks to ensure that maternally derived immunity has not interfered with vaccine response.<sup>249</sup> In the face of an outbreak, kittens can be vaccinated as early as 4 weeks with a MLV vaccine to provide rapid onset of immunity. Current guidelines recommend re-vaccination at 1 year of age followed by vaccination every 3 years. Vaccination of pregnant queens or

neonates (<4 weeks) with a MLV vaccine is not recommended, because the live attenuated virus may infect and produce lesions in the fetus or neonate.

## FELINE CORONAVIRUS

Appearing for the first time in the 1950s, feline infectious peritonitis (FIP) continues to be a significant disease in domestic cats. Approximately 1 out of every 200 new feline cases seen at veterinary medical teaching hospitals are cats diagnosed with FIP.<sup>252</sup> The pathogenesis of FIP is complex, involving feline coronavirus (FCoV) and an inappropriate humoral response to the virus. A minority of FCoV-infected cats develops the lethal disease, and both host and virus genetic factors are believed to play a role.

Feline coronavirus is a member of the Coronaviridae, and is antigenically related to canine enteric coronavirus as well as transmissible gastroenteritis virus of swine. It is an enveloped virus, which is unusual for an enteric pathogen. There is a relatively large amount of glycoprotein embedded in the envelope in the form of peplomers, such as the spike protein, and this may contribute to the virus' stability. The virus may survive in the environment for up to 7 weeks under dry conditions<sup>264</sup> but is readily inactivated by common detergents and disinfectants.

The spike protein is used for cellular attachment and may play a role in cellular tropism of the virus as well as the pathogenesis of FIP. The genome of FCoV is single-stranded RNA and is one of the largest RNA genomes of the animal viruses. The coronaviruses have a high mutation rate, including point mutations, deletions, and recombination with heterologous coronaviruses. For example, FCoV serotype 2 is a recombinant between FCoV and canine enteric coronavirus, specifically, in the gene encoding the spike protein. Thus this serotype of FCoV is more antigenically related to canine coronavirus than to FCoV serotype 1.<sup>195,300</sup>

As alluded to above, there are two antigenically distinct serotypes of FCoV, based primarily on the antigenicity of the viral spike protein. Viruses capable of causing FIP may be of either serotype; however, the majority of field strains are serotype 1.<sup>19</sup> Feline coronaviruses are also characterized according to virulence, referred to as virus biotype. The most common biotype is that of mild or no disease associated with enteric infection by the virus, and it is often referred to as feline enteric coronavirus (FECV). This is actually a misnomer, because even in asymptomatic infections, the virus can spread systemically, albeit at relatively low levels. The biotype associated with FIP (FIPV) occurs in only a small percentage of infected cats. The viral properties responsible for the difference in biotype are the subject of intense research.



Virus factors are important to disease development, because virus strains vary in virulence. It has been theorized that a viral mutation is responsible for the change in biotype of the virus, leading to disease production. Speculation on the genomic locale of this mutation has involved the gene encoding the spike protein, as well as genes encoding several nonstructural proteins including 3c, 7a, and 7b. However, no consistent genetic difference between virulent and avirulent biotypes has been found. In fact, one study found 100% homology in the 3-prime one third of the genome when comparing the enteric and nonenteric forms of the virus from a cat with FIP.<sup>65</sup> Recently, genetic analysis of 56 isolates from cases of FIP (n=8) and asymptomatic FCoV infection (n=48) revealed biotype-specific genotypes in the gene encoding the membrane (M) protein.<sup>30</sup> In addition, phylogenetic clustering of virulent isolates was observed when based upon the genes encoding the spike structural protein and 7b nonstructural protein. These researchers concluded that based on their analyses, cases of FIP arise from infection with a distinct strain rather than *in vivo* mutation.

Other studies have shown that the product of the nonstructural 3c gene may also play a role in pathogenesis.<sup>38,225</sup> One research group found deletions within this gene occurring in the majority of FIPV isolates examined (n=28) but intact in all FECV isolates (n=27).<sup>38</sup> They speculate that these deletions lead to poor replication of the virus in the intestines of cats and may explain, at least in part, why FIP outbreaks are uncommon. Another research group had similar findings when analyzing the virus in eight cats that died of FIP, in that extraintestinal virus from FIP lesions in the majority of cases (74%) had deletional mutations in the 3c gene, leading to truncation of the protein, while fecal virus in all cats had an intact 3c gene and presumably functional 3c gene product.<sup>225</sup>

One phenotypic change in the virus associated with FIP disease production appears to be efficiency of replication in monocytes and macrophages, in that viruses causing FIP have acquired significant tropism for macrophages. Although FECV may spread beyond the intestines, it does so at relatively low levels, probably because of a poor ability to replicate in monocytes and macrophages.<sup>144,192,269</sup> The virus of FIP, on the other hand, replicates at high levels in macrophages and may disseminate throughout the body. Macrophage tropism appears to reside in a region of the spike protein.<sup>254</sup> Quantitative differences in viral RNA levels in the blood of cats with and without FIP have been found.<sup>143</sup> Rising amounts of viral RNA in the blood seen in end-stage disease may indicate enhanced viral replication and disease progression. This increased viral replicative capacity may be a key element of FIP pathogenesis. It is likely that the viral properties responsible for development of FIP do not lie in a single or, even necessarily, the same mutation in all cases, but instead lie in the high mutability of the virus

and multiple genetic changes. It is also likely that each virulent isolate arises individually.

## Transmission and Pathogenesis

Feline enteric coronavirus is spread by the fecal–oral route because the virus is primarily shed in feces and rarely in saliva or other body fluids. Virus may infect intestinal epithelial cells from the lumen after ingestion. From there, systemic spread by infection of monocytes/macrophages may occur. In multicat environments, kittens are infected at a young age, typically at 4 to 6 weeks as maternally derived antibodies wane.<sup>8</sup> However, infection as young as 2 weeks of age has been documented.<sup>176</sup> Fecal shedding occurs within 1 week following infection and may continue for weeks, months, or even lifelong. Two types of shedding patterns are observed: cats that shed virus almost continuously and cats that shed virus only intermittently.<sup>77</sup> In addition, a small number of cats are seropositive for FCoV, but never shed virus in feces, apparently having a high degree of immunity.<sup>77</sup> Chronic carriers are an important source of the virus for other cats within the household. Virus persists primarily in the colon; it may also persist in tissue macrophages, giving rise to recurrent viremia.<sup>147</sup> It is important to note that although FECV (the benign biotype) is highly infectious, FIPV (the virulent biotype) is infrequently spread in a horizontal manner.<sup>221</sup> FIPVs are strongly cell-associated and tissue-associated so that shedding into feces would not normally be possible.

Enzootic disease is common in multicat environments, such as catteries, where losses are sporadic and unpredictable. Overall mortality during a period of years is usually less than 5%.<sup>221</sup> Very occasionally, epizootics with high mortality have been reported that generally last less than 12 months. Epizootics are probably multifactorial, involving factors such as population stresses, overcrowding, high kitten birth rates, and genetically predisposed breeding cats. Risk factors for FIP in catteries include individual cat age, individual cat coronavirus titer, overall frequency of fecal coronavirus shedding, and the proportion of cats in the cattery that are chronic shedders.<sup>78</sup>

In addition to changes in viral properties causing the shift from a benign to virulent biotype, the pathogenesis of FIP also involves host factors. Genetic predisposition along familial lines has been observed, and breeds in certain countries or areas appear to have a predisposition for FIP development.<sup>75,206,229</sup> However, the incidence of FIP in cat breeds can vary greatly among countries, suggesting that susceptibility to disease is more related to bloodlines than the breed itself.<sup>221</sup> These host factors may manifest in the immune response of the cat to systemic spread of FCoV. In cats that develop FIP, a strong humoral response to infection occurs, with inadequate cell-mediated response by cytotoxic T lymphocytes.<sup>220</sup>

The antibody production is ineffective in clearing the virus and contributes to the immune-mediated disease.<sup>138</sup> The factors responsible for this unsuccessful immune response are unknown, but various mechanisms appear to be at work. As stated above, they seem to involve the immune response to FCoV infection, in particular, a shift from a T-helper lymphocyte type I (Th1) to a T-helper lymphocyte type 2 (Th2) response to the infection. The former is important in coordinating cell-mediated immunity, which is protective against FIP, while the latter is important in humoral response. This shift results in an exaggerated humoral response that is not protective, and, in fact, actually enhances the disease progression as the virus-specific antibody opsonizes the virus for phagocytosis by monocytes and macrophages.

Another finding in cats with FIP is lymphocyte depletion, particularly T lymphocytes,<sup>106</sup> through apoptosis. The resultant depletion of T lymphocytes contributes to enhanced viral replication, because these cells are important in cell-mediated immunity. At least one group of investigators propose that the virus-driven T-lymphocyte depletion occurring in infected cats that do not mount a quick and effective cell-mediated immune response leads to loss of immune control and unchecked viral replication.<sup>51</sup> The virus does not replicate in lymphocytes; so, some other mechanism must be responsible for this process.

Because lymphocytes are not target cells of FCoV, it is theorized that secreted factors, including cytokines, are critical to these lymphocyte effects, including the Th2 response and T-lymphocyte depletion. In fact, the T-lymphocyte response appears to be the decisive factor in disease progression. Monocytes and macrophages are major cytokine producers and are the target of FIPV infection. The cytokine secretion patterns from these cells thus determine the magnitude and direction of the immune response. Cytokines associated with cell-mediated immunity, such as IL-10, IL-12, and IFN-gamma have been found to decrease in cats that develop FIP. Elevations in cytokines IL-1beta and IL-6 have also been found in affected cats, which may contribute to the humoral response.<sup>79</sup> An increase in tumor necrosis factor-alpha (TNF-alpha) has been observed in some studies, and may contribute to the T-lymphocyte apoptosis.<sup>282</sup> A recent study has shown that FCoV-infected macrophages produce factors that promote B-lymphocyte differentiation into plasma cells.<sup>281</sup> This may contribute to the exaggerated humoral response.

Much focus has been placed on interferon-gamma, because of its role in enhancing the cell-mediated immune response. Although serum interferon-gamma concentrations were not found to differ between cats with FIP and healthy cats with feline coronavirus in catteries with a low incidence of FIP, higher serum concentrations were seen in healthy cats with feline coronavirus compared with cats with FIP in catteries with a high

prevalence of FIP.<sup>91</sup> In addition, higher interferon-gamma concentrations were associated with FIP lesions, indicating that, at least at the tissue level, cell-mediated immunity may contribute to lesion development.<sup>91</sup> In particular, it indicates that local activation of macrophages by interferon-gamma may be occurring, leading to enhanced viral replication.<sup>22</sup> In contrast, a systemic increase in interferon-gamma concentrations, as indicated by elevated expression in blood, may protect infected cats from disease.<sup>89,91</sup>

## Clinical Signs

The disease of FIP is predominantly immune mediated. Lesions are distributed along the vasculature, particularly along veins.<sup>146</sup> Vasculitis is the hallmark lesion of FIP, whether the effusive or noneffusive form. Emigration of infected monocytes/macrophages from blood vessels into perivascular regions incites local inflammatory responses. Type II and type III hypersensitivity responses occur with complement activation and cellular destruction. This may occur widely throughout an infected cat's tissues, leading to increased vascular permeability, extensive pyogranulomatous lesions, and the classic signs of the effusive, or wet, form of FIP. Alternatively, focal lesions may be confined to one or more organ systems in the noneffusive, or dry, form of FIP. The cells involved in the inflammatory process are primarily macrophages and neutrophils; however, B lymphocytes play a critical role in producing disease.<sup>145</sup>

The incubation period for FIP is unknown, but is probably weeks or months, in some cases, even years.<sup>221</sup> The cat with FIP generally presents with weight loss, fever, and inappetence. The fever may wax and wane and is not responsive to antibiotics. Kittens are often underweight and unthrifty compared with normal littermates (Figure 33-13). Icterus may be seen with both effusive and noneffusive forms (Figure 33-14). Abdominal palpation of affected cats may reveal thickened bowel loops, mesenteric lymphadenopathy, or irregular serosal surfaces of abdominal organs. Cats with the effusive form characteristically present with significant abdominal ascites. In fact, FIP is the leading cause of ascites in young cats, proving a more common cause than cardiac disease, neoplasia, and hepatic or renal disease.<sup>307</sup> The enlarged abdomen can contain a surprising amount of fluid and may be mistaken for pregnancy by owners of female cats. Typically, the abdominal distension is nonpainful, and a fluid wave can be palpated. Effusion in the thorax and/or pericardial sac may also occur. If pleural effusion occurs, the primary clinical signs may include dyspnea, tachypnea, open-mouth breathing, and cyanotic mucous membranes. Heart sounds will be muffled on thoracic auscultation.

With the noneffusive form, signs may be referable to virtually any organ, singly or in combination (Table



**FIGURE 33-13** Kittens with FIP are often underweight and unthrifty compared with normal littermates. Pleural effusion may cause dyspnea (A), and ascites may cause abdominal enlargement (B).

33-2). Thoracic or abdominal effusions are either absent or too scant to be appreciated clinically. Granulomatous lesions may occur in the eye, including retinal changes, iritis, an irregular pupil, and uveitis with hyphema, hypopyon, aqueous flare, miosis, and keratic precipitates.<sup>58</sup> Ocular disease may be the sole manifestation of FIP in affected cats, or it may be combined with CNS or abdominal involvement. CNS lesions may be single or multifocal and may involve the spinal cord, cranial nerves, or meninges, causing seizures, ataxia, nystagmus, tremors, depression, behavior or personality changes, paralysis or paresis, circling, head tilt, hyperesthesia, or urinary incontinence.<sup>149</sup> FIP is the most common inflammatory disease of the CNS in cats<sup>28</sup> and is a leading cause of spinal disease.<sup>185</sup> Affected cats are typically



**FIGURE 33-14** A Burmese kitten with noneffusive FIP. Icterus may be seen with both effusive and noneffusive forms of FIP.

**TABLE 33-2** Variability in Clinical Signs of Noneffusive Feline Infectious Peritonitis

Clinical Signs Referable to Involvement of:	% of Affected Cats
Peritoneal cavity	32.0
CNS	23.0
Eyes	15.0
CNS and eyes	8.5
Peritoneal cavity and eyes	7.4
Peritoneal and pleural cavities	4.3
Peritoneal and pleural cavities, CNS	3.2
Peritoneal and pleural cavities, eyes	2.1
Peritoneal cavity, CNS, eyes	2.1
Pleural cavity	1.1
Pleural cavity, CNS, eyes	1.1

Adapted from Table 2 in Pedersen NC: A review of feline infectious peritonitis virus infection: 1963-2008, *J Feline Med Surg* 11:225, 2009.

young (less than 2 years old) and come from multicat environments.<sup>76</sup> In one study of 24 cats with FIP and neurologic involvement, 75% had hydrocephalus at necropsy.<sup>149</sup> The occurrence of seizures indicates extensive brain damage and is an unfavorable prognostic sign.<sup>288</sup>

Abdominal involvement with FIP may include granulomas in mesenteric lymph nodes, kidneys or liver, as well as adhesions throughout the omentum and mesentery that may be palpable as masses and visible with ultrasonography (Figure 33-15). With intramural intestinal involvement, diarrhea and vomiting may be observed. Focal granulomas may be found in the ileum, ileocecolic junction, or colon. Involvement of the cecum and colon produces a distinct form of FIP with signs of colitis (soft stools containing blood and mucus).<sup>116</sup>



**FIGURE 33-15** Abdominal involvement with FIP may include granulomas on the serosal surface of the intestines (A); in mesenteric lymph nodes, kidneys, or liver (B); as well as adhesions throughout the omentum and mesentery (B) and accumulation of straw-colored fluid (C).

Uncommon manifestations of noneffusive FIP include cutaneous lesions, such as intradermal papules.<sup>57</sup> Skin lesions resulting from coronavirus-induced vasculitis have been reported in a cat with FIP and concurrent feline immunodeficiency virus infection.<sup>33</sup> Scrotal



**FIGURE 33-16** A Siamese kitten with FIP. Scrotal enlargement may occur because of extension of the peritonitis to the tunics surrounding the testes or because of chronic fibrinous necrotizing orchitis.

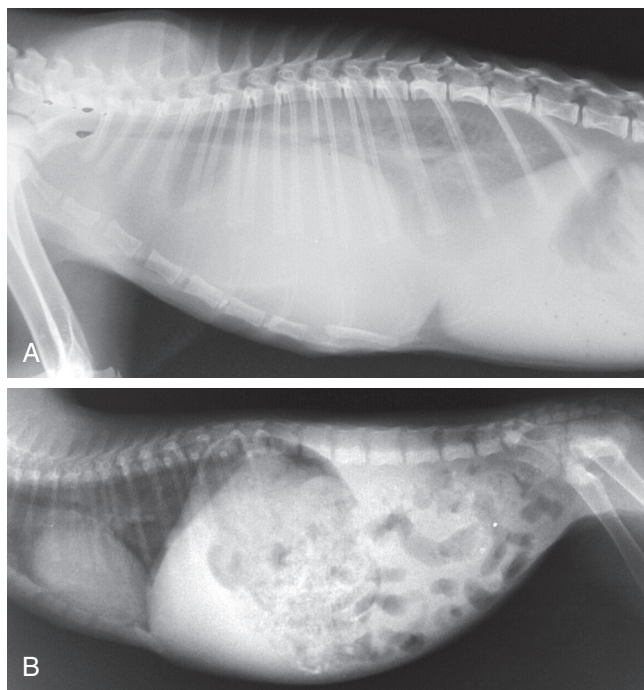
enlargement may occur because of extension of the peritonitis to the tunics surrounding the testes or because of chronic fibrinous necrotizing orchitis (Figure 33-16).<sup>81,268</sup>

In addition, a combination of effusive and noneffusive forms may occur, and transition between the two can occur in any given cat with FIP. The onset of FIP may be acute or insidious. For the former, rapid development of effusion may occur, and the disease course may be short. For the latter, a subclinical state may exist for sometime or may be preceded by months or even years of vague illness and poor growth.<sup>221</sup>

## Diagnosis

Diagnosis of FIP can be challenging, especially for the noneffusive form. Clinical signs of FIP, particularly the noneffusive form, are often vague; in addition, changes in clinical parameters are not pathognomonic for FIP. The effusive form of FIP is the easiest to diagnose, but only about 50% of cats that present with effusions will have FIP. The most common diseases that produce effusions similar to FIP include lymphocytic cholangitis and malignancies.<sup>273</sup> Feline coronavirus infection is common, thus evidence of infection is not diagnostic for FIP. Though diagnosis of FIP is critical, given the poor prognosis, ante mortem diagnosis of FIP can be a challenge, requiring a combination of evidence gathered from patient signalment, medical history, physical examination, imaging, and laboratory findings. There is no single test, other than histopathology and immunohistochemistry, that will confirm a diagnosis of FIP.

Diagnosing FIP starts with obtaining an animal's history and noting its signalment. Most cases occur in young cats (usually <1 year of age); it occurs more frequently in purebred than it does in mixed-breed cats; and affected cats usually originate from or are currently housed in multicat situations.<sup>9</sup> In breeding catteries, examination of records may reveal a genetic connection

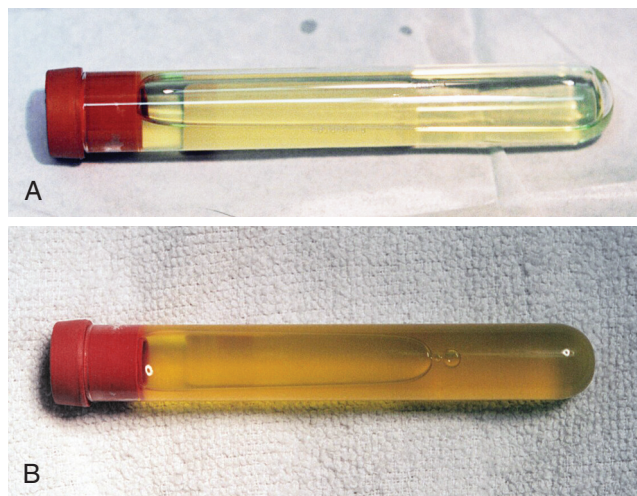


**FIGURE 33-17** A, A thoracic radiograph of the kitten with pleural effusion shown in Figure 33-13, A. B, An abdominal radiograph of the kitten with ascites shown in Figure 33-13, B.

among cases. A history of a stressful event, such as spay or neuter, adoption from a shelter, or trauma, may precede the onset of signs by several weeks. An event that qualifies as a stressor may also be more subtle, such as a change of social hierarchy within the population.

Imaging, such as radiography and ultrasonography are useful to rule out other diseases and identify effusions, especially in cats with abdominal enlargement or dyspnea. A recent study of abdominal ultrasonographic findings in 16 cats with FIP identified a variety of non-specific changes, such as renomegaly, irregular renal contour and hypoechoic subcapsular echogenicity, abdominal lymphadenopathy, peritoneal or retroperitoneal effusion, and diffuse changes within the intestines.<sup>171</sup> However, a normal abdominal ultrasonograph does not exclude a diagnosis of FIP.

For cats with effusion, evaluation of this fluid can be informative. Tests on effusions have greater diagnostic reliability than tests on blood or serum. Therefore the first step should be evaluation of the patient for evidence of effusion using radiographs and/or ultrasonography if necessary (Figure 33-17). The fluid has been described as straw-colored (Figure 33-18) and is usually viscous because of the high-protein content (Box 33-2). It usually has a relatively low cellular content that is pyogranulomatous (macrophages and neutrophils—usually no toxic changes in the latter) in nature. Detection of feline coronavirus antigen by immunofluorescence within inflammatory cells (macrophages) in effusive fluid correlates with a diagnosis of FIP.<sup>112,217</sup> Viral antigen detection by



**FIGURE 33-18** The effusion characteristic of FIP is straw to golden yellow in color, viscous, clear (A) to slightly cloudy (B), depending on cell count, often frothy when shaken.

