Outbreaks of severe enteric disease associated with *Eimeria furonis* infection in ferrets (*Mustela putorius furo*) of 3 densely populated groups

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**Case Description**—3 unrelated, densely populated, dynamic ferret populations with severe outbreaks of enteric coccidiosis were evaluated.

**Clinical Findings**—In each outbreak, morbidity rate was high, there were an appreciable number of deaths, and ferrets of all ages were affected. Affected individuals had acute onset of diarrhea, and feces often contained frank or digested blood. Other clinical signs included dehydration, weakness, lethargy, and weight loss. Fecal examinations of affected ferrets revealed sporadic and inconsistent shedding of coccidial oocysts. Necropsy findings included moderate to marked atrophic enteritis associated with numerous intraepithelial and fewer extracellular coccidial life stages. Sporulated oocysts isolated from feces were consistent with *Eimeria furonis*. A PCR assay was performed on formalin-fixed, paraffin-embedded sections of intestine for the gene encoding the small subunit of rRNA yielding products with sequences identical to those described for *E furonis*.

**Treatment and Outcome**—Supportive care and treatment with sulfadimethoxine over the course of these outbreaks was palliative, but long-term treatment was required and failed to completely eradicate infection as identified by the subsequent finding of oocysts in fecal samples.

**Clinical Relevance**—Enteric coccidiosis due to infection with *E furonis* has typically been reported to be subclinical rather than to cause severe gastrointestinal disease in ferrets. This report indicated that infection with *E furonis* may have contributed to severe enteric disease with high morbidity and mortality rates in 3 densely populated, dynamic groups of ferrets. Furthermore, long-term treatment with anti-coccidials may be required in outbreak situations, but may be ineffectual in completely eradicating infection. (J Am Vet Med Assoc 2011;239:1584-1588)

From June 2005 until December 2009, 3 outbreaks of severe diarrhea with substantial mortality rates were identified in 3 high-density populations of ferrets that were dynamic groups in that new ferrets were regularly introduced. All 3 outbreaks had similar rates of morbidity and mortality, similar clinical signs of enteric disease in affected ferrets, and a similar overall course of disease. Ages of affected ferrets in each group ranged from < 1 year to > 6 years, with ferrets of all ages being affected similarly. There was no apparent sex predilection as sexually intact and neutered jills and hobs were equally affected. All ferrets in each group had been previously vaccinated against canine distemper virus and rabies virus. In each of the 3 groups, chronic underlying diseases such as hyperadrenocorticism or insulinemia presumably due to insulin-producing pancreatic islet tumors affected small numbers of ferrets; however, such ferrets were not disproportionately affected.

The first outbreak began in June 2005 and involved a ferret rescue group (group 1) in the Detroit area. At the time of the outbreak, there were 42 ferrets at the shelter that ranged from < 1 year to > 5 years of age. Over the course of the outbreak, greater than half of the ferrets at the shelter were clinically affected and 7 died. Of the 7 ferrets that died during this outbreak, 4 were submitted for a complete necropsy to the Diagnostic Center for Population and Animal Health at Michigan State University. Most clinical affected ferrets were identified in the first 2 to 3 weeks of the outbreak. Occasionally, individual ferrets had coccidial oocysts identified on fecal examination or developed diarrhea sporadically over the next 4 months despite attempts to quarantine affected ferrets and thorough environmental cleanings. No additional affected ferrets were identified at this shelter from the end of October 2005 until June 2008, when there was a similar but more isolated outbreak of bloody diarrhea. In the June 2008 outbreak, the first ferret affected was a 1-year-old female with several broken ribs that had been introduced to the shelter 2 weeks earlier. Within the next 2 weeks, 5 cage mates developed diarrhea. At that time, the 35 other ferrets at the facility had no clinical signs of enteric disease.
The second affected group (group 2) was composed of 63 ferrets from a private ferret breeder and shelter in western Pennsylvania. Most of the ferrets at this site included ferrets used for breeding; however, introduction of rescued ferrets was common practice. Ferrets were separated into 3 groups, with each group being confined to 1 of 3 floors of the shelter. Ferrets on any given floor had no contact with ferrets in the other groups and did not share bowls, bedding, or other supplies with ferrets on other floors. Between November and December of 2008, 13 ferrets died and at least another 21 had clinical signs of enteric disease but recovered. A gross necropsy was performed on all 13 ferrets that died. Histologic examination of tissue specimens was performed for 3 of these ferrets.

