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Rational Approach to Diagnosing and Managing Infectious Causes of Diarrhea in Kittens

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Diarrhea in kittens is a frequent disorder facing veterinarians and managers of feline shelters and catteries;[1] however, there is scant literature providing specific information on causes and management of this problem. An innumerable number of kittens are abandoned or relinquished shortly after birth to be fostered by 4,000 to 6,000 American animal shelters (Humane Society of the United States report, www.humanesociety.org), and a recent survey of the Association of Shelter Veterinarians identified kitten diarrhea as one of the top two concerns of veterinarians treating shelter cats, second only to upper respiratory infections (K. Hurley, personal communication). Infectious disease was reported by Cave et al. to be the most common cause (55%) of kitten mortality identified from the necropsy findings of 274 kittens from private homes and rescue centers within the United Kingdom, with 25% of kitten mortality being attributed specifically to feline parvovirus (FPV).[2]

Knowledge of the most common causes of diarrhea in kittens is integral to formulating appropriate diagnostic and therapeutic plans, as well as guiding the veterinarian when standard therapeutic recommendations fail (Table 1-1). *Diarrhea in kittens is often associated with the effects of stress,[3] dietary intolerance, primary intestinal disease (congenital short colon, intussusception,[4] or inflammatory bowel disease [IBD]), and infections with enteropathogenic bacteria, viruses,[5] parasites, and protozoa.[6]* Although bacterial enteropathogens have been associated with diarrhea in kittens, identifying a causal relationship is difficult because potentially pathogenic enteric organisms can frequently be isolated in clinically healthy kittens. Routine bacterial examination of 57 clinically healthy kittens presented for initial vaccination revealed bacterial enteropathogens and intestinal parasites in 45% of the kittens.[7] These findings were substantiated by a study that documented a significantly higher incidence of *Campylobacter* spp. in 28% of 54 nondiarrheic cats compared to 10% of 219 diarrheic cats.[8] In addition, this study demonstrated that

there was no significant difference in the prevalence of intestinal parasites between diarrheic and nondiarrheic cats.[8] Determination of the frequency of enteropathogens in 100 cats entering an animal shelter in Florida confirmed that cats with diarrhea were no more likely to be infected with one or more (84%) enteropathogen than were cats with normal feces (84%).[9] Only feline coronavirus (FCoV) was significantly more prevalent in cats with diarrhea (58%) compared with cats with normal feces (36%).[9]

The high prevalence of enteropathogens in healthy feline populations underscores the challenges faced by veterinarians when trying to attribute causality in diarrheic kittens infected with the same enteropathogens. These studies highlight the importance of establishing practical guidelines for the treatment of the most common and important enteropathogens, as it is challenging and cost-prohibitive to test all shelter cats for all possible infections.

PARASITIC CAUSES OF DIARRHEA

Trichomoniasis
Over the past 15 years the protozoan parasite *Tritrichomonas blagburni* (formerly known as *Tritrichomonas foetus*) has emerged as an important cause of feline diarrhea worldwide (Figure 1-1).[10,11] Experimental cross-infection studies between cats and cattle using both feline and bovine isolates of the parasite, the differences in pathogenicity between *T. foetus* in cattle and *T. blagburni* in domestic cats, and molecular gene sequencing differences between parasites obtained from domestic cats and parasites obtained from cattle have resulted in characterization and differentiation of this new species of *Tritrichomonas* infecting cats.[12] Infected cats are generally young, but have ranged in age from 3 months to 13 years (median 9 months). The pathogenicity

Table 1-1	Parasitic, Bacterial, and Viral Causes of Diarrhea in Kittens				
Disease	**Clinical Signs**	**Diagnostic Test(s)**	**Infectious Agent**	**Comments**	**Treatment**
Parasitic Causes of Diarrhea in Kittens					
Trichomoniasis	Large bowel diarrhea Subclinical infection is common	1. Direct fecal smear 2. InPouch fecal culture 3. Fecal PCR	*Tritrichomonas blagburni* trophozoites identified on fecal smear and culture DNA detected via PCR	Fecal PCR testing is the most sensitive method. Fecal InPouch culture can take up to 10 days to yield a positive result	Ronidazole 30 mg/kg PO every 24 h for 14 days. Isolate infected cats. Retest.
Cryptosporidiosis	Subclinical infection is common Small bowel or mixed-bowel diarrhea	1. Acid-fast stain on fecal cytology 2. DFA testing 3. Fecal PCR	*Cryptosporidium felis* oocysts	More common in kittens and immunocompromised cats. Infection can be self-limiting.	Treatment can be challenging. No drug is FDA approved. Azithromycin (7-10 mg/kg PO every 12 h for 10 days) is recommended.
Giardiasis	Small bowel diarrhea Subclinical infection is common	1. Direct fecal smear 2. Fecal flotation (centrifugation) 3. Fecal ELISA 4. Fecal DFA 5. Fecal PCR (recommended for determination of *Giardia* assemblages if warranted)	*Giardia intestinalis* (has 8 assemblages [A-H] that determine its zoonotic potential) Flotation and DFA detect cysts via microscopy and ELISA detects soluble antigen	Fecal flotation combined with fecal ELISA has a combined sensitivity of > 97% PCR testing has a lower sensitivity than ELISA and flotation ELISA testing should be used for baseline screening only	None of the treatments are FDA approved. Metronidazole 25 mg/kg PO every 12 h for 7 days or Fenbendazole 50 mg/kg PO every 24 h for 5 days. Environmental control is important.
Coccidiosis	Subclinical infection is common Large bowel to mixed-bowel diarrhea	Fecal flotation (centrifugation)	*Cystoisospora felis* oocysts *Cystoisospora rivolta* oocysts	Coccidiosis is typically a disease of kittens, and diarrhea can be self-limiting.	Sulfadimethoxine is approved but is coccidiostatic, label dose is 55 mg/kg PO initial dose followed by 27.5 mg/kg every 24 h for up to 14 days. Ponazuril 50 mg/kg PO every 24 h for 4 days. Environmental control.
Whipworms	Large bowel diarrhea	Fecal flotation (centrifugation)	*Trichuris serrata*	Rare in domestic cats.	Fenbendazole 50 mg/kg PO every 24 h for 5 days (not FDA approved).
Roundworms	Small bowel diarrhea, failure to thrive, "pot-bellied" appearance	Fecal flotation (centrifugation)	*Toxocara cati* *Tocascaris leonina*	Common in kittens < 6 months old.	Pyrantel pamoate 20 mg/kg PO beginning at 2 wks of age, or Fenbendazole 50 mg/kg PO every 24 h for 5 days.

Hookworms	Small bowel diarrhea, melena, iron-deficiency anemia, failure to thrive	Fecal flotation (centrifugation)	*Ancylostoma tubaeforme, Ancylostoma braziliense, Uncinaria stenocephala Ancylostoma caninum*	Relatively uncommon in cats.	Selamectin, moxidectin, milbemycin oxime, emodepside. Fenbendazole and pyrantel pamoate are not FDA approved but are used off-label.

Bacterial Causes of Diarrhea in Kittens

Clostridium perfringens	Subclinical infection is occasionally seen Diarrhea can be small bowel, large bowel, or mixed in nature	1. ELISA test for *C. perfringens* enterotoxin 2. Fecal PCR for enterotoxin gene (should not be used alone to make a diagnosis)	*C. perfringens* enterotoxin	The pathogenicity of *C. perfringens* is unclear in cats, and detection of the enterotoxin via ELISA in diarrheic kittens and cats is far less common compared with dogs. A stained fecal smear for detecting endospores is highly insensitive and is not recommended. Fecal culture alone is of no diagnostic utility.	Supportive treatment is sufficient in most cases. In cats with systemic illness, metronidazole (10 mg/kg PO every 12 h for 5-7 days), amoxicillin (22 mg/kg PO every 12 h for 5-7 days), or tylosin (10 mg/kg PO every 24 h for 5-7 days) is recommended.
Clostridium difficile	Subclinical infection is occasionally seen Diarrhea can be small bowel, large bowel, or mixed in nature	1. Fecal culture (negative culture rules out infection) 2. ELISA test for *C. difficile* toxins A&B	*C. difficile* toxins A&B	Detection of *C. difficile* toxins A&B in asymptomatic kittens is not uncommon.	Supportive treatment is sufficient in most cases. In cats with systemic illness, metronidazole (10 mg/kg PO every 12 h for 5-7 days) is the drug of choice.
Campylo-bacteriosis	Subclinical infection is commonly seen with nonpathogenic species *C. jejuni* can cause large bowel diarrhea	1. Fecal culture 2. Fecal PCR 3. Stained fecal smear is extremely unreliable and insensitive	Over 14 species described in dogs and cats *C. jejuni* is pathogenic and zoonotic	Most *Campylobacter* spp. are non-pathogenic. Prevalence rates of *Campylobacter* spp. are higher in non-diarrheic cats vs. diarrheic cats. PCR is helpful to differentiate *Campylobacter* species.	Avoid injudicious antimicrobial therapy. Supportive treatment and appropriate barrier control is optimal. Azithromycin (5-10 mg/kg PO every 24 h for 5-21 days) is warranted in immunocompromised cats or cats with systemic illness.

Continued

Table 1-1	Parasitic, Bacterial, and Viral Causes of Diarrhea in Kittens—cont'd				
Disease	**Clinical Signs**	**Diagnostic Test(s)**	**Infectious Agent**	**Comments**	**Treatment**
Salmonellosis	Subclinical infection is uncommonly seen. Diarrhea is typically small bowel. Other clinical signs include fever, lethargy, anorexia, vomiting	1. Fecal culture 2. Fecal PCR	Two main species, *Salmonella enterica* and *Salmonella bongori*, each of which contains multiple serotypes	Infection of cats with *Salmonella* has been associated with feeding of raw meats. Outbreaks of *S. enterica serovar* Typhimurium infection in cats have been associated with seasonal song bird migrations ("songbird fever").	Avoid injudicious antimicrobial therapy. Supportive treatment and appropriate barrier control is optimal. Amoxicillin (22 mg/kg PO every 12 h for 7 days with enrofloxacin 5 mg/kg PO every 24 h for 7 days) is warranted in immunocompromised cats or cats with systemic illness.
Anaerobiospirillum infection	Large bowel diarrhea	1. Histopathology of colon with special stains	*A. succuniciproducens*	Infection is relatively rarely documented in cats.	Amoxicillin-clavulanic acid 15 mg/kg PO every 12 h for 14 days.
Gastric and intestinal *Helicobacter* infections	Subclinical infection is very common. Clinical signs can range from vomiting to anorexia to diarrhea and lethargy depending on which species and organ system are involved	1. PCR of gastric biopsies (helpful for determination of species) 2. Serology (only determines exposure) 3. Cytology of impression smears or biopsies 4. Rapid urease testing of gastric biopsies 5. Culture is of low sensitivity	Over 15 species of *Helicobacter* described in dogs and cats	Majority of cats show no clinical signs.	Treatment is not routinely administered in infected cats. A 2-3 wk course of omeprazole with metronidazole and clarithromycin PO has been used with varying success in eradicating the infection.
Tyzzer's disease	Small and large bowel diarrhea, hepatic disease	1. PCR of affected intestinal or liver biopsies 2. Histopathology and special stains of intestine and liver	*Clostridium piliforme*	Infection can be rapidly fatal.	Amoxicillin 22 mg/kg PO every 12 h for 10 days.
Viral Causes of Diarrhea in Kittens					
Feline Panleukopenia Virus	Fever, lethargy, inappetence, vomiting, diarrhea, sudden death. Cerebellar signs can also occur	1. Canine parvovirus fecal antigen (ELISA) 2. Histopathology (usually necropsy) 3. PCR of feces, tissue samples 4. Fecal electron microscopy 5. Virus isolation (feces, tissues)	FPV, occasionally infection with related mink enteritis virus, CPV-2a, CPV-2b, or CPV-2c	Pathogenesis of FPV is similar to that of CPV infection. Subclinical infection is probably widespread. Marked variation in the sensitivity and specificity of fecal ELISA tests. Disinfection of the environment with bleach or potassium peroxymonosulfate is important.	Supportive care, IV crystalloids and parenteral antimicrobials (ampicillin and fluoroquinolone), antiemetics, dextrose, colloids, antacids (H_2-blockers or proton pump inhibitors).