### BOX 33-2

#### Characteristics of the Effusion Found in Feline Infectious Peritonitis<sup>217</sup>

- Nonseptic exudate
- Straw to golden yellow color, viscous, clear to slightly cloudy, frothy when shaken
- High specific gravity (1.017 to 1.047)
- High protein (typically >3.5 g/dL, often 5 to 12 g/dL)
- Albumin:globulin ratio less than 0.45
- Low to moderate cellularity (<5000 cells/ $\mu$ L)

immunofluorescence is offered by many diagnostic laboratories and can be performed on sediment from submitted abdominal fluid. RT-PCR has been shown to differentiate FIP effusions from effusions because of other causes.<sup>112</sup> High levels of protein and a low albumin to globulin ratio in the fluid are also indicative of FIP.<sup>112,217</sup>

The Rivalta test is a simple and inexpensive supportive test on effusions in the diagnosis of FIP. It distinguishes between exudates and transudates. A test tube is filled with distilled water and one drop of 98% acetic acid is added, followed by one drop of effusion sample. If the effusion drop dissipates in the solution, the test is negative and not supportive of FIP. If the drop retains its shape, the test is positive and supportive of FIP. In one large retrospective study, the positive predictive value of the Rivalta test was 86%, and the negative predictive value was 97%.<sup>112</sup>

Serum chemistry profiles reveal that many cats with FIP have elevated serum total protein concentrations, because of the high globulin concentrations; however, even with normal total protein concentrations, a decreased albumin to globulin ratio may be evident. As

**TABLE 33-3** Specificity, Sensitivity, Positive Predictive Value, Negative Predictive Value, and Optimum Cutoff Value of Different Total Protein Concentrations, Gamma-Globulin Concentrations, and Albumin to Globulin Ratios in Effusions

Total Protein (g/dL)	Total Protein				Gamma-Globulin					Albumin to Globulin Ratio				
	SP	SE	PPV	NPV	Gamma-Globulin (g/dL)	SP	SE	PPV	NPV	Albumin to Globulin Ratio	SP	SE	PPV	NPV
5.0	0.10	1.00	0.56	1.00	0.5	0.47	0.94	0.67	0.87	0.5	0.89	0.62	0.86	0.76
6.0	0.33	0.88	0.60	0.71	0.1*	0.83	0.82	0.84	0.80	0.6	0.85	0.67	0.83	0.70
7.0	0.53	0.82	0.66	0.72	1.5	0.93	0.65	0.91	0.70	0.7	0.82	0.69	0.81	0.71
8.0*	0.90	0.55	0.78	0.62	2.0	0.97	0.44	0.94	0.61	0.8	0.79	0.78	0.80	0.68
9.0	0.93	0.32	0.84	0.55	2.5	0.99	0.35	0.98	0.57	0.9*	0.74	0.86	0.79	0.82
10.0	0.95	0.23	0.85	0.52	3.0	1.00	0.26	1.00	0.55	1.0	0.65	0.94	0.75	0.91
11.0	0.98	0.12	0.87	0.50										
12.0	0.99	0.07	0.89	0.49										

SP, Specificity; SE, sensitivity; PPV, positive predictive value; NPV, negative predictive value.

\*Optimum cutoff value as determined by differential positive rate analysis.

Adapted from Table 3 in Hartmann K, Binder C, Hirschberger J et al: Comparison of different tests to diagnose feline infectious peritonitis, *J Vet Intern Med* 17:781, 2003.

this ratio approaches 0.5, a diagnosis of FIP becomes more likely (Table 33-3).<sup>112</sup> Other abnormalities may be evident depending on the tissues involved (e.g., elevated hepatic enzyme activities, azotemia, hyperbilirubinemia, hyperbilirubinuria).<sup>271,273</sup>

Complete blood count (CBC) results are variable and nonspecific but may include neutrophilia with a mild left shift, lymphopenia (<1500/ $\mu$ l), and anemia of chronic disease.<sup>214,271,273</sup> Lymphopenia may be present in the face of an elevated total white blood cell count. Immunophenotyping shows that the T lymphocytes, in particular, are depleted; in fact, a normal T-lymphocyte count has a significant negative predictive value for FIP. Immunophenotyping or flow cytometry is often offered by laboratories associated with academic institutions.<sup>51</sup> Results of serum chemistries and CBC may also be normal in cats with FIP.

In addition to high serum globulin concentrations, elevation in acute phase proteins also occurs. Elevations in alpha-1 acid glycoprotein (AGP) in serum have been noted in cats with FIP and may aid diagnosis. In one study that evaluated the usefulness of measuring AGP to diagnose FIP, it was found that high AGP concentrations (>1.5 g/L) in serum, plasma, or effusion samples are a discriminating marker for FIP.<sup>64,216</sup> Measurement of AGP can be specifically requested from some but not all commercial labs and is more commonly available in Europe than North America. However, it must be remembered that many other inflammatory conditions, such as lymphoma and FIV, can lead to an increase in serum AGP; so, it is not diagnostic for FIP by itself.

FIP is one of the most frequent causes of neurologic disease in the cat, especially in cases with

multifocal clinical signs. Examination of cerebrospinal fluid from cats with neurologic FIP reveals a marked pleocytosis (>100 cells/mL) primarily consisting of neutrophils, high protein content (>200 mg/dL), and coronavirus antibody titer greater than 1:25.<sup>241</sup> Magnetic resonance imaging (MRI) is useful to confirm the presence of inflammatory disease and demonstrate abnormalities consistent with FIP, such as periventricular contrast enhancement, ventricular dilatation, and hydrocephalus.<sup>76,202</sup>

### Serum Antibody and Virus Detection Assays

Feline coronavirus-specific assays can generally be categorized as FCoV-specific antibody measurement or virus detection assays. Because of the inability to identify a consistent viral mutation correlating with FIP, no FIP virus-specific test exists. Serologic analysis detects only antibody to the coronavirus and does not reflect the virus' biotype. Unfortunately, some commercial diagnostic laboratories use the misnomer "FIP test" for coronavirus antibody titer. Although a high antibody titer is consistent with a diagnosis of FIP, it is not confirmatory; in addition, some cats with FIP have low antibody titers or are seronegative.<sup>9</sup> This latter situation may occur in fulminant cases or may be due to high virus levels that bind antibody, making it undetectable in the serologic assay. Therefore serology should only be used as an aid to rule in or rule out the possibility of FIP, and a diagnosis of FIP should never be made on antibody titers alone.

Serologic assays for antibody to a single virus-specific protein (as opposed to antibody to multiple virus proteins) have been developed. In particular, a serologic test

for antibody to the 7b protein has been offered as a diagnostic aid to FIP. This protein is a viral nonstructural protein whose function is unknown, but, as described above, it may play a role in disease development. It has been theorized that this protein is not expressed in all feline coronavirus infections; when expression does occur, perhaps because of a viral mutation allowing 7b expression, FIP may develop. Cats with high concentrations of antibody to the 7b protein would, by definition, be infected with the FIP viral biotype. However, subsequent studies have shown that 7b expression occurs in most infections; 7b-specific antibodies, although consistently present at high concentrations in cats with FIP, are also present in healthy cats with feline coronavirus.<sup>142</sup> Thus, although 7b seronegative status would lessen the likelihood of a diagnosis of FIP, this test cannot be used to confirm FIP.

Because of the problems associated with serology, it is difficult to use FCoV antibody testing to control or eliminate FIP from catteries.<sup>221</sup> In most cases, it is not possible to interpret the results of FCoV testing cats in catteries. Most catteries with an active breeding program, and having at least six cats, will have endemic FECV, and 50% or more of the cats will have FCoV titers of 1:100 or greater at any given time.<sup>221</sup> Unfortunately, antibody titers do not provide the type of information the breeder requires, such as whether any cats have FIP, whether a particular cat will develop FIP, and which cats are shedding FECV.

Virus detection assays also suffer from a lack of specificity for FIP virus. That is, finding the virus by antigen detection (e.g., immunofluorescent staining of ascitic macrophages) or genetic detection (e.g., real-time polymerase chain reaction testing of whole blood) is consistent with a diagnosis of FIP but is not necessarily confirmatory. At least one commercial laboratory (Auburn University College of Veterinary Medicine) offers a RT-PCR assay that quantitates the level of viral messenger RNA (mRNA) in the monocytes of cats. Although it is not known precisely how the cutoff levels were determined, high levels of viral mRNA do reflect efficient viral replication in circulating monocytes.<sup>269</sup> However, in a recent study, FCoV mRNA was detected in 14 of 26 blood samples, yet only one of these cats had clinical signs compatible with FIP.<sup>32</sup> As stated above, high viral loads in the blood are consistent with FIP, especially in the end stage; however, high viral loads in the blood are also found in healthy cats in endemically infected populations.<sup>148,192</sup> In addition, absence of circulating virus detectable by PCR has been observed in noneffusive, localized forms of FIP (Dr. Alfred Legendre, personal communication). Virus detection and quantitation is thus not confirmatory for FIP but does offer diagnostic information. In general, results of any single assay claiming specificity for the virus of FIP must be interpreted with great caution.

The gold standard for FIP diagnosis remains histopathology and immunohistochemistry for feline coronavirus antigen.<sup>221,283</sup> Granulomatous lesions are vascular and perivascular, primarily involving small and medium veins. Cellular composition is mainly monocytes and macrophages with a minority of neutrophils. B lymphocytes and plasma cells may be found at the periphery of lesions, while T lymphocytes are few. Detection of viral antigen (immunohistochemistry) or nucleic acid (in situ hybridization) in infected cells within lesions is found and is confirmatory; this testing is offered by some pathology laboratories.

## Treatment

In the past, treatment has focused on two areas: suppressing the immune response or modulating the immune response. The former generally involves administering immunosuppressive drugs to inhibit the immune response, while the latter attempts to enhance the cell-mediated response through the administration of cytokines such as interferon. Immunosuppression by using prednisolone or cyclophosphamide will sometimes slow disease progression but will not provide a cure.<sup>114</sup> Antibiotics are not justified unless neutropenia occurs as a result of cytotoxic drug therapy. Good nutritional support and avoidance of stressors are also recommended.

Although human and feline recombinant interferon has been shown to inhibit feline coronavirus replication in vitro, in vivo studies have shown no effect on survival time or quality of life. Recombinant feline interferon-omega (Virbagen Omega, Virbac, Carros, France) had showed some initial promise in a small, uncontrolled clinical trial.<sup>135</sup> However, a larger placebo-controlled double-blind trial found no statistically significant difference in the survival time of cats treated with recombinant feline interferon-omega versus a placebo.<sup>250</sup>

Recently, a new drug tested in three cats with the dry form of FIP demonstrated efficacy in prolonging life and alleviating signs.<sup>162</sup> The drug, a polyprenyl immunostimulant, is an investigatory veterinary biologic and acts by upregulating mRNA expression of T-helper lymphocytes responsible for effective cell-mediated immunity. In this study, two cats with FIP were still alive 2 years after diagnosis, while one cat survived 14 months. As of this writing, further studies are underway to assess its potential for FIP treatment.

Finally, it is rarely necessary to isolate a cat with FIP from other cats in the home, particularly if the other cats are healthy adults. Transmission of FIP directly from cat to cat is the exception, not the rule. Isolation of an already sick kitten or young cat simply provides another stressor that may further impair the immune response.

## Prevention and Control

Preventing FIP is challenging, because the only effective means of control is preventing infection with feline coronavirus. The widespread nature of the virus and its ease of transmission, as well as the existence of persistent infections, make this difficult in a multicat situation. If one cat in a multicat population dies of FIP, the other members are likely already infected with the circulating virus. The likelihood that other cats in the population will develop FIP is not high, but it can occur, especially if there are genetic links to the affected cat. There may be some risk to introducing a new cat to this population, but generally, outbreaks of FIP are not observed. In most pet cat homes, where the number of cats is small, there should be little risk to introduction of a new cat after a resident cat has died of FIP. To decrease the risk, owners should consider adopting older (greater than 16 weeks) rather than younger kittens, or even a young adult cat.

Various strategies have been used to eliminate or prevent feline coronavirus infection in a cat population (Box 33-3). In breeding catteries, isolating pregnant queens nearing parturition and queens and kittens after parturition, as well as early weaning at 5 to 6 weeks of age, has been advocated (Table 33-4).<sup>5,6</sup> This prevention method, which requires strict quarantine measures and low (<5) numbers of cats in the population, is designed to delay infection until the kitten is older and can more easily eliminate the virus after exposure. One of the most important measures that can be used in a breeding cattery is to maintain complete breeding records. Heritability of FIP susceptibility is known to exist; thus continued breeding of parents, particularly sires that have produced kittens that developed FIP, is not recommended.

Other means of control involve removing chronic shedders from the population. Detection of virus in the

feces by using PCR testing is the optimal method for identifying viral shedding in multicat environments. PCR testing without quantitation is offered at many commercial laboratories. Testing multiple samples from an animal over time can identify chronic shedding.<sup>120</sup> Because these animals may shed the virus intermittently, at least two fecal samples (preferably more), collected at weekly to monthly intervals should be tested. An example regimen would be three samples collected daily, followed by three samples daily 1 month later. Some laboratories may offer pooling of samples to reduce costs. Serology may also be helpful, because cats that maintain high antibody levels are likely shedding high levels of virus.<sup>10</sup> However, it may be almost impossible to maintain a group of cats free of FCoV without strict quarantine measures and barrier nursing techniques that are typically beyond the capabilities of most breeding catteries.

### BOX 33-3

#### Methods for Control of Feline Infectious Peritonitis in Multicat Environments<sup>221</sup>

1. Eliminate overcrowding: maintain no more than six breeding cats, keep cats in stable small groups
2. Maintain cats 3 years and older as a larger proportion of the population
3. Manage litter boxes properly: have adequate numbers of litter boxes, limit spread of litter and dust, scoop boxes regularly, empty and disinfect boxes at least weekly
4. Have a selective breeding program: produce the least number of kittens necessary, do not use any tom cat in a breeding program that has produced kittens that have developed feline infectious peritonitis, preferably do not use such queens either

**TABLE 33-4** Protocol for Early Weaning and Isolation to Prevent Coronavirus Infection of Kittens

Step	Description
Prepare kitten room	<ol style="list-style-type: none"> <li>1. Remove all cats and kittens 1 week before introducing new queen.</li> <li>2. Disinfect room using 1:32 dilution of sodium hypochlorite (bleach).</li> <li>3. Dedicate separate litter trays and food and water bowls to this room, and disinfect with sodium hypochlorite.</li> <li>4. Introduce single queen 1-2 weeks before parturition.</li> </ol>
Practice barrier nursing	<ol style="list-style-type: none"> <li>1. Work in the kitten room before tending other cats.</li> <li>2. Clean hands with disinfectant before going into kitten room.</li> <li>3. Have shoes and coveralls dedicated to the kitten room.</li> </ol>
Wean and isolate kittens early	<ol style="list-style-type: none"> <li>1. Test queen for FCoV antibodies either before or after she gives birth.</li> <li>2. If queen is seropositive, she should be removed from the kitten room when the kittens are 5-6 weeks old.</li> <li>3. If the queen is seronegative, she can remain with the kittens until they are older.</li> </ol>
Test kittens	<ol style="list-style-type: none"> <li>1. Test kittens for FCoV antibodies after 10 weeks of age.</li> </ol>

Adapted from Table 11-5 in Addie DD, Jarrett O: Feline coronavirus infections. In Greene CE, editor: *Infectious diseases of the dog and cat*, ed 3, St Louis, 2006, Saunders Elsevier, p 101.



At least one commercially available vaccine for feline coronavirus exists. It is an intranasal vaccine containing a temperature-sensitive mutant of feline coronavirus allowing replication in the upper respiratory tract but not systemically. The vaccine is given as two doses, 3 or more weeks apart, but is not started until 16 weeks of age or older. Although this vaccine appears to be safe, its efficacy has been questioned. A small reduction in the number of FIP cases was noted in one study when the vaccine was given to seronegative cats.<sup>244</sup> However, in cats with preexisting antibody, the vaccine showed no protection. In another field study, the vaccine failed to prevent FIP in kittens with preexisting FCov antibodies in a cattery.<sup>72</sup>

In households in which feline coronavirus is endemic or in which FIP has occurred, most cats are seropositive and thus not aided by vaccination. Kittens at highest risk for FIP are those born into colonies in which the virus is endemic, where infection often occurs by 4 to 6 weeks of age. However, the vaccine is not given until 16 weeks of age; thus the vaccine is of dubious usefulness in those situations in which the risk is greatest. It may provide some protection for seronegative cats entering an infected population, but currently, this vaccine is not recommended as part of core vaccines for routine use.<sup>5,50,249</sup>

## RABIES

Rabies is a member of the Rhabdoviridae family and belongs to the *Lyssavirus* genus along with European bat lyssaviruses 1 and 2. Rabies virus particles (rhabdovirions) are a characteristic bullet shape because of the cylindrical form of the nucleocapsid core. Rabies is an enveloped single-stranded RNA virus. Although all warm-blooded animals are susceptible to infection with rabies, mammals are the only known vectors and reservoirs. Species susceptibility varies considerably; for example, cats, foxes, and raccoons are highly susceptible while domestic dogs, horses, and goats are moderately susceptible, and birds have low susceptibility. Younger animals are generally more susceptible than older animals. Rabies virus is neurotrophic, traveling quickly to the CNS after infection. Salivary glands have high concentrations of virus, thus allowing efficient transmission through bites or saliva-contaminated scratches. An infected cat will be able to transmit the disease in saliva about 3 days before clinical signs appear. Environmental transmission by fomites is rare and infected animals are not viremic, so blood is not infectious. As an enveloped virus, rabies is inactivated by many disinfectants and labile when exposed to ultraviolet light and heat. The virus can remain viable in a carcass for several days or longer, depending on temperature.

Large parts of Europe are now rabies-free because of wildlife vaccination programs. Strict quarantines have

kept several countries free of rabies, such as Japan and the United Kingdom. Worldwide, the majority of human rabies cases are due to dog bites, because dog rabies is endemic in many developing countries. In Canada and the United States, domestic dogs have been effectively eliminated as a reservoir. However, rabies virus continues to be a concern for cat owners; in 2008, 294 cases of rabies in cats were reported in the United States and Puerto Rico compared with 75 cases in dogs.<sup>27</sup> The risk of exposure to outdoor cats from infected wildlife is significant. Raccoons, skunks, and bats are the main reservoirs in the United States, but other species such as foxes, coyotes, and bobcats may also be infected. In addition, importation of rabies infected animals from areas of endemic infection, such as Africa, poses a risk and requires practitioners to be aware of potential cases, even in rabies-free areas.

Following exposure an incubation period of weeks to months may follow, but once symptoms appear, death occurs within days. The typical incubation period in cats is 2 to 24 weeks (average 4 to 6 weeks) before CNS signs appear.<sup>102</sup> Once rabies virus enters the CNS, damage to lower motor neurons (LMN) causes the typical ascending paralysis. After replicating in the CNS, the virus spreads via peripheral, sensory, and motor nerves. Virus reaches the salivary glands via cranial nerves. Virtually any tissue may be infected, but spread outside the CNS does not occur in every case.

Classically, rabies has been divided into two clinical presentations—furious and paralytic. However, rabies is variable in its presentation and atypical signs are common. Usually the initial history includes a bite wound. The prodromal phase in cats lasts up to 2 days and is characterized by behavior changes, erratic behavior, and fever spikes. In the furious form, cats show erratic and unusual behavior, aggression, restlessness, muscle tremors, and weakness or incoordination. The paralytic phase typically follows as LMN paralysis progresses. Mandibular and laryngeal paralysis is less common in cats than dogs. However, increased frequency of vocalization and a change in voice pitch are common in cats.<sup>73</sup> Ascending paralysis terminates in coma and death, usually after as little as 3 to 4 days.<sup>255</sup> There is no effective therapy for animals with rabies, and supportive care is not recommended. Clinically normal cats with suspected exposure to rabies should be quarantined as recommended by local authorities. Postexposure vaccination is forbidden in most countries.

Rabies should be considered as a differential diagnosis in any cat with profound behavior changes and/or LMN paralysis, particularly if there is a history of contact with wildlife. The definitive diagnostic test is demonstration of rabies virus antigen by direct fluorescent antibody testing of brain tissue. No ante mortem tests are considered sensitive enough for rabies diagnosis. Handling live cats suspected of rabies must be done with

extreme care, using heavy protective gloves, cages, catchpoles, and other equipment. The animal must be humanely euthanized and the head removed and refrigerated until the brain can be examined. Specimens should be transported according to the specifications of the individual laboratory and should always be identified as hazardous.

Rabies titer testing may be required to export a pet to a rabies-free country. Although no “protective” titer is known in animals, a titer of less than or equal to 0.5 IU/mL detected by the fluorescent antibody virus neutralization (FAVN) method is accepted by most countries. Depending on the country, other requirements must be fulfilled, such as identification with microchip or tattoo.

Virus neutralizing antibody is critical for protection against rabies following exposure. Rabies is considered a core vaccination in countries where the disease is endemic.<sup>50,82,249</sup> Current vaccines provide excellent protection; the presence of neutralizing antibody at the time of exposure eliminates the virus prior to neuronal infection. In the unvaccinated cat, this immune response occurs too late to prevent neuronal spread. Rarely, rabies can occur in vaccinated animals; thus any animal exhibiting signs compatible with rabies should be handled as such, regardless of vaccination history.<sup>199</sup> Any cat dying from neurologic disease for which antemortem diagnosis was not obtained should be submitted for rabies testing.

## FELINE RETROVIRUSES

The feline retroviruses, feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), are members of the Retroviridae and are among the most common and important infectious diseases of cats. FeLV and FIV are found worldwide, with variable seroprevalence depending on geography and risk factors (Table 33-5). Although infected cats may remain clinically well for prolonged periods (especially with FIV infection), retroviruses are associated with a wide variety of clinical problems, such as anemia, lymphoma, chronic inflammatory diseases, and secondary and opportunistic infections. Testing for FeLV and FIV should be part of the minimum database for all sick cats, even if previously tested negative.

Certain risk factors for infection are common to both FeLV and FIV worldwide. Sick cats are more likely to be seropositive than healthy cats, with sick feral cats having the highest risk. Other risk factors include age (>6 months), male gender, and access to outdoors. Low-risk groups include juvenile cats (<6 months) and spayed or neutered cats.<sup>94,170,172,196</sup> However, in one large study in the United States, seroprevalence in healthy feral cats was similar to that of healthy outdoor pet cats.<sup>170</sup> An important risk factor is bite wounds. In a study of over 900 cats with bite wounds or abscesses, 19.3% of cats

**TABLE 33-5** Seroprevalence of FeLV and FIV in Selected Areas and Populations of Cats

Location	FeLV%	FIV%	Population
Japan <sup>137</sup>	N/A	28.9	3,323 cats
Belgium <sup>59</sup>	3.8	11.3	346 stray cats
Istanbul <sup>1308</sup>	5.8	22.3	103 owned, outdoor cats
United Kingdom <sup>196</sup>	3.5	10.4	517 stray cats
Finland <sup>278</sup>	1.0	6.6	196 stray cats
Germany <sup>92,94</sup>	3.7	3.2	17,462 cats
South Africa <sup>259</sup>	12.3	22.2	454 sick cats; 3.5% co-infected
Australia <sup>205</sup>	N/A	8	340 owned/feral cats
United States <sup>170</sup>	2.3	2.5	18,038 cats; 0.3% co-infected
Canada <sup>172</sup>	3.4	4.3	11,144 cats; 0.5% co-infected

FeLV, Feline leukemia virus; FIV, feline immunodeficiency virus; N/A, not available.

were seropositive for one or both viruses (FeLV 8.8%, FIV 12.7%, co-infected 2.2%) at the time of treatment.<sup>95</sup>

FeLV and FIV share several important properties. They are single-stranded diploid RNA viruses with a cone-shaped capsid made up of the core protein. They possess a lipid envelope in which the glycoproteins needed for attachment and entry into the host cell are embedded. Outside of the host animal, these viruses are very labile, lasting only minutes in the environment; thus direct contact between animals is the most efficient mode of spread.

