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Enteric coronavirus infection in a juvenile dromedary (*Camelus dromedarius*)

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Abstract. A case of an enteric coronavirus infection in a 6-week-old dromedary calf is described. The animal had diarrhea for 5 days and died despite symptomatic treatment. Numerous viral particles, approximately 140 nm in diameter, with club-like projections were detected in the feces by electron microscopy. These characteristics were consistent with a coronavirus. Immunohistochemical reactivity with 2 antigenic group II coronavirus-specific antibodies confirmed the presence of viral antigen in colonic epithelial cells. The death of the animal was attributed to a neutrophilic and emphysematous colitis that likely was caused by an infection with a *Clostridium* sp.

A 6-week-old female dromedary (*Camelus dromedarius*) calf with a history of acute diarrhea died 5 days after the initial symptoms. According to the owner, the calf was born in Missouri on a pasture that was also inhabited by zebras. The camel nursed from its mother for 1 week after birth. At 1 week of age, the calf was separated from its mother and shipped to a farm in Wisconsin. Upon arrival, the calf was contained indoors and initially fed approximately 1 liter of warm calf milk replacer[a] containing oxytetracycline and neomycin 4 times per day. The amount of milk replacer was gradually increased to approximately 2 liters per feeding. Clover grass hay was available at all times. The calf had contact with miniature horses, zebras, and reindeers that were kept in the same barn in different stalls. The calf was healthy for approximately 4 weeks. Its weight was approximately 75 kg. Five days before death, the calf developed watery diarrhea after initially being bloated. The owner administered penicillin (approximately 20,000 units/kg) intramuscularly. The calf was presented to the referring veterinarian for the first time approximately 1 day after the initial signs were noted by the owner. The animal appeared to be mildly dehydrated. The calf was treated by the veterinarian with butorphanol,[b] dipyrone,[c] and flunixin meglumine[d] intravenously and received electrolytes per os because it was still drinking. At the second visit 2 days later, the rectal temperature was approximately 35 C (reference range[7]: 36–40 C). The hematocrit was slightly elevated (37.5%; reference range[13]: 26–31%). The calf was tachycardic (60 heart beats/minute; reference range[7]: 40–50 beats/minute). The white blood cell count was 16,000 leukocytes/ml (reference range[13]: 13,000–24,000) with neutrophilia (83%; reference range[13]: 53–74%). The anti-inflammatory and antibiotic treatment was continued but the calf died and was submitted to the Department of Veterinary Diagnostic Medicine, University of Minnesota, St. Paul, Minnesota, for postmortem examination. Tissue samples, including small and large intestine, lung, brain, liver, kidney, spleen, heart, intestinal lymph node, and adrenal gland, were fixed in 10% buffered formalin and embedded in paraffin. Sections cut at 4 μm were stained with hematoxylin and eosin (HE). Additional sections of the intestine were stained with a modified Gram stain. Fresh samples of lung and liver were submitted for aerobic culture. The intestine was cultured under aerobic and anaerobic conditions by routine laboratory procedures. Feces were submitted for routine parasitologic examination by flotation techniques. Fecal samples were examined for viruses by using direct negative-contrast transmission electron microscopy as previously described.[8]

Avidin–biotin–peroxidase complex method was used for immunohistochemical demonstration of coronaviral and rotaviral antigens as previously described.[4,19] Two monoclonal antibodies against ruminant coronavirus (bovine and elk coronavirus) spike protein[e] (clone Z3A5) and nucleocapsid protein[f] (clone 8F2), 1 monoclonal antibody against porcine transmissible gastroenteritis virus[g] (TGE; clone 14-E3), and 1 monoclonal antibody against bovine rotavirus[h] (clone 9-10) served as primary antibodies.[3,16,19]

At necropsy, the calf was in a good nutritional state. The colon and cecum were moderately distended and filled with yellow–brown watery fluid. The mucosa of the proximal colon and cecum had multiple, slightly raised, well-demarcated, red–white mottled areas, which were up to 10 cm in length by 5 cm in width (Fig. 1). The mesentery was edem-

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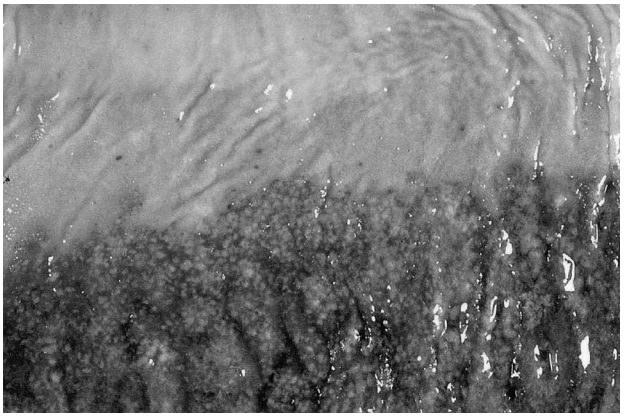

Figure 1. Section of the opened colon with a well-demarcated, mildly raised, reddened area (bottom) that had numerous, whitish, emphysematous foci.