Organism	Clinical Signs	Diagnosis	Agent/Type	Comments	Treatment
Feline Coronavirus	Enteric FCoV subclinical infection is common or may result in diarrhea. If FIP develops, may see fever, lethargy, inappetence, vomiting, diarrhea, icterus, uveitis, neurologic signs, abdominal distension (effusion)	1. RT-PCR for detection of FCoV in feces (enteric FCoV) 2. Serology for detection of antibodies to FCoV indicates exposure only 3. For FIP, immunohistochemical staining for coronavirus antigen within lesions characterized by pygranulomatous or granulomatous vasculitis 4. Diagnosis also supported by analysis of abdominal effusion (high protein exudate that contains low numbers of nucleated cells [<5000 cells/µL] 5. RT-PCR for detection of FCoV in effusion or tissue samples	FCoV	FCoV is commonly detected in healthy and diarrheic cats with a prevalence ranging from 36-75%. Interpretation of positive FCoV serological or PCR-based tests must be made cautiously.	No cure exists for FIP. Prednisolone therapy with or without chlorambucil has been associated with prolongation of life span and improved quality of life. Several immunomodulators and antiviral drugs have been tried, but none has shown convincing benefit *in vivo*.
Feline Leukemia Virus	Extremely variable and depends on the strain involved, challenge dose, host immune function, age, and coinfections. Uncommonly, FeLV causes enteritis that clinically and histologically resembles that caused by FPV, except that lymphoid depletion is absent	1. Screening via ELISA or related immunochromatographic in-house assays for free FeLV antigen (targets FeLV p27 antigen in serum or blood) 2. IFA on serum or bone marrow targets FeLV antigen in blood cells 3. PCR on blood, bone marrow, or tissue targets FeLV RNA or proviral DNA	FeLV-A (present in all cats with FeLV) FeLV-B FeLV-C	The use of tears or saliva is suboptimal compared to serum for ELISA testing. Infected cats should be housed indoors to prevent spread of infection to other cats. Avoid feeding raw-food diets to infected cats.	Supportive care with management of opportunistic infections when warranted. Antiviral agents and immunomodulators are of limited benefit for treatment of cats with FeLV infections.

CPV, Canine parvovirus; DFA, direct fluorescent antibody; ELISA, Enzyme-linked immunosorbent assay; FCoV, feline coronavirus; FDA, U.S. Food and Drug Administration; FeLV, feline leukemia virus; FIP, feline infectious peritonitis; FPV, feline panleukopenia virus; IFA, indirect immunofluorescent antibody assay; IV, intravenous; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction
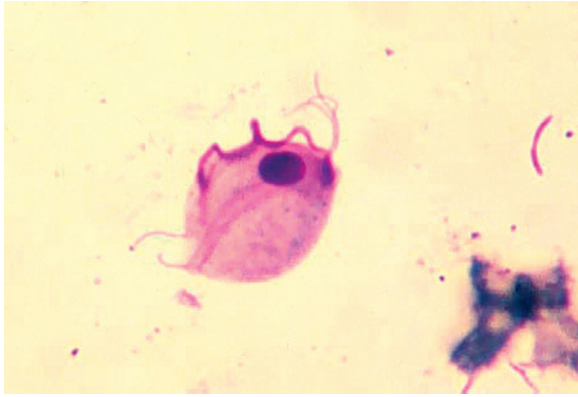
Figure 1-1: Giemsa-stained fecal smear showing characteristic appearance of *Tritrichomonas foetus* with its three anterior flagellae and long undulating membrane (magnification ×1000).

of *T. blagburni* for cats was demonstrated when eight cats were experimentally infected with a *T. blagburni* strain isolated from a diarrheic kitten.[13] Trophozoites were cultured from the feces of all eight cats within 1 week following oral inoculation, with infection persisting throughout the entire 203 days of the study, even when stools became normal.

Prevalence of *Tritrichomonas blagburni*

Natural *T. blagburni*-associated intestinal disease has been described mainly in younger cats (<2 years old) from multi-cat environments such as catteries, shelters, or cat shows.[14-16] The prevalence of *T. blagburni* infection at an international cat show was found to be 31% (36 out of 117 cats), with 28 out of 89 catteries affected.[11] Coinfection by *T. blagburni* and *Giardia* spp. was common and was documented in 12% of cats.[11] Xenoulis and colleagues documented coinfections with *T. blagburni* and *Giardia* in 22% of 104 cats, underscoring the importance of differentiating these enteropathogens.[16] Improper treatment of *T. blagburni* infection with metronidazole is common in cats because the trophozoites can be confused with those of *Giardia* spp. Risk factors for protozoal shedding and exacerbation of diarrhea included concurrent infection with *Cryptosporidium* spp. and cats living in close proximity with one another.[11] The predominance of infection in young cats from dense housing conditions may reflect an increased opportunity for exposure, or enhanced susceptibility to infection because of environmental stress or immunologic immaturity.

Clinical Signs

T. blagburni infection in cats can be associated with a chronic or recurrent large intestinal diarrhea characterized by increased mucus, tenesmus, occasional hematochezia, and increased frequency.[16] The median duration of diarrhea was 135 days, with a range of 1 day to 7.9 years.[16] The anus frequently is red, swollen, and painful, and fecal incontinence is not uncommon. Most cats usually are bright, alert, and responsive, and in good body condition with a normal appetite. *T. blagburni* can be isolated from the feces of asymptomatic cats, many of whom will not develop diarrhea.

Diagnosis

Multiple Direct Fecal Smears on Diarrheic Fecal Specimens. Direct fecal smears are indicated for the recovery of motile trophozoites of *Giardia* spp. and trichomonads such as *T. blagburni*. The procedure should be performed with saline (0.9%) on fresh feces (body temperature, <2 hours old). Trophozoites in older specimens lose their motility, degenerate, and become unrecognizable. The survival of trichomonads can be prolonged by adding 3 mL of 0.9% saline to 2 g of feces. A small amount of feces (size of a match head) is placed on a warm slide and a drop of 0.9% saline is mixed with the feces. Alternatively, a miniscule amount of fresh feces can be collected by insertion of a cotton-tipped swab into the rectum. The smear must not be too thick because trophozoites will be missed easily. A simple rule of thumb is that the observer should be able to read the fine newsprint of a newspaper through the smear.

After application of a coverslip, the smear is evaluated for motile organisms by examining at 100× magnification, with confirmation at 400× magnification. After the wet preparation has been checked thoroughly for motile trophozoites, a drop of D'Antoni's iodine can be placed at the edge of the coverslip, or a new wet mount can be prepared with iodine alone for morphological identification of the organism. A weak iodine solution that resembles "strong tea" is recommended.

The main limitation of direct fecal smears is sample size, with the result that negative smears are not uncommon with low fecal parasite burdens. The sensitivity of direct fecal smear examination for diagnosis of *T. blagburni* is relatively low in cats with spontaneous disease (14%).[17] In addition, the trophozoites of *T. blagburni* can be very difficult to distinguish from those of nonpathogenic intestinal trichomonads such as *Pentatrichomonas hominis* in the absence of fixation and staining. *T. blagburni* should also be distinguished from *Giardia* spp. *Giardia* trophozoites have a concave ventral disc and motility that mimics a falling leaf. In contrast, trichomonads are spindle shaped, have an undulating membrane that courses the entire length of the body, and move in a more irregular and jerky fashion. In contrast to *Giardia* spp., trichomonads do not have a cyst stage, which underscores the limitations of the fecal flotation technique for diagnosis of trichomoniasis. Trichomonads will not survive refrigeration and are rarely found in formed fecal specimens.

Fecal Cultures Performed with an InPouch TF Feline Test Kit. A commercially available system marketed for diagnosis of *T. blagburni* infection in cats (InPouch TF Feline, Biomed Diagnostics, White City, Oregon) should be considered if multiple direct fecal smears are negative for trophozoites.[17] Approximately 0.05 g (match head size) of freshly voided feces can be placed in the InPouch for culture, or alternatively, a saline-moistened cotton-tipped swab can be placed in the rectum and then gently agitated in the InPouch for culture. The InPouch should be incubated at room temperature in an upright position in the dark and examined every 48 hours for up to 10 days for motile trophozoites with use of a 20× or 40× objective (Figure 1-2). Incubation of the InPouch at
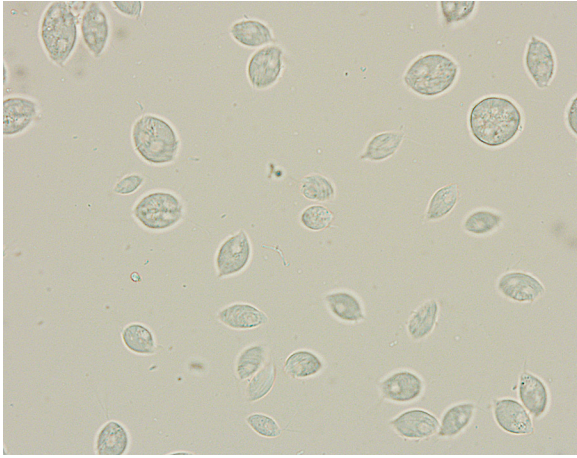
Figure 1-2: *Tritrichomonas* trophozoites in culture medium (InPouch Feline TF) isolated from a diarrheic cat (magnification ×400).

Figure 1-3: Zinc sulfate fecal flotation showing *Cystoisospora* spp. oocysts recovered from a diarrheic kitten (magnification ×400).

37°C (98.6°F) for 24 hours before incubation at room temperature can facilitate an earlier diagnosis because the warmer temperature is more conducive to replication of the trophozoites. Before microscopic evaluation, it is easiest to place the pouch in a plastic clamp provided by the manufacturer that facilitates mounting the pouch onto the stage of a light microscope. Possible reasons for negative fecal culture results include the use of old, desiccated, nondiarrheic, or refrigerated feces in which trophozoites are unlikely to survive; the use of bacteriostatic lubricant to collect the feces; or the placement of excessive feces in the InPouch resulting in overgrowth of bacteria or yeast in the culture medium.

Polymerase Chain Reaction (PCR). PCR is the most sensitive test for detecting *T. blagburni* in cats, but also the most expensive of the three options.[18] The PCR test is more sensitive than fecal culture and resulted in positive tests in 55% of cultures that were negative for *T. blagburni*, even when feces were normal. Fresh viable fecal specimens should be used for PCR testing whenever feasible.

Treatment

A nitroimidazole drug, ronidazole, is the drug of choice for the treatment of *T. blagburni* in cats.[19] The dosage is 30 mg/kg orally (PO) once a day for 14 consecutive days. In a retrospective study of 104 cats infected with *T. blagburni,* 64% of cats treated with ronidazole had a good response to treatment, while about 36% of cats had an inadequate response or a relapse shortly after treatment.[16] It is important that the amount of ronidazole be accurately calculated for each cat on the basis of its body weight. There is no evidence that higher doses of ronidazole are more effective, and they could increase the risk of neurotoxicity.[20] Clinical signs of neurotoxicity include lethargy, inappetence, ataxia, and seizures. These signs generally resolve upon cessation of drug therapy, but can last for 1 to 2 weeks. The drug must be immediately discontinued if signs of neurotoxicity are observed.

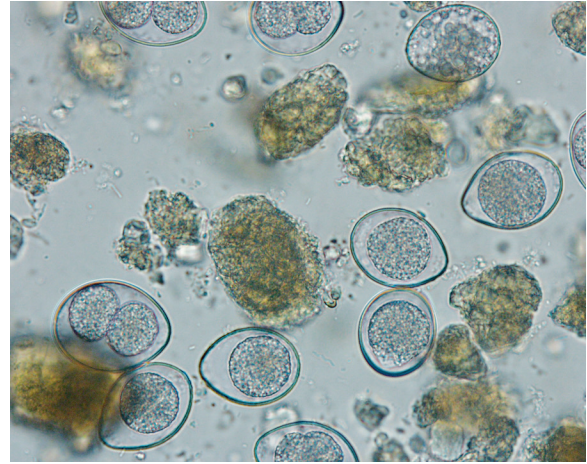Cats that have persistent diarrhea despite ronidazole administration should be further evaluated for other enteropathogens or a persistent *T. blagburni* infection. Additional considerations for a suboptimal response to ronidazole therapy include quality control problems at the compounding pharmacy or inadequate dosing of the ronidazole. Consider repeating the treatment regime with an appropriate dose and formulation of ronidazole only if the cat is not exhibiting any signs of neurotoxicity.