During replication of the retroviruses, the RNA genome is converted into double-stranded DNA (provirus) by the viral enzyme reverse transcriptase. This enzyme has no proofreading ability and is mistake-prone. As a result, the retroviruses have a high mutation rate, and even within a host, the population is heterologous, differing slightly from one another; thus each animal is infected with a cloud of variants, rather than a single genotype. These mutations may lead to changes in phenotype, which will be discussed with the individual viruses, as well as antigenicity. After conversion to DNA, the viral genome becomes incorporated into the host cell DNA. This integration is permanent; thus for total elimination of the virus, all infected cells must be removed. This viral DNA then serves as the template for new viral RNA genomes, which are ultimately packaged and released from the infected cell.

### Feline Leukemia Virus

Feline leukemia virus was first described in 1964 in a cat with lymphoma and was formerly classified in the

subfamily Oncovirinae, referring to its oncogenic ability. The subfamilies of the Retroviridae were renamed, and FeLV is now classified as a gammaretrovirus. Within the gammaretroviruses, FeLV is classified into A, B, C, and T subgroups based on antigenicity and host cell target (Table 33-6). Subgroup A viruses are generally mildly pathogenic and are the forms horizontally transmitted. Subgroups B, C, and T arise by point mutations of subgroup A members, and in the case of subgroup B, by recombination with endogenous retroviruses. All cats have endogenous retroviral genetic material that is normally present in the genome and is inherited. These pieces of endogenous DNA are not pathogenic themselves and do not produce infectious virus particles. However, they can recombine with exogenous retroviruses, such as FeLV-A, and increase the pathogenicity of the infecting virus.

Although FeLV was only “discovered” in 1964, genomic analysis has determined that it evolved from a virus in an ancestor of the rat.<sup>21</sup> This event likely took place up to 10 million years ago in the North African desert, an area where both cats and rats lived.

### Transmission and Pathogenesis

Viremic FeLV-infected cats shed virus in many body fluids, including saliva, feces, milk, and urine. FeLV transmission occurs through sustained close contact among cats. Behaviors such as mutual grooming, sharing of food and water bowls and litter boxes, and fighting can contribute to transmission, primarily through saliva. Resistance to persistent infections increases with age, although the degree of natural resistance is unknown. Kittens less than 16 weeks of age are most likely to remain persistently infected after exposure. However, adult cats may be susceptible to FeLV infection after long-term exposure.<sup>100</sup>

FeLV can be transmitted to kittens by various routes from infected queens. Infected pregnant queens may suffer reproductive loss; kittens that survive to term are generally born viremic and fade quickly. Up to 20% of vertically infected kittens may survive to become persistently infected adults.<sup>111</sup> Transmission to kittens may also occur through the milk from an infected queen or through saliva when the queen cleans the kittens.<sup>111</sup>

FeLV subgroups are associated with distinct pathologies: Subgroup B is associated with lymphomas, C with nonregenerative anemia, and T with immunosuppression. This reflects the distinct syndromes that may be seen with FeLV infection—proliferative (cancer), degenerative (blood cell line depletion), and immunosuppression. The pathogenesis of FeLV infection can be thought of as occurring in six stages:

1. Virus enters through the oral cavity (e.g., by mutual grooming), where it infects and replicates in mononuclear white blood cells in the tonsils.
2. Transient cell-associated lymphatic and viremic spread of the virus to regional lymphatics occurs.
3. Spread of the virus to systemic lymphoid tissue occurs.
4. Infection of the blood cell precursors in the bone marrow occurs.
5. Secondary viremia disseminates the virus.
6. Virus replicates in many epithelial cells, including those of salivary glands, intestines, and conjunctiva.

In the past, it was thought that about one third of cats would become persistently viremic and two thirds would eventually clear infection.<sup>125</sup> Depending upon several factors, including immune status, age, dose, and strain of the virus, infection may be eliminated in the first three stages. Once bone marrow infection occurs, it becomes much less likely that the cat will clear the virus.

TABLE 33-6 Classification of FeLV Subgroups

Viral Subgroups	Frequency of Isolation in FeLV-Positive Cats	Associated Disease	Comparison by Species of In Vitro Replication
A	100% viremic cats, mildly pathogenic but highly contagious, mildly cytopathogenic	Hematopoietic neoplasia, experimentally may cause hemolysis	Cat, rabbit, pig, mink, human
B	Occurs with subgroup A in 50% or more of cats with neoplastic disease (lymphoma)	Not pathogenic alone, virulent in recombination with subgroup A, noncontagious	Cat, dog, cow, hamster, pig, human
C	Rarely isolated, arises by mutation from FeLV subgroup A	Nonregenerative anemia and erythremic myelosis, nonreplicating and noncontagious	Cat, dog, guinea pig, human
T*	Highly cytopathic, T-cell tropic virus; affinity for two host cell proteins: Pit1 and FeLIX; evolved from FeLV subgroup A	Lymphopenia, neutropenia, fever, diarrhea	Cat

\*Subgroup T is a variant of subgroup A. Changes in the envelope protein result in increased cytopathogenicity of T strains.

Adapted from Table 13-2 in Hartmann K: Feline leukemia virus infection. In Greene CE, editor: *Infectious diseases of the dog and cat*, ed 3, St Louis, 2006, Saunders Elsevier, p 107. Modified from Jarrett O: Feline leukemia virus subgroups. In Hardy WD, Essex M, McClelland AJ, editors: *Feline leukemia virus*, New York, 1990, Elsevier; and Nakata R, Myiazawa T, Shin YS, et al: Reevaluation of host ranges of feline leukemia virus subgroups, *Microbes Infect* 5:947-950, 2003.

**TABLE 33-7** Outcomes of Feline Leukemia Virus Infection

Outcome of FeLV Exposure	FeLV p27 Antigen in Blood	Viral Blood Culture	Viral Tissue Culture	Viral RNA in Blood	Proviral DNA in Blood	Viral Shedding	FeLV-Associated Disease
Progressive infection	Positive	Positive	Positive	Positive	Positive	Positive	Likely
Regressive infection	Negative or transiently positive	Negative or transiently positive	Negative or transiently positive	Transiently or persistently positive	Positive	Negative	Unlikely
Abortive exposure	Negative	Negative	Negative	Not tested	Negative	Negative	Unlikely
Focal infection	Negative	Negative	Positive	Not tested	Not tested	Variable	Unlikely

Adapted from Table 1 in Levy J, Crawford C, Hartmann K et al: 2008 American Association of Feline Practitioners' feline retrovirus management guidelines, *J Feline Med Surg* 10:300, 2008.

Evaluation of the FeLV-host relationship has been evaluated using real-time PCR, which provided new insight and evolving ideas on infection with FeLV.<sup>289</sup> This technology detects viral genetic material and can be designed to detect viral RNA or DNA (provirus—the DNA-integrated form of the virus). Researchers examined FeLV infection in vaccinated and unvaccinated cats and were able to define four separate classes of infection: abortive, regressive, latent, and progressive (Table 33-7):

1. Abortive infections are those in which the exposed cat produces an effective and early immune response preventing viral replication and eliminating virus-infected cells. These cats are negative for circulating viral antigen (core protein) and viral genetic material.
2. Regressive infections are those in which viral replication is limited, and a small population of virus-infected cells remain. These cats are also antigen negative, but viral genetic material can be detected in a small percentage of blood cells by PCR. These cats may go on to eliminate the virus completely. Regressively infected cats are not viremic (and therefore not contagious), but proviral DNA may be infectious through blood transfusion.<sup>39</sup>
3. Latent infection refers to those in which a moderate amount of proviral-infected cells remain. These cats are antigen negative but PCR positive. These latently infected cells have the potential for reactivation of virus replication but are not contagious as long as the infection remains latent.
4. Progressive infections are those in which virus replication is not eliminated; both viral antigen and genetic material can be detected in the blood of these cats, and they are actively shedding virus primarily in saliva and feces.<sup>96,98</sup> These cats are likely to become ill with FeLV-related disease.

In this study, these classifications were attained in the exposed cats within 4 to 8 weeks post-infection; however, these classifications are likely dynamic, especially in the

intermediate stages. Interestingly, vaccination was not found to prevent provirus integration; thus vaccinated cats that are exposed to FeLV may become latently infected. Finally, focal infections were reported in early studies, describing FeLV infection restricted to certain tissues.<sup>117</sup>

These and other results suggest that many cats may remain infected with FeLV for life following exposure but may revert to a regressive state.<sup>97,122,227</sup> One study of 597 Swiss cats found that 10% of cats negative on ELISA for p27 antigen were positive for FeLV provirus by PCR.<sup>122</sup> The provirus is integrated into the cat's genome, and so, it may not be possible to clear infection.<sup>36</sup> The clinical significance of antigen-negative, PCR-positive cats is unclear. One study evaluated 152 necropsied cats, with various disorders, that were negative for viral antigen but positive for FeLV provirus in bone marrow. A significant association with anemia, panleukopenia, and purulent inflammation, but not lymphosarcoma, was found.<sup>280</sup>

### Clinical Signs

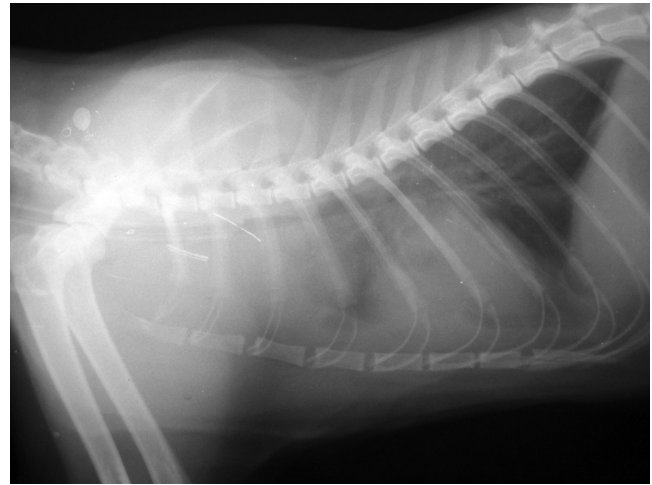
Following exposure, cats may exhibit mild clinical signs, such as fever and malaise, or may remain asymptomatic. For cats that remain persistently infected, this acute phase is followed by a period of asymptomatic infection that may last months or years. Ultimately, persistently infected cats develop one of several FeLV-associated disorders (Box 33-4).

The B subgroup variants, which as stated above, arise in about 50% of infected cats by recombinational mutation between the infecting A subgroup with endogenous retroviruses, are oncogenic primarily by insertional mutagenesis. The provirus integration into the host cell genome activates a cellular oncogene or disrupts a tumor suppressor gene.<sup>84</sup> Some of these genomic loci for cellular integration have been identified, such as those for lymphomas,<sup>84</sup> the most common tumor of cats. The most common malignancies associated with FeLV are lymphomas and leukemias, but nonhematopoietic

## BOX 33-4

## Retrovirus-Associated Illnesses in Cats

- Common illnesses associated with feline leukemia virus (FeLV) infection
  - Hematologic disease: anemia (most commonly nonregenerative), neutropenia, thrombocytopenia
  - Lymphoma: common sites include mediastinum, eye, and multicentric forms
  - Myelopathy: gradually progressive neurologic dysfunction; abnormal vocalization and behavior, hyperesthesia, paresis progressing to paralysis
- Common illnesses associated with FIV infection
  - Stomatitis: variable severity, often refractory to conservative treatment
  - Neoplasia: most commonly lymphoma, but also other tumor types, including sarcomas and carcinomas
  - Ocular disease: most commonly uveitis and chorioretinitis
  - Central and peripheral neurologic disease: abnormal behavior, nystagmus, ataxia, seizures, paresis, and paralysis
  - Hematologic disease: anemia and leukopenia; often more than one cell line involved
  - Renal disease: similar to nephropathy in human immunodeficiency virus (HIV) patients
- Common secondary diseases in retrovirus-infected cats
  - Systemic infections: *Toxoplasma*, *Cryptococcus*, *Mycoplasma haemofelis*, feline infectious peritonitis
  - Gastrointestinal: stomatitis/gingivitis, parasitism (*Giardia*, coccidia, *Cryptosporidia*), bacterial infection (*Salmonella*, *Campylobacter*), chronic diarrhea
  - Dermatologic: *Demodex*, ringworm
  - Respiratory/ocular: herpesvirus keratitis, chronic upper respiratory infections/sinusitis, uveitis, chorioretinitis, spastic pupil syndrome (FeLV associated)
  - Urinary tract: pyelonephritis, bacterial cystitis



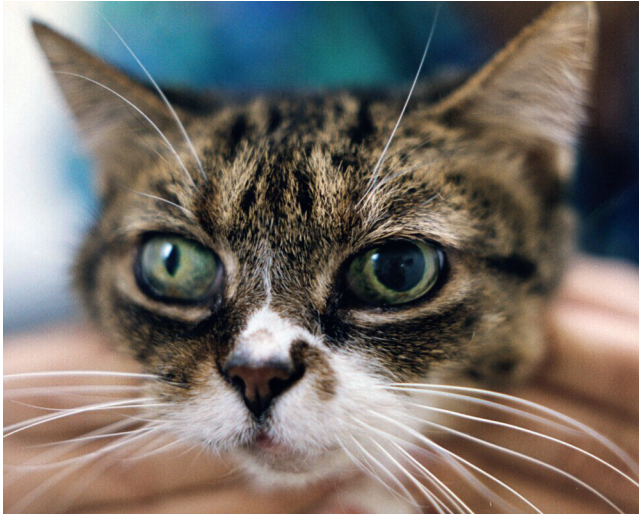
**FIGURE 33-19** Radiograph of a mediastinal mass in a young cat with FeLV.

malignancies are occasionally seen. Prior to the 1980s, about 80% of feline lymphomas were FeLV-related. A dramatic shift has occurred, where now only a small percentage of cats with these malignancies are FeLV-positive. For example, only 8% of cats with lymphoma treated at the Animal Medical Center in New York between 1988 and 1994 were FeLV positive.<sup>44</sup> PCR detection of proviral DNA in tumor tissue may uncover more cases than FeLV antigen testing alone.<sup>111,303a</sup> Lymphomas are classified based on anatomic locale as mediastinal (thymic; **Figure 33-19**), alimentary, multicentric (lymph nodes), or extranodal (kidneys, CNS, skin). Leukemias are generally classified as to the cellular origin (e.g., erythroid, granulocytic, myelocytic).

Anemias, mainly nonregenerative, are one of the most common clinical problems in FeLV-infected cats. Occasionally, regenerative anemia associated with *M. haemofelis* or immune-mediated destruction is seen. FeLV-infected cats may also develop anemia of chronic disease. The C subgroup variants are rare and are associated with fatal red cell aplasia. These variants arise from mutations in the envelope glycoprotein gene of the infecting subgroup A virus. This mutation leads to a change in cell receptors used by the virus from the thiamine transporter to the heme exporter.<sup>265</sup> This switch in host receptors is believed to disrupt early erythropoiesis, leading to a nonregenerative anemia, typically with a hematocrit less than 15%, that is resistant to therapy.

Immunosuppression is one of the most common manifestations of FeLV infection, and is very complex. Some viral proteins, particularly the transmembrane protein p15e, are directly immunosuppressive. The p15e protein affects the interleukin 2 signaling pathway.<sup>155,191</sup> In addition, FeLV infection may lead to lymphopenia, especially a decrease in CD8+ cytotoxic T lymphocytes, which are critical for viral immunity. Granulocytopenia may also occur, as well as effects on neutrophil function. The result is recurrent or chronic infections with other pathogens (e.g., poxvirus, *M. haemofelis*, *Cryptococcus*, *Toxoplasma gondii*), including agents that are usually of little clinical significance, such as *Salmonella* or *Listeria*.<sup>175,240</sup> Concurrent infection with feline coronavirus may lead to FIP development. Other infections, such as abscesses, rhinitis, and stomatitis, may be slow to resolve.<sup>175</sup>

FeLV may be neuropathogenic.<sup>60</sup> Neurologic disease not associated with malignancy has been described and manifests clinically as anisocoria (**Figure 33-20**) and mydriasis, Horner syndrome, urinary incontinence, abnormal vocalization, hyperesthesia, as well as paresis and paralysis.<sup>29,34</sup>



**FIGURE 33-20** FeLV may cause neurologic disease not associated with malignancy that may manifest as anisocoria.

### Diagnosis

Diagnosis of FeLV-related disorders is multifaceted. Clearly, confirmation of FeLV infection is of primary importance, but since FeLV-infected cats may be concurrently infected with other pathogens that are treatable, such as *M. haemofelis*, identification of any concomitant pathogen is critical as well. A minimum database (CBC, serum chemistry panel, urinalysis) is important for the investigation of any sick cat, including cats that may have retroviral infections. Low neutrophil and thrombocyte levels are commonly seen in FeLV-infected cats, as well as anemia.<sup>92,93</sup> No consistent abnormalities are seen in serum chemistry panels from FeLV-infected cats.

Confirmation of FeLV infection relies on detection of the virus. There are several in-clinic screening ELISA-based kits available that detect the core protein of the virus (p27). These tests vary in sensitivity and specificity, but most have high negative and positive predictive values.<sup>113,256</sup> In-clinic kits may be used with anticoagulated whole blood, serum, or plasma, although the test kit should be checked for the manufacturer's recommendations. Most cats will test positive for soluble FeLV antigen with ELISA early in primary viremia, within 30 days of exposure. The exception is those ELISA tests using saliva and/or tears; these tests do not detect viral antigen until the epithelial cells are infected, at stage 6, and are thus not ideal for routine screening. As well, they are known to have a high rate of false results.<sup>165</sup>

Because ELISA tests for FeLV detect viral antigen, and not antibody to the virus, maternal immunity will not interfere with testing. Vaccination generally does not interfere with testing; however, blood samples drawn immediately following vaccination may contain detectable FeLV vaccine antigen.<sup>166</sup> It is unknown how long this type of test interference lasts; FeLV testing should always be performed before vaccination. Confirmation

of a positive result obtained by ELISA is recommended, particularly in healthy cats. False-positive results are possible, and may be due to a number of factors, including improper testing or kit storage and hemolyzed samples. A false-negative test result can occur if the cat is tested too early in the course of infection for detection of soluble antigen (less than 4 weeks). In addition, because ELISA testing on serum can detect early infection, it does not distinguish transient from persistent infection. The confirmatory test recommended is the immunofluorescence assay (IFA), which detects virus-infected cells, primarily neutrophils. It is best performed on a smear prepared from fresh whole blood, because anticoagulants can interfere with results.<sup>15</sup> This assay can detect infection only after the blood cell precursors in the bone marrow have been infected (stage 4), 6 to 8 weeks after exposure. Thus an IFA positive result indicates that the cat is likely persistently infected. False-negative IFA results may occur in leukopenic cats. False-positive results may occur if the smear is too thick, if background fluorescence is high, or if the test is performed and interpreted by inexperienced personnel.<sup>166</sup>

Early in infection a cat may be ELISA positive and IFA negative; these cats should be rechecked with ELISA in 1 to 3 months to determine their status. If the ELISA result remains positive, a second IFA test should be performed to confirm. Uncommonly, a cat may remain discordant (ELISA positive, IFA negative); the reason for this is unclear but may be seen in a latently infected cat that is periodically antigenemic. In these cases, PCR may be used to detect proviral DNA. PCR can also be used to detect provirus DNA in antigen-negative cats that are regressively or latently infected. PCR is recommended only for cats that are to be used as blood donors, cats with persistently discordant ELISA/IFA results, or antigen-negative cats that are believed to have a FeLV-related disorder, like lymphoma.

Recently, a novel PCR for detection of FeLV viral RNA in saliva has been described.<sup>97</sup> The diagnostic sensitivity and specificity, as well as positive and negative predictive values for the PCR, were very high when compared with conventional ELISA. In situations where the cost of testing is a barrier, such as shelters and multicat households, it is possible that pooled saliva samples could be used for screening. The method is sensitive enough to detect one infected cat in a pool of up to 30 samples.<sup>98</sup>

### Treatment

Illness in the FeLV-infected cat requires prompt attention for accurate diagnosis and institution of appropriate treatment. Because of the immunosuppression associated with FeLV, identification of secondary infections and treatment, which may be prolonged compared with noninfected cats, is required. Immunosuppressive drugs, such as corticosteroids, should be avoided unless specifically indicated.<sup>175</sup> For FeLV-associated anemias,

transfusions may be necessary. Treatment of FeLV-associated neoplasia should follow established regimens. Symptomatic and supportive treatment may also be required for FeLV-associated diseases.

Immunomodulatory treatment has been investigated for the FeLV-infected cat. *Staphylococcus* protein A (SPA), a cell wall component of the bacterium with immunoenhancing activity has been evaluated in FeLV-infected cats. In at least one study, although subjective assessment of owners indicated improvement with SPA treatment, objective parameters did not differ from those in cats given the saline control.<sup>190</sup> Several other treatments, such as acemannan and *Propionibacterium acnes*, have been evaluated and either failed to demonstrate efficacy or suffer from poorly-designed studies.

Interferon, an important antiviral cytokine has also been evaluated in FeLV-infected cats. The effects on the virus in cell culture were induction of apoptosis in infected but not uninfected cells, decreasing the amount of viral replication overall.<sup>41</sup> In vivo studies have had conflicting results; at least one study showed no improvement.<sup>190</sup> However, this study used human recombinant interferon. Treatment with feline recombinant interferon (Virbagen Omega, Virbac) has shown evidence of improved clinical picture and survival, but evaluation of virologic parameters was not performed in these studies.<sup>52,189</sup> A suggested treatment protocol is 1 million U/kg, SC, every 24 hours for 5 consecutive days.

Lymphocyte T-cell immunomodulator (LTCI; ProLabs, St. Joseph, Mo.) is a product of the thymic stromal epithelial cells that is now commercially available for feline retrovirus-infected cats. It enhances interleukin-2 and interferon production by T-helper lymphocytes, which, in turn, enhances cytotoxic T-lymphocyte activity.<sup>90</sup> LTCI is supplied in 1-mL single dose vials for SC injection, and the recommended protocol is an initial three-dose regimen given at weekly intervals, with further treatments as necessary. LTCI is reported to improve clinical and hematologic parameters. However, controlled clinical trials have not been published.

The antiretroviral drug 3'-azido-2'3'-dideoxythymidine (AZT) has shown some positive effects but can have serious side effects at higher dosages (recommended dose is 5 to 10 mg/kg, PO, every 12 hours).<sup>175</sup> Careful monitoring of the patient's CBC is necessary, because AZT can cause bone marrow suppression, especially anemia. Many other antiretroviral drugs are too toxic for use in the cat or are not effective against FeLV or FIV.

