Suspected *Clostridium difficile*-associated diarrhea in two cats

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- *Clostridium difficile* is an anaerobic bacterium that has been associated with diarrheic disease in humans and several other species.
- Diagnosis of *C difficile*-associated disease is dependent on detection of bacterial toxins in fecal samples.
- Metronidazole is often an effective treatment for *C difficile*-associated diseases.

A 6.1-kg (13.4-lb) 9-year-old castrated male Siamese (cat 1) was referred to Burnhamthorpe Animal Hospital for evaluation of diarrhea of 48 hours' duration. Three other mature cats were present in the household; problems with these cats were not reported. All cats lived indoors, and new animals had not been introduced into the house within 18 months. All cats had been vaccinated against viral rhinotracheitis, calicivirus infection, panleukopenia, FeLV infection, and rabies within 1 year but had not been treated with an anthelmintic during this period. Canned cat food of the same brand that was usually fed but with a different flavor had been fed 24 hours prior to the onset of diarrhea. Vomiting had not been observed. At the time of referral, the cat was bright, alert, and in good body condition. Rectal temperature, heart rate, and respiratory rate were within reference ranges. Watery diarrhea with mucus was the only abnormality detected by physical examination. A CBC was performed, and mild neutrophilia (19,200 cells/µl; reference range, 5,500 to 15,400 cells/µl) with a left shift (band neutrophils, 1,800 cells/µl; reference range, 0 to 300 cells/µl) and mild lymphopenia (1,300 cells/µl; reference range, 1,500 to 7,000 cells/µl) were detected. Packed cell volume (47%; reference range, 24 to 45%) and total plasma protein concentration (8.2 g/dl; reference range, 6.0 to 8.0 g/dl) were slightly greater than reference ranges, which is consistent with mild dehydration. There were no other important serum biochemical abnormalities. Thyroxine concentration (2.4 µg/dl) was within reference range (1.0 to 4.0 µg/dl). Empirical treatment with metronidazole (8 mg/kg [3.6 mg/lb] of body weight, PO, q 12 h for 7 days) was prescribed, and the cat was discharged with instructions to feed small amounts of a recuperative diet[a] during the next 48 hours.

The cat was returned for reevaluation 2 days later. The owner reported that the cat had been resistant to drug administration, and only 1 dose of metronidazole had been successfully administered. The owner reported an initial improvement in the cat's appetite and attitude; however, it then developed progressive signs of depression and anorexia. Brown watery diarrheal fluid without any evidence of mucus or blood was passed. Because the cat was approximately 7% dehydrated, IV administration of a balanced electrolyte replacement solution supplemented with 20 mEq of potassium chloride per liter was started. Broad-spectrum antimicrobial treatment with trimethoprim-sulfadiazone (28 mg/kg [12.7 mg/lb], SC, q 24 h) was prescribed because of the neutrophilia, left shift, and worsening clinical signs. Metronidazole treatment was continued but was administered IV (7.5 mg/kg [3.4 mg/lb], q 12 h). A selection of food was offered, but the cat did not eat. The following morning (day 4), diarrhea was still evident, but frequency of defecation was decreased. Appetite continued to be poor. A fecal sample was collected for zinc sulfate floatation and saline (0.9% NaCl) solution wet mount; gastrointestinal parasites were not detected. Results of selective microbiologic culture of feces for *Salmonella* spp were negative. Nonselective aerobic and anaerobic culture did not yield recognized pathogens. The fecal sample was also tested by use of ELISA for *Clostridium difficile* toxins A and B[b] and *C perfringens* enterotoxin[c]; results were positive for *C difficile* toxins. Results of selective culture for *C difficile* by use of cycloserine-cefoxitin fructose agar were negative. Because fecal toxin detection is considered diagnostic for ***C difficile*-associated disease (CDAD)** in other species,[1] a tentative diagnosis of CDAD was made. Because metronidazole is considered the first-line treatment for CDAD in humans[2,3] and anecdotally appears to be effective in horses, the treatment regimen was not changed. Feces were loose but formed the following morning, and the cat's appetite had improved. Intravenous administration of fluid was discontinued, and the cat was able to maintain normal hydration. Oral metronidazole administration was resumed, and the cat was discharged with instructions to continue metronidazole treatment until feces remained normal for 48 hours. The cat responded to treatment, and normal feces were passed within 7 days. At follow-up 6 months after discharge, the cat was clinically normal.