Ronidazole is not approved by the United States Food and Drug Administration (FDA) for use in companion animals, and veterinarians are advised to obtain informed consent before using this drug. The drug is obtained from compounding pharmacies and is best compounded into capsules because of the drug's bitter taste. In light of the poor host specificity of *T. blagburni* and the intimate association between infected cats and their human companions, the potential for zoonotic transmission should be considered. A single case of human infection with *T. blagburni* has been documented in the literature to date. In that case, the infection presented as epididymitis and meningoencephalitis following immunosuppression and peripheral blood stem cell transplantation.[21]

Environmental Control of *Tritrichomonas blagburni*

T. blagburni is extremely fragile because of its inability to form a cyst. Desiccation, refrigeration, exposure to temperatures above 40.6°C (105°F), and prolonged exposure to oxygen will kill the organism. Litter should be replaced and boxes disinfected to prevent cats from getting reinfected with *T. blagburni* during the treatment period.

Coccidia Species

Coccidia are obligate, intracellular protozoan parasites commonly found in the gastrointestinal tract of dogs and cats. They include the *Cryptosporidium* spp. described later. The most commonly diagnosed coccidial infections in cats are *Cystoisospora felis* and *Cystoisospora rivolta* (Figure 1-3).[22] Coccidiosis is typically a disease of puppies and kittens less than 6 months old; parasite recurrence is rare in animals greater than 1 year of age. In most cases, diarrhea, if present, is self-limiting or rapidly responsive to treatment for coccidiosis.

The presence of enteric protozoans in diarrheic stool does not denote a causal association, and reinfection with *Cystoisospora* spp. is common. Immunity in kittens to *C. rivolta* is not complete, and some oocysts are shed after challenge.[23] Four-week-old kittens are most susceptible to infection with *C. felis*. Enteritis, emaciation, and death can occur after inoculation of 10^5 oocysts,[24] although older kittens can exhibit large- or mixed-bowel diarrhea and abdominal discomfort.

Diagnosis

Fecal flotation with zinc sulfate is the recommended method for diagnosis. Examination of stools for infectious agents that cause disease in these animals is important because coccidiosis usually is asymptomatic. Cats can have oocysts in their fecal specimens from ingestion of prey. These should be recognized as pseudoparasites. The most common of these are *Eimeria* species from ruminants, rabbits, or rodents. These oocysts will not be in the two-celled stage as is common for *Cystoisospora* species. They often will have ornamentations such as micropyle caps or dark thick walls that are not found on *Cystoisospora* oocysts.

Treatment

Sulfadimethoxine is the only drug that has been approved for the treatment of coccidiosis in dogs and cats, but because sulfonamides are coccidiostatic, a low level of persistent infection is possible after treatment. Sulfadimethoxine given at 50 mg/kg PO every 24 hours for 10 to 14 days eliminates oocyst excretion in most animals,[26] but doses and duration of treatment do vary; the label dose of sulfadimethoxine specifies 55 mg/kg PO as an initial dose followed by 27.5 mg/kg PO thereafter, for up to 14 days. Trimethoprim and sulfonamide, furazolidone, and amprolium are also commonly used drugs. Cats do not like the taste of trimethoprim sulfa and will drool profusely. Providing the drug as a capsule or some other compounded dosage form may facilitate ease of administration.

Ponazuril is currently the treatment of choice for many clinicians and shelter managers for eradication of *Cystoisospora* spp. infections in dogs and cats. It is available in the United States in paste form (Marquis paste, Bayer Animal Health; ponazuril 150 mg/g concentration) as a treatment for *Sarcocystis neurona* infection in horses. The drug appears to be well tolerated even in very young kittens and puppies; the dosage is 30 to 50 mg/kg PO every 24 hours for 3 consecutive days. A study in shelter-housed cats and dogs revealed that a dose of 50 mg/kg every 24 hours for 3 consecutive days was not always effective at reducing fecal oocyst counts to levels below the detection limit by 3 to 4 days after the initiation of treatment.[27] Single doses of less than 50 mg/kg do not appear efficacious. Future studies should evaluate increasing the dose rate or continuing treatment for a longer period. Toltrazuril (Baycox, Bayer Animal Health) has been used successfully for the management of kittens infected with *Cystoisospora* spp. in Canada, Australia, and the United Kingdom. The drug is not available in the United States. In addition, environmental oocyst contamination should be reduced by cleaning contaminated surfaces thoroughly, preferably with 10% ammonia with 10 minutes contact time, and by bathing infected animals.

Cryptosporidium Species

Coccidia of the genus *Cryptosporidium* are small, obligate intracellular protozoan parasites that replicate in the microvillous borders of the intestinal and respiratory epithelium of many vertebrates, including birds, mammals, reptiles, and fish.[28] The *Cryptosporidium* genus currently contains at least 20 species and over 40 genotypes, most of which are host adapted and have a narrow host range (e.g., *Cystoisospora canis* in dogs, *C. felis* in cats, and *Cystoisospora hominis* in human beings).[29] The zoonotic risk of feline cryptosporidiosis is relatively low, as most human cases of cryptosporidiosis are associated with *C. hominis* and *Cystoisospora parvum*.[30]

Clinical Signs

Infection with *Cryptosporidium* spp. in kittens and immunosuppressed cats causes a spectrum of disease ranging from an asymptomatic carrier state to mild, transient diarrhea, cholera-like illness, or prolonged and severe life-threatening malabsorption syndrome.[31] The organism has also been associated with diarrhea in adult cats without obvious evidence of immunosuppression. In addition, *Cryptosporidium* spp. infection has been diagnosed in association with intestinal cellular infiltrates indistinguishable from those seen with IBD in cats.[32] Caution should be taken against overinterpretation of the presence of the organism with these infiltrates, because other co-factors, including diet, might be associated with these cellular infiltrates. *Cryptosporidium* spp. was identified in 10 of 50 nondiarrheic cats entering an animal shelter (20%) and in 5 of 50 diarrheic cats (10%), illustrating the fact that many cats can be subclinically infected with *Cryptosporidium* spp.[9]

Diagnosis

Despite the relatively high seroprevalence rates of *C. parvum*-specific immunoglobulin G (IgG) in cats (8.3% to 87%),[33-35] the laboratory detection of this ubiquitous protozoan parasite in spontaneously infected diarrheic cats is difficult, predominantly because the organism is so small (average 4.6 × 4.0 μm), is difficult to find in fecal specimens via light microscopy,[36] and because fecal shedding may be intermittent. Current laboratory protocols for detection of *Cryptosporidium* oocysts in fecal specimens include microscopic examination of smears stained with Giemsa stain, the modified Ziehl-Neelson (ZN) technique (Figure 1-4), the modified Kinyoun acid-fast technique, or an immunofluorescent detection procedure (Figure 1-5).[37,38] Immunofluorescent detection procedures are more sensitive and specific than acid-fast stains and are generally the method of choice for morphological diagnosis in human beings.[39] Microscopic techniques work well when clinical signs are present and oocyst numbers are relatively high; however, once clinical signs abate and oocyst numbers are greatly decreased, the sensitivity of tests relying
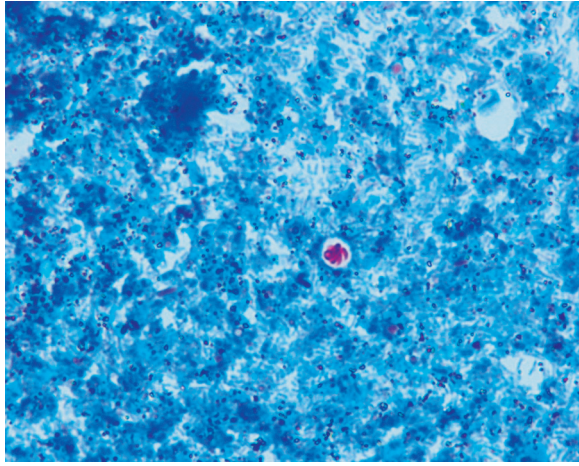
Figure 1-4: Fecal smear showing a single acid-fast (modified Ziehl-Neelson) staining *Cryptosporidium* oocyst from a diarrheic cat (magnification ×1000).
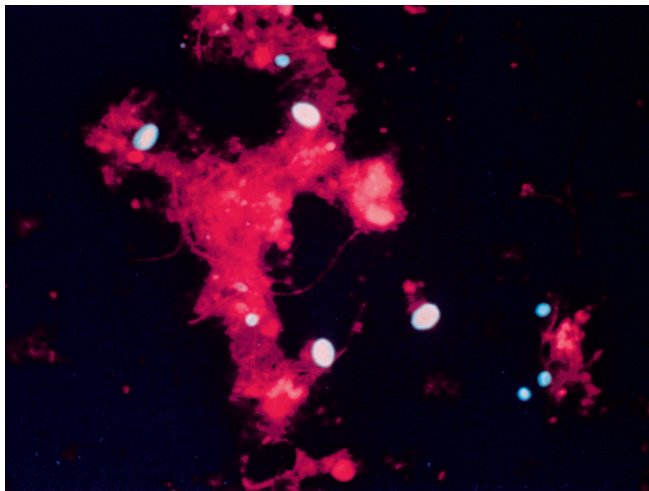

Figure 1-5: Direct immunofluorescent assay (Merifluor *Cryptosporidium/Giardia* direct Immunofluorescent kit, Meridian Diagnostics Inc, Cincinnati, Ohio) showing fluorescent *Giardia* cysts (*larger, oval*) and *Cryptosporidium* oocysts (*smaller, round*) (magnification ×400).

on morphological identification is reduced and diagnosis often requires examination of multiple fecal specimens. In these cases, the newer enzyme immunoassays designed to detect *Cryptosporidium* antigens in feces have proven more sensitive.[40] Difficulties in detection and enumeration of oocysts in fecal specimens are compounded by variation in consistency among individual fecal specimens, the amount of specimen used, and oocyst losses incurred during recovery processes. Real-time PCR for diagnosis of *Cryptosporidium* spp. infection is readily available in large reference laboratories, and studies utilizing this diagnostic modality have demonstrated a significantly higher prevalence of *Cryptosporidium* spp. in infected cats compared with microscopic evaluation and immunoassay methods.[6]

A study compared the performance characteristics of a ZN stain, direct fluorescent antibody technique, and three enzyme-linked immunosorbent assay (ELISA) tests (Table 1-2).[21] It revealed that the Remel ProSpecT Microplate ELISA (Thermo Fisher Scientific, Lenexa, Kansas) was the most sensitive diagnostic test for *Cryptosporidium* spp. on a single day, whereas the ProSpecT Rapid ELISA was highly insensitive and should not be utilized by veterinary diagnostic laboratories.

Treatment

Eradication of this parasite has proven difficult, and many putatively effective drugs are either toxic or ineffective in cats. The aminoglycoside paromomycin is potentially nephrotoxic[41] and ototoxic in cats, and preferably should not be used. Although the benzamide antimicrobial nitazoxanide has been shown to eradicate *Cryptosporidium* spp. in humans and cats, its use in cats is associated with unacceptable adverse effects (i.e., vomiting and anorexia). One report stated that tylosin was effective in eradicating *Cryptosporidium* infection in a cat[32]; however, this drug failed to eradicate infection in a prospective double-blind study conducted by the author in naturally infected cats. Azithromycin is used in humans for management of cryptosporidiosis, and the author has used this drug successfully in cats, administered at a dosage of 7 to 10 mg/kg PO every 12 hours for 7 days. Veterinarians should make every effort to identify and treat underlying causes of immunosuppression and/or concurrent disorders in

Table 1-2	Cumulative Sensitivities of Five Methods of Detection of *Cryptosporidium* Species in Fecal Specimens Collected Once Daily over 4 Consecutive Days from 104 Naturally Exposed Kittens		
Detection Method	**Day 1 (%)**	**Day 3 (%)**	**Day 4 (%)**
Ziehl-Neelson technique	72	91	94
Direct immunofluorescence detection	50	83	84
Premier ELISA* (Meridian Bioscience, Inc., Cincinnati, Ohio)	80	93	93
Remel ProSpecT Microplate ELISA (Thermo Fisher Scientific, Lenexa, Kansas)	89	94	95
Remel ProSpecT Rapid ELISA (Thermo Fisher Scientific, Lenexa, Kansas)	15	43	49

*Enzyme-linked immunosorbent assay

infected kittens because infection with the parasite is often self-limiting.