In this second outbreak, the initial ferrets that were affected were confined to the first floor and newly affected ferrets were identified on this floor over a 3-week period. One week following the last clinically affected ferret identified on the first floor, several ferrets on the second floor developed diarrhea. Newly affected ferrets on the second floor were identified for 2.5 weeks. A week after the last ferret was affected on the second floor, several ferrets on the third floor and a single ferret on the first floor developed diarrhea. New affected ferrets on the third floor were identified over the next 2.5 weeks.

The third affected group (group 3) was from a shelter in eastern Pennsylvania that housed 62 ferrets at the onset of the outbreak. This facility had opened in 2005 and, since opening, had seen sporadic instances of ferrets with diarrhea attributed to inflammatory bowel disease. From October through mid-December of 2009, an outbreak of severe diarrhea occurred. Over this time, at least 29 ferrets had clinical signs of enteric disease and 4 died. Similar to the initial outbreak in group 1, most of the affected ferrets were identified in the first 3 weeks of the outbreak. Two of the 4 ferrets that died were necropsied by the referring veterinarian, and histologic examination of collected tissue specimens was performed at the Diagnostic Center for Population and Animal Health. An additional ferret that died was submitted whole to the Diagnostic Center for Population and Animal Health for necropsy.

Clinical signs of enteric disease were similar for all 3 groups. The most prominent abnormality was diarrhea. Diarrheic feces were foul smelling and ranged from beige and pasty or gelatinous to dark black and tarry, consistent with melena. Occasionally, ferrets had sunken, dull eyes, tenting of the skin, dry mucous membranes, and capillary refill times of 2 to 3 seconds. In addition to gastrointestinal signs, affected ferrets rarely had signs of respiratory disease, including sneezing, coughing, ocular and nasal serous to mucoid discharge, and reddening of the conjunctival membranes. Clinical signs of illness in an individual ferret generally persisted for 5 to 10 days prior to recovery or progressively worsened over a similar time frame leading to death. Few ferrets were found dead with few to no previously observed clinical abnormalities.

Complete blood counts and serum biochemical analyses were performed on multiple ferrets from each of the groups at various points during the outbreaks. Abnormalities were consistent with dehydration and regenerative anemia and included moderately high BUN concentration with serum creatinine concentration within reference range, serum protein concentration in the high or upper end of reference range, mild reticulocytosis, and mild to moderate thrombocytopenia.

Fecal samples of affected ferrets from all groups were examined for parasites by a direct smear technique and fecal flotation testing by referring veterinarians. In group 1 ferrets, few coccidial oocysts were observed sporadically in fecal samples pooled from diarrhetic feces. Identification of oocysts, however, was inconsistent and often took multiple attempts. In group 2 and 3 ferrets, coccidian oocysts were not observed in fecal samples.

Gross necropsies were performed on 20 ferrets that died during the 3 outbreaks. Histologic examination was performed on formalin-fixed tissue specimens, which variably included sections of heart, liver, lung, spleen, adrenal glands, pancreas, multiple sections of intestine, and brain, from 10 of these ferrets. Gross and histopathologic lesions were similar in all ferrets. On necropsy, affected ferrets were thin and moderately to markedly dehydrated. The perineal regions were variably stained by diarrhetic feces. The small and large intestines were moderately dilated and thin walled. Intestinal lumens of the distal aspect of the small intestine and large intestine contained moderate amounts of pasty tan to dark black and tarry digesta. There were, however, no appreciable changes in the mucosa of the intestines by gross examination. Several ferrets had few (1 to 3) small superficial gastric or duodenal ulcers and variable amounts of blood within the stomach or proximal aspect of the small intestine. Additionally, the spleens of at least 5 ferrets were moderately enlarged and dark red, and the livers of a few ferrets were slightly enlarged and pale tan.