Three days after initial referral of cat 1, a 5.1-kg (11.2-lb) 12-year-old spayed female domestic shorthair from the same household (cat 2) was referred for evaluation of diarrhea and lethargy of 24 hours' duration. At time of referral, the cat was inactive but responsive. Rectal temperature (39.6 C [103.3 F]) was slightly

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greater than reference range. Other abnormalities were not detected, and diagnostic tests were not performed. Empirical treatment with metronidazole (8 mg/kg, PO, q 12 h for 5 days) was prescribed, and the cat was discharged. The cat's clinical condition deteriorated during the next 12 hours, and it was taken to an emergency clinic that evening because of substantial lethargy. The cat was pyrexic (rectal temperature, 40.5 C [104.9 F]) and 5 to 8% dehydrated. Heart rate was within reference range, but respiratory rate was high (80 breaths/min). Signs of abdominal pain associated with palpation, particularly in the midcaudal region of the abdomen, were reported. A CBC was performed, and abnormalities were not detected. An IV catheter was placed, and treatment with a balanced electrolyte solution (7 ml/kg [3.2 mg/lb]/h, IV), enrofloxacin (5 mg/kg [2.3 mg/lb], IM), sodium ampicillin (20 mg/kg [9.1 mg/lb], IV), and dipyrone (25 mg/kg [11.4 mg/lb], IM) was initiated. Watery diarrheal fluid was passed overnight, and the cat vomited once. Prochlorperazine mesylate (0.13 mg/kg [0.06 mg/lb], IV) was administered as an antiemetic. The cat was returned to the primary care clinic for reevaluation the next morning; hydration and physical examination findings (other than the diarrhea) were considered normal. The cat appeared to resent abdominal palpation. Intravenous administration of fluids (6 ml/kg/h) was continued. Antibiotic treatment initiated at the emergency clinic was discontinued, and treatment with metronidazole (9 mg/kg [4.1 mg/lb], IV, q 12 h) was resumed. Food was offered but not consumed. Examination of a fecal sample yielded negative results for gastrointestinal parasites. Selective bacteriologic cultures for *Salmonella* spp and *C difficile* were performed, and no pathogens were isolated; however, a delay of 24 to 48 hours from sample collection to submission was encountered. Positive results were obtained for *C difficile* toxins A and B, and negative results were obtained for *C perfringens* enterotoxin. Intravenous fluid administration and metronidazole treatment were continued. Appetite, attitude, and fecal consistency improved during the next 2 days, at which time IV administration of fluid was discontinued. Oral administration of metronidazole (10 mg/kg, q 24 h for 10 days) was resumed, and the cat was discharged. Normal feces were passed within 7 days, and diarrhea did not recur. At follow-up 6 months after discharge, the cat was clinically normal.

A fecal sample was collected from cat 2 approximately 1 month after resolution of clinical signs of disease. A fecal sample was also collected from 1 of the other 2 cats in the household that had normal feces. Both samples were processed within 24 hours of collection, and neither *C difficile* nor *C difficile* toxins were detected.

Diarrhea is a commonly encountered problem in small animal veterinary practice and was the reason for referral in 1.8% of cats referred to primary care veterinary clinics.[4] Recognized causes of enterocolitis in cats are numerous and include infectious, neoplastic, allergic, inflammatory, and nutritional diseases. As in other species, a causative agent is not identified in a substantial percentage of cats with enterocolitis. *Clostridium difficile* is an anaerobic gram-positive spore-forming bacterium that has been associated with enteric disease in several species. It is the most common cause of nosocomial and antibiotic-induced diarrhea in humans.[5-7] *Clostridium difficile* has been recognized as an important cause of sporadic and antibiotic-induced enterocolitis in various animals, including horses,[8-10] pigs,[11,12] laboratory mammals,[13,14] prairie dogs,[15] a Kodiak bear,[16] and a penguin.[17] Administration of almost any antimicrobial may be associated with CDAD, although clindamycin, cephalosporins, and penicillins are most commonly implicated in humans.[18-20] *Clostridium difficile* is believed to cause disease after colonization of the gastrointestinal tract with toxigenic strains, proliferation of the organism, and production of toxins. *Clostridium difficile* produces at least 5 toxins, although only toxin A and toxin B have been thoroughly studied.[21] Toxin A is a potent enterotoxin that also possesses cytotoxic properties and causes fluid accumulation in animal intestinal models.[22,23] Toxin B possesses up to 1,000 times the cytotoxicity of toxin A but has no detectable effect on intestinal permeability, neutrophil migration, or intestinal morphologic features.[23-25] These toxins may act individually or synergistically to induce characteristic signs of disease. The other 3 toxins include an unstable enterotoxin, a high molecular weight protein, and an actin-specific ADP-ribosyl-transferase[21]; the roles of these toxins are not understood. Not all strains of *C difficile* possess the genes that encode for toxin production, and such strains are not considered to be clinically relevant.[21,26]