FeLV-infected cats that are otherwise healthy can be maintained, sometimes for years, without problems. Data on survival of retroviral infected cats indicate that the lifespan of FeLV-infected cats is generally shorter than that of uninfected cats. In one study conducted in the United States, records of 67,963 cats that were tested for FeLV and FIV in 2000, and that had outcome information available 6 years later, were analyzed.<sup>169</sup> Survival of

### BOX 33-5

#### Client Education for Owners of Retrovirus-Infected Cats

- Confine retrovirus-infected cats indoors to prevent disease transmission to other cats and to protect the infected cat from trauma and infectious disease.
- Spay and neuter intact cats.
- Whenever possible, isolate infected cats from uninfected cats to prevent disease transmission.
  - Feline leukemia virus (FeLV) is primarily spread by close, intimate contact (i.e., between friendly cats); vaccination of any FeLV-negative in-contact cats is recommended.
  - Feline immunosuppressive virus (FIV) is primarily spread by bite wounds (i.e., between unfriendly cats); transmission is unlikely in socially stable households, and a decision to vaccinate any FIV-negative in-contact cats should be taken with care because of vaccine interference with testing.
- Feed a high-quality commercial diet.
- Avoid raw meat and eggs, and unpasteurized milk as potential sources of bacterial or parasitic infections.
- Monitor infected cats closely for potential signs of illness, such as
  - Changes in social interactions with people or other pets.
  - Changes in activity level and sleeping habits.
  - Changes in food or water consumption.
  - Unexpected weight loss or weight gain.
  - Bad breath odor.
- Consult a veterinarian promptly at the earliest sign of illness.

infected cats was compared with age-matched and sex-matched uninfected cats. The 6-year survival rates were 90% for uninfected cats, 65% for FIV-positive cats, and 51% for FeLV-positive cats. Most deaths in cats with FeLV or FIV occurred in the first year after diagnosis, probably because of the illness that prompted the original veterinary visit or because of euthanasia for purposes of infection control. A study of 17,289 cats in Germany tested for FeLV and FIV from 1993 to 2002 included survival data on 100 randomly selected cats: 19 FIV positive, 18 FeLV positive, and 63 uninfected.<sup>94</sup> The mean survival time of FeLV-positive cats (312 days) was significantly shorter than that of FeLV-negative cats (732 days).

Owners should be advised that certain precautions should be instituted with FeLV-infected cats, including isolation from uninfected cats (Box 33-5). This protects not only the uninfected cats from FeLV, but it also serves to limit the risk of exposure of the FeLV-infected cat to other feline pathogens.<sup>175</sup> Given that many

**BOX 33-6****Wellness Examination Procedures for Retrovirus-Infected Cats**

- Obtain a detailed medical, dietary and behavior history.
- Perform a thorough physical examination, with special attention to the lymph nodes, skin, eyes, and oral cavity.
- Weigh the patient accurately.
- Perform a complete blood cell count, serum chemistries, and urinalysis (cystocentesis collection) at least once yearly.
- Feline leukemia virus (FeLV)-infected cats should have a complete blood cell count at least every 6 months.
- Perform fecal examinations if the patient is at risk of intestinal parasite infection or has signs of gastrointestinal disease.

retrovirus-infected cats will survive for years after diagnosis, veterinarians should be familiar with guidelines for management of infected cats.<sup>166,175</sup> Wellness exams should be performed every 6 to 12 months to detect problems early (Box 33-6). Otherwise healthy retrovirus-infected cats require routine veterinary care, including surgical sterilization and dental prophylaxis. Simple precautions in the veterinary hospital will enable these patients to receive appropriate care safely (Box 33-7). FeLV is not considered a zoonotic disease; one study of 204 veterinarians and others with potential exposure to retroviruses, including needle sticks, failed to detect retrovirus infection using serologic and molecular methods.<sup>31</sup>

The necessity of vaccination of healthy FeLV-infected cats with core vaccines should be evaluated on an individual basis. Inactivated vaccines are often recommended because modified live virus vaccines have a theoretical risk of reversion to virulence in an immunosuppressed animal. However, definitive clinical evidence to support this recommendation is not available. FeLV vaccination is of no benefit and should not be administered to FeLV-infected cats.

**Prevention and Control**

FeLV testing may be performed for a variety of reasons (Box 33-8). The American Association of Feline Practitioners (AAFP) has stated that the retrovirus status of all cats should be known because the consequences of infection are important to the patient and any in-contact cats.<sup>166</sup> Preventing exposure of healthy cats to FeLV-infected cats by test and removal or isolation is an important way to prevent spread of the disease and is not replaced by vaccination as a control method.<sup>253</sup>

**BOX 33-7****Prevention of Retrovirus Transmission in Veterinary Hospitals**

- Feline leukemia virus (FeLV) and feline immunosuppressive virus (FIV) are fragile viruses that do not persist in the environment and are susceptible to all common detergents and disinfectants.
- Ensure routine infection-control measures are in place.
  - Ensure routine handwashing.
  - Disinfect equipment, cages, instruments, food/water bowls, litter boxes, and so forth.
  - Do not re-use dental or surgical instruments without sterilization.
  - Do not re-use needles/syringes or share bags of intravenous fluids among patients.
  - Avoid multidose vials of medication and vaccines.
  - Feed all cats individually; do not share dishes of food.
  - Carefully handle/dispose of infected body fluids (blood, urine, saliva, feces).
- House infected cats individually but not in special isolation or contagious disease areas.
- Screen blood donors appropriately for blood type and infectious diseases.

Kittens can be tested for FeLV at any age, because passively acquired maternal antibody does not interfere with testing for viral antigen. Newborn kittens infected from FeLV-positive queens may not test positive for weeks to months after birth. Although it may be tempting to test only a queen and not her kittens in an attempt to conserve resources in shelter or rescue settings, it is inappropriate to test one cat as a representative for others. Even young kittens may be exposed to cats other than the dam; for example, feral queens often share mothering of kittens. If a queen or any one of her kittens tests FeLV positive, all should be considered potentially infected and isolated, with follow-up testing to resolve status.<sup>111</sup> If a queen or one kitten in a litter tests negative, it cannot be guaranteed that the others are also negative. Shelters or rescue groups sometimes test pooled blood samples from litters of kittens in order to save money; the reliability of this method is unknown and cannot be recommended.

Certain populations of cats require tailored recommendations for control of retrovirus infections. Retrovirus testing and management for multicat environments such as shelters is discussed in Chapter 46. Breeding catteries have a low prevalence of FeLV infections, since the advent of test and removal programs over 30 years ago. However, these multicat environments require



**BOX 33-8****Summary of Feline Leukemia (FeLV) and Feline Immunosuppressive Virus (FIV) Testing Recommendations**

1. Cats that should be tested for FeLV and FIV include
  - a. At-risk cats: sick cats, cats with bite wounds or oral disease, cats with known exposure to a retrovirus-infected cat, cats in multicat environments where the status of all cats is not known, cats entering shelters or rescue organizations
    - Sick cats should be tested regardless of a negative FeLV or FIV test result in the past
  - b. Newly acquired cats and kittens
  - c. Cats about to be vaccinated for FeLV or FIV
2. Test for FeLV antigen and FIV antibody at presentation with in-clinic or referral laboratory enzyme-linked immunosorbent assay (ELISA)
  - a. Cats that test positive for FeLV and/or FIV
    - If FeLV positive, confirm with immunofluorescence assay (IFA)
    - If FIV positive and greater than 6 months of age
      - i. If not FIV vaccinated, confirm with Western blot
      - ii. If known or possibly FIV vaccinated, confirm with an alternative test methodology, such as a validated polymerase chain reaction (PCR) test

If FIV positive and less than 6 months of age, re-test at intervals of 30 days until the kitten tests negative or is greater than or equal to 6 months of age
  - b. Cats that test negative for FeLV and FIV
    - Ideally, all cats should have confirmatory testing performed to ensure negative status; however, it is most important for sick cats and cats with bite wounds
      - i. Although FeLV retesting alone can be performed in a minimum of 30 days, it may be more practical and cost effective to re-test for both viruses in a minimum of 60 days with in-clinic or referral laboratory ELISA
3. Cats at ongoing risk of infection (e.g., cats with access to outdoors) should be tested annually for FeLV and for FIV, if not FIV vaccinated, with in-clinic or referral laboratory ELISA
4. Cats used for blood or tissue donation in practice or in shelters should have negative screening tests for FIV antibody, as well as FeLV antigen and FeLV provirus by serology and real-time PCR, respectively

ongoing disease surveillance because factors such as group living and introduction of new cats favor transmission of infectious diseases. The retrovirus status of all cats in a breeding cattery should be known, and ideally negative test results should be confirmed.

**BOX 33-9****Retrovirus Infection Prevention Recommendations for Breeding Catteries**

- Any newly acquired cats should be isolated and tested before introduction into the population.
- Queens sent outside the cattery for breeding should only be mated to toms known to be retrovirus negative. Upon return, the queen should be isolated and tested in 60 days.
- Cats that have left the cattery for a cat show do not need to be re-tested, because cat shows are very low-risk environments for retrovirus transmission.
- Catteries that maintain retrovirus-negative status do not require vaccination of cats against feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV), as long as no cats have access to outdoors.
- Catteries that rely heavily on sending queens to an outside stud service should consider FeLV vaccination of queens in addition to testing.
- Vaccination against FIV is not recommended because FIV is uncommon in catteries and vaccination interferes with common testing methods.

Adapted from Levy J, Crawford C, Hartmann K et al: 2008 American Association of Feline Practitioners' feline retrovirus management guidelines, *J Feline Med Surg* 10:300, 2008.

Infected cats should be removed from the cattery. Additional recommendations for breeding catteries are found in [Box 33-9](#).

Vaccination against FeLV is not considered a core vaccine but is recommended for cats at risk of exposure<sup>50,175,249</sup> (e.g., cats with access to outdoors, cats living with known FeLV-infected cats, multicat environments where the status of all cats is not known). In addition, vaccination of all kittens has been recommended,<sup>249</sup> because a kitten's status (indoor vs. outdoor; low risk vs. high risk) may change, and susceptibility to persistent infection is highest in kittenhood. Several vaccines are available, including whole inactivated virus, subunit, and recombinant canarypox vector vaccines (which may be administered subcutaneously or intradermally). Testing of cats prior to vaccination is recommended to ensure negative status. Inadvertent use of FeLV vaccine in a cat infected with FeLV is not harmful, but it is also of no benefit. However, vaccination of a cat that is unknowingly retrovirus-infected gives false expectations to the owner and will give rise to unnecessary questions of vaccine efficacy when the infection is eventually discovered.

The efficacy of the available vaccines is controversial.<sup>272</sup> Many of the published efficacy trials have been conducted or supported by the vaccine manufacturer, and most studies do not evaluate more than one vaccine.

Other factors hamper interpretation of vaccine efficacy, such as lack of standard challenge and testing protocols, and the difficulty of infecting adult cats for a trial. Generally, inactivated whole virus vaccines have been recommended. In addition, a recombinant FeLV vaccine provided protection against persistent antigenemia equivalent to an efficacious inactivated whole virus vaccine.<sup>104</sup> One study using whole inactivated virus vaccines found that, after challenge, vaccinated cats had no detectable viral antigen, RNA, proviral DNA, or infectious virus.<sup>290</sup> Other studies have shown that vaccines do not prevent the persistence of proviral DNA following exposure.<sup>123</sup> Despite these findings, several current vaccines are efficacious at preventing persistent virus persistence and replication, as well as FeLV-associated disease.<sup>121</sup>

### Feline Immunodeficiency Virus

Feline immunodeficiency virus (FIV) was discovered in 1986 in a California cattery where cats had immunodeficiency-like illnesses.<sup>224</sup> FIV is a member of the Retroviridae, along with FeLV, but is classified in a different subfamily, Lentivirinae, along with HIV, equine infectious anemia virus, and ovine progressive pneumonia/caprine arthritis encephalitis viruses. The immunodeficiency viruses of domestic cats are classified into several subtypes or clades, designated A to E, based on the antigenicity of the envelope glycoprotein, gp120. Some authorities also recognize a sixth clade (F) that is found predominantly in Texas. Prevalence of the various clades varies geographically, although most field isolates belong to clades A or B. In the United States, clades A, B, C, and F have been identified, with clade B being predominant.<sup>303</sup> In Canada, clades A, B and C have been identified.<sup>246</sup> In the United Kingdom, only clade A is found, and in Japan, all clades have been identified. In general, clade A is thought to be less pathogenic than clades B and C. Within a clade, variations in genotype as well as phenotype may occur, including emergence of more pathogenic subtypes.<sup>53,230</sup> Recombination between two distinct isolates may also occur with co-infection, leading to new strains as well.<sup>118</sup>

Infection with lentiviruses related to FIV has also been documented in many nondomestic cat species around the world, such as the lion, puma, and Florida panther. In general, the isolates from nondomestic cats are less pathogenic than domestic cat FIV. This suggests that nondomestic cats have been living with the virus for a long time and that infection of domestic cats is more recent. Domestic cats can be infected with isolates from nondomestic felids, but do not develop the same clinical and immunologic abnormalities found in FIV-infected cats.<sup>292,297</sup>

Variations in the envelope glycoprotein affect cross-reactivity and cross-protection among virus strains. The

virus structure, stability, genomic characteristics, and replication at the cellular level are similar to FeLV. One of the main target cells of FIV is the CD4+ T-helper lymphocyte, which is essential for both cell-mediated and humoral immunity. Dysfunction and destruction of these cells are critical to the pathogenesis of disease. But FIV has a relatively broad cell tropism, and it is not restricted to cells expressing CD4; it may also use chemokine receptors for cellular attachment and entry. The virus also replicates in B lymphocytes, monocytes and macrophages, salivary gland epithelia, and fibroblast and neural cell lines.

FIV has a high mutation rate because of an error-prone reverse transcriptase enzyme, leading to the circulation of many heterologous strains, even within a single host. Some of these mutations may lead to changes in virulence or antigenicity.<sup>53,230</sup> This tremendous variation has an impact on diagnostics, therapeutics, and vaccine development.

### Transmission and Pathogenesis

The virus is present in saliva of infected cats, and FIV infection is most likely to occur in male cats and free-roaming cats, reflecting the efficient transmission by bite wounds. Queens may be infected during mating if bitten by an infected tom cat. However, transmission through sustained contact among infected and uninfected cats, as with FeLV, may occur.<sup>7</sup> In addition, *in utero* and lactogenic transmission to kittens from queens may occur, especially if the queen is experiencing high levels of viremia.<sup>12,208</sup> *In utero* transmission may lead to fetal resorption, abortion, or stillbirth and is likely due to placental inflammation.<sup>40</sup> However, experimental evidence suggests that not all kittens in a litter will acquire infection *in utero* from an FIV-infected queen.<sup>207,208,251</sup> When the pregnant queen is acutely infected and has a high viral load, most of the kittens will become infected. However, when the pregnant queen is chronically infected and healthy, with low a viral load, few kittens will become infected. Complicating the picture is the fact that some kittens born to FIV-infected queens have FIV provirus detected in tissues, but not blood, and are negative for FIV antibody in blood.<sup>11</sup> In experiments, queens can be infected through semen, but it is unknown how important this mode of transmission is in nature.<sup>139</sup>

After inoculation of FIV, the virus replicates in T-helper (CD4+) lymphocytes, which are critical cells for appropriate and adequate immune responses to infecting pathogens. The virus binds through CD134 molecules and may also use a chemokine receptor (CXCR4) for attachment on the cell surface.<sup>68</sup> CD134 protein is upregulated on activated T lymphocytes, thus making these cells the primary target of FIV. Viral infection of these cells leads to disruption of normal function, as well as cell death. In addition, during the acute phase of infection, infection of a subset of T-helper cells, the

T-regulatory lymphocytes, contributes to the disease process. These cells have an immunosuppressor function. It has been shown that infection of these cells with FIV leads to activation, and, by definition, immunosuppression. This may contribute to the ineffective FIV clearance with resultant chronic infection, as well as immunodeficiency.<sup>194</sup> In addition to T lymphocytes, the virus infects macrophages and dendritic cells.

Following cellular infection, as part of the replication cycle, viral RNA is transcribed to double-stranded DNA by the viral enzyme reverse transcriptase. The DNA product then integrates into the cellular DNA as a provirus. In activated lymphocytes, viral RNA is transcribed using the DNA template by the cellular RNA polymerase. This is followed by viral protein synthesis, assembly of the virion, and release of the infectious virus. In nonactivated cells, the replication cycle may stop at the provirus stage; this is referred to as a latent infection, which may be reactivated with activation of the lymphocyte, allowing completion of the viral replication cycle.<sup>126</sup> This ability of the virus to persist integrated into the cellular genome makes treatment as well as prevention through vaccination challenging.

Most infected cats will mount an immune response to the virus, which leads to decreased virus replication and viral load in infected cats, but not elimination of infection. This generally occurs within 1 to 3 months postinfection, and the cat will then enter an asymptomatic phase. Virus replication continues, but at very low levels. This phase may last for months or years. Initially, levels of both CD4+ and CD8+ lymphocytes decline. As the cat mounts an immune response, a rebound of CD8+ lymphocytes above preinfection levels occurs. This causes an inversion of the CD4+:CD8+ lymphocyte ratio (the normal ratio is 2:1) that is persistent. Over time, the level of both CD4+ and CD8+ lymphocytes may gradually decline, ultimately leading to immunodeficiency in the infected cat.

### **Clinical Signs**

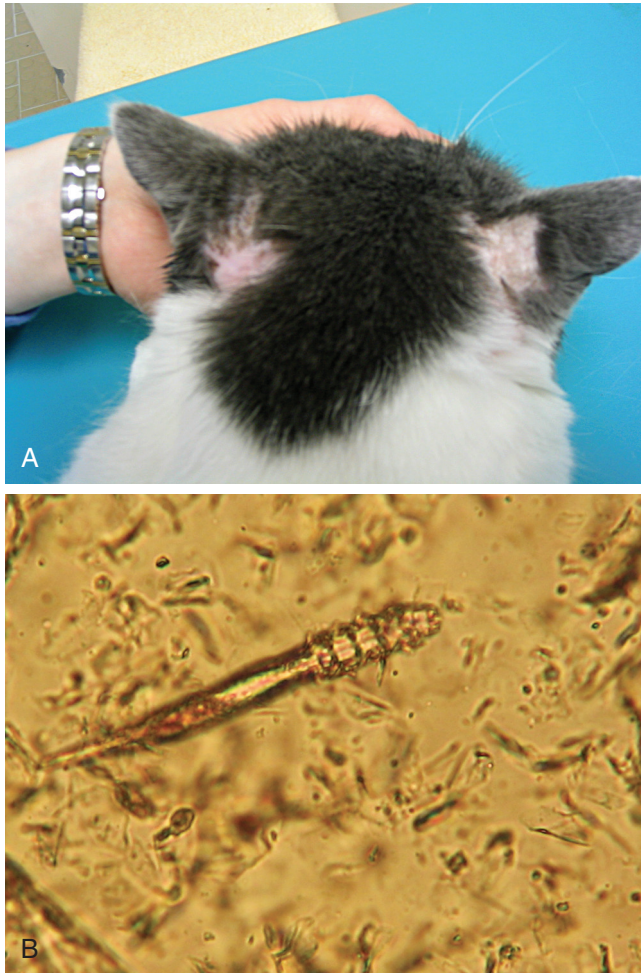
FIV infection can be categorized into clinical stages similar to HIV infection, and various schemes for staging cats have been devised.<sup>99,136,222</sup> A simplified and useful categorization for the practicing clinician is as follows, bearing in mind there may not be clear distinction between phases and not all cats will demonstrate each phase:

1. Acute phase: Clinically, cats may present in the acute phase of infection with signs such as depression, anorexia, fever, and lymphadenopathy. Some cats, however, remain asymptomatic immediately following infection.
2. Clinically latent phase: A significant period of asymptomatic infection follows that may last for months or years. During this asymptomatic stage,

however, changes in blood cell values may occur. Although hematologic abnormalities are less common than in FeLV-infected cats, FIV infection of bone marrow cells may lead to peripheral cytopenia of one or more cell lines.<sup>83,93</sup> In addition, FIV-infected cats have higher serum total protein and globulin concentrations than uninfected cats. In one study, FIV-infected cats also had lower serum aspartate transaminase (AST) and glutamate dehydrogenase than uninfected cats.<sup>93</sup>

3. Acquired immunodeficiency syndrome (AIDS)-related complex (ARC) phase: As the cat progresses to the immunodeficient state, secondary infections may occur. In addition, immune-mediated diseases resulting from immune cell activation may also occur. These manifestations generally occur later in life, perhaps years after initial FIV infection. Cats may present with single or combinations of infectious agents, including viral, bacterial, fungal, protozoal, and parasitic, and clinical signs may involve any system. Infections may be chronic or intermittent/recurring in nature.
4. Acquired immunodeficiency syndrome (AIDS) phase: This terminal phase of infection is characterized by neurologic disorders, neoplasia, multiple concurrent infections, and serious opportunistic infections. Survival time is no more than a few months.

The clinical signs and illnesses associated with FIV are varied and nonspecific (see [Box 33-4](#)) and are usually not a direct effect of the virus but resulting from secondary infections that may be treatable, such as *Demodex*-associated skin disease ([Figure 33-21](#)). One of the most common clinical presentations is chronic gingivostomatitis ([Figure 33-22](#)),<sup>126</sup> though the precise pathogenic mechanisms at work are unclear. Histologic findings include lymphocytes, plasma cells, and variable neutrophilic and eosinophilic infiltrates. Ocular disease has been well characterized in cats with FIV, with abnormalities in both the anterior (uveitis [[Figure 33-23](#)], glaucoma) and posterior (pars planitis, retinal degeneration, retinal hemorrhage) segments.<sup>70,158,306</sup> Neoplasia is also common in cats with FIV and includes various tumors, such as lymphomas (primarily B lymphocyte) and leukemias. FIV may infect neural tissue, causing neurologic disease, affecting central or peripheral nerves. Clinical signs reported include seizures, behavior changes, cognitive difficulties, and paresis.<sup>126</sup> Renal disease has also been associated with FIV infection and may be similar to HIV-associated nephropathy. Affected cats have glomerular and tubulointerstitial lesions, elevations in BUN and creatinine, and proteinuria.<sup>232,233</sup> In one group of 155 cats with FIV, azotemia and proteinuria were more common than in age-matched uninfected cats.<sup>164</sup> In an Australian case-control study of 73 cats with chronic



**FIGURE 33-21** Secondary causes of disease are common in FIV-infected cats, such as this cat with alopecia and pruritus (A). Skin scrapings revealed infection with *Demodex cati* (B).

kidney disease (CKD) and 69 control cats, cats less than 11 years of age with CKD were significantly more likely to be FIV positive than cats of similar age without CKD.<sup>304</sup>

### Diagnosis

Routine diagnosis of FIV infection currently relies on detection of virus-specific antibody. Rapid screening for viral antigen is not possible, because the amount of circulating virus is low after the acute stage of infection. FIV produces a persistent, lifelong infection so that the detection of antibodies is sufficient for diagnosis as long as the cat has not been vaccinated for FIV. Detection of FIV-specific antibodies is initially performed using a point-of-care ELISA or immunochromatography kits. Using these kits, most cats will have detectable FIV antibody within 60 days of infection, but some cats take up to 4 months to seroconvert.<sup>15</sup> Comparison of several diagnostic kits that are commercially available indicate a high sensitivity and specificity and significant (>90%) negative and positive predictive values in cats with no



**FIGURE 33-22** One of the most common clinical presentations in cats with FIV is chronic gingivostomatitis.



**FIGURE 33-23** Ocular disease has been well characterized in cats with FIV, such as the anterior uveitis seen in this cat.

history of FIV vaccination.<sup>113,168</sup> Despite these results, confirmation of positive ELISA results when screening a healthy cat is recommended. Although virus culture is considered the gold standard for FIV infection, it is not readily available in many countries, and it is time-consuming and labor-intensive. A different soluble antibody test has been recommended as a confirmatory test,<sup>166</sup> but to date, only one FIV antibody test is commercially available in Canada and the United States. Western blot and immunofluorescent antibody assays are available in many countries. These assays detect antibodies against an increased number of viral antigens and are suggested as confirmatory tests in seropositive cats with no history of FIV vaccination.