Giardia Species

Giardia spp. are an important cause of outbreaks of waterborne infection resulting from contamination of raw municipal water, backcountry streams, and lakes with human effluent or infected animal feces.[42] The overall prevalence of *Giardia* in cats in North America has been reported at about 4%, with much higher levels in kittens and in cats housed in shelters.[43] Fourteen percent of cats entering an animal shelter in Florida tested positive for *Giardia* spp.[9] Interestingly, adult cats with diarrhea were significantly more likely (odds ratio [OR] 5.00) to be infected with *Giardia* spp. (10/15 [67%]) than were juveniles with diarrhea (10/35 [29%]).[9]

Epidemiological studies have focused on the transmission route of *Giardia* spp. and have sought to determine their zoonotic potential. *Giardia intestinalis* assemblages A-H have been defined by DNA sequence analysis so far, of which assemblages A and B are mainly virulent for humans and are often referred to as "zoonotic assemblages."[44] A study comparing mammalian *G. intestinalis* assemblages studied 13 feline isolates, of which seven were assemblage F, two were assemblage D, three were assemblage A, and one contained both assemblages C and D.[45] These results support the notion that *Giardia* spp. isolated from infected cats can be zoonotic, although transmission from cats to humans appears to be rare.

Clinical Signs

Giardia infections in adult cats are often subclinical or associated with a transient softening of the stool early in the infection; however, acute diarrhea tends to occur in kittens shortly after infection. Feces are often malodorous and pale, and may contain mucus.

Diagnosis

Giardiasis is commonly misdiagnosed or underdiagnosed because of intermittent shedding and difficulty identifying cysts and trophozoites. The Companion Animal Parasite Council (CAPC; http://www.capcvet.org/) recommends testing symptomatic cats with a combination of direct smear, fecal flotation via centrifugation, and a sensitive, specific ELISA optimized for use in companion animals. A commercially available dual (*Cryptosporidium* spp. and *Giardia* spp.) direct fluorescent antibody (DFA) assay is available and is more sensitive than fecal flotation for detection of *Giardia* spp.; however, the procedure requires a fluorescent microscope to evaluate the fecal specimen. Fecal PCR is commonly performed at reference laboratories, although the author recommends the use of conventional testing (i.e., fecal flotation via centrifugation, ELISA testing, and DFA) whenever feasible. The author combines the use of the dual DFA test with a fecal flotation and wet mount preparation in dogs and cats with diarrhea.

Fecal PCR for *Giardia* should not be used in lieu of fecal flotation or other tests because the sensitivity of the currently available PCR assays is low. *Giardia* PCR fails to amplify DNA from approximately 20% of samples that are positive for *Giardia* cysts or antigens in other assays.[46] This finding likely results from the presence of PCR inhibitors in feces. PCR should only be used for *Giardia* if genotyping of the previously detected *Giardia* spp. is desired to determine the *Giardia* assemblage. The latter assay can be performed at the Veterinary Diagnostic Laboratory at Colorado State University (http://dLab.colostate.edu/).

The diagnosis of *Giardia* spp. infection traditionally has depended on microscopic identification of trophozoites (Figure 1-6) or cysts (Figure 1-7) in feces from affected animals. However, microscopic diagnosis of giardiasis can be difficult, because cysts may be shed intermittently and the cysts are so delicate. Many artifacts (e.g., grass pollen, yeast) mimic the morphology of *Giardia* cysts to varying degrees, and care must be exercised in differentiating these from *Giardia* spp. A survey evaluated the sensitivity of fecal

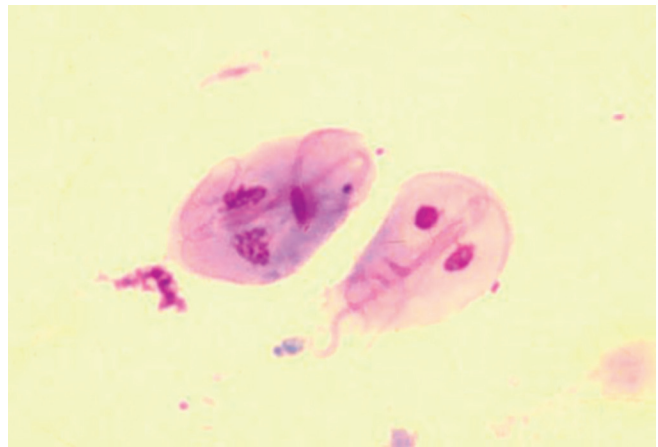**Figure 1-6:** Giemsa-stained fecal smear showing two *Giardia* trophozoites exhibiting the characteristic pear, or tear-drop, shape with bilateral symmetry when viewed from the top, two nuclei, and fibrils running the length of the parasite (magnification ×400).

Figure 1-7: Zinc sulfate fecal flotation showing *Giardia* cysts with distinctive fibrils (axonemes) coursing the length of the cyst (magnification ×400).

flotation for detection of *Giardia* spp. in dogs and confirmed the poor performance of current in-house microscopy testing for *Giardia* spp. compared with microplate ELISA. In that study, microscopy following fecal flotation identified only half of the infected dogs and falsely diagnosed up to 25% of uninfected animals.[47]

Many veterinarians and reference laboratories have resorted to using ELISA tests that rely upon detection of *Giardia* cyst wall protein 1 (GCWP 1).[48] The ELISA tests are advantageous because they are generally easy to perform and results are easy to interpret. In addition, the test does not rely upon morphological identification of cysts via microscopy, which saves technician time and potentially avoids false-negative interpretations. The ELISA tests also can detect GCWP 1 in the absence of detectable cysts.[48] However, the SNAP *Giardia* Test (IDEXX Laboratories, Westbrook, Maine) is the only commercially available *Giardia* ELISA assay approved for patient-side testing of giardiasis in dogs and cats. The SNAP *Giardia* Test is a rapid in-house enzyme immunoassay that can be performed on fresh feces, previously frozen feces, or feces stored at 2° to 7°C (35.6 to 44.6°F) for up to 7 days. The test has the added advantages of simplicity, rapid availability of results (8 minutes after mixing of the conjugate solution with feces), and low cost. However, such *Giardia* antigen assays should be supplemental tests because they only detect *Giardia* spp. and should not replace fecal flotation and wet mount examination to detect a wide variety of intestinal parasites, including *Giardia* spp. In addition, the *Giardia* antigen test is best used as a baseline supplemental test to diagnose new infections in animals and should not be used to assess efficacy of therapy because antigen can persist for up to 4 weeks or longer in the absence of *Giardia* cysts.

The performance characteristics of the SNAP *Giardia* test were evaluated in 304 diarrheic and nondiarrheic shelter cats that had also undergone fecal testing via direct immunofluorescence, fecal flotation via centrifugation, and four other human-based immunoassays.[49] Both the sensitivity and specificity of the SNAP Giardia test were 85.3%. When the SNAP *Giardia* test was used in parallel with fecal flotation, the sensitivity of the combined tests increased to 97.8% for detection of *Giardia* spp.[49]

Treatment

In the United States, there is no drug that is FDA approved for treating giardiasis in dogs and cats, and the use of different drugs has been extrapolated from use in humans. The majority opinion of the CAPC is that *asymptomatic* cats may not require treatment. A cat without clinical signs found to be infected with *Giardia* may be treated with a single course of antigiardial therapy. If other cats or dogs live with an infected kitten, all those of the same species may also be treated with a single course of antigiardial therapy. *Repeated courses of treatment are not indicated in dogs and cats without clinical signs.*

Metronidazole was shown to be highly effective and safe when given at 25 mg/kg PO every 12 hours for 7 days to cats with experimental infections.[50] Albendazole also is relatively

effective when dosed at 25 mg/kg PO every 12 hours for 5 days; however, the drug has been associated with pancytopenia and is teratogenic. A trial evaluating the efficacy of fenbendazole (50 mg/kg PO every 24 hours for 5 days) in cats coinfected with *C. parvum* revealed that the drug was safe; however, it was relatively ineffective (50%).[51] Fenbendazole may be administered in combination with metronidazole in refractory cases, and the combination may result in better resolution of clinical disease and cyst shedding. A combination of febantel, pyrantel, and praziquantel (Drontal Plus, Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, Kansas) was shown to be relatively safe and effective in experimentally infected kittens when given at twice the dose recommended for dogs. The dose of febantel used was 56.5 mg/kg PO every 24 hours for 5 days.[52] If treatment combined with bathing (see Control of *Giardia* Infection) does not eliminate infection, as evidenced by testing feces for persistence of cysts in a diarrheic kitten, treatment with either fenbendazole alone or in combination with metronidazole may be extended for another 10 days.

Control of *Giardia* Infection

The following four fundamental steps should be taken to control *Giardia* infection and minimize reinfection of treated animals:

1. The environment is decontaminated. Simultaneous treatment of animals with medication and decontamination of the environment with quaternary ammonium-based (QUAT) disinfectant such as Roccal-D Plus (Zoetis, Florham Park, New Jersey), Quatsyl 256 (Lehn & Fink Products, Montvale, New Jersey), or Aqua Quat 400 (Arysta LifeScience, South Africa) should improve effectiveness of treatment and maximize the possibility of eliminating *Giardia* spp. from the cattery or shelter. Specifically, gross fecal contamination should be removed as much as possible on a daily basis. Runs should be rinsed with water, after which a layer of disinfectant foam (e.g., Roccal-D Plus) should be applied. After 10 to 20 minutes, the foam should be rinsed away with fresh water. Cages should be sponged clean on a daily basis with a dilute disinfectant or mix of bleach (e.g., Clorox, The Clorox Company, Oakland, California) diluted at 1:32 and Quatsyl 256 at 1:256.
2. The animal is treated with effective drugs.
3. The animal is bathed to clean cysts from the coat.
4. Reintroduction of infection is prevented.

Unfortunately, the last three recommendations have limitations and inherent challenges in a cattery or shelter environment. There are no consistently effective antigiardial drugs, and it is difficult to bathe cats. Reinfection is common, so decontamination of the environment in shelters is paramount.

Whipworms

Domestic cats rarely acquire whipworm infections in North America, although they are a possibility in animals with
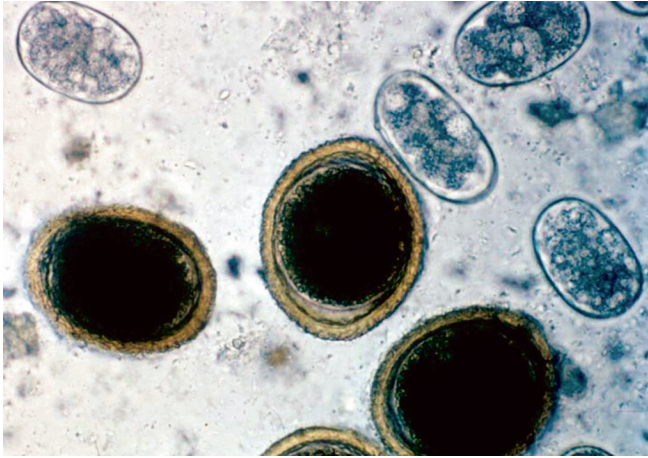
Figure 1-8: Fecal flotation showing large, thick-walled ova of *Toxocara cati* and *Ancylostoma caninum* ova (magnification ×400).

clinical signs of colitis. The adult worms burrow into the colonic and cecal mucosa and may cause inflammation, hematochezia, and intestinal protein loss.