Clostridium difficile may be isolated from the feces of 2 to 5% of clinically normal humans[27,28]; asymptomatic carrier rates increase dramatically after antimicrobial treatment and hospitalization.[29] Detection of nontoxigenic strains and asymptomatic carriers of toxigenic strains indicates that bacteriologic culture is not adequate to determine the role of this potential pathogen in animals with diarrhea.[6,29,30] As a result, toxin detection in feces is the standard for clinical diagnosis of this condition.[6,20,31] The cell cytotoxicity assay, which detects the cytopathic effects of toxin B in feces, is considered to be the gold standard; however, this test is expensive, time-consuming, technically demanding, and of limited availability.[31] Fortunately, a variety of rapid, cost-effective, and easy-to-use ELISA are available for the detection of toxins A and B. Because the ELISA used for the cats reported here has not been validated for use in feline feces, test results should be interpreted with caution. Although this ELISA has good sensitivity and specificity in human feces[31] and has been used clinically in horses,[32] it should be validated in feline feces to ensure that there are no species-specific differences that could interfere with test results.

Although antimicrobial administration is the most recognized risk factor for CDAD, hospitalization, administration of enemas and stool softeners, use of indwelling nasogastric tubes, and administration of antacids are also considered risk factors in humans.[20,33,34] Risk factors have not been studied extensively in nonhuman species, although antimicrobial administration has been associated with CDAD in

horses.[35] It is unclear whether factors that put humans at risk for development of CDAD apply to cats; no such factors were identified in the cats of this report.

The role of *C difficile* in enteric disease in cats has not been thoroughly investigated. *Clostridium difficile* has been isolated from the feces of 2 to 30% of cats with normal feces.[36-38] Madewell et al[39] reported the isolation of *C difficile* from 23 of 245 hospitalized cats with normal feces, although carriers were not identified among 56 clinically normal outpatient cats or 49 cats housed in a research facility. Toxigenic strains of *C difficile* were isolated from 8 cats in that study; all of these cats had 1 or more recognized risk factors, and 4 had diarrhea.[39] High incidence of the carrier state in hospitalized animals is not surprising, because the bacterium may be isolated from veterinary hospital environments.[36,39,40] Studies based on toxin detection have not been reported in cats; therefore, the role of *C difficile* in enteric diseases of cats is poorly understood.

The association between *C difficile* and enteric disease in the cats reported here was based on detection of *C difficile* toxins in feces. Isolation of *C difficile* would have been desirable, but negative culture results do not exclude this organism as the causative agent. *Clostridium difficile* has poor tolerance for aerobic conditions, and recovery of *C difficile* from equine feces stored under aerobic conditions declines dramatically during the first 24 to 72 hours of storage.[41] For samples obtained from both cats reported here, delays of 24 to 48 hours from sample collection to processing occurred, which may have resulted in false-negative culture results. In addition, treatment with metronidazole was instituted prior to collection of fecal samples for bacteriologic culture. Metronidazole treatment may have eliminated the active infection or inhibited growth of *C difficile* in vitro but not have affected toxins that were already in the gastrointestinal tract.

Metronidazole is the drug of choice for treatment of CDAD in humans[2,3] and is used with anecdotal success in horses. Vancomycin is equally effective in humans[42] but is usually reserved for severe or recurrent cases because of cost and concerns regarding development of vancomycin-resistant enterococci.[43] Metronidazole resistance is uncommon in *C difficile* isolates from humans and other species but has occasionally been reported.[44,45] There is limited information regarding efficacy of metronidazole in treatment of CDAD in cats. Madewell et al[39] reported that 4 of 4 diarrheic cats with toxigenic strains of *C difficile* in feces responded to metronidazole treatment, whereas 1 other metronidazole-treated cat developed chronic intermittent diarrhea. Metronidazole appeared to be effective in the cats reported here, but it cannot be proven that the clinical signs were not self-limiting.

The origin of *C difficile* infection in these cats was unclear. One or more of the cats may have been carriers, with *C difficile* proliferating secondary to a disruption of the normal protective gastrointestinal microflora. It is possible that the dietary change was a predisposing factor for colonization or proliferation of *C difficile*. Judged on the basis of the time frame of the 2 cases, it is possible that cat 2 was infected by contact with cat 1, but this cannot be proven.

In our experience, metronidazole-responsive diarrhea is commonly encountered in companion animals. Although it cannot be proven that such diarrhea in these animals actually resolves in response to metronidazole administration, it is possible that diarrhea in some of these animals is associated with *C difficile* infection. The origin of infection, risk factors, and possibility of interspecies transmission are all unknown. The potential for zoonotic transmission is particularly concerning in situations in which domestic animals are in contact with high-risk individuals such as those undergoing antimicrobial administration or those in chronic-care facilities.

[a]Hill's Prescription Diet A/D, Hill's Pet Nutrition Inc, Topeka, Kan.
[b]*Clostridium difficile* TOX A/B Test, TechLab Inc, Blacksburg, Va.
[c]*Clostridium perfringens* enterotoxin ELISA, TechLab Inc, Blacksburg, Va.

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