Kittens born to infected or FIV-vaccinated queens may acquire FIV antibodies in colostrum. In a study of such kittens, FIV antibodies persisted past 8 weeks of age in more than 50% of kittens (n=55) born

to FIV-vaccinated queens (n=12), but were no longer detectable at 12 weeks of age.<sup>177</sup> In another study, passively acquired antibodies in five kittens from infected queens declined to undetectable levels only by 17 weeks of age.<sup>237</sup> None of the routine testing methods can distinguish passively acquired maternal antibodies from antibodies produced by infected kittens; thus kittens less than 6 months of age testing positive using these assays should be retested when maternal antibodies wane. For example, kittens can be retested at intervals of 30 days until negative for FIV antibody. Though infection of kittens, even those born to infected queens, is uncommon, one must assume a kitten testing positive is contagious until a negative result is achieved. Kittens greater than 6 months of age with FIV antibodies are more likely to be infected. Because of concerns regarding detection of passively acquired FIV antibodies, it is tempting to delay testing kittens for FIV until after 6 months of age. Because they are a low-risk group, most kittens test negative and can reliably be considered clear of infection. However, infected kittens could be a source of infection for other cats if they are not identified and isolated. Compliance of both owners and veterinarians with retroviral testing recommendations was low in one published study so that delaying testing of newly acquired kittens until 6 months of age would potentially result in many cats that never undergo FIV testing at all.<sup>95</sup>

The recent development of a vaccine for FIV has complicated testing in countries where it is available (e.g., Canada, United States, Australia, New Zealand, and Japan, but not Europe), because the current technology used for screening tests cannot distinguish natural infection from vaccination.<sup>168</sup> Antibodies derived from vaccination persist for more than 1 year, and possibly for more than 4 years.<sup>166,168</sup> In some cats, it may be difficult to determine if a positive FIV antibody test means the cat is truly infected with FIV, is vaccinated against FIV but not infected, or is vaccinated against FIV and also infected. Detection of viral nucleic acid by PCR has been proposed as an alternative testing method for vaccinated cats. However, both false-negative and false-positive results may occur.<sup>25,46</sup> For the former, the most common cause is the inherent genetic variability of FIV strains, making development of a genetic assay that can detect all strains challenging. False-positive results have been observed in vaccinated cats.<sup>46</sup> In one study, the sensitivity and specificity of PCR testing for FIV varied tremendously among laboratories. The most accurate test was the real-time PCR, but it had a sensitivity of only 76%.<sup>46</sup> Newer PCR technologies, such as dual-emission fluorescence resonance energy transfer (FRET) real-time PCR, may prove more reliable for discrimination of FIV-vaccinated from FIV-infected cats.<sup>301</sup>

Because of the limited sensitivity of currently available assays, PCR is not useful as a screening tool for FIV and will not replace in-clinic or referral laboratory ELISA

tests. Rather, PCR testing should be reserved for FIV antibody-positive cats that have an unknown vaccination history or that have been vaccinated against FIV but where infection is still suspected. PCR test results must be interpreted with caution. A positive FIV PCR result from a laboratory with stringent quality control should confirm FIV infection and should not be affected by FIV vaccination. However, a negative FIV PCR result does not rule out infection, but may reflect a level of viral nucleic acid below the limit of detection, or a strain of FIV that is not detected by the test.

Recently, a study has shown that cats vaccinated for but not infected with FIV may not produce antibodies to all FIV epitopes. The process of inactivation of the virus for vaccine production leads to alteration of the native structure of some virus proteins.<sup>154</sup> This alteration leads to loss of certain viral epitopes. As a result, cats infected with FIV would have antibody able to recognize these epitopes, while vaccinated cats would not, which has been shown in one study.<sup>167</sup> Use of these viral proteins to distinguish vaccine response from FIV infection may become commercially available.

### Treatment

As for FeLV infection, sick FIV-infected cats require prompt attention to diagnosis and appropriate treatment. Retrovirus-infected cats may respond to treatment as well as uninfected cats, although in some cases, longer or more intensive courses of therapy may be needed. It is important for both the veterinarian and owner to allow enough time for response to treatment and not become discouraged too quickly. Treatment of the ill FIV cat requires a full health evaluation, including a minimum database (CBC, chemistry panel, urinalysis) and identification of any secondary infecting agent. When secondary infections are identified, institution of appropriate treatment (e.g., doxycycline for *M. haemofelis* infection) may resolve the clinical problem. Supportive treatment may also be indicated depending on the severity of the illness. Treatment of chronic stomatitis with corticosteroids is controversial because of adverse effects with long-term use. Griseofulvin should never be used in FIV-infected cats, because it causes bone marrow suppression<sup>266</sup>; newer azole drugs are safe and effective for treatment of fungal infections.

Treatment of the FIV infection itself has focused primarily on drugs developed against HIV. Reverse transcriptase (RTase) inhibitors, such as AZT (zidovudine; Retrovir, GlaxoSmithKline), have been used in cats, both alone and in combination with other drugs. Reduced viral load and improved clinical status have been observed with AZT treatment (5 mg/kg, PO, every 12 hours).<sup>126</sup> Side effects are possible, including nonregenerative anemia, and cats should be monitored carefully during treatment, including complete blood cell counts. One study has shown that AZT alone or in combination

with another inhibitor of RTase was effective in preventing infection after exposure (treated cats that were exposed to the virus did not become infected), but did not have therapeutic value in chronically infected cats.<sup>14</sup> Another study showed in vitro viral replication was inhibited by AZT in combination with other nucleoside analogs.<sup>26</sup> AZT is best reserved for treatment of severe stomatitis/gingivitis or neurologic disease.<sup>166</sup> Another RTase inhibitor, stampidine, has shown antiretroviral activity in FIV-infected cats and is well tolerated.<sup>294,295</sup>

Other drugs that may potentially be valuable for FIV treatment but have not yet been tested in cats include other reverse transcriptase inhibitors, as well as inhibitors of viral protease, integrase, and envelope fusion.<sup>47,210,257</sup> A selective antagonist (AMD3100) to the cellular co-receptor for FIV (chemokine receptor CXCR4) has shown in vitro and in vivo activity against the virus, leading to reduced viral load.<sup>115</sup> In addition, side effects were not observed. A protease inhibitor that showed in vitro activity against FIV (TL-3) was shown to prevent and even counteract changes in the CNS from FIV.<sup>131</sup> As research continues, additional medications may become available and useful for FIV treatment.

Immune modulation has also been attempted for the FIV-infected cat. Cytokines, such as granulocyte colony-stimulating factor (G-CSF) and erythropoietin have been used to stimulate blood cell production in cases of neutropenia and anemia, respectively. As they are human in origin, antibodies to these cytokines are produced in treated cats, reducing the drugs' effectiveness within a few weeks.<sup>231</sup> Interferons, both human and feline origin, have also been used in FIV-infected cats. To date, only one study has been published on the use of recombinant feline interferon (rFeIFN; Virbagen Omega, Virbac) for FIV, and the study population consisted of 24 cats co-infected with FeLV.<sup>52</sup> In this multicenter, double-blind, placebo-controlled trial, rFeIFN-treated cats (1 million U/kg/day SC for 5 days in three courses: days 0 to 4, days 14 to 18, days 60 to 64) had improved clinical scores in the first 4 months, minor improvement in hematologic parameters, and lower rates of mortality. However, evaluation of virologic parameters was not performed and the study is difficult to interpret, because the data was not broken down by infection type (FeLV-infected only versus FeLV/FIV-infected). Ironically, one study evaluating human interferon (10 IU/kg, PO, once daily using a 7-day on, 7-day off treatment schedule) in 24 FIV-infected cats did show clinical improvement and improved survival in infected cats compared with six placebo-treated cats despite no change in viral loads.<sup>226</sup> However, the control group was small, and all cats were treated with antiparasitic drugs and antibiotics as needed.

To modulate the lymphocyte activation and proliferation, which may play a role in chronic inflammation associated with FIV (e.g., stomatitis), the

antiinflammatory product bovine lactoferrin may affect lymphocyte proliferation and cytokine production, and may provide clinical improvement.<sup>151</sup> Investigation of in vivo effects is ongoing.

As for FeLV, lymphocyte T-cell immunomodulator (LTCI; ProLabs) has been evaluated in cats with FIV. Limited data from placebo-controlled trials in small groups of cats has been published, reporting increased lymphocyte counts, more rapid recovery from respiratory infections, and reduced viral load in LTCI-treated cats.<sup>90</sup>

Data on survival of retroviral infected cats indicate that the lifespan of FIV-infected cats appears similar to that of uninfected cats. FIV-infected cats may have a long disease-free period, especially if wellness care is provided and exposure to other infectious diseases is limited. In one study conducted in the United States, records of 67,963 cats that were tested for FeLV and FIV in 2000, and that had outcome information available 6 years later, were analyzed.<sup>169</sup> The 6-year survival rates were 90% for uninfected cats and 65% for FIV-positive cats. Most deaths in cats with FIV occurred in the first year after diagnosis, probably because of the illness that prompted the original veterinary visit or because of euthanasia for purposes of infection control. A study of 17,289 cats in Germany tested for FeLV and FIV from 1993 to 2002 included survival data on 100 randomly selected cats, including 19 FIV-positive cats. There was no statistically significant difference in the mean survival time of FIV-positive cats (785 days) compared with FIV-negative cats (625 days).<sup>94</sup> In a study of 1,205 cats tested for FeLV and FIV in western Canada, FIV-positive/FeLV-negative cats were compared with randomly selected, age-matched and sex-matched FIV/FeLV-negative cats. The median survival time for FIV-positive cats (n=39, 3.9 years) was not significantly different from that of FIV-negative cats (n=22, 5.9 years).<sup>242</sup>

Management of the FIV-infected cat is similar to the FeLV-infected cat, including client education about isolation (again, not only to prevent spread of FIV, but to protect the FIV-infected cat from infectious agents carried by other cats) and other management issues (see [Box 33-5](#)). Guidelines for management of retrovirus-infected cats have been published.<sup>126,166</sup> Wellness exams should be performed every 6 to 12 months to allow early detection, diagnosis, and treatment of health problems (see [Box 33-6](#)). FIV-infected cats will require hospitalization both for treatment of illness and for routine wellness care (e.g., surgical sterilization, dental prophylaxis); simple infection control precautions should be instituted (see [Box 33-7](#)). Administration of perioperative broad-spectrum antibiotics should be considered for surgical and dental procedures. As for FeLV, FIV is not considered a zoonotic disease, and one study of 204 veterinarians and others with potential exposure to retroviruses failed to detect infection using serologic and molecular methods.<sup>31</sup>

Vaccination of healthy FIV-infected cats against core diseases should be evaluated on a case-by-case basis, taking into account individual risk factors. As for FeLV, inactivated vaccines are often recommended but no data exist to support the recommendation. Healthy cats with FIV have adequate immune responses to vaccination.<sup>48,163</sup> Concern exists that activation of infected lymphocytes by vaccinations may increase viral replication<sup>48</sup>; however, the clinical significance is unclear.<sup>126</sup> FIV vaccination is of no benefit and should not be administered to FIV-infected cats.

### Prevention and Control

Control of FIV is aimed primarily at preventing infection. FIV testing may be performed for a variety of reasons (see [Box 33-8](#)), including identification of infected cats to prevent disease transmission. It has been recommended that the retrovirus status of all cats should be known.<sup>166</sup> Neutering may limit aggressive behavior, thus limiting spread by fighting and bite wounds. Restricting contact with cats outside the household, especially feral cats that are at risk for FIV infection, is the ideal method of prevention.

A vaccination for FIV is commercially available, and contains inactivated whole virus isolates from clades A and D with an adjuvant. It has been found to induce antibodies as well as cell-mediated responses.<sup>211</sup> Studies of the currently available vaccine (Fel-O-Vax FIV; Boehringer Ingelheim Vetmedica, Inc.) conducted by the inventor or manufacturer have demonstrated efficacy when vaccinated cats were challenged with subtypes A and B.<sup>127,128,153,236</sup> One independent study showed that the vaccine was not able to protect cats when they were challenged by a subtype A field strain from the United Kingdom.<sup>63</sup> Although it offers some protection to some cats at high risk, its use remains controversial, and it is listed as noncore or not recommended by the major vaccine advisory groups.<sup>50,126,249</sup> An informed decision to use the vaccine requires local knowledge about prevalent FIV subtypes, which is typically not available to the practitioner, as well as better evaluation of vaccine efficacy against FIV field strains. Another important concern is that current screening/testing methods cannot distinguish naturally infected from vaccinated cats. In addition, because high-risk cats are those that are free roaming, these animals may be more likely to be seized by animal control authorities. Without identification and access to vaccination records, these cats may be inappropriately euthanized if tested FIV positive at the receiving facility.

Cats should be tested for FIV infection prior to vaccination. The AAEP guidelines recommend clients be informed of the difficulties interpreting FIV test results in vaccinated cats, the lack of knowledge about vaccine efficacy, and that vaccinated cats should be permanently identified, such as with a microchip, tattoo, and/or

collar.<sup>166</sup> Microchip databases can be used to record FIV vaccination histories.

As for FeLV, certain populations of cats require tailored recommendations for control of FIV. Retrovirus testing and management for multicat environments is discussed in Chapter 46. Although FIV is uncommon in breeding catteries, the retrovirus status of all breeding cats should be known. Vaccination against FIV is generally not required in catteries. Additional recommendations for control of retrovirus infections in breeding catteries are found in [Box 33-9](#).

## MISCELLANEOUS VIRUSES

### Other Viral Enteritis Agents

Agents of viral enteritis in cats other than coronavirus include astrovirus, rotavirus, reovirus, enterovirus, and calicivirus/norovirus.\* These agents, all nonenveloped RNA viruses, may survive for extended periods in contaminated environments. They are transmitted orally, and unlike parvoviruses, infect the intestinal epithelia from the lumen. They target mature epithelia at the villus tips, leading to intestinal villus atrophy. Disease, which manifests as diarrhea without blood, is typically only seen in very young animals, where turnover/replacement of intestinal epithelia is slower than in adults. The most serious consequence of disease in affected kittens is dehydration. Because these are not systemic infections, changes in leukocyte levels and other signs of systemic disease, such as depression and fever, may not be seen. Diagnosis can generally only be accomplished using electron microscopy. Treatment is supportive, with fluids being the key component. Environmental decontamination involves cleaning with a detergent to remove all organic matter followed by disinfection with an appropriate product with oxidizing activity (e.g., 6% sodium hypochlorite, potassium peroxymonosulfate). Zoonotic transmission of rotavirus and perhaps norovirus is possible; thus owners should take appropriate precautions when handling affected cats.<sup>43,263</sup>

### Bornavirus

Borna disease was named after a town in Saxony, Germany where, in 1895, an outbreak of fatal neurologic disease occurred in horses. The causative virus was identified in 1925 and named Bornavirus. Since that report, Bornavirus has been identified in a number of species, including cattle, donkeys, dogs, wild birds, and ostriches, and occurs virtually worldwide.<sup>140</sup> It is a cause of encephalomyelitis in many species. In the mid-1990s,

\*References 42, 43, 188, 248, 263, 275.

Bornavirus was isolated from cats experiencing a neurologic disease in Sweden called “staggering disease.”<sup>174</sup> Since that report, evidence of potential Bornavirus-associated disease has been described in cats in Australia, the United Kingdom, and Japan.<sup>141,201,213,245</sup> Evidence of infection with Bornavirus, but not necessarily disease, has been found in several countries. FIV-infected cats may have a higher prevalence of infection.<sup>119,130</sup>

Bornavirus is a single-stranded RNA virus in the family Bornaviridae with a helical capsid and a lipid envelope. Its genome is nonsegmented and approximately 9000 bases in length. Interestingly, the virus does not appear to be cytolytic. In affected animals, the disease is a nonsuppurative meningoencephalitis, and pathology includes an inflammatory response in the CNS and demyelinating lesions.<sup>103</sup> Asymptomatic infection has also been documented.<sup>204</sup>

The most characteristic clinical sign is hindlimb paraparesis and ataxia (staggering); other clinical signs include behavioral changes, lumbosacral pain, anorexia, hypersalivation, hypersensitivity to light and sound, visual impairment, seizures, and inability to retract the claws.<sup>103</sup> The clinical signs progress over 1 to 4 weeks until the patient either deteriorates to the point of death or euthanasia, or stabilizes. Recovered cats may be permanently affected with motor dysfunction or personality changes.<sup>103</sup>

The mode of transmission of Bornavirus is unclear, though vectorborne transmission has been postulated because of its seasonality, with most cases occurring in spring and summer.<sup>103</sup> Transmission by bodily fluids has also been proposed, and rodents and birds have been postulated to be reservoirs.<sup>103</sup> The virus is believed to reach the CNS from its site of entry by axonal migration. The immune response is believed to play a role in disease development and is primarily mediated by CD8+ T lymphocytes.<sup>23</sup>

Diagnosis is problematic and controversial, because the presence of antibody is not confirmatory and virus is present at low levels, even in affected tissue. Ante mortem, it is a diagnosis by elimination of other causes. As well as postmortem examination, histopathology, and immunohistochemistry of CNS gray matter for Bornavirus antigen may be required for diagnosis. Treatment is largely supportive; however, given the immunopathologic component, corticosteroids may be beneficial.

## Papillomavirus

Papillomaviruses are members of the Papovaviridae family and cause cutaneous warts in a number of animal species, including domestic and nondomestic cats. They are small, nonenveloped DNA viruses that are highly species specific, although there is one report in the literature of a feline papilloma associated with human



**FIGURE 33-24** Papillomavirus lesions in cats appear distinct from those in other species and are locally extensive, often multiple, and can appear on the skin or in the oral cavity. Cutaneous papillomas may be rough, raised, pigmented (A) or nonpigmented (B), scaly plaques. (A courtesy Kelly St. Denis. B courtesy Lisa Henderson, Veterinary Information Network.)

papillomavirus type 9.<sup>198</sup> In cats, though infection appears to be infrequent, papillomaviruses have been associated with papillomas, fibropapillomas, and squamous cell carcinomas.\* Papillomavirus skin lesions have also been reported in a cat with FIV infection.<sup>66</sup>

Papillomas most likely develop after introduction of the virus through skin lesions or abrasions. The papillomaviruses have a specific tropism for squamous epithelial cells. Lesions in cats appear distinct from those in other species and are locally extensive, often multiple, and can appear on the skin or in the oral cavity.<sup>279</sup> Oral papillomas are small, soft, light pink, oval, slightly raised, flat, and appear on the ventral lingual surface.<sup>279</sup> Cutaneous papillomas may be rough, raised, pigmented or nonpigmented, scaly plaques (Figure 33-24).<sup>279</sup> Histologic examination reveals pigmented, hyperplastic

\*References 35, 67, 108, 173, 197, 261.



epidermal plaques without evidence of inflammation.<sup>67</sup> Papillomavirus DNA has been identified in plaques and invasive squamous cell carcinomas. The virus causes hyperplasia of epithelia and contributes to epithelial proliferation in cutaneous neoplasms.<sup>197</sup>

The histology of feline fibropapillomas is very similar to equine sarcoid, and in one report, 17 of 19 tumors were positive for a papillomavirus most similar to bovine papillomavirus type 1.<sup>108,261</sup> Fibropapillomas appear to be most common in outdoor cats living in rural areas and cats with known exposure to cattle. Like equine sarcoids, local recurrence after excision is common and metastasis has not been reported.

Definitive diagnosis of feline papillomatosis is by immunohistochemical staining of tissue obtained during biopsy or surgical resection. PCR can also be used to demonstrate viral DNA in lesions. No specific treatment has been identified; surgical excision is rarely warranted. Spontaneous regression has occurred in other species, such as dogs.

## Poxvirus

Cats are most commonly infected with cowpox,<sup>200</sup> an orthopoxvirus in the Poxviridae family that is only found in Europe and Asia. Orthopoxviruses are enveloped DNA viruses that are relatively stable in the environment, surviving under dry conditions for months to years. They are readily inactivated by common disinfectants. The reservoir hosts are small rodents, such as voles and wood mice. Cowpox infection is seen primarily in rural cats that hunt rodents, and cases are typically seasonal, occurring in the summer and fall.<sup>20</sup>

The virus is probably inoculated under the skin through a bite wound. The virus replicates locally, producing a skin lesion, and then spreads systemically, causing more widespread skin lesions within 1 to 3 weeks. The skin lesions are small nodules at first, but form well-circumscribed ulcers that become scabbed.<sup>20</sup> The lesions gradually exfoliate after 4 to 5 weeks, and new hair growth occurs, although some lesions may result in permanent bald patches. Signs of systemic illness occur early in infection in some cats; they are generally mild and include pyrexia, anorexia, and depression. Severe or fatal disease is rare and is typically associated with immunosuppression, such as from retrovirus infection or administration of immunosuppressive drugs.<sup>20,262</sup>

Feline cowpox virus infection is diagnosed by culturing dried scab material, electron microscopy, or PCR. Serum antibodies can also be detected. Histologic examination of lesions reveals epithelial hyperplasia, vesicle formation and ulceration. Infected cells may contain intracytoplasmic eosinophilic inclusion bodies. No specific treatment has been identified; therapy is primarily supportive, such as broad-spectrum antibiotics for

secondary bacterial infections. Corticosteroids should be avoided.

Cat-to-cat and cat-to-human transmission has been documented.<sup>16,71,107,262</sup> Human cowpox infection is rare in the United Kingdom, but more than half of cases are due to transmission from cats.<sup>16</sup> Cowpox causes skin lesions in humans, as well as systemic infections. Basic hygiene precautions will help prevent transmission from infected cats to humans, and euthanasia of infected cats is not warranted.

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## BACTERIAL INFECTIONS

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Primary bacterial diseases are less common in cats compared with other domestic species and humans. Important canine bacterial diseases, such as leptospirosis and Lyme disease (borreliosis), are not clinically important in cats even though seroconversion after exposure has been documented. Secondary bacterial infections, however, are common complications of many conditions, such as viral diseases, trauma, and surgery. The most common bacterial diseases are discussed elsewhere in this book along with the relevant body system. This chapter addresses bartonellosis, mycobacterial infections, and nocardiosis. A summary of less common bacterial diseases is found in [Table 33-8](#).