Diagnosis. *Trichuris serrata* should be considered in cats with evidence of colonic disease. A fecal flotation by centrifugation test should allow recognition of the bioperculate ova. However, intermittent shedding of *Trichuris vulpis* and *Trichuris campanula* has been well documented in dogs; therefore cats with a negative fecal flotation should be dewormed empirically.

Treatment. Fenbendazole is a safe broad-spectrum anthelminthic. The drug is administered at 50 mg/kg PO every 24 hours for 5 consecutive days, and the regime is repeated at 3 weeks and 3 months after initiation of therapy. Despite its reported safety in cats even when administered at 5 times the recommended dosage and 3 times the duration approved for use in dogs, fenbendazole is not approved for use in cats in the United States (although it is in other countries), so the drug is typically prescribed off-label to treat cats.

Roundworms

Roundworms (*Toxocara cati* and *Toxascaris leonina*) are particularly common in kittens <6 months old and can cause diarrhea, failure to thrive, a poor quality coat, and a "potbellied" appearance. Vomiting is observed occasionally when the roundworms gain access to the stomach.

Diagnosis. The large ova (approximately 80 μm) with a characteristic thick wall are easy to recognize on fecal flotation (Figure 1-8).

Treatment. Pyrantel pamoate at 20 mg/kg PO is safe in kittens over 2 weeks of age. The treatment should be repeated at approximately 2 weeks. Fenbendazole also is an effective anthelminthic and can be administered to kittens from 4 weeks of age at 50 mg/kg PO for 5 days to kill more than 90% of prenatal larvae. Because the prepatent period of *T. cati* is 8 weeks, kittens do not need to be treated for roundworms until 6 weeks of age. However, given the concern

about hookworm infection, all kittens should be routinely dewormed with pyrantel pamoate beginning at 2 weeks of age and then placed on a monthly heartworm preventative with efficacy against *Toxocara* spp.

Hookworms

Cats can be infected with *Ancylostoma tubaeforme*, *Ancylostoma braziliense*, *Uncinaria stenocephala*, and less commonly, the canine hookworm *Ancylostoma caninum*. The worms are voracious blood suckers and attach to the mucosa of the small intestine. Hookworm infections in cats are relatively uncommon with reported prevalences of 0.9% and 1.1%.[53] Kittens are infected by ingestion of larvae from a contaminated environment, larval skin penetration, or ingestion of larvae in the tissues of vertebrate hosts (usually rodents). Infected kittens occasionally can have life-threatening blood loss or iron-deficiency anemia, melena, hematochezia, and failure to thrive.

Diagnosis. Fecal flotation should be positive because the worms produce a large amount of eggs.

Treatment. Effective drugs that are FDA approved in the United States include selamectin (Revolution, Zoetis, Florham Park, New Jersey), moxidectin (Advantage Multi, Bayer Animal Health Division, Germany), milbemycin oxime (Milbemax, Novartis Animal Health, New York, New York), and emodepside (Profender Bayer Animal Health Division, Germany). Fenbendazole and pyrantel are not FDA approved, but are frequently used off-label in cats at the same doses as those used for treatment of roundworms.

BACTERIAL CAUSES OF DIARRHEA

Diagnosis of bacterial-associated diarrhea in kittens is challenging for two reasons: (1) the isolation rates for putative bacterial enteropathogens often are similar in diarrheic and nondiarrheic animals and (2) the incidence of bacterial-associated diarrhea is extremely variable. Caution should be heeded in interpretation of the results of fecal ELISAs for *Clostridium perfringens* enterotoxin (CPE) and *Clostridium difficile* toxin A and/or B in neonatal kittens because of the high incidence of positive ELISAs (up to 40%) observed in apparently healthy kittens by the author. Although testing of human infants is not recommended, data have shown that 26% of children hospitalized with *C. difficile* infections (CDIs) were younger than 1 year, and 5% were neonates.[54] What cannot be determined from these data are whether the rates of hospitalization for CDIs represent true disease or asymptomatic carriage. *C. difficile* carriage rates average 37% for infants 0 to 1 month of age and 30% between 1 and 6 months of age.[55] The rate of carriage is similar to that of nonhospitalized adults (0% to 3%) by 3 years of age. It is plausible that neonates and infants may lack the cellular machinery to bind and process the toxins of *Clostridium* species. This phenomenon is underscored in neonatal puppies that have been shown to have a high incidence of carriage of toxigenic

C. difficile (up to 58% of puppies) with no demonstration of pathogenicity.[56] These findings highlight the potential concerns with overinterpreting fecal PCR panels that detect the genes for the CPE or *C. difficile* toxins A and B.

The indications for performance of fecal enteric panels on diarrheic kittens are poorly defined, which results in indiscriminate testing and misinterpretation of results. Fecal PCR and toxin analysis for specific bacteria should be reserved for (1) kittens that develop diarrhea after kenneling or show attendance once parasitic and viral (feline panleukopenia virus [FPV]) causes for diarrhea have been ruled out, (2) kittens with an acute onset of bloody diarrhea in association with evidence of sepsis, (3) outbreaks of diarrhea occurring in more than one household pet, and (4) screening for enteropathogens (*Campylobacter jejuni* or *Salmonella* spp.) when zoonotic concerns are present. The prevalence of five groups of potentially zoonotic enteric infections (*Salmonella* spp., *Campylobacter* spp., *Cryptosporidium* spp., *Giardia* spp., and *T. cati*) in fecal samples from cats under 1 year of age that were either housed in humane shelters or presented to primary-care veterinarians in central New York State was studied.[57] Possible associations of these organisms with the cat's source or with the presence of diarrhea were evaluated. The presence of diarrhea was not significantly associated with the number of organisms identified. Of the 74 cats with diarrhea, 35% (26/74) had one or more types of organisms identified, but of the 189 without diarrhea, 43% (81/189) had one or more types of organisms identified. The proportion of fecal samples with one or more zoonotic organisms was 35.1% among client-owned cats and 44.2% among shelter cats. The prevalence of *Salmonella* spp. was 0.8%, which is similar to the reported prevalence of *Salmonella* spp. in cats in Colorado[58] and in kittens from shelters in Japan (1.1%).[59]

Campylobacter spp. was isolated from significantly fewer diarrheic (21 of 219 or 9.6%) versus nondiarrheic cats (15 of 54 or 27.8%) in a study evaluating the prevalence of bacterial and parasitic agents in feces from diarrheic and healthy cats from northern California.[8] Caution should be heeded in overinterpreting the isolation of *Campylobacter* spp. from diarrheic kittens, because many species are nonpathogenic. In addition, fecal cultures are relatively insensitive for isolation of *Campylobacter* spp. compared with PCR-based testing. In the study by Queen and colleagues[8] only 13.2% of cats were positive for *Campylobacter* spp. via fecal culture versus 56.5% via PCR. It has been well documented that biochemical and phenotypical characterization of *Campylobacter* spp. in cat feces is insufficient to characterize the infection. Molecular-based testing allows differentiation of enteric *Campylobacter* spp. from *Helicobacter* spp., and also allows identification of multiple *Campylobacter* spp. in individual animals.[60] Molecular-based testing allows the clinician to detect zoonotic enteropathogens such as *Campylobacter jejuni* and avoid injudicious antimicrobial therapy for kittens infected with *Campylobacter helveticus,* an organism of questionable pathogenicity given its high prevalence in healthy cats.

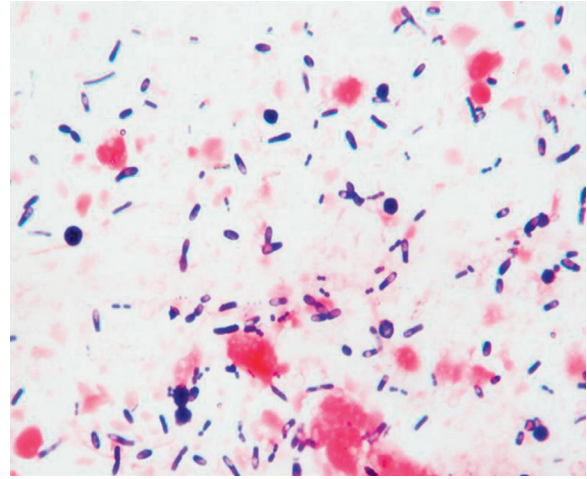Wright- or Gram-stained fecal smears have been suggested as a tool to diagnose enterotoxigenic *C. perfringens-*

Figure 1-9: Stained fecal smear (modified Wright's stain) from a healthy, nondiarrheic cat showing numerous endospores of *Clostridium perfringens* (magnification ×1000).

associated disease as well as infection with *Campylobacter* spp. (Figure 1-9). Several studies in dogs have reported no association between fecal endospore counts and the presence of diarrhea, or between spore counts and the detection of the CPE in fecal specimens.[61,62] In addition, the identification of spiral-shaped bacteria on fecal smears is suboptimal for diagnosis of *Campylobacter* spp. infection for two reasons: (1) most *Campylobacter* spp. infections in cats are nonpathogenic and observation of stained fecal smears does not allow differentiation of pathogenic versus nonpathogenic species and (2) *Campylobacter* spp. cannot be differentiated from other spiral-shaped bacteria such as *Arcobacter* spp., *Anaerobiospirillum* spp., and *Helicobacter* spp. PCR testing of oral swabs collected from 85 cats in Southern Italy documented carriage of *Arcobacter* spp. in 78.8% of the cats,[63] highlighting the limitations of stained fecal smears for identifying spiral-shaped bacteria.

A recent study demonstrated an association between mortality in kittens and a shift in ileum mucosa-associated enterococci from *Enterococcus hirae* to *Enterococcus faecalis* and adherent *Escherichia coli*.[64] In addition, the *E. faecalis* isolates obtained from these kittens were characterized as carrying multiple genotypic and phenotypic attributes of virulence. However, whether the colonization of ileum-mucosa-associated microbiota by *E. faecalis* was a contributing cause or consequence of gastrointestinal disease and terminal illness in the sick kittens is unknown.

Miscellaneous Bacterial Causes of Diarrhea
Anaerobiospirillum Species
Anaerobiospirillum spp. are motile, spiral-shaped, anaerobic gram-negative rods that were first identified by Malnick and colleagues in 1983 in two human patients with diarrhea.[64] Since then, *Anaerobiospirillum succiniciproducens* and *Anaerobiospirillum thomasii* have been recognized as causes of septicemia, particularly in immunocompromised humans, and have been isolated from the throat and feces of healthy dogs
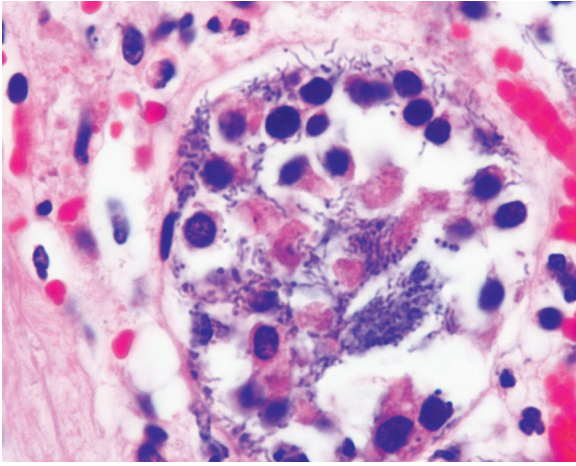
Figure 1-10: Light photomicrograph of colon obtained from a cat, showing spiral-shaped *Anaerobiospirillum* bacteria inside the lumen of a dilated crypt (Steiner stain) (magnification ×1200).

and cats.[65,66] The author has identified three cats (one of which was a 6-month-old kitten) with clinical signs of either acute onset of vomiting, diarrhea, or abdominal pain that progressed rapidly to systemic disease characterized by lethargy and collapse. On necropsy, an acute to subacute ileocolitis was found in association with abundant spiral-shaped organisms confirmed as *Anaerobiospirillum* spp.[67] (Figure 1-10). *Anaerobiospirillum* spp. and *Campylobacter* spp. are morphologically similar and can be confused. *Anaerobiospirillum* spp. are oxidase- and catalase-negative, whereas *Campylobacter* spp. usually are oxidase- and catalase-positive. *Anaerobiospirillum* demonstrate corkscrew motility, whereas *Campylobacter* display darting motility. *Anaerobiospirillum* has bipolar tufts of flagella, whereas *Campylobacter* has a single flagellum on one or both poles. Although the organisms have been isolated from the rectal swabs of asymptomatic dogs and cats, they have not been isolated from the feces of asymptomatic human beings. Most human patients infected with *Anaerobiospirillum* spp. are immunocompromised, and the organism is a rare cause of bacteremia in people. According to the National Committee for Clinical Laboratory Standards breakpoints for anaerobes, the isolates are susceptible to amoxicillin-clavulanic acid, cefoxitin, imipenem, and penicillin, intermediately susceptible to metronidazole, and resistant to clindamycin.