On histologic examination, lesions were most consistently seen in the intestines. There was moderate to severe blunting and occasional fusion of intestinal villi in the jejunum and ileum (Figure 1). The superficial mucosal epithelium overlying villus tips was locally attenuated and eroded. In the most severe areas of erosion, there was exudation of fibrin, neutrophils, and blood into the intestinal lumen. The intact superficial mucosal epithelial cells of the villus tips and occasional sloughed epithelial cells in the intestinal lumen contained numerous intracytoplasmic coccidia representing multiple life stages, including meronts, macrogametocytes, microgametocytes, and oocysts (Figure 2). The lamina propria of the associated small intestine and, to a lesser degree, the large intestine was moderately infiltrated by lymphocytes, plasma cells, and occasional neutrophils adjacent to areas of epithelial erosion. Blood vessels of the lamina propria were often congested. In the most severely affected areas, there was marked hemorrhage into the mucosa. Other histopathologic changes seen less consistently in affected ferrets included congestion and moderate to marked extramedullary hematopoiesis in the spleen, mild to moderate hepatic lipodosis, and superficial to moderate erosions in the stomach.

Speciation of coccidia was done on the basis of morphology of sporulated oocysts (feces from group 1 of the third floor were identified over the next 2.5 weeks.
ferrets only) and by PCR amplification of a fragment of the gene encoding the SSUrRNA of *Eimeria* spp (formalin-fixed tissue specimens from ferrets in groups 1, 2, and 3). Sporulation of oocysts was accomplished by incubation of feces at 24°C for 2 to 5 days. The sporulated oocysts were spherical, measured approximately 12 × 13 μm in diameter, and contained 8 sporozoites/sporocyst. Such morphological features are consistent with previous reports of *Eimeria furonis*.

Polymerase chain reaction assays were performed on formalin-fixed, paraffin-embedded intestinal specimens from each group of ferrets that had lesions and contained several life stages of coccidia. The protocol for purification of total DNA from animal tissues described previously by Bolin et al. was followed with minor modifications. Briefly, a 20-μm-thick section of paraffin-embedded tissue was placed in a 1.5-ml microcentrifuge tube, and 200 μl of digestion buffer (10mM Tris-HCl, 50mM KCl, 0.5% Tween 20, and 200 μg of proteinase K/mL; pH, 8.3) was added to the tube. The tube was incubated at 42°C until the paraffin melted and the embedded tissues were lysed. The digested tissue lysate was centrifuged briefly and incubated at 100°C for 10 minutes, chilled on ice for 5 minutes, and centrifuged at 18,000 × g for 3 minutes. Clarified supernatant was aspirated from under the melted paraffin for PCR assay. The PCR primers used were designed from a reported sequence of the gene encoding SSUrRNA of *E furonis* (GenBank accession No. AB329724). The PCR primers were ACA ATT GGA GGG CAA GTC TG and GGC GAC AAG CCT GCT TGA AAC, which produce an amplicon of 247 base pairs. The PCR reaction mixture consisted of 2 μL of DNA, 12.5 μL of a commercial PCR mixture, 9.7 μL of molecular biological analysis–grade water, and 0.4 μL (25 pmol/μL) of each PCR primer. The reaction conditions were 95°C for 5 minutes; 40 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds; and then a final extension step of 72°C for 5 minutes. The 247-bp PCR products were visualized by ethidium bromide staining after electrophoresis through a 1.5% agarose gel. The PCR amplicons were cut from gels, purified with the use of a gel-extraction kit, and submitted to the Research Technology Support Facility at Michigan State University for nucleic acid sequencing. Sequences were edited by use of sequencing software, trimmed to remove primer sites, and analyzed by use of a bioinformatics search tool. The PCR amplicons from each group of ferrets were 100% identical to nucleic acid sequences from the gene encoding SSUrRNA of *E furonis*.

Tissue specimens from each group of ferrets were additionally tested for other pathogens. A PCR assay for the coronavirus associated with epizootic catarrhal enteritis in ferrets and for rotavirus was performed on formalin-fixed, paraffin-embedded intestinal specimens from ferrets of each of the 3 outbreaks by use of previously described methods. Immunohistochemistry testing for ferret enteric coronavirus was performed on formalin-fixed, paraffin-embedded intestinal specimens from affected ferrets of groups 1 and 2 as previously described. A PCR assay and immunohistochemistry testing for influenza virus were performed on formalin-
fixed, paraffin-embedded intestinal specimens from affected ferrets of group 2 as previously described. Test results for viruses were negative in all instances. In addition, bacterial cultures of sections of intestine obtained during necropsy from ferrets in group 1 and of fecal samples obtained during the outbreak from ferrets in group 2 failed to yield pathogenic organisms.