## BARTONELLOSIS

*Bartonella* spp. are receiving increasing attention both in veterinary and human medicine. Historically, their role in feline disease has been unclear and without consensus. As more is learned about these bacteria, their function as disease-causing agents will become clarified. The current understanding of *Bartonella* in cats is discussed here.

*Bartonella* spp. are very small gram-negative bacteria that can survive and replicate intracellularly in their mammalian hosts, not unlike rickettsial organisms. Their primary targets are vascular endothelial cells and red blood cells, and spread to various tissues is facilitated through infection of macrophages.<sup>11</sup> There are many



**TABLE 33-8** Less Common Bacterial Diseases of Cats

Disease	Agent	Clinical Signs	Diagnosis	Treatment	Comments
Tetanus <sup>57,20</sup>	<i>Clostridium tetani</i> Motile, gram-positive, anaerobic, spore-forming bacillus	Appear within 5 to 21 days; localized and generalized forms; limb stiffness, stiff gait, dorsally curved tail, muscle rigidity, hyperthermia, cranial nerve signs, facial and masticatory muscle spasms, reflex muscle spasms/tonic contraction, seizures, death from respiratory compromise	History of a recent wound, clinical signs; serum antibody titers to tetanus toxin	Tetanus antitoxin, penicillin G or metronidazole; chlorpromazine, barbiturates, benzodiazepines; muscle relaxants, such as methocarbamol; nursing care	Transmission by environmentally resistant spores introduced into wounds; disease caused by neurotoxin formed during vegetative growth; localized forms have better prognosis, mortality from complications in generalized disease is high
Tularemia <sup>4,28,70,73</sup>	<i>Francisella tularensis</i> Gram-negative, non-spore-forming bacillus	Fever, depression, generalized lymphadenomegaly, hepatomegaly, splenomegaly, oral ulcers, icterus, draining abscesses, panleukopenia	Serology for microscopic agglutinating antibody	No validated treatment; suggested antibiotics include aminoglycosides, tetracyclines, fluoroquinolones	Zoonotic; type A and B strains isolated from cats in United States; transmission through contact with wildlife reservoirs (rodents, rabbits), tick vectors or contaminated environment
Plague <sup>24,61</sup>	<i>Yersinia pestis</i> Gram-negative, non-spore-forming, facultative anaerobe coccobacillus	Bubonic form: fever, dehydration, lymphadenomegaly, cervical/submandibular abscesses, hyperesthesia; septicemic form: septic shock, rapidly fatal; pneumonic form can develop by hematogenous or lymphogenous spread; 50% die acutely	Clinical signs, epidemiologic information; contact public health laboratory for guidance on submission of samples for culture (fluids, tissues, aspirates, blood), direct fluorescent antibody testing, serology	Strict barrier nursing techniques required; eliminate fleas; recommended antimicrobials include aminoglycosides, penicillins	Zoonotic; transmitted by ingestion of infected rodents or rabbits, bites from infected fleas on prey

species of *Bartonella*, and many of them can infect humans. Various species of mammalian hosts are adapted to the various *Bartonella* spp. and maintain a bacteremia for long periods without any effects. The organisms are spread by a variety of vectors, including sand flies, lice, and fleas; ticks may be a vector, but this has not been definitively proven.<sup>11</sup>

Several *Bartonella* species have been identified in cats. *B. clarridgeiae* causes asymptomatic bacteremia of cats. Other species have been found in isolated cases, but the primary species of concern in cats is *B. henselae*, the agent of cat scratch disease.<sup>35</sup>

## Epidemiology

*Bartonella*-infected cats have been found throughout the world, but prevalence appears to be highest in warm, humid climates.<sup>35</sup> In the United States, prevalence studies have shown rates from 5% to 40%. In addition to transmission by biting insect vectors, cats may become infected by a bite or scratch from another infected animal.<sup>10</sup> Infection of domestic and nondomestic felids with *B. henselae* has been documented. Interestingly, the genetic variation among *B. henselae* isolates is significant, which may account for the bacteria's ability to persist in an infected animal as well as the fact that infected cats may be reinfected with heterologous strains.<sup>35</sup>

*B. henselae* is naturally transmitted among cats by cat fleas (*Ctenocephalides felis felis*), specifically by flea excrement. Cat-to-cat transmission, even by transplacental exposure, is rare to nonexistent.<sup>35</sup> The resultant bacteremia is often chronic in nature though it may be intermittent.

## Pathogenesis and Clinical Signs

After infection, the bacteria enter red blood cells and endothelial cells where they are protected from the immune response. In experiments, infection of bone marrow progenitor cells has been documented and may be the mechanism for red blood cell infection.<sup>10</sup> The intraerythrocyte locale facilitates transmission throughout the host tissues, as well as vector transmission. Transient fever has been associated with primary infection as well as recurrence of bacteremia in chronically infected cats following a stressor, such as surgery.<sup>10</sup> Lethargy, anorexia, and lymphadenomegaly have been reported following experimental infection, and less commonly, transient mild neurologic manifestations, such as nystagmus and tremors, have been noted.<sup>35</sup> In natural infection, however, clinical signs are uncommon. *Bartonella* infection has been associated with gingivostomatitis, but causation has not been shown.<sup>67</sup> In fact, a lack of association of *Bartonella* infection and chronic gingivostomatitis has also been documented.<sup>23</sup> No association with other disease syndromes, including kidney,

pancreatic, neurologic, nasal, or ocular diseases, has been proven. More severe disease has been seen in immunocompromised humans infected with *Bartonella*, but no such enhancement of disease has been found with concurrent FIV or FeLV infection in cats. However, an association between FeLV and *B. henselae* co-infection has been observed, indicating that infection with the former may predispose to infection with the latter.<sup>14</sup> Large epidemiologic studies are needed to determine what role, if any, *B. henselae* plays in feline disease.

## Diagnosis

Veterinarians may be asked to test pet cats because of a diagnosis of *Bartonella*-associated disease in the cat owner. Diagnosis of active infection is difficult, and will likely rely on multiple assays. Finding the organism in red blood cells on smears is notoriously insensitive and is not considered a viable method for diagnosis.<sup>35</sup> Serology alone is also difficult, because both false-negative and false-positive results occur. Antibody levels may remain elevated for prolonged periods, even if the organism is cleared; in addition, the latter is difficult to document. Antigen preparations vary among the different serologic assays, affecting results. Having said this, although the positive predictive value of a positive test is low, the negative predictive value of a seronegative result is much higher.<sup>35</sup>

Definitive diagnosis of infection should be performed with culture and/or PCR on blood samples. Generally, multiple samples must be tested because of the intermittent nature of bacteremia. Culture is done by sterile blood collection in ethylenediaminetetraacetic acid (EDTA)-containing tubes. Tubes should be kept chilled until reaching the diagnostic laboratory. Because special conditions and enriched media are required, labs having experience with this organism should be selected and consulted for optimal collection and transport methods.<sup>35</sup>

Nucleic acid detection by PCR is much more rapid, but is no more sensitive than culture, and reveals nothing about organism viability. The same care as required for sample collection for culture is required for PCR.

## Treatment

The efficacy of treatment is difficult to assess because of the intermittent nature of the bacteremia. Because of concern over antibiotic resistance, only treatment of clinically ill cats is recommended.<sup>34</sup> Optimal treatment regimens have not been determined. Currently, recommendations are that doxycycline or amoxicillin-clavulanate should be used initially; if no response is seen in 7 days, and other diagnoses are ruled out, treatment with azithromycin or fluoroquinolones may be needed.<sup>13</sup> In addition, prolonged treatment (at least 4 weeks) is recommended. Owners should be made aware

of the limitations of diagnostics as well as the caveats of positive results.

## Prevention

The emphasis for control of *Bartonella* infections should focus on year-round flea control.<sup>13</sup> Cats that are seropositive for *Bartonella* should not be used as blood donors.<sup>35</sup> No vaccine is available.

## MYCOBACTERIAL INFECTIONS

*Mycobacterium* is a genus of aerobic, non-spore-forming, nonmotile, gram-positive, pleomorphic bacterial rods with wide variations in host affinity and pathogenic potential. Traditionally, mycobacteria are classified by their growth in culture (slow, difficult to cultivate, rapid), whether they produce tubercles or lepromatous or granulomatous disease, and whether or not there is dissemination (Table 33-9). More recently, DNA sequencing of various genomic regions has provided more insight into taxonomy and discovered three new fastidious mycobacterial species. In cats, this diverse class of bacteria produces various, seemingly unrelated syndromes that this chapter will address as

1. Slow-growing organisms that do or do not produce tubercles
  - a. Tuberculous mycobacteria
  - b. *Mycobacterium avium* complex (MAC) and other slow-growing saprophytes
2. Leproid granuloma-producing organisms that cannot be cultured using standard methods
  - a. Feline leprosy
3. Rapidly growing mycobacteria that are easily cultivated

### *Mycobacterium tuberculosis* Complex

Feline tuberculosis is caused by the *Mycobacterium tuberculosis* complex, primarily *M. microti* and *M. bovis*. Tuberculous mycobacteria survive within mammalian hosts. The only reservoir hosts for *M. tuberculosis* are humans, cattle are the predominant host for *M. bovis*, and *M. microti* is prevalent in small rodents, such as voles, shrews and field mice in the United Kingdom. Cats are naturally more resistant to *M. tuberculosis* than to *M. bovis* or *M. microti*.

### Epidemiology

Feline infections with *M. tuberculosis* are considered an anthroponosis; the direction of transmission is from human to animal. Spread of infection back from cats to people has not been reported. In general, the tubercle bacilli are not as transmissible as other bacterial

pathogens, requiring frequent exposure or exposure to a large dose of pathogen to produce disease.

The primary mode of transmission of *M. tuberculosis* is by inhalation of aerosolized droplets of about 3 to 5  $\mu\text{m}$  diameter that are able to reach the alveoli. The prevalence of human and animal *M. tuberculosis* infections has been decreasing in developed countries because of effective infection control measures in people, although unanticipated increases in prevalence have occurred in certain human populations because of many factors, such as immunosuppression from HIV infection and illicit drug use. Multidrug-resistant tuberculosis has thus emerged in these populations because of poor compliance with drug therapy. This may increase the risk of infection in cats in contact with these human populations.

*M. bovis* infects many species of animals as well as people, and is found worldwide, although bovine tuberculosis has been eradicated in most industrialized countries. The most common route of infection for *M. bovis* is via the gastrointestinal tract through the consumption of contaminated milk or meat from cattle. Bovine tuberculosis has become established in wildlife hosts in many countries (e.g., white-tailed deer in Michigan,<sup>45</sup> badgers in the United Kingdom,<sup>21</sup> and brushtail possums in New Zealand<sup>15</sup>) so that cats may continue to become infected even in areas where infection of domestic animals is uncommon. In this situation, cats are most likely to be infected by eating secondarily infected small wild mammals.<sup>20,22</sup>

Cats are more commonly infected with *M. bovis* than dogs, and can excrete the organism in feces and thus disseminate and maintain infection on farms. In the United States, cats are rarely responsible for transmission of infection to humans.<sup>71</sup> However, in some areas of the world, such as Buenos Aires, *M. bovis* infection of cats may be a significant human health hazard.<sup>75</sup>

*M. microti* infection is most commonly seen in rural cats in Great Britain. Infection is most likely transmitted by hunting and ingesting prey species, such as mice and voles.<sup>33,41</sup>

### Pathogenesis

Tubercle bacilli enter the body through either the respiratory or alimentary tract or by skin penetration. In cats, *M. bovis* infection is more common than *M. tuberculosis* infection so that tonsils, mandibular lymph nodes, and ileocecal lymph nodes are often infected. The ileocecal nodes are the most common sites for localization and shedding of *M. bovis* organisms.

Cats with mucocutaneous infections caused by *M. tuberculosis* or *M. avium*-complex organisms develop a pyogranulomatous infiltrate with variable amounts of necrosis, presence of multinucleated giant cells, and degrees of lymphoid infiltration.<sup>47</sup> *M. tuberculosis* organisms are frequently extracellular, whereas *M. avium* complex organisms are usually intracellular.

TABLE 33-9 Species of *Mycobacterium* Infecting Cats

Organism	Environmental Factors	Clinical Features	Drug Susceptibility or Reported Successful Therapy*
<b>SLOW-GROWING TUBERCULOUS: TUBERCLES AND LYMPHADENITIS, OCCASIONAL DISSEMINATION</b>			
<i>M. tuberculosis</i>	Urban, close contact with affected person	Usually respiratory, pulmonary localization, can disseminate systemically	Isoniazid, rifampin, ethambutol, pyrazinamide
<i>M. bovis</i>	Rural cats, ingest raw beef or dairy products or infected wildlife	Usually alimentary disorders; may get respiratory, cutaneous, or lymphatic involvement, sometimes systemic dissemination	Rifampin, clarithromycin, fluoroquinolones, ethambutol, isoniazid, surgical excision of skin lesions
<i>M. microti</i>	Rural, suburban, hunter, bite wounds, prey exposure, ingestion of rodents	Nodular cutaneous lesions draining, ulceration, peripheral lymphadenomegaly, local myositis, arthritis, osteomyelitis, sometimes pneumonia, peritoneal infection, or systemic dissemination	Clarithromycin/azithromycin, fluoroquinolones + rifampin; rifampin, isoniazid, ethambutol
<b>LEPROMATOUS: CUTANEOUS NODULAR DERMATOSIS</b>			
<i>M. lepraemurium</i>	Cooler wet climates, winter months, cats less than 3 years of age exposed to infected rodent prey	Single to multiple cutaneous and subcutaneous dermal nodules on head and extremities, ulcers, fistulas, abscesses regional spread only	Clarithromycin, clofazimine, doxycycline or minocycline, rifampin, surgical removal
Feline leprosy, <i>Mycobacterium</i> sp. strain Tarwin	Central coast New South Wales, Australia, New Zealand, older cats greater than 10 years of age, feline immunodeficiency virus predisposes	Multiple subcutaneous dermal nodules, no ulceration, sometimes dissemination	Clarithromycin, clofazimine, rifampin
"Candidatus <i>M. visibile</i> "	Western Canada and United States, environmental exposure?	Cutaneous and disseminated	Clofazimine
<b>NONTUBERCULOUS: PYOGRANULOMATOUS</b>			
<b>Saprophytic Slow Growing: Cutaneous Lesions, Lymphadenitis, Dissemination in Immunocompromised Hosts</b>			
<i>M. avium</i> complex	Exposure to infected soil, water or dust; acidic soils contaminated with bird feces or carcasses, most prevalent in Siamese and Abyssinian cats	Dermal and regional lymph node granulomas, alimentary infiltration, corneal granulomas, systemic dissemination	Clarithromycin, clofazimine, doxycycline or minocycline, rifabutin, ethambutol; rifampin preferred for better penetration if central nervous system involvement
<i>M. genavense</i>	Environmental exposure in immunocompromised host	Disseminated lymphadenitis	Clarithromycin, ethambutol, fluoroquinolones, clofazimine
<i>M. terrae</i> complex	Environmental exposure	Cutaneous lesions	Clarithromycin, fluoroquinolones, rifampin
<i>M. simiae</i>	Environmental exposure	Cutaneous and disseminated	Clarithromycin, fluoroquinolones, rifampin?
<i>M. ulcerans</i>	Environmental exposure	Cutaneous	Surgical removal, clarithromycin
<b>Saprophytic Rapidly Growing Mycobacteria (RGM): Cutaneous and Subcutaneous Pyogranulomatous Infections</b>			
Mycobacterial panniculitis: <i>M. smegmatis</i> (Australia), <i>M. fortuitum</i> (United States)	Soil and water exposure; bite and puncture wounds; immunocompromised host	Cutaneous and subcutaneous granulomas, especially inguinal region, ulcers, drainage, with regional spread only; secondary wound infections	Surgical removal, wide excision, variable susceptibility to fluoroquinolones, doxycycline, aminoglycosides, clofazimine, clarithromycin, trimethoprim-sulfonamide

\*A minimum of two and often three drugs should always be used in combination.

Adapted from Table 50-2 in Greene CE, editor: *Infectious diseases of the dog and cat*, ed 3, St Louis, 2006, Elsevier.

### Clinical Signs

Feline tuberculosis is frequently a subclinical disease, often acquired through contact with *M. tuberculosis* or *M. bovis*-infected people.<sup>69</sup> When clinical signs are present, they are similar whether infection is by *M. tuberculosis*, *M. microti*, or *M. bovis* and typically reflect the site of granuloma formation. Cats may develop localized cutaneous infections with *M. bovis* and *M. microti* seen as dermal nodules and nonhealing, draining ulcers at the site of a bite or scratch wound or a penetrating injury. Regional lymphadenomegaly may develop.<sup>33</sup> Pulmonary infection causes dyspnea and cough.<sup>33</sup> Dysphagia, retching, hypersalivation, and tonsillar enlargement may result from ulcerated and chronically draining oropharyngeal lesions. Localized intestinal lesions may cause weight loss, anemia, vomiting, and diarrhea, as well as enlarged mesenteric lymph nodes and abdominal effusion.

Disseminated disease resulting from *M. bovis* or *M. microti* may develop from cutaneous lesions and cause respiratory dysfunction.<sup>33</sup> Other signs of disseminated disease include abdominal masses, organ enlargement, generalized lymphadenomegaly, anorexia, weight loss, and fever. *M. bovis* may be associated with tuberculous choroiditis and retinal detachment.<sup>25</sup> Sudden death can also occur.

### *Mycobacterium avium* Complex

The *M. avium* complex (MAC) organisms are opportunistic mycobacteria that survive in soil and water (i.e., are saprophytic). Other slow-growing saprophytic mycobacteria, such as *M. genavense*, *M. simiae*, *M. xenopi*, *M. terrae*, and *M. kansasii*, have a similar environmental niche and can cause similar clinical disease in cats and thus should be considered in the same context. Clinical infection with these organisms results in granulomas but not true tubercles. In cats, localized lymphadenitis can occur, but disease can disseminate if the cat does not mount an appropriate immune response. Disseminated MAC infection occurs, therefore, in immunocompromised animals, such as cats receiving immunosuppressive therapy after renal transplantation<sup>31</sup> and cats with retroviral infections;<sup>40</sup> congenital immune deficiencies are also considered to be a possible predisposing factor.<sup>6</sup>

### Epidemiology

MAC and other slow-growing saprophytic mycobacteria are ubiquitous worldwide in soil and water when conditions are acidic (pH 5.0 to 5.5) and soils are high in organic matter, such as swamps, coastal plains, and brackish coastal waters.<sup>48</sup> MAC is found in large numbers in the feces of infected birds. Infection of cats occurs by ingestion of infected meat or contact with infected soil or contaminated fomites. Despite the widespread nature

of MAC organisms in the environment, infections in cats have been uncommon because of natural resistance. No evidence has been found for spread of MAC organisms from animals to people.

### Pathogenesis

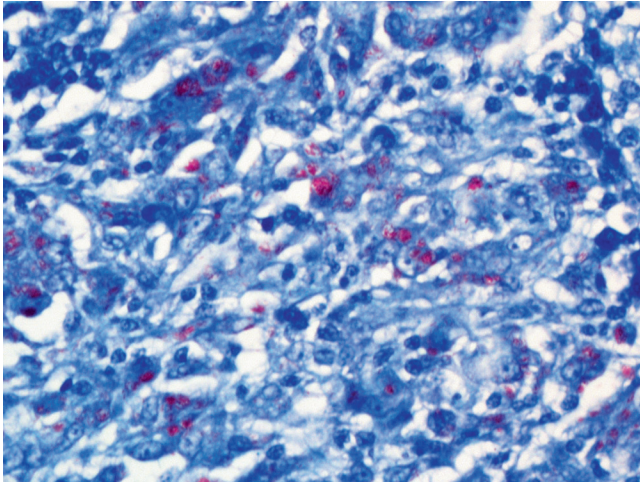
Infection with MAC organisms begins with ingestion of contaminated food or contact with the organism in the environment. MAC infections in cats are often disseminated through many tissues and are due to organisms closely related to the *M. avium* subspecies *paratuberculosis* that causes chronic granulomatous enteritis in ruminants (Johne's disease). It is thought that animals with Johne's disease acquire the infection as neonates, although it initially becomes quiescent. Stress or immunosuppression later in life allows the organisms to replicate and produce disease. A similar scenario may occur in predisposed cat breeds (Siamese, Abyssinian) in which disseminated infections typically develop while cats are young, possibly because of defects in cell-mediated immunity.<sup>6,44</sup>

### Clinical Signs

Localized infections often follow bite or scratch wounds so that clinical signs include enlarged regional lymph nodes and subcutaneous swellings, especially around the head and face. Other signs include weight loss, anorexia, and fever. Disseminated infection can occur with clinical signs reflecting the areas involved.<sup>6,8</sup> Clinical findings may include thickened intestinal loops, hepatomegaly, splenomegaly, and lymphadenomegaly; in one series of 12 cats, 10 of 12 cats had enlarged mesenteric lymph nodes, and 6 of 12 had enlarged popliteal lymph nodes.<sup>6</sup> Pulmonary nodular interstitial infiltration is commonly recognized radiographically but does not necessarily result in respiratory signs (Figures 33-25

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**FIGURE 33-25** Lateral radiograph of a 1-year-old neutered male Abyssinian. The diffuse pulmonary interstitial pattern was caused by disseminated MAC infection. (From Baral RM, Metcalfe SS, Krockenberger MB et al: Disseminated Mycobacterium avium infection in young cats: overrepresentation of Abyssinian cats, J Feline Med Surg 8:23, 2006.)



**FIGURE 33-26** A high-power photomicrograph of a lymph node from the cat shown in Figure 33-25. The Ziehl-Neelsen stain shows intracellular acid-fast bacilli (staining pink with the carbol fuchsin) in macrophages. (Courtesy Dr. Randolph Baral.)

and 33-26). Disseminated infection is particularly noted in Abyssinians, where signs of illness develop before 5 years of age.<sup>6,66</sup>

### **Diagnosis of *M. tuberculosis* Complex and MAC Infections**

Clinical laboratory findings in mycobacterial infections are typically nonspecific. Non-regenerative anemia may be seen, and has been reported to be macrocytic in some cats with intestinal infections.<sup>44</sup> Other findings include neutrophilic leukocytosis, hyperglobulinemia, and hypercalcemia.<sup>1,6,59</sup> Imaging studies may reveal masses in various organ systems. Tracheobronchial lymphadenomegaly, interstitial pulmonary infiltrates, calcified pulmonary lesions, and pleural or pericardial fluid may be seen with thoracic radiography. Hepatomegaly, splenomegaly, solitary abdominal masses, and ascites may be seen on abdominal radiography or ultrasonography.

Specific diagnostic methods include acid-fast staining, mycobacterial culture, biopsy with histopathologic examination and direct detection of organisms. Intradermal tuberculin testing is not reliable in cats, unlike other species, including dogs.