Helicobacter Species

Helicobacter spp. are gram-negative, microaerophilic spiral-shaped, motile bacteria that colonize the gastrointestinal tract of several mammalian and avian hosts. Although *Helicobacter* spp. are better known as gastric pathogens, accumulating reports describe enteric pathogenic helicobacters in dogs, humans, and birds. *Helicobacter canis* was isolated from two adult Bengal cats and two 8-month-old Bengal kittens with and without chronic diarrhea.[68] Because the cats were coinfected with other potential pathogens, including *C. helveticus*,

and because *H. canis* was isolated from nondiarrheic cats, the causal role of *H. canis* in production of the diarrhea could not be proven.[69] Histologically, the colons of the four affected cats were characterized by mild to moderate neutrophilic, plasmacytic, and histiocytic infiltrates in the lamina propria, with crypt abscesses.

A 4-month-old male British Blue cat with catarrhal to hemorrhagic enteritis showed massive colonization of the stomach, small intestine, and cecum with spiral-shaped bacilli that strongly resembled *Flexispira rappini*, a spiral-shaped *Helicobacter* species known as a normal intestinal colonizer in dogs and mice.[70] Inflammatory infiltration was moderate and dominated by T cells. In the intestine, bacilli were found in the gut lumen, between villi, in crypt lumina, and within epithelial cells. Degeneration of crypt epithelial cells was observed, in addition to crypt dilation and moderate to massive macrophage-dominated infiltration of the mucosa and submucosa.

A morphologically, ecologically, and genetically unique helicobacter was recovered from an 8-week-old domestic shorthaired stray kitten with severe diarrhea.[69] A Gram stain of the fecal smear showed large numbers of *Helicobacter*-like curved, gram-negative rods. The kitten was ultimately euthanized and necropsied. Histopathologic assessment of the intestine revealed a thick layer of densely packed bacteria that covered the mucosal surface of the cecum and colon. The bacteria stained strongly with Warthin-Starry stain. The appearance of the duodenum, jejunum, and ileum were within normal limits. The organism could not be cultured but was described on the basis of the 16S ribosomal ribonucleic acid gene sequence analysis and morphology, and appeared to be a new species, with *H. canis* being the most genetically similar species. The new helicobacter organism was proposed as a candidate species, with the specific designation *Helicobacter colefelis*.[69] It is unclear how pathogenic *H. colefelis* is, and attempts to transfect other cats did not induce diarrhea after inoculation, despite the cats becoming PCR-positive.

There are a plethora of protocols that have been utilized in an effort to eradicate *Helicobacter* spp. from infected cats, and most protocols incorporate a gastric protectant agent in combination with one or two antimicrobials. Only a few controlled, randomized, blind therapeutic studies in cats have been published. Twenty-three cats naturally infected with *Helicobacter heilmannii* were randomized to four treatment groups to receive a placebo (group 1); azithromycin, tinidazole, ranitidine, and bismuth once daily for 4 days (group 2); clarithromycin, metronidazole, ranitidine, and bismuth twice daily for 4 days (group 3); or clarithromycin, metronidazole, ranitidine, and bismuth twice daily for 7 days (group 4).[71] Ten days after treatment, all of the cats in the placebo group were infected with *H. heilmannii* following testing utilizing a urea breath test. The urea breath test is the most reliable noninvasive test for *Helicobacter pylori* infection in humans and has been used in natural and experimental animal *Helicobacter* infections.[72] Four of 6 cats in group 2 and all the cats in groups 3 and 4 had a negative result for the urea breath test. Forty-two days after treatment, 0 of 4, 3 of 6, 7 of 11, and 4

of 8 cats in groups 1 to 4, respectively, still had a negative result, underscoring the challenges of maintaining a definitive long-term cure in cats naturally infected with *Helicobacter* spp. A recent study in 13 asymptomatic cats with naturally acquired *Helicobacter* spp. infection evaluated the efficacy of a quadruple therapy protocol utilizing a regime of omeprazole, amoxicillin, metronidazole, and clarithromycin for 14 days.[73] Molecular analysis of gastric biopsies revealed persistence of *Helicobater* spp. DNA in four cats that were negative on quantitative urease testing in biopsies, cytology, and histopathology. These results suggest that antibiotic regimes that are effective against *H. pylori* in people are less effective at eradicating *Helicobacter* spp. in cats with naturally acquired infection.

Clostridium piliforme

Tyzzer's disease caused by *C. piliforme* infection has been reported in immunocompromised kittens with feline infectious peritonitis (FIP),[74] feline leukemia virus infection (FeLV),[75] or FPV infection.[76] Histopathologic lesions caused by *C. piliforme* are characterized by necrotizing enteritis or multifocal hepatic necrosis, and special stains (toluidine blue, Giemsa, periodic acid-Shiff, and Warthin-Starry methods) reveal large filamentous bacilli in bundles or crisscross patterns in the cytoplasm of the epithelial cells. PCR of affected intestinal biopsies can be used to detect the 196-bp bands specific to 16S rDNA of *C. piliforme*.

Although the liver is the most commonly involved organ, necrotizing enterocolitis has been well documented in infected kittens. *C. piliforme* has been reported to be susceptible to penicillin, tetracycline, and erythromycin in studies using infected embryonated eggs; however, the author is unaware of any studies evaluating the efficacy of antimicrobial therapy in infected cats. Avoiding contact with environments contaminated by rodents is important to minimize transmission of the organism.

Treatment of Enteropathogenic Bacteria in Kittens

The lack of well-scrutinized therapeutic guidelines for veterinarians that provide objective recommendations for implementing fecal bacterial testing, combined with the clinical documentation of enteropathogenic bacteria in diarrheic and healthy kittens, has resulted in indiscriminate testing and misinterpretation of results. In addition, antimicrobial therapy is commonly administered injudiciously to diarrheic kittens, with cessation of diarrhea erroneously equated with eradication of the putative enteropathogen. Veterinarians should be cognizant of the fact that most bacterial enteropathogens are associated with self-limiting diarrhea, and the injudicious administration of antimicrobials could be more harmful than beneficial. Supportive therapy and appropriate hygiene control should be considered in all kittens with suspected or confirmed bacterial-associated diarrhea, and antimicrobials should only be administered to immunocompromised kittens or kittens manifesting systemic signs of illness.

Management of *Clostridium perfringens*-Associated Diarrhea

Kittens that are systemically ill (e.g., fever, hemorrhagic gastroenteritis, inflammatory or toxic leukogram) merit appropriate antimicrobial therapy. There is no documented evidence for the benefits of antimicrobial therapy in dogs or cats with uncomplicated diarrhea associated with *C. perfringens*. Antibiotics that have been recommended for the treatment of canine *C. perfringens*-associated diarrhea include ampicillin (22 mg/kg PO every 12 hours for 5 to 7 days), erythromycin (10 to 15 mg/kg PO every 8 hours for 5 to 7 days), metronidazole (10 to 15 mg/kg PO every 12 hours for 5 to 7 days), and tylosin (10 mg/kg PO every 24 hours for 5 to 7 days). Tylosin is an extremely bitter-tasting powder that should be compounded into empty gelatin capsules or into a palatable suspension before administration to cats.

Management of *Clostridium difficile*-Associated Diarrhea

In general, CDI is treated like any other diarrheal disease. Supportive therapy should be administered. If CDI is suspected to be antimicrobial associated, antimicrobial therapy should be discontinued if possible. Metronidazole (10 to 15 mg/kg PO every 12 hours for 5 to 7 days) is commonly used, although it is unclear whether it is needed in all cases. Other treatment options that have been used with a lack of objective scrutiny in kittens include intestinal adsorbents such as di-tri-octahedral smectite (Bio-Sponge, Platinum Performance, Buellton, California), probiotics, and dietary modification with increased soluble fiber.

Management of *Salmonella*-Associated Diarrhea

It is widely accepted (although supportive scientific evidence is lacking) that the administration of antimicrobials is not warranted for uncomplicated episodes of *Salmonella* infection, and only supportive therapy is recommended. In the event of systemic disease or an immunocompromised patient, antimicrobials may be necessary and a combination of ampicillin and a fluoroquinolone for 5 to 7 days is advocated as empirical therapy. If culture results are available, antimicrobial susceptibility testing should be performed to optimize antimicrobial therapy.

Management of *Campylobacter*-Associated Diarrhea

The majority of cases are uncomplicated, self-limiting, and will resolve with supportive therapy alone. Because isolation of *Campylobacter* does not necessarily imply causation of clinical signs, treatment may not be warranted and may further disrupt the intestinal microbiota. However, in immunocompromised or febrile kittens, or in kittens with evidence of hemorrhagic diarrhea, antimicrobial treatment may be indicated. Macrolides and fluoroquinolones are most commonly used to treat *Campylobacter* infections, although the macrolides are the drugs of choice in light of the increasing resistance to fluoroquinolones observed in people and dogs. Erythromycin administered at 10 to 15 mg/kg PO every 8 hours or azithromycin at 5 to 10 mg/kg PO every 24 hours

can be given for 5 to 21 days as treatment. Azithromycin is better tolerated, but to the author's knowledge, no published studies regarding efficacy of azithromycin for treatment of campylobacteriosis in cats or its comparison with other macrolides or fluoroquinolones are available.

Enteropathogenic Bacteria Zoonotic Considerations

All kittens with idiopathic diarrhea or a diagnosis of infection with any of the bacteria described here should be considered potentially contagious. Salmonellosis and campylobacteriosis are diseases of major zoonotic importance, and contact with diarrheic animals has been identified as a risk factor for diarrhea in humans. Nosocomial transmission of *C. difficile* and *Salmonella* has been identified in small animal clinics and outbreaks of human salmonellosis in clinic personnel have been documented. The risk of nosocomial and zoonotic transmission of *C. perfringens* probably is minimal, but cannot be dismissed.

Basic practices such as isolation, use of appropriate personal protective equipment, and proper cleaning and disinfection practices are the main control measures. Handwashing is preferred over alcohol-based hand sanitizers because spores of *C. difficile* and *C. perfringens* are alcohol resistant. Litter boxes should be cleaned and disinfected regularly. Gloves should be worn when handling litter boxes and hands washed after glove removal. *C. difficile* and *C. perfringens* spores are highly resistant to most disinfectants but susceptible to bleach (1:10 to 1:20 dilution of regular household bleach) and some oxidizing agents such as accelerated hydrogen peroxide.

VIRAL CAUSES OF DIARRHEA

Feline viral enteritis is usually diagnosed in younger unvaccinated animals. The animal's signalment, history, clinical signs, and hematologic findings are important in ranking a viral etiology as a likely cause of the animal's diarrhea. The two most common viral enteropathogens in cats are FPV and FCoV.