Identification of affected ferrets for treatment during the course of all 3 outbreaks was based on clinical signs of enteric disease. In all groups, coccidosis was not initially suspected as a clinically relevant cause of the observed clinical signs. Before a diagnosis of coccidiosis was established in each group, ferrets that developed diarrhea were treated empirically with various combinations of prednisolone (0.5 mg/kg [0.2 mg/lb], PO, q 12 h), amoxicillin (25 mg/kg [11.4 mg/lb], PO, q 12 h), enrofloxacin (5 mg/kg [2.3 mg/lb], PO, q 12 h), metronidazole (25 mg/kg, PO, q 12 h), famotidine (0.5 to 1.0 mg/kg [0.2 to 0.5 mg/lb], SC, q 12 to 24 h), omeprazole (0.7 mg/kg [0.3 mg/lb], PO, q 24 h), or sucralfate (25 to 50 mg/kg [11.4 to 22.7 mg/lb], PO, q 12 to 8 h). Fluid therapy included administration of lactated Ringer’s solution (22 mL/kg [10 mL/lb], SC). Severely affected ferrets in group 2 also received fluid therapy via IP or intraosseous routes of administration. Other supportive care included supplemental feeding and use of heating pads. For ferrets that survived, such treatment was administered for approximately 2 weeks and resulted in mild to moderate improvement in clinical signs of enteric disease. In addition to treatment of affected ferrets, the environment of affected animals, including feeding bowls, litter boxes, and other supplies, were thoroughly cleaned and disinfected multiple times over the course of each outbreak.

In groups 1 and 3, coccidia were never found through either fecal examination or histologic examination of tissue specimens while outbreaks were occurring. Once coccidia were identified, ferrets in these groups were treated with sulfadimethoxine (25 mg/kg, PO, q 24 h) for 21 days in addition to supportive care. Initially, all ferrets at the shelters were treated with sulfadimethoxine. Clinical signs in ferrets in both of these groups that had been affected at the start of treatment were largely alleviated. In group 1, however, the series of sulfadimethoxine treatments was repeated twice owing to identification of coccidial oocysts on fecal examinations performed 2 and 5 months after the initial treatment. Ferrets in group 2 were never treated with sulfadimethoxine because coccidia were only identified by histologic examination of tissue specimens following the resolution of the outbreak. Given that few ferrets in group 2 had signs of respiratory disease, infection with influenza virus was considered a differential diagnosis. Late in the course of the outbreak in this group, all ferrets at the facility were treated by administration of oseltamivir (2.2 mg/kg [1 mg/lb], PO, q 12 h) in an extralabel manner. When used in veterinary species, oseltamivir administration constitutes extralabel drug use according to AMDUCA.

Discussion

In all 3 groups, coccidial organisms were identified in ferrets that had clinical signs of enteric disease. Lesions of atrophic enteritis with intralesional coccidia, often in high numbers, were observed in the small intestine of ferrets from each outbreak. No other infectious agents commonly observed in ferrets with diarrhea (eg, Aleutian mink disease virus, rotavirus, coronavirus, and bacteria) were identified in these 3 outbreaks by histologic examination or other diagnostic testing. Coccidia were speciated as *E furonis* through morphology of sporulated oocysts and sequencing of the gene encoding SSUrRNA. These findings strongly suggest that infection with *E furonis* played a causative role in the observed enteric disease in all 3 outbreaks.

Severe disease due to intestinal coccidiosis is most commonly seen in intensively managed, young, naive animals of many species. Infections with low numbers of organisms that are clinically inapparent are much more common in domestic animal species than are infections with high numbers of organisms that are accompanied by severe manifestations of disease. Enteric coccidiosis, however, can lead to pronounced diarrhea, dehydration, and sometimes death in cases of overwhelming exposure to sporulated oocysts or immunosuppression of moderately affected animals.