Acid-fast (Ziehl-Neelsen) staining of cytologic specimens obtained by tissue aspirates or impressions smears from biopsy samples is a widely available and useful method of diagnosis. Acid-fast organisms may also be demonstrated within lesions on histopathologic examination of tissue biopsy samples or in direct smears of exudates or fluids. Intracellular tubercle bacilli have a clubbed shape and beaded appearance. *M. tuberculosis* bacilli may be found in extracellular locations.<sup>47</sup> MAC organisms are generally smaller and present in high numbers within infected cells. When biopsy samples are obtained from lesions where mycobacterial infection is

suspected, the sample should be divided into three pieces. One piece is fixed in formalin for histopathology and acid-fast staining, and one is sent for routine bacterial culture. A third piece is placed in a sterile container and frozen. If the first sample is acid-fast positive, the frozen sample can be submitted for mycobacterial culture. PCR testing can be performed on formalin-fixed samples.

Finding acid-fast staining organisms confirms mycobacterial infection, but culture is required to determine the species in order to evaluate zoonotic risk, sources of infection, and treatment options. Unfortunately, mycobacterial organisms are very slow-growing and may fail to culture. A specialized laboratory should be consulted for advice on specimen preparation and transport media.

Specific detection methods for mycobacterial organisms in body fluids and tissue specimens include enzyme-linked immunosorbent assay, radioimmunoassay and PCR. PCR appears to be highly sensitive when organisms are abundant in the specimen, but false-negative results are possible when organisms are few because the nucleic acid is difficult to extract and purify. Therefore methods based on detection of nucleic acid should not replace conventional mycobacterial isolation methods but may be complementary, such as to identify organisms found in culture of clinical specimens.

At necropsy, generalized emaciation is a common finding. Multifocal granulomas appear in many organs as grayish-white to yellow, circumscribed, nodular lesions. The primary lesion sites in cats are ileocecal and mesenteric lymph nodes. Disseminated infection may lead to lesions in the mesenteric lymph nodes, spleen, and skin. Uncommon sites for lesions include bones, joints, genitals, and conjunctiva. Histologically, granulomas consist of focal necrosis surrounded by plasma cells and macrophages in a connective tissue capsule.

Precautions should always be taken whenever handling potentially tuberculous material to prevent human infection. In many countries, specific laws govern the diagnosis and reporting of suspected tuberculosis cases.

### **Therapy of *M. tuberculosis* Complex and MAC Infections**

Treatment of tuberculous mycobacterial infections should be considered separate from treatment of disseminated infections with slow-growing saprophytic mycobacteria. This is because of the zoonotic potential of *M. tuberculosis*; *M. bovis*-infected cats do not appear to be a major risk for their owners,<sup>18,19</sup> and reports of *M. microti* infections in people appear to be associated with direct contact with rodents.<sup>60</sup> Additional considerations are the need for long-term (sometimes indefinite) drug administration that is expensive and can make patient compliance uncertain; and whether immunosuppressed people may be exposed. Also, increasing antimicrobial resistance to drugs used to treat human tuberculosis

must be considered, because the routine treatment of animal infections might contribute to the development of resistance. Infections with saprophytic mycobacteria, such as MAC and *M. microti*, are the appropriate mycobacterial infections to consider treating.

When a decision to treat a cat has been made, treatment should be started based on cytologic or histopathologic diagnosis, because results of mycobacterial culture and species identification typically take several weeks if the organism grows in culture at all. Treatment of mycobacterial disease poses several difficulties. To be effective, antimicrobials must reach therapeutic concentrations within phagocytes in various tissues but with minimal toxicity to the host. Importantly, there is a propensity for mycobacteria species, in general, and MAC organisms, in particular, to spontaneously and rapidly develop antibiotic-resistant mutants.<sup>58</sup> Multiple agents should therefore be used to reduce the chance of resistant clones developing. Using several agents concurrently, however, increases the likelihood of adverse drug reactions, because each agent has a potential toxicity profile. Furthermore, some of these toxicity profiles overlap.<sup>58</sup>

*M. bovis* and *M. microti* infections in cats have been successfully treated with a combination of rifampin, plus enrofloxacin or marbofloxacin, plus clarithromycin or azithromycin.<sup>18,33</sup> It is appropriate to treat localized MAC infections (or other slow-growing saprophytes) with surgical excision followed by combination antibiotic therapy with clarithromycin and doxycycline.<sup>46</sup> Disseminated MAC infections (or other slow-growing saprophyte) are best treated with clarithromycin in combination with at least one other agent, such as clofazimine or rifampin.<sup>6</sup> There is widespread resistance of MAC strains to the traditional fluoroquinolones,<sup>2</sup> but newer agents, such as moxifloxacin, may have some role in treating these organisms. More details about these drugs including dosages are included in [Table 33-10](#).

### **Prevention of Mycobacterium tuberculosis Complex and MAC Infections and Public Health Considerations**

Cats (and dogs) should be evaluated as temporary sources for dissemination of infection when *M. tuberculosis* is identified in people and when outbreaks of *M. bovis* in cattle occur on farms. Prevention of infection in cats involves discouraging the hunting of prey and avoiding the feeding of potentially infected meat and milk. MAC organisms may be acquired from the environment by both cats and people.

Mycobacteria are more resistant to heat, pH changes, ultraviolet light, and routine disinfection than are other pathogenic bacteria. Contaminated equipment should always be manually cleaned with a neutral detergent before disinfection. Mycobacteria are killed by 5% household bleach within 15 minutes and 2% glutaraldehyde

for 10 minutes at room temperature. Ethyl and isopropyl alcohols can be used as a terminal rinse.

### **Feline Leprosy**

Feline leprosy was first described in the 1960s and consists of solitary or multiple, well-circumscribed, nodular granulomas in the skin and/or subcutis, resulting from mycobacterial infection. Unfortunately, the causative species are fastidious and typically cannot be grown using routine mycobacteriologic techniques, even in specialist laboratories. Recent studies incorporating PCR methodologies have led to the recognition that there are numerous agents associated with feline leprosy, including *M. lepraemurium* as well as at least three novel mycobacterial agents.<sup>4,7,27</sup> In Australia and New Zealand, feline leprosy has typically been considered to be composed of two syndromes: one caused by *M. lepraemurium* (affecting younger, mostly immunocompetent cats) and one caused by a novel mycobacterial species currently described as *Mycobacterium* sp. cat (affecting older, immunocompromised cats).<sup>52</sup> However, a recent study has recognized another organism from a specific regional area, described as *Mycobacterium* sp. strain Tarwin, with no obvious age or sex predisposition in affected cats.<sup>27</sup> In a study of 26 cases in New Zealand and British Columbia, Canada, various species were identified, such as *M. lepraemurium*, *M. intracellulare*, *M. mucogenicum*, *M. septicum*, as well as one case of *Mycobacterium* sp. cat.<sup>17</sup> Three cats from the northwestern United States and western Canada have been diagnosed with diffuse cutaneous and disseminated disease similar to diffuse lepromatous leprosy in people caused by *M. visibile*.<sup>7</sup>

Feline leprosy has been reported from many areas of the world, including New Zealand, Australia, the United Kingdom, the Netherlands, the United States, Canada, and Italy. Many cases originate in temperate coastal areas, suggesting that the route of infection may be rodent or insect bites or contamination of cat fight wounds with soilborne organisms.<sup>56</sup>

Feline leprosy is characterized by single or multiple nodules of the skin and/or subcutis, often on the head, face, limbs or trunk. The nodules are painless, well circumscribed, moveable, and firm or soft on palpation. Overlying skin may be intact or may ulcerate if lesions are large. In advanced disease, regional lymph nodes and local tissues may become involved as well as the liver or spleen.

Diagnosis is similar to that for other mycobacterial infections; an index of suspicion on the part of the clinician is essential. Other causes of cutaneous and subcutaneous nodular lesions must be ruled out (see Chapter 22). Samples obtained for cytology and histopathology by fine-needle aspiration or biopsy can be stained with Ziehl-Neelsen to demonstrate acid-fast organisms surrounded by granulomatous to pyogranulomatous

**TABLE 33-10** Antimicrobial Drug Therapy for Slow-Growing Mycobacterial Infections

Drug	Dose (mg/kg) <sup>a</sup>	Route	Interval (Hour)	Toxicities
<b>TUBERCULOUS MYCOBACTERIA: M. TUBERCULOSIS, M. BOVIS, M. MICROTI</b>				
<i>Treatment (minimum of two, and preferably three, of different classes of the following drugs in combination)<sup>b</sup></i>				
Isoniazid	10-20 <sup>c</sup>	PO	24	Hepatotoxic, seizures, acute renal failure, peripheral neuritis
Rifampi(ci)n	10-20 <sup>d</sup>	PO	24	Hepatotoxic; discolors mucosae, tears, and urine
Ethambutol	10-25	PO	24	Optic neuritis
Dihydrostreptomycin	15	IM	24	Ototoxic
Pyrazinamide <sup>e</sup>	15-40	PO	24	Hepatotoxic, GI signs, arthralgia
Clarithromycin	5-15	PO	12	GI signs, hepatotoxic, cutaneous erythema, allergic reactions
	62.5 total	PO	12	
Azithromycin	7-15	PO	24	GI signs?
Enrofloxacin	5	PO	24	Vomiting, retinal toxicity
Marbofloxacin	2	PO	24	Retinal toxicity?
<b>SLOW-GROWING SAPROPHYTIC MYCOBACTERIA: M. AVIUM COMPLEX, M. TERRAE, M. SIMIAE, M. ULCERANS</b>				
Clarithromycin	7.5-15	PO	12	Cutaneous erythema, hepatotoxicity
Clofazimine <sup>f</sup>	8-10	PO	24	Orange staining body fluids, hepatotoxic, GI signs, photosensitization
	25 mg total <sup>g</sup>	PO	24	
Rifampi(ci)n	10-20	PO	24	Hepatotoxic, cutaneous erythema, discolors body fluids
	75 mg total	PO	24	
Doxycycline	5-10 <sup>h</sup>	PO	12	Vomiting, esophagitis

<sup>a</sup>Dose per administration at specified interval. After daily dosing for weeks to months, switch to twice weekly administration for 6 to 9 months.

<sup>b</sup>Treatment for 2 months minimum with three drugs in combination (e.g., rifampin with a fluoroquinolone [e.g., marbofloxacin] and with either clarithromycin or azithromycin). Maintenance therapy for 4 months thereafter consists of the same dosages of any two of the three drugs.

<sup>c</sup>Maximum 300 mg daily.

<sup>d</sup>Maximum 600 mg daily.

<sup>e</sup>Ineffective for *M. bovis* strains.

<sup>f</sup>Only available from a compounding pharmacist in most countries.

<sup>g</sup>Alternatively, 50 mg total can be given every 48 hours.

<sup>h</sup>Can increase dosage up to 10 mg/kg for improved efficacy, but only if this level is tolerated; give with food or administer water to avoid esophageal injury; if possible, use monohydrate salt to minimize gastrointestinal irritation.

GI, Gastrointestinal; IM, intramuscular; PO, by mouth.

Adapted from Table 50-4 in Greene CE, editor: *Infectious diseases of the dog and cat*, ed 3, St Louis, 2006, Elsevier.

inflammation. With Romanowski stains, mycobacterial rods are “negatively” stained and are typically located within macrophages and giant cells. Culturing these organisms is usually unsuccessful, but they are readily detected using PCR methodologies in laboratories with mycobacterial expertise.

Feline leprosy is divided into two forms: lepromatous and tuberculoid, which correspond to the host’s immune response to infection.<sup>65</sup> The lepromatous form corresponds with a poor cell-mediated immune response. Histopathologic findings are primarily pyogranulomatous, and lymphocytes and plasma cells are absent. Large numbers of mycobacterial organisms are present. The tuberculoid form is associated with a more effective cell-mediated immune response and is characterized by pyogranulomatous dermatitis and panniculitis.

Histopathologic findings are primarily epithelioid histiocytes with moderate numbers of lymphocytes and plasma cells but moderate to few mycobacterial organisms. The tuberculoid form accounts for about two-thirds of cases in Canada,<sup>17</sup> most cases in New Zealand and the Netherlands, but few cases in Australia.<sup>52</sup> Invasion of peripheral nerves, a feature of human leprosy, is not usually found in feline patients.

Because the organisms responsible for feline leprosy cannot be grown in culture, therapy cannot be guided by susceptibility testing, and no firm guidelines exist to direct therapy. Aggressive surgical resection of lesions with wound reconstruction when required is often recommended, especially when the disease is diagnosed early and lesions are localized.<sup>50,62,72</sup> Adjunctive antibiotic therapy is recommended to prevent local recurrence



and should be continued for at least 2 months.<sup>24,52</sup> The most commonly recommended drugs are a combination of clarithromycin, rifampicin, and/or clofazimine.<sup>52</sup> Monotherapy is avoided to prevent development of resistance.

Human leprosy is acquired from the environment and is caused by *M. leprae*. *M. lepraemurium* has no zoonotic potential.

### Rapidly Growing Mycobacteria

Rapidly growing mycobacteria (RGM) were formerly called Runyon group IV or atypical mycobacteria and are characterized by the ability to form colonies in solid media culture within 1 week. These mycobacteria are ubiquitous in the environment, including soil and water sources. The taxonomy of RGM has been redefined based on molecular methods and now includes the *Mycobacterium chelonae-abscessus*, *Mycobacterium fortuitum*, and *Mycobacterium smegmatis* groups among others. RGM are not known to be transmissible among animals.

RGM cause opportunistic disease in both healthy and immunocompromised cats. Disseminated disease typically occurs only in cats with underlying immunosuppression. The most common presentation is localized infection in healthy cats, most typically chronic panniculitis<sup>72</sup>; there have been occasional reports of pneumonia caused by RGM.<sup>16,26</sup> Many individual case reports are in the veterinary literature as well as a case series of 29 affected cats in Australia.<sup>17</sup> Other countries with reported cases include the United States,<sup>49</sup> Canada,<sup>17,74</sup> New Zealand,<sup>17</sup> France,<sup>68</sup> Finland,<sup>1</sup> the Netherlands,<sup>43</sup> and Switzerland.<sup>3</sup> In Australia, organisms from the *M. smegmatis* group account for most feline cases,<sup>53,57</sup> while in the United States, most cases are caused by members of the *M. fortuitum* group.<sup>39,42</sup>

Infection typically starts in the inguinal fat pad, possibly after a cat-fight wound has become contaminated,<sup>56</sup> and it may spread to the abdominal wall, perineum, and tail base. Other penetrations of the integument (such as through bite wounds, penetrating foreign bodies, injections, surgical wounds) may also allow RGM infections to become established in subcutaneous tissues, especially in fat. RGM organisms appear to prefer tissues rich in lipid so that certain areas of the body are more likely to be affected, and overweight or obese cats are at most risk. Initial lesions are circumscribed plaques or nodules at the site of injury, although trauma to the skin is not always reported.<sup>39</sup> Many patients are initially treated for a cat fight abscess with surgical drainage and antibiotics, although the lesions do not have a fetid odor or the typical bacterial discharge. Wound breakdown and development of a nonhealing suppurating tract then occurs. Later, the subcutaneous tissue becomes thickened, and the overlying skin becomes adherent and alopecic, with watery exudate discharging from fistulas.

Gradually, the affected area may involve the entire ventral abdomen, adjacent flank areas, and limbs. Lesions typically remain localized, and most cats have few signs of systemic illness. In severe cases, depression, fever, inappetence, weight loss, and reluctance to move may be noted. Hypercalcemia of granulomatous disease develops only occasionally.

Diagnosis of mycobacterial panniculitis is similar to other mycobacterial infections; an index of suspicion on the part of the clinician is essential. Specimens for cytology (acid-fast staining) and culture are best obtained by fine-needle aspiration of pockets of purulent material through intact skin that has been disinfected with 70% ethanol (to eliminate skin-dwelling mycobacterial species).<sup>55</sup> Material from draining tracts is usually unsuitable, because of the high numbers of contaminating secondary bacteria. Tissue homogenates from surgically collected samples can also be used for cytology and culture. The diagnostic laboratory should be consulted in advance for advice on sample submission and supplies.

The medical and surgical management of mycobacterial panniculitis is well described.<sup>55</sup> However, recommendations continue to evolve as new drugs, such as fourth-generation fluoroquinolones and tetracycline derivatives, become available.<sup>29</sup> The use of appropriate antimicrobial agents based on susceptibility data and aggressive surgical resection, when warranted, improves outcome. However, some cases, especially those caused by *M. fortuitum* in the United States remain frustrating to treat.<sup>39</sup> Initial treatment should be with one or two oral antimicrobials chosen empirically, then adjusted based on susceptibility data. In Australia, doxycycline and/or a fluoroquinolone (such as pradofloxacin or moxifloxacin) are appropriate choices for first-line therapy, whereas in the United States, clarithromycin is the drug of choice for empiric therapy. Treatment durations are typically 3 to 12 months, and agents should be administered for at least 1 to 2 months after affected tissues look and feel completely normal. Surgical resection of persistently nonhealing tissue may be necessary.<sup>63</sup> In human medicine, RGM infections may develop resistance to quinolones (but not doxycycline or clarithromycin) during therapy.<sup>12</sup> For this reason, many veterinary dermatologists in Australia routinely use combination therapy with doxycycline and a fluoroquinolone from the outset. Although some RGM strains show in vitro susceptibility to amoxicillin-clavulanate, this drug combination has no efficacy in vivo.

Once susceptibility data is obtained, drug therapy may be refined. The highest possible doses are used because of the poor perfusion of affected tissues. Patients should be reassessed every 3 to 4 weeks to determine response to treatment and whether surgical resection is required. If surgery is required, it is most important to remove as much abnormal subcutaneous tissue as

possible. Some cases require removal of large portions of infected tissue followed by reconstruction to close the wound without tension. Latex or closed suction drains must be used in large areas of dead space. Vacuum-assisted wound closure has been used in some challenging cases.<sup>32</sup>

## NOCARDIOSIS

Nocardiosis is caused by several species of gram-positive aerobic actinomycetes that are ubiquitous soil saprophytes. These facultative intracellular pathogens with a propensity to erode blood vessels grow in branching filaments, often in tangles, and fragment into rods and cocci. They are somewhat acid fast. The most commonly isolated species are the *Nocardia asteroides* complex (including *N. farcinica*, *N. nova*), but infections in cats have also been reported to be caused by *N. brasiliensis*, *N. otitidiscaviarum*, *N. elegans*, *N. tenerifensis*, and *N. africana*.<sup>9,36-38,51,54,64</sup> Infections are opportunistic and are introduced primarily through scratches and bite wounds. Males are overrepresented in the published cases. The most common clinical scenario is infection of the skin and subcutis following penetrating wounds, with lesions typically located in regions subjected to cat bite or scratch injuries, including limbs, body wall, inguinal panniculus, and the nasal bridge (Figure 33-27). Pneumonia and pyothorax, possibly following aspiration of plant material, such as grass awns, and disseminated disease associated with immunodeficiency have also been documented.



**FIGURE 33-27** Lesion on the paw of a 13-year-old neutered male Devon Rex. Tangles of gram-positive branching filaments were seen on cytologic examination, and pyogranulomatous inflammation was seen on histopathologic examination. *Nocardia nova* infection was confirmed on culture. Treatment with clarithromycin (62.5 mg/cat, PO, every 12 hours) for 1 month resolved the lesion. (Courtesy Dr. Randolph Baral.)

Chronic nonhealing wounds often start as an abscess but spread circumferentially as well as by development of satellite lesions. The clinical appearance may be very similar to those caused by rapidly growing mycobacteria. In a series of 17 cases from eastern Australia, the majority of cats presented with spreading lesions of the skin and subcutis associated with draining tracts.<sup>54</sup> Most of the cats were male (14 of 17) and random bred (14 of 17). About half (9 of 17) were 10 years old or older. The majority of infections were due to *N. nova*. Several of the cats had conditions predisposing to immunosuppression, such as renal transplantation, chronic corticosteroid administration, postsurgical status, and FIV infection. The prognosis was considered to be guarded, and factors predisposing to treatment failure included delayed treatment resulting from misdiagnosis and insufficient duration of treatment.

Differential diagnoses include other infections associated with pyogranulomatous inflammation, such as mycobacterial panniculitis, *Rhodococcus* spp., *Corynebacterium* spp., and sporotrichosis. Clinical laboratory findings are nonspecific, such as nonregenerative anemia, neutrophilic leukocytosis with a left shift, monocytosis, and hyperproteinemia. Hypercalcemia associated with granulomatous disease has been reported,<sup>59</sup> but localized infections may show no clinical laboratory changes. Analysis of fluids and aspirates of abscesses demonstrates a suppurative to pyogranulomatous inflammation. The causative agent may be observed as a gram-positive, partially or weakly acid-fast, branching filamentous organism, either individually or in groups. Sulfur granules are not common. Diagnosis is confirmed by culture, usually within a few days, although 2 to 4 weeks of incubation may be necessary if samples have a high bacterial load or the patient was receiving antibiotics. Species identification is important for determining optimal antimicrobial treatment. Although traditionally species have been identified by phenotypic features, DNA-based techniques provide more rapid and reliable detection methods.

The primary drugs for treatment of nocardiosis are sulfonamides, such as trimethoprim-sulfamethoxazole (15 to 30 mg/kg, PO, every 12 hours). Prolonged courses of treatment, such as 3 to 6 months, are required to prevent recurrence. Sulfonamides may not be well tolerated for such long treatment durations because of drug reactions (anemia, leukopenia) and gastrointestinal side effects. *N. nova* infections are often susceptible to ampicillin, sulfonamides, clarithromycin, tetracyclines, amikacin, and imipenem but resistant to fluoroquinolones and amoxicillin clavulanate.<sup>38,54</sup> One recommended treatment regime for *N. nova* infections is amoxicillin (20 mg/kg, PO, every 12 hours) and/or erythromycin (10 mg/kg, PO, every 8 hours) or clarithromycin (62.5 mg/cat, PO, every 12 hours). Extensive débridement of skin lesions may be necessary in some cases.

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## MOLECULAR ASSAYS USED FOR THE DIAGNOSIS OF FELINE INFECTIOUS DISEASES

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Infectious agents of cats are associated with many clinical disease syndromes evaluated by practicing veterinarians. A definitive diagnosis is best made by documenting current infection, which can be achieved with a variety of techniques that vary by the body system; these include fecal flotation, cytology, histopathology, immunohistochemistry, culture, antigen tests, and molecular diagnostic assays. For some agents, antibody test results are also used to help make a clinical diagnosis. However, presence of antibodies may only document prior exposure, not current infection.

Sensitivity is the ability of an assay to detect a positive sample; specificity is the ability of an assay to detect a negative sample. Sensitivity and specificity vary with each assay. Positive predictive value (PPV) is the ability of a test result to predict presence of disease; negative predictive value (NPV) is the ability of a test result to predict absence of disease. Many of the infectious agents encountered in feline practice infect a large percentage of the population, resulting in positive organism detection techniques or serum antibody production. However, they only induce disease in a small number of cats in the infected group. Classic examples include coronaviruses, *Toxoplasma gondii*, and *Bartonella* spp. For these agents, even though assays with good sensitivity and specificity are available, the predictive value of a positive test is actually very low.