Feline Panleukopenia Virus

Feline panleukopenia is the prototype parvovirus of carnivores and is environmentally stable, highly contagious and spread by direct contact with the feces, urine, and blood of infected cats. Without thorough disinfection with an appropriate disinfectant suitable for nonenveloped viruses such as bleach, potassium peroxymonosulfate (Trifectant, Tomlyn Products, Division of Vétoquinol, USA, Buena, New Jersey) or Virkon-S (DuPont Animal Health Solutions), environmental contamination can remain infectious for many months. Bleach must be applied to a clean surface to be effective. Five percent household bleach should be freshly diluted 1:32 (½ cup per gallon). Correct dilution is very important for maximizing effectiveness. Historically, feline panleukopenia was

caused exclusively by FPV; however, it has now been confirmed that feline panleukopenia can be caused by canine parvovirus (CPV) 2a, 2b, and 2c.[77] Because of the widespread use of highly effective vaccines against FPV, the disease has become much less prevalent over the last 20 years, particularly in private practice.[78] The disease seems more prevalent in animal shelters which are home to a continual influx of cats of unknown vaccination status, particularly during the summer and fall when large numbers of kittens with waning maternal immunity are admitted.[79] Because the incubation period is 2 to 14 days, exposed cats that are clinically healthy but incubating the infection might not show clinical signs until days after they have arrived at a shelter or an adoptive home.

Clinical Signs

The hallmark of FPV is diarrhea caused by marked shortening of the intestinal villi with impaired regeneration of the enterocytes. In the peracute form, kittens can die within 12 hours due to septic shock, dehydration, and hypothermia, and clinical signs can be minimal or absent. The more common acute form is characterized by fever for 3 to 4 days, lethargy, anorexia, vomiting, and diarrhea. The disease has an acute self-limiting course and cats that survive infection for longer than 5 days usually recover over the course of several weeks.[78] Intrauterine or perinatal infection may affect the central nervous system of the fetus, leading to cerebellar ataxia and intention tremor in affected kittens.

Diagnosis

Diagnosis is supported on the basis of the cat's history, physical examination findings, and results of a hemogram (neutropenia and lymphopenia). In clinical practice, virus isolation from blood and feces is impractical, and most veterinarians rely upon detection of FPV in feces using either ELISA or immunochromatographic technology. The ELISA tests marketed for detection of CPV-2 antigen can be used for detection of FPV antigen due to cross-reactivity between the two viruses. *FPV infection should never be ruled out on the basis of a negative fecal ELISA.* Reference laboratories offer PCR-based testing of whole blood or feces, facilitating the diagnosis of FPV in those cats that are ELISA negative. In-house parvovirus antigen tests may be positive up to 2 weeks after administration of modified live vaccines; therefore, in recently vaccinated cats, positive results do not necessarily equate with infection.[80]

Management

A cat diagnosed with FPV should be kept in isolation. Treatment is supportive and virtually identical to that described for the dog with parvovirus. Restoration of fluid, electrolyte, and acid-base balance with intravenous (IV) fluid and electrolyte therapy is indicated, with particular attention given to potassium repletion. The intraosseous route can be utilized in kittens, because the subcutaneous route is likely to be inadequate. Enteral administration of dextrose solution (2.5% to 5%) is recommended if the kitten is hypoglycemic. Parenteral administration of dextrose should be reserved for kittens with intractable vomiting. Plasma or colloids

(hetastarch) are indicated if the serum albumin concentration drops below 2.0 g/dL (20 g/L), and whole blood transfusions can be used if the cat is anemic with concurrent severe hypoalbuminemia.

The compromised intestinal mucosal barrier facilitates bacterial translocation, and the presence of bacteremia in combination with neutropenia may lead to sepsis in these immunocompromised patients. Prevention of sepsis is important and a broad-spectrum parenterally administered antibiotic with efficacy against gram-negative and anaerobic bacteria is recommended. Examples include ampicillin (20 mg/kg IV every 8 hours) or piperacillin in combination with aminoglycosides, fluoroquinolones (despite not being FDA-approved for parenteral administration in cats in the United States), or cephalosporins. Human granulocyte colony-stimulating factor at 5 µg/kg every 24 hours subcutaneously (SC) will increase neutrophil numbers, but may not influence outcome. Antiemetics such as prochlorperazine, metoclopramide, ondansetron, dolasetron, or maropitant are indicated if the kitten is vomiting. Maropitant is FDA approved for parenteral (SC) administration in kittens older than 16 weeks of age at a dose of 1 mg/kg administered once daily for up to 5 consecutive days. Use of refrigerated product may reduce the pain response associated with the injection. Metoclopramide is a less effective centrally acting antiemetic in cats compared to dogs because chemoreceptor trigger zone D_2-dopamine receptors may not be as important in mediating humoral emesis in the cat. In addition, the drug has an extremely short half-life (90 minutes in dogs) necessitating administration via a constant rate infusion at a dose of 1 mg/kg every 24 hours to maximize its efficacy.

Gastric protectants including the H2-receptor antagonists famotidine at 0.5 to 1 mg/kg PO every 12 to 24 hours (Pepcid, Alchemy Importers, Inc.); ranitidine at 1 to 2 mg/kg PO, IV, SC every 12 hours (Zantac, SmithKline Beecham); sucralfate at 0.25 to 0.3 g PO every 6 to 8 hours (Carafate, Nostrum Laboratories, Inc.); and proton pump inhibitors such as omeprazole at 0.7 to 1 mg/kg PO every 12 to 24 hours (Prilosec, AstraZeneca) are indicated if there is evidence of secondary esophagitis or gastrointestinal bleeding. Broad-spectrum anthelminthics to treat concurrent intestinal parasites should be administered when the cat is no longer vomiting. Oral intake of water and food should be restricted only if vomiting persists, and enteral feeding should be restarted as soon as possible. Beneficial effects of early enteral nutrition have been documented in dogs with CPV.[81] Cats with persistent vomiting, diarrhea, or anorexia will require parenteral nutrition, preferably via a central venous catheter in the jugular or the saphenous vein depending on the size of the cat.

Feline recombinant interferon-omega (Virbagen Omega, Virbac) is effective in the treatment of CPV and also inhibits replication of FPV in cell culture.[82] Interferon-omega was administered to cats in a cattery at the onset of an outbreak of FPV infection.[83] A dose of 1 MU/kg SC once daily for 3 days was given to some of the cats, whereas the remaining control cats were untreated. Although clinical signs and survival were similar for both cat groups, treated cats had lower levels of α-1 globulins and higher mean levels of γ-globulins. Following recovery and subsequent modified live virus vaccination, treated cats had higher levels of γ-globulin and anti-FPV–specific IgG as compared to untreated control cats.

In a disease outbreak, passive immunization can be used to protect susceptible young kittens with an incomplete vaccination history or unvaccinated adult cats. Homologous antisera from cats with a high titer to infection can be used to provide immunity. The recommended dose is 2 mL per kitten given SC or intraperitoneally. Because administered immunoglobulins persist for up to 2 to 4 weeks, the neonatal vaccination series must be delayed. Passive administration of antisera is recommended for use only in exposed susceptible (unvaccinated) cats that require immediate protection or in colostrum-deprived kittens.

Feline Enteric Coronavirus

Feline coronavirus is an enveloped single-stranded RNA virus that occurs as two pathotypes: feline enteric coronavirus (FECV), defined as the "ubiquitous enteric biotype," and feline infectious peritonitis virus (FIPV), the "virulent biotype" that causes FIP in individual cats.[84] Feline coronavirus is transmitted via the fecal-oral route and primarily infects enterocytes. Cats can become persistently infected and continuously or intermittently shed virus with the feces. They generally remain healthy despite systemic infection, indicating that healthy FECV carriers play a key role in the epidemiology of FIP. Feline enteric coronavirus is generally regarded as the avirulent pathotype of FCoV and in older cats oral FECV infection only leads to mild, nonspecific clinical signs such as transient anorexia. However, in young kittens after waning of maternal antibodies, oral FECV infection can induce severe enteritis. There have also been reports of fatal coronavirus enteritis in naturally infected juvenile and adult cats. Affected cats presented with catarrhal to hemorrhagic enteritis, and immunohistopathology confirmed that the virus infected the fully differentiated villous epithelial cells.[85] Infected cats can seroconvert and test positive on FCoV serological testing. Feline coronavirus is commonly detected in healthy and diarrheic cats with a prevalence ranging from 36% to 75%.[6,7] Interpretation of positive FCoV serological or PCR-based test results must be made cautiously because most cats that are infected with FECV have mild clinical signs, unless the animal is coinfected with other enteropathogens. There is no specific treatment for coronaviral enteritis in cats; treatment is symptomatic and supportive. Please refer to Table 1-1 for a summary of the parasitic, bacterial, and viral infections of kittens.

DIAGNOSTIC APPROACH TO THE KITTEN WITH SUSPECTED INFECTIOUS DIARRHEA

The widening array of recognized enteropathogens in kittens and the increasing demand for cost-containment in the face of a need for rapid turnaround of results increases the need for judicious implementation of fecal testing. Thorough

clinical and epidemiological evaluation must define the severity and type of illness (e.g., febrile, hemorrhagic diarrhea, nosocomial infection, inflammatory leukogram), exposure (travel history, ingestion of raw or undercooked meat products, contacts that are ill, recent antibiotic use), and determination of whether the animal or owner is immunocompromised to facilitate fecal testing and optimization of antimicrobial therapy.

A rational understanding of the indications and limitations of different fecal examination techniques is of paramount importance for optimizing the diagnosis of infectious diarrhea in the kitten. Specific fecal examination techniques for the diagnosis of intestinal parasites that should be considered in all diarrheic kittens include the fecal wet mount (direct smear) for motile protozoan trophozoites of *Giardia* spp. and *T. blagburni*; fecal flotation via centrifugation for parasitic oocysts, cysts, and ova; acid-fast staining of a fecal smear to assess for the presence of *Cryptosporidium* spp. oocysts; fecal ELISA for *Giardia* spp.; and fecal DFA for *Giardia* spp. and *Cryptosporidium* spp. Stained fecal smears using Wright-Giemsa or Diff-Quik to assess feces for endospores, Campylobacter-like organisms, and white blood cells are of limited diagnostic utility. A rectal scraping to evaluate the colorectal mucosa for inflammatory cells, neoplastic cells, or infectious agents can be performed in cats with clinical signs of colitis or dyschezia. Fecal culture for *T. blagburni* is somewhat time-consuming to perform and is less sensitive than the commercially available fecal PCR. A detailed overview of fecal flotation via centrifugation, fecal culture for enteropathogenic bacteria, fecal immunoassays, and fecal PCR is provided later. The author does not advocate fecal fat assessment with Sudan IV stain because the test is highly insensitive and nonspecific.

Fecal Flotation via Centrifugation

Fecal flotations are indicated to find cysts, oocysts, and ova in feces. Different flotation procedures have been described but not all provide optimal conditions for parasite identification. For example, the duration and speed of centrifugation together with the amount of time the coverslip sits on the tube after centrifugation are important.

Fresh feces should be examined whenever possible, or a fresh specimen can be refrigerated for up to 72 hours for detection of cysts, oocysts, or ova via a concentration technique. Fresh feces also can be placed in 10% buffered formalin if evaluation will be delayed more than 72 hours. Specimens fixed in formalin are suitable for concentration techniques, acid-fast stains, and immunoassays. Although standing (gravitational) flotation methods are easier and quicker to perform than centrifugation flotation (Figure 1-11), the latter clearly has superior sensitivity (up to eight times).[86] Animals with low parasite burdens in feces could have a false-negative result if the gravitational method is utilized. Fecal flotations have limitations and should not be used to detect heavy ova that do not float (*Paragonimus* spp.) or larvae (*Aelurostrongylus* spp.).

Figure 1-11: Centrifuge with free swinging buckets showing a coverslip in place before centrifugation.

The type of flotation solution used and its specific gravity are important considerations. The author recommends zinc sulfate with a specific gravity of 1.18 or 1.20 for flotations. This solution and specific gravity are optimal for flotation of ova and *Giardia* cysts, while the structural detail of the *Giardia* cyst is maintained.

Procedure for Centrifugal Flotation

1. A fecal emulsion is prepared with use of 2 to 5 g of feces and 5 to 10 mL of flotation solution.
2. The emulsion is strained through a tea strainer or cheesecloth with 10- to 15-mL flotation solution into a 15- to 20-mL conical centrifuge tube.
3. The tube is filled with flotation medium to create a positive meniscus.
4. A coverslip is placed on top of the tube.
5. The tube is balanced in the centrifuge.
6. The tubes are centrifuged for 5 minutes at 1200 rpm ($280 \times g$).
7. Remove the tube and let it stand with the coverslip for 10 minutes.
8. The coverslips are removed carefully from the tubes by lifting straight up; they are placed on a clean slide.
9. Systematically examine the entire area under the coverslip at 100 diameters (i.e., 10× magnification). The 40× objective lens can be used to confirm the diagnosis and make measurements.