Infection of the intestinal tract of ferrets may occur from numerous species of coccidia, including *Isospora laidlawi, Isospora eversmanni, Eimeria icidex, E furonis*, and *Eimeria vision*. Of these, *E furonis* is the most commonly reported and is generally thought to cause subclinical infections. Infected ferrets are typically clinically unremarkable, but low numbers of oocysts can be found in fecal samples. Overt disease, including dehydration and moderate to severe diarrhea, has rarely been reported in individual young animals. Although enteric coccidiosis has previously been reported in ferrets, to our knowledge, this is the first report of outbreaks of severe enteric disease associated with *E furonis* in multiple ferrets at individual sites. Furthermore, morbidity and death occurred in ferrets of all ages, and severity was seemingly not dependent on preexisting health conditions.

The large number of ferrets of various ages within confined spaces likely allowed for rapid accumulation of oocysts of *E furonis* in the environment as the number of infected ferrets increased. The regular introduction of new members into these populations through rescue events would likely have allowed for a constant supply of naive animals that would have been particularly susceptible to coccidial infection. Thus, a high population density and dynamic population would have predisposed these groups for outbreaks of coccidiosis.

This series of outbreaks suggests that the initial diagnosis, the management of outbreaks, and the treatment of individual ferrets with intestinal coccidiosis can be challenging. Fecal examinations by referring veterinarians were positive for oocysts in group 1 only, suggesting that shedding of oocysts may be intermittent or may occur in low numbers that are not easily detected by routine means. Identification of oocysts in the face of outbreaks of enteric disease may require multiple fecal examinations and examination of pooled fecal samples from ferrets acutely and chronically affected. At all sites, disease spread rapidly throughout the ferret populations despite containment of affected indi-
individuals and environmental decontamination. However, the exact methods for quarantine and environmental decontamination were not explicitly described. Therefore, it is unclear how stringently quarantines were followed, how long quarantines were implemented, or what methods of disinfection or types of disinfectants were used. It remains likely that stringent quarantine and fecal examination of any ferrets being introduced to a new population, isolation of ferrets with clinical signs of enteric disease, and thorough cleaning of the environment along with the use of disinfectants such as quaternary ammonium compounds or bleach are practices that will help to avoid future outbreaks and help to control any outbreak that may occur.

Specific treatment for coccidia (sulfadimethoxine) was moderately effective in controlling clinical signs of enteric disease in groups 1 and 3 but failed to eliminate fecal shedding of oocysts in group 1 despite multiple rounds of treatment. Furthermore, aggressive supportive treatment was necessary in a substantial proportion of ferrets, and in some cases, ferrets died despite treatment. No specific treatment for coccidia was used in group 2, but the course of the outbreak in this group was similar to that of the other affected groups. Resolution of the group 2 outbreak suggests that, over time, resistance to overt disease developed in ferrets that survived. Ferrets in group 2 were treated with oseltamivir® in an extralabel manner as a precautionary treatment; however, such use of oseltamivir is not recommended without specific confirmation of infection with influenza virus. While sulfadimethoxine was the only specific treatment for coccidia that was attempted in these groups, it is plausible that other anticoccidial drugs such as ponazuril or sulfamethoxazole may be used with similar or better results in future outbreaks of diarrhea associated with intestinal coccidiosis.

The possibility of infection with other enteric pathogens cannot be completely ruled out in any of the 3 groups of the present report. None of the examined tissues had histopathologic evidence of other causes for diarrhea, including Aleutian mink disease virus, and results of all testing for other infectious agents, including coronavirus, rotavirus, pathogenic bacteria, and, in group 2, influenza virus, were negative. However, only a small proportion of the total number of affected ferrets were examined or tested. In particular, pathogens with a short time course of infection may not have been detected by testing ferrets that were chronically affected. The consistent association of large numbers of coccidia with both clinical signs of enteric disease and histopathologic lesions, however, suggests that infection with E furonis was at least a pivotal contributor to enteric disease in the high-density, dynamic populations of ferrets described in this report. On the basis of the data presented here, E furonis should be considered an important cause of diarrhea in ferrets, especially in high-density populations.

References