## MOLECULAR ASSAYS

Types of molecular assays used in cats were recently reviewed.<sup>33</sup> Molecular assays rely upon detection of the nucleic acids deoxyribonucleic (DNA) and ribonucleic (RNA) acid. Nucleic acids are part of the genetic makeup of the organism and consist of four nucleotides in varying sequences. Many portions of DNA and RNA are highly

conserved among organisms, while others are specific to the organism on a family, genus, species, or even strain level. The sequence specificity is used to detect the organisms within clinical samples using some form of complementary sequence and sometimes a signaling molecule. Signaling molecules are often some form of a fluorescent molecule in order to improve sensitivity.

### Detection of Pathogens Without Amplification

The simplest application of molecular tools for detection of infectious organisms is to apply a complementary nucleic acid sequence, termed a probe, which has been tagged with a fluorescent molecule. This probe is then applied directly to a clinical sample and hybridizes to a target sequence in an organism if present. Probes with different fluorescent tags can be applied to a single sample, allowing for detection of several organisms. However, sensitivity is poor compared with other molecular techniques, because the target DNA is not amplified. When probes are designed for use with tissues, it is termed *in situ* hybridization. Use of this technique can allow for detection of the organisms of interest in association with inflammatory lesions or specific areas of tissue. Fluorescent molecules are the most common signaling mechanism used with this technique,

which is abbreviated FISH (fluorescent *in situ* hybridization).

### Detection of Pathogens with Amplification: Polymerase Chain Reaction

The polymerase chain reaction (PCR) was first described in 1985.<sup>28</sup> This technique results in the cyclic amplification of a single strand of DNA to produce an exponential number of identical copies that then can be easily detected, usually on a gel (conventional or end-point PCR), to determine if it is the predicted size for the reaction (Figure 33-28). PCR is superior in sensitivity to probe hybridization techniques because of this amplification step. The great sensitivity of these assays requires strict adherence to good laboratory practice to avoid false-positive results from contamination within the laboratory.

Detection of microbial nucleic acids in a feline sample does not prove the organism is alive, capable of replication, or actually causing clinical signs in the host. Correlation with clinical signs of a known syndrome associated with the organism and/or a response to therapy must be used in conjunction with results of PCR. False-negative reactions can occur with PCR on some tissues or fluids that may have PCR inhibitors present.

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**FIGURE 33-28** Traditional polymerase chain reaction. **A**, Short sequences of nucleotides called primers are annealed to the target DNA after the separation of the double strands. A proprietary enzyme is used to produce complementary strands of DNA during the synthesis step. Denaturation is repeated, and replication of the newly formed DNA strands, as well as the original target DNA, is repeated. **B**, The DNA produced in the reaction is then visualized using gel electrophoresis. The size of the product is compared with a standard to confirm that the predicted product has been obtained. (From Veir JK, Lappin MR: *Molecular diagnostic assays for infectious diseases in cats*, Vet Clin North Am Small Anim Pract 40:1189, 2010.)

This problem varies by the syndrome as well as the assay and should be considered in each case. Finally, in order to prevent false negatives, samples tested should be obtained prior to treatment, which may decrease organism load below the level of detection of the assay even though the organism is still present in the host.

The enzyme used in PCR can only duplicate strands of DNA, and so, to detect RNA, the sample must first have a reverse transcription (RT) step to create a complementary strand of DNA from the target RNA. Amplification of the complementary DNA by polymerase chain reaction is then performed; this method is commonly known as RT-PCR.

PCR is used most commonly in veterinary medicine to detect infectious disease agents: The primers used in PCR can be designed to amplify the nucleic acids only of members of a certain genus, species, or even strain of organism. When a single organism is targeted in an assay, it is termed a singleplex PCR. If multiple targets can be detected in a single assay, it is termed a multiplex assay. It is clearly most attractive to investigate the presence of multiple organisms in a single assay. However, each target sequence competes with the others for the common building blocks in the PCR assay: the enzyme, nucleotide, and various buffers and ions that allow the reaction to proceed. Therefore multiplex reactions can be less sensitive than singleplex assays.

The use of broad-range or degenerate primers amplifying members of an entire genus or even kingdom can be used, targeting highly conserved regions of the nucleic acids. The most common application of this is for rapid detection and identification of eubacteria or fungi in clinical samples.<sup>18,29</sup> Subsequent analysis of the PCR product may then be used to identify the infecting organism much more rapidly than traditional microbiologic techniques and may be more sensitive for detection of fastidious organisms. It must be noted that antimicrobial sensitivity is not available using this technique; therefore it is complementary to traditional culture techniques. However, the use of PCR for detection of certain genes that encode for antimicrobial resistance genes is starting to gain clinical use as well and may provide additional rapid information prior to sensitivity results being available.<sup>23</sup>

It is difficult to acquire quantification information using traditional end-point PCR. Real-time PCR or quantitative PCR (qPCR) is the most recent application of PCR.<sup>12</sup> In this technique, production of DNA is monitored during each amplification cycle so that the original starting quantity could be extrapolated by identification of the logarithmic amplification phase of each individual reaction. This technique uses fluorescent dyes or probes that produce a signal after formation of the product (Figure 33-29). During each amplification cycle, a detector records the amount of fluorescence in the sample. Pathogen detection and load are one of the many

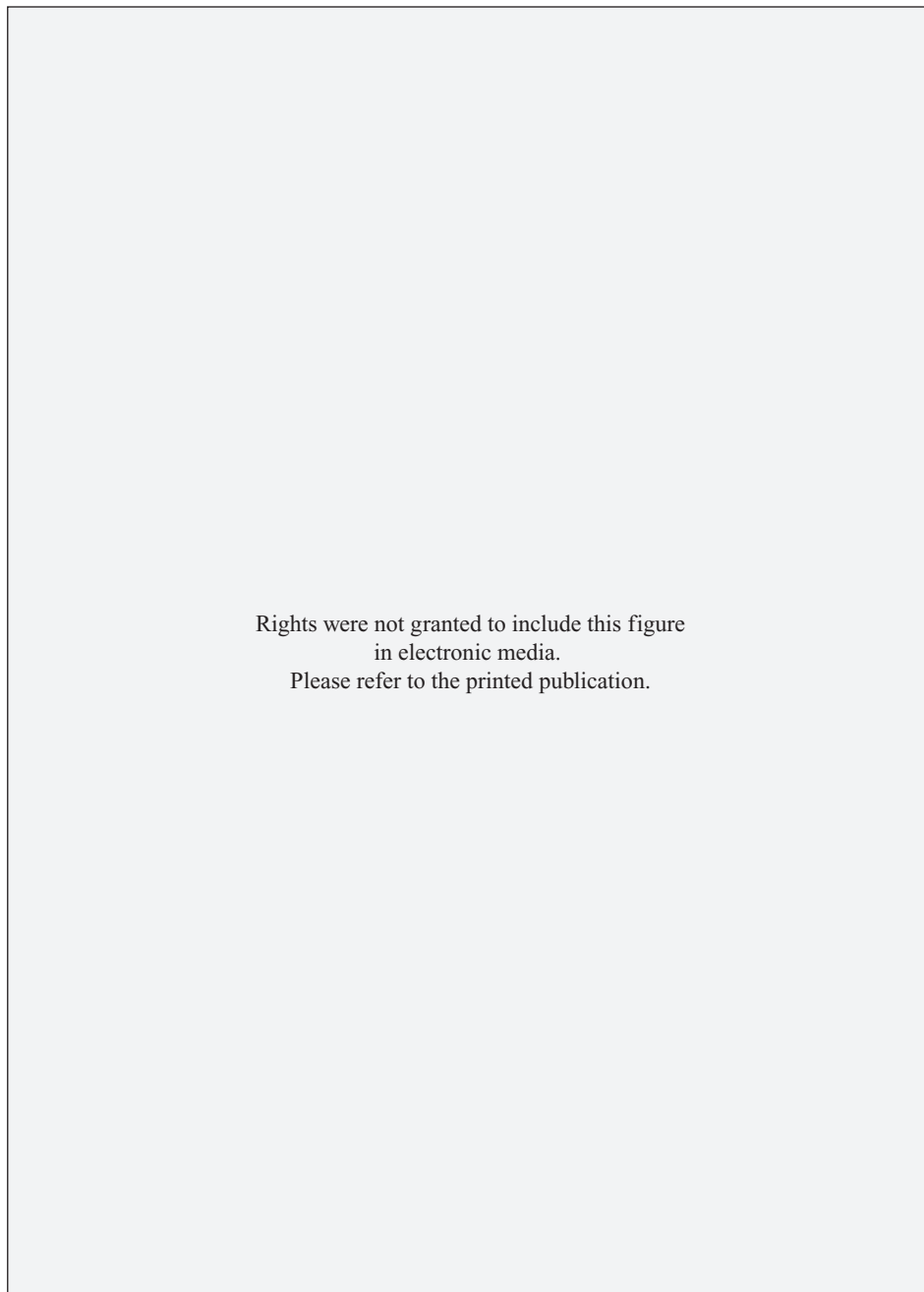
applications of this technology. This assay has all the advantages of traditional end-point PCR (good sensitivity, specificity) but also offers a more rapid result and the ability to quantify microbial DNA or RNA load and so can be used to monitor therapy in some cases (see the following sections of the chapter). Because qPCR is very sensitive, strict quality control must be maintained. In addition, accuracy of quantification is reliant upon the availability of a reproducible, high-quality, standard curve. Although minimum laboratory standards have been proposed and are generally met for published protocols,<sup>4</sup> many diagnostic laboratories use proprietary reactions that are not subject to peer review. Thus all laboratories providing PCR assays may not be equivalent, and so, use of laboratories that have published results of their assays may be prudent.

## CURRENT CLINICAL APPLICATIONS OF MOLECULAR ASSAYS IN FELINE MEDICINE

In the following subsections, a brief review of the benefits and problems associated with PCR assays currently used in feline medicine is presented.

### Respiratory Agents

Feline calicivirus (FCV) is a common differential diagnosis for cats with clinical evidence of rhinitis and stomatitis. Less commonly, FCV is associated with conjunctivitis, polyarthritis, and lower airway disease in kittens. Virus isolation can be used to document current infection but takes at least several days for results to return. Because of widespread exposure and vaccination, the positive predictive value of serologic tests is poor. Reverse transcriptase (RT)-PCR assays can be used to amplify the RNA of FCV, and results can be returned quickly. However, these assays also amplify vaccine strains of FCV.<sup>27</sup> FCV RNA can be amplified from samples collected from normal carrier cats as well as clinically ill cats and so have poor positive predictive value.<sup>24</sup> For example, in one study in our laboratory, presence of FCV RNA failed to correlate with the presence or absence of stomatitis in cats.<sup>26</sup> In addition, amplification of FCV RNA cannot be used to prove virulent systemic calicivirus infection. The negative predictive value for FCV RT-PCR assays is currently unknown. Feline caliciviruses, as RNA viruses, have genetic variability among the different strains. Depending on the viral genetic region targeted by the assay, the degree of genetic variation among strains at that site will vary. Most laboratories design their assays to target conserved regions of the viral genome, but even this cannot guarantee that all strains are detectable by any individual assay.



**FIGURE 33-29** Quantitative polymerase chain reaction. **A**, The standard PCR assay is enhanced by using a fluorescent probe that fluoresces only after the removal of a quencher dye in close proximity to the reporter dye. The quencher dye is removed by the enzyme that synthesizes new strands of DNA as in traditional PCR. At each step, fluorescence is measured, allowing for the extrapolation of the amount of product present during each replication phase. **B**, The change in fluorescence is then plotted against time (number of cycles), and a starting quantity can be calculated by the extrapolation of the signal produced during the exponential replication phase. (From Veir JK, Lappin MR: *Molecular diagnostic assays for infectious diseases in cats*, Vet Clin North Am Small Anim Pract 40:1189, 2010.)

FHV-1 is a common differential diagnosis for cats with clinical evidence of rhinitis, stomatitis, conjunctivitis, keratitis, and facial dermatitis. Because of widespread exposure and vaccination, the positive predictive value of serologic tests is poor. FHV-1 can be documented by direct fluorescent staining of conjunctival scrapings, virus isolation, or PCR. FHV-1 DNA can be

amplified from conjunctiva, nasal discharges, and pharynx of healthy cats, and so, the positive predictive value of conventional PCR assays is low.<sup>34</sup> Currently used PCR assays also detect vaccine strains of FHV-1, further lessening the positive predictive value of the assays.<sup>21</sup> In one study in our laboratory, presence of FHV-1 DNA failed to correlate with the presence or

absence of stomatitis in cats.<sup>26</sup> Quantitative PCR may ultimately prove to correlate with the presence or absence of disease, but it failed to correlate with the presence of conjunctivitis in one study.<sup>20</sup> The negative predictive value of 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 a hypersensitivity reaction. Tissue biopsies have greater sensitivity than conjunctival swabs but do not necessarily have greater predictive value. FHV-1 DNA can be amplified from aqueous humor of some cats, but whether this indicates FHV-1 associated uveitis is unknown.<sup>22</sup>

*Mycoplasma* spp., *Chlamydomphila felis*, and *Bordetella bronchiseptica* are other common respiratory pathogens in cats. As for FHV-1 and FCV, PCR-positive test results for these organisms cannot be used to distinguish a carrier from a clinically ill cat. However, in one recent study, *Mycoplasma* spp. DNA was amplified from conjunctival swabs from more kittens with conjunctivitis than control cats in the same shelters, suggesting the organism can be pathogenic in some cats.<sup>36</sup> In addition, PCR assays do not provide antimicrobial drug susceptibility testing, and so, for cats with potential bordetellosis, culture and sensitivity is the optimal diagnostic technique, especially if an outbreak is occurring. *Toxoplasma gondii* DNA has been amplified from airway washings of some cats with lower respiratory tract disease, and so, PCR is an option for evaluation of samples from diseased animals from which the organism is not identified cytologically.

## Gastrointestinal Agents

The diagnosis of *Giardia* spp. infection is generally made with the combination of fecal flotation techniques and wet mount examination. Fecal antigen tests are also accurate, and there are several assays available for point-of-care use, including one labeled for veterinary use. Fecal PCR assays are often falsely negative because of PCR inhibitors in stool, and so, PCR should not be used as a screening procedure for this agent. However, *Giardia* spp. PCR can be used to determine whether the infective species is a zoonotic assemblage, which is the primary indication for this technique. However, it now appears that assemblage determination should be performed on more than one gene for most accurate results.<sup>30</sup>

Although *Cryptosporidium* spp. infection is common, it is unusual to find *C. felis* oocysts after fecal flotation in cats. Acid-fast staining of a thin fecal smear is cumbersome and insensitive. Antigen assays titrated for use with human feces are inaccurate when used with cat feces. Thus PCR may be aid in the diagnosis of cryptosporidiosis in dogs and cats and has been shown to be more sensitive than immunofluorescence assay (IFA) in cats.<sup>31</sup> *Cryptosporidium* spp. PCR assays are indicated in

IFA-negative cats with unexplained small bowel diarrhea and when the genotype of *Cryptosporidium* is to be determined. However, *C. felis* infection in cats is common, and so, positive tests results do not always prove that the agent is the cause of the clinical disease. No drug is known to eliminate *Cryptosporidium* spp. infections, and small animal strains are not considered significant zoonotic agents; so, PCR is never indicated in healthy animals.

PCR assays are also available for detection of DNA of *Tritrichomonas foetus*, *Salmonella* spp., *Campylobacter* spp., *Clostridium* spp., parvoviruses, and *T. gondii*, and a RT-PCR assay is available for coronaviruses. Trophozoites of *T. foetus* can often be detected on wet mount examination of fresh feces, which can be completed as an in-clinic test. PCR for *T. foetus* DNA is indicated if wet mount examination is negative and results return more quickly than culture. However, DNA of *T. foetus* can be detected in healthy carrier cats, and so, positive results do not always prove illness from the organism.<sup>8</sup> Cases with suspected salmonellosis or campylobacteriosis should be cultured rather than assessed by PCR to determine the anti-microbial susceptibility patterns. In dogs, the PPV of *Clostridium* spp. PCR assays on feces is low and, if used, should be combined with enterotoxin assays. Information in cats is currently lacking. There is no current evidence that parvovirus PCR on feces is superior to currently available antigen assays. It was recently shown that cats vaccinated with modified live panleukopenia-containing vaccines shed parvovirus DNA in feces within several hours.<sup>7</sup> Thus parvovirus PCR testing should not be used to diagnose panleukopenia virus outbreaks in recently vaccinated cats. *Toxoplasma gondii* is only shed for about 7 to 10 days, and millions of oocysts are generally shed during this time, making the organism very easy to identify. Thus PCR assays are usually not needed to diagnosis this infection. Because virus isolation is not practical clinically, RT-PCR is used most frequently to detect coronavirus RNA in feces. However, positive test results do not differentiate FIP-inducing strains from enteric coronaviruses. Additionally, in one study, presence of coronavirus RNA did not correlate to the presence of diarrhea in shelter cats.<sup>7</sup>

## Bloodborne Agents

*Mycoplasma haemofelis* (Mhf), “*Candidatus* *Mycoplasma haemominutum*” (Mhm), and “*Candidatus* *M. turicensis*” (Mtc) all can be found in cats. In experimentally infected cats, Mhf is apparently more pathogenic than Mhm. It appears that Mtc has intermediate pathogenicity. Diagnosis is based on demonstration of the organism on the surface of erythrocytes on examination of a thin blood film or PCR assay. Organism numbers fluctuate, and so, blood film examination can be falsely negative



up to 50% of the time. The organism may be difficult to find cytologically, particularly in the chronic phase. Thus PCR assays are the tests of choice because of sensitivity.<sup>13</sup> Primers are available that can amplify all three hemoplasmas. Real-time PCR assays can be used to monitor copy numbers during and after treatment but do not have greater sensitivity, specificity, or predictive value than conventional PCR assays.<sup>32</sup> PCR assays should be considered in the evaluation of cats with unexplained fever or anemia that are cytologically negative. In addition, the American College of Veterinary Internal Medicine (ACVIM) recommends screening cats for use as blood donors by PCR assays for hemoplasmas.<sup>35</sup> Many cats (approximately 15%) are carriers of the relatively nonpathogenic *Candidatus M. haemominutum*, and so, positive test results may not always correlate with the presence of disease (poor PPV).

Cats can be infected by *E. canis*-like organism<sup>2</sup> and *Anaplasma phagocytophilum*.<sup>15</sup> Little is known about the other agents in these genera regarding cats. Because the organisms are in different genera, serologic cross reactivity is variable. Thus although the clinical syndromes can be similar, there is no one serologic test to document infection, and there is currently no standardized serology for cats. In addition, some cats with *E. canis* infection do not seroconvert, and so, PCR assay is superior to serology in cats. PCR assays can be designed to amplify each organism. Alternatively, primers are available to amplify all of the organisms in a single reaction, and then sequencing can be used to determine the infective species. However, positive test results do not always correlate with the presence of disease. *Anaplasma phagocytophilum* DNA has been amplified from the blood of healthy cats for more than 10 weeks after experimental infection by exposure to *Ixodes* ticks (MR Lappin, unpublished data, 2011).

Cats can be infected by *Rickettsia felis* and have been shown to have antibodies against *R. rickettsii*. Fever, headache, myalgia, and macular rash in humans have been attributed to *R. felis* infection in several countries around the world. In recent study in our laboratory, we assayed 92 pairs of cat blood and flea extracts from Alabama, Maryland, and Texas, using PCR assays that amplify a region of the citrate synthase gene (*gltA*) and the outer membrane protein B gene (*ompB*). Of the 92 pairs, 62 of 92 (67.4%) flea extracts and none of the cat blood samples were positive for *R. felis* DNA.<sup>11</sup> In another study, we showed *R. felis* and *R. rickettsii* antibody prevalence rates in cats with fever to be 5.6% and 6.6%, respectively, but neither organism was amplified from blood.<sup>1</sup> These results prove that cats are sometimes exposed, but further data are needed to determine the significance of disease associations. Whether *Rickettsia* spp. PCR assays are indicated for use in cats at this time is unknown.

Blood culture, PCR assay on blood, and serologic testing can be used to assess individual cats for *Bartonella*

spp. infection.<sup>3</sup> Cats that are culture negative or PCR negative and antibody negative, and cats that are culture negative or PCR negative and antibody positive, are probably not a source of flea, cat, or human infection. However, bacteremia can be intermittent, and false-negative culture or PCR results can occur, limiting the predictive value of a single battery of tests.<sup>17</sup> Although serologic testing can be used to determine whether an individual cat has been exposed, both seropositive and seronegative cats can be bacteremic, limiting the diagnostic utility of serologic testing. Thus testing healthy cats for *Bartonella* species infection is not currently recommended.<sup>3,14</sup> Testing should be reserved for cats with suspected clinical bartonellosis. Because *Bartonella* spp. infection is so common in healthy cats, even culture-positive or PCR-positive results do not prove clinical bartonellosis. For example, although we detected *Bartonella* spp. DNA in more cats with fever than in pair-matched cats without fever, the healthy cats were still commonly positive.<sup>16</sup> Combined serology with PCR in evaluation of cats with suspected bartonellosis is likely to give the best predictive value.

*Cytauxzoon felis* is usually easily identified on cytologic examination of blood smears or splenic aspirates during evaluation of clinically ill cats. Serologic testing is not commercially available at this time. PCR can be used to amplify organism DNA from blood from cats that are cytologically negative.<sup>9</sup>

Antibodies against feline immunodeficiency virus (FIV) are detected in serum in clinical practice most frequently by enzyme-linked immunosorbent assay (ELISA). Comparisons among different tests have shown the results of most assays are comparable.<sup>10</sup> Results of virus isolation or RT-PCR on blood are positive in some antibody-negative cats. False-positive reactions can occur using ELISA; hence, positive ELISA results in healthy or low-risk cats should be confirmed using Western blot immunoassay. Kittens can have detectable, colostrum-derived antibodies for several months. If antibodies persist at 6 months of age, the kitten is likely infected. Virus isolation or RT-PCR on blood can also be performed to confirm infection. However, FIV is not present in the blood at high levels, and so, false-negative results are common. In addition, there are variable results among laboratories.<sup>6</sup>

Most cats with feline leukemia virus infection are viremic, and so, molecular diagnostic assays are not usually needed in clinical practice. However, use of newer sensitive real-time PCR assays has been used to accurately characterize the stages of infection.<sup>19</sup> However, these assays are not commonly available commercially.

RNA of both FIPV and FECV can be amplified from the blood 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 had mixed

results in two studies performed to date. In the one study, 13 of 26 apparently normal cats were positive for FECV mRNA in blood, suggesting that the positive predictive value of this assay for the diagnosis of FIP was low.<sup>5</sup>

## Ocular Agents

*Toxoplasma gondii*, *Bartonella* spp., FHV-1 and coronavirus are the organisms for which DNA or RNA has been amplified most frequently from the aqueous humor of cats with endogenous uveitis.<sup>22,25</sup> Although little is known about the predictive value of these assays when used with aqueous humor, the combination of molecular assays with local antibody production indices may aid in the diagnosis of some cases.

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