Modification

With a centrifuge that is fixed-angle and does not have free-swinging buckets, the above procedure should be followed but the centrifuge tube is filled to within an inch or so from the top, and a coverslip is not added for the final spin. When the final centrifugation step is complete, the tube is set upright carefully in a test tube rack. A pipette is used to gently run additional flotation solution down the side of the tube while disturbing the contents as little as possible. A positive meniscus is created and a coverslip set on top. This preparation should be allowed to stand for 10 minutes only.

The coverslip is removed to a slide and examined as described in step 8.

Fecal Culture for Enteropathogenic Bacteria

The indications for performing fecal enteric panels on diarrheic dogs and cats are poorly defined, resulting in indiscriminate testing and misinterpretation of results. Fecal cultures for *C. difficile*, *C. perfringens*, *Campylobacter* spp., and *Salmonella* spp. can be time-consuming and insensitive. In addition, isolation of a putative bacterial enteropathogen does not denote causation. The author discourages the use of bacterial culture for isolation of *C. perfringens* and *C. difficile* in cats in the clinical setting, as both enteropathogens are infrequently associated with disease based upon detection of enterotoxins and toxins, respectively, the organisms are of dubious pathogenicity, and isolation can take up to 72 hours. Most clinicians prefer real-time PCR for detection of *Salmonella* spp. and *Campylobacter* spp. and for differentiation of pathogenic from nonpathogenic *Campylobacter* spp. Most regional veterinary reference laboratories are able to use molecular-based methods to differentiate *Campylobacter* spp.

Fecal Immunoassays for Parasitic, Viral, and Bacterial Enteropathogens

A DFA has been validated for concurrent detection of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts in dog and cat feces. This assay requires a fluorescent microscope and is available at commercial reference laboratories or universities. A variety of highly sensitive and specific CPV antigen tests are commercially available for the detection of FPV; however, antigen shedding can be intermittent thus limiting the sensitivity of the test as a screening tool. Animal shelter veterinarians should select fecal tests for FPV detection that have high sensitivity for FPV and low frequency of vaccine-related test interference. The SNAP Parvo test (IDEXX Laboratories, Westbook, Maine) had the lowest incidence of positive results in sixty-four 8- to 10-week-old specific-pathogen-free kittens inoculated with a modified-live (MLV) or inactivated FPV vaccine. The AGEN CPV (AGEN Biomedical Ltd, Brisbane, Queensland, Australia) and in particular the WITNESS CPV (Synbiotics Corp, San Diego, California) have a much higher frequency of vaccine-related interference.[74] Detection of FeLV antigen is warranted in kittens failing to respond to conventional therapy, although detection of the antigen denotes exposure of the kitten to the virus, but does not prove that clinical disease is due to the virus. The *Giardia* ELISA test has been validated in both dogs and cats, and is an excellent in-house immunoassay that should be used in conjunction with fecal flotation and wet mounts to increase the diagnostic yield for *Giardia* spp. Commercially available enterotoxin and toxin ELISAs are available for diagnosis of *C. perfringens* and *C. difficile* infections; however, none of the immunoassays have been validated in cats or dogs to date, and caution should be heeded in interpreting these results as these organisms are of dubious pathogenicity.

Polymerase Chain Reaction for Parasitic, Viral, and Bacterial Enteropathogens

Diagnosis of *Giardia* spp. infection is generally made with the combination of fecal flotation technique, wet mount, and fecal antigen tests (ELISA or DFA). Fecal PCR assays for *Giardia* can have false-negative results because of PCR inhibitors in feces, and PCR should not be used as a screening procedure for this organism. Large commercial reference laboratories that routinely perform PCR have incorporated a number of controls to ensure quality at each step in the process: quantitative DNA/RNA controls to assess the quality of each clinical sample; extraction controls for every DNA/RNA extraction cycle to ensure the absence of contamination; and internal positive and negative controls to verify each real-time PCR test for optimal performance and the absence of contamination. The primary indication for *Giardia* spp. PCR is for determining whether the infective species is a zoonotic assemblage. The latter assay can be performed at the Veterinary Diagnostic Laboratory, Colorado State University (http://dLab.colostate.edu/). This PCR test is different from the RealPCR Feline Diarrhea Panel (IDEXX Laboratories, Westbrook, Maine) or the FastPanel PCR Feline GI Profile Panel (Antech Diagnostics, Irvine, California) performed at commercial reference laboratories. PCR can be used to diagnose *Cryptosporidium* spp. in kittens; however, the author prefers using a DFA test that allows direct visualization of oocysts under a fluorescent microscope. Detection of *C. felis* and *C. canis* do not always prove that the agent is the cause of the clinical disease. Fecal PCR testing is recommended for the diagnosis of *T. blagburni* infection in cats; however, DNA of *T. blagburni* can be detected in healthy carrier cats and so positive results must be interpreted in the context of the animal's history, physical examination findings, and environment. Polymerase chain reaction is a sensitive method for detecting DNA of *Salmonella* spp. and *Campylobacter* spp., but positive results do not inherently necessitate antimicrobial therapy as discussed previously. In cats, the positive predictive value of *Clostridium* spp. PCR assays on feces is low, and should best be combined with toxin immunoassays to increase the diagnostic yield. Reverse transcriptase–PCR is used to detect coronavirus RNA in feces; however, positive test results do not differentiate FIP-inducing strains from FECV, and the prevalence of coronavirus in healthy, nondiarrheic cats is high.

It is incumbent upon the clinician to be aware of the limitations and benefits of each of the fecal diagnostic tests, and to recognize that the mere detection of DNA from a putative enteropathogen or the detection of *Giardia* spp. cysts or *Cryptosporidium* spp. oocysts in a fecal specimen do not denote a cause-and-effect phenomenon. It should be recognized that a kitten demonstrating signs of colitis (tenesmus, hematochezia, increased fecal mucus, scant fecal volume with a marked increase in frequency) with evidence of *Giardia* spp. on fecal flotation or ELISA has another cause for the colitis signs, because *Giardia* is a small bowel pathogen. Further investigation for known causes of colitis in kittens (e.g.,

T. blagburni, *Cystoisospora* spp., *Campylobacter* spp., *C. perfringens*, *C. difficile*, food intolerance) should be undertaken.

EMPIRICAL THERAPY FOR KITTENS WITH DIARRHEA OF UNKNOWN CAUSE

The most common causes of diarrhea in neonatal and juvenile kittens are the rapid introduction of milk replacer or rapid transition from formula to commercial diets (weaning period) and infectious causes of diarrhea, specifically parasites (e.g., *Cystoisospora* spp., *Giardia* spp.) and viral enteropathogens (e.g, FCoV). The stress of changing the kitten's environment can exacerbate the diarrhea. The author deworms kittens with simple diarrhea routinely using a broad-spectrum anthelminthic (e.g., fenbendazole) even in the face of a negative fecal flotation or negative *Giardia* ELISA. Metronidazole administration at 10 to 15 mg/kg PO every 12 hours for 5 to 7 days often is associated with partial to complete amelioration of diarrhea, possibly because of altering the intestinal microbiota, dampening cell-mediated immunity, or activity against a specific pathogen such as *C. difficile* or *C. perfringens*. Dietary modification should be considered in kittens that fail to respond to empirical antiparasitic therapy and metronidazole administration. One can temporarily dilute the milk replacer with an oral electrolyte solution such as Pedialyte (Abbott Laboratories, Abbott Park, Illinois) to facilitate acclimation to the formula. One can also feed a highly digestible therapeutic intestinal diet for kittens that have been weaned, and there is compelling evidence documenting the benefits of canned therapeutic diets for the management of adult cats with naturally occurring chronic diarrhea.[87]

Dietary fat restriction does not appear to be of benefit in adult cats with chronic diarrhea, according to a study that compared the effects of a high-fat (45.1% of calories from fat) versus a low-fat (23.8% of the calories from fat), highly digestible diet.[88] Caution should be heeded in extrapolating the results of these studies in adult cats to kittens, because similar studies have not been undertaken to date. Kittens that fail to improve on a commercial diet can be fed a cooked turkey or chicken diet (without carbohydrates) for 5 to 10 days to provide a highly digestible meal containing moderate amounts of fat. Home-cooked diets are not complete and balanced, and should not be fed to kittens for more than 10 days. Probiotics containing *Enterococcus faecium*, *Lactobacillus* spp., or *Bifidobacterium bifidum* can be used in kittens with simple diarrhea, and several studies have shown benefit for the use of these nutraceuticals.[89] Kittens that have failed to respond adequately to administration of fenbendazole, metronidazole, and dietary therapy are given a 3-day course of ponazuril at 50 mg/kg PO. The author has observed many diarrheic neonatal kittens diagnosed with *Cystoisospora* spp. on fecal flotation at 6 weeks of age that had negative fecal flotations at 2 to 3 weeks of age, due to the long incubation period, and intermittent shedding of the parasite is also well documented. Ronidazole is only administered for the treatment of *T. blagburni* infection.

Kittens with complicated diarrhea characterized by worsening of clinical signs in the face of hematochezia or melena should undergo fecal testing (PCR or ELISA) for FPV and PCR testing for enteropathogenic bacteria, in particular *C. jejuni* and *Salmonella* spp. A complete blood cell count should be done, and the kitten should undergo serological screening for FeLV and feline immunodeficiency virus if this has not been done before. Metronidazole administered for 5 to 7 days should effectively treat *C. perfringens* and *C. difficile*, and kittens infected with *C. jejuni* and showing evidence of systemic clinical signs should be managed with a macrolide antibiotic such as azithromycin at 7 to 10 mg/kg PO every 12 hours for 10 days. Fecal PCR testing can also detect DNA of *C. felis*, and kittens infected with this protozoan can be treated with azithromycin at the same dose.

Inflammatory bowel disease primarily is a disease of middle-aged to older cats, and kittens are more likely to have diarrhea resulting from an infectious cause. The author discourages the administration of prednisolone to diarrheic kittens unless a comprehensive work-up, including intestinal biopsies, warrants this therapy. Kittens with chronic ileitis could have secondary deficiencies of vitamin B_{12} (cobalamin), an important micronutrient for DNA replication in the intestinal crypts. Vitamin B_{12} can be administered empirically to kittens at 100 µg per kitten, given SC once weekly for 6 weeks. Repeat injections should be based on determination of serum cobalamin concentrations. Cobalamin is safe, easy to administer, and inexpensive.

CONCLUSION

Comprehensive fecal exams are important in the diagnostic evaluation of kittens with diarrhea. The diagnostic yield will be markedly increased with the examination of fresh fecal specimens, the use of a centrifugation technique with zinc sulfate solution, and the timely incorporation of immunoassays for diagnosing *Giardia* and *Cryptosporidium* spp. Diagnosis of *T. blagburni* is enhanced with the utilization of PCR, although InPouch culture kits facilitate the growth and direct visualization of motile trophozoites. The author recommends using PCR over InPouch cultures because of the PCR test's increased sensitivity over culture and rapid turnaround.

The clinical documentation of enteropathogenic bacteria that cause diarrhea in kittens is clouded by the presence of many of these organisms existing as normal constituents of the indigenous intestinal microbiota. Attributing disease to a putative bacterial enteropathogen(s) in kittens should be made only after considering the animal's signalment, predisposing factors, clinical signs, fecal assays for toxins, fecal culture, and/or PCR. Relying on results of fecal culture alone is discouraged, because *C. perfringens*, *C. difficile*, *Campylobacter* spp., and pathogenic and nonpathogenic *E. coli* are commonly isolated from apparently healthy kittens.

Accurate diagnosis of infections may require diagnostic laboratories to incorporate PCR-based assays using genus- and species-specific primers to facilitate detection of toxin

genes and differentiation of species that appear phenotypically and biochemically similar. In assessment of a diarrheic kitten not responding to therapy and for which a diagnosis has not been made, repeating previously negative diagnostic tests frequently is more helpful than performing endoscopy and biopsy.

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