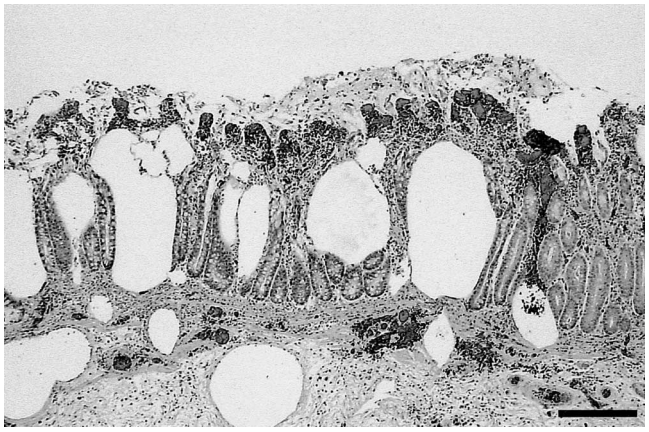

Figure 2. Histologic section of the colon with emphysematous mucosa. The mucosa and submucosa were infiltrated with neutrophils and lymphocytes. Occasional vessel walls had neutrophilic infiltration. Moderate acute mucosal edema was present. HE. Bar = 200 μm.

atous. Histologically, marked acute mucosal and submucosal emphysema was found in the grossly affected colon and cecum. The submucosa and mucosa were moderately infiltrated with neutrophils (Fig. 2). The submucosa was edematous and had mild, multifocal hemorrhages. Numerous crypts contained cellular debris. A moderate number of gram-positive bacilli, approximately 3–5 μm in length, were attached to the multifocally denuded intestinal surface.

Mixed nonhemolytic coliforms and nonhemolytic *Streptococcus* sp. were cultured from colon, liver, and lungs. *Clostridium* sp. were not isolated. Parasites, parasite ova, or oocysts were not detected in the fecal sample.

Bovine coronavirus–specific immunohistochemistry demonstrated brown cytoplasmic staining in the epithelial cells of multiple colonic crypts (Fig. 3). Crypt epithelial cells appeared to be the target cell in grossly altered colonic areas. In contrast, the superficial epithelial cells were almost exclusively infected in the macroscopically normal areas of the colon. More epithelial cells were infected in macroscopically altered colonic areas than in macroscopically normal areas. Bovine coronavirus antigen–specific immunohistochemistry of jejunum and lungs and immunohistochemistry for porcine transmissible gastroenteritis virus antigen and rotavirus antigen in the colon yielded negative results.

Numerous viral particles approximately 140 nm in diameter with club-like surface projections were detected by electron microscopy of the feces (Fig. 4). Enteric coronaviruses are common pathogens in pigs and cattle and are frequently associated with fatal neonatal diarrhea.[2,14] A magnitude of 100,000 virus particles per milliliter of feces are thought to be necessary to detect virus particles by direct negative-contrast electron microscopy, as performed in the present case.[6] This high number of virus particles suggests an acute infection in the present case.

The source of the infection is uncertain in the present case. Coronaviruses are divided into 3 antigenic groups with antigenic cross-reactivity within the groups. Rat coronavirus, mouse hepatitis virus, murine enteric coronavirus, human respiratory coronavirus, elk coronavirus, equine coronavirus, porcine hemagglutinating encephalomyelitis virus, and bovine coronavirus belong to the antigenic group II.[2,4,9,12] The immunohistochemical results indicate that the virus of the camel calf may be closely related, if not identical, to members of group II. Although both monoclonal antibodies used for immunohistochemistry have a high specificity for coronaviral spike protein and nucleocapsid protein, they are not able to discriminate between coronaviruses of group II.[4] Coronavirus has been isolated from wild living sambar deer, white-tailed deer, and waterbuck with diarrhea.[15] These viruses were antigenetically indistinguishable from bovine co-

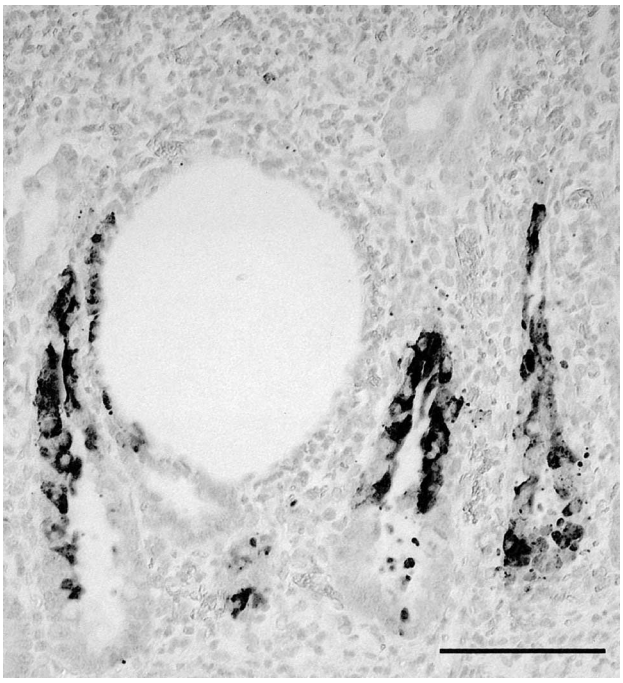

Figure 3. Immunohistochemical section of the colon. Multiple epithelial cells of the crypts contained a reddish precipitate indicating the presence of coronavirus antigen. Avidin–biotin–peroxidase method; anti-bovine coronavirus antibody (clone 8F2). Bar = 100 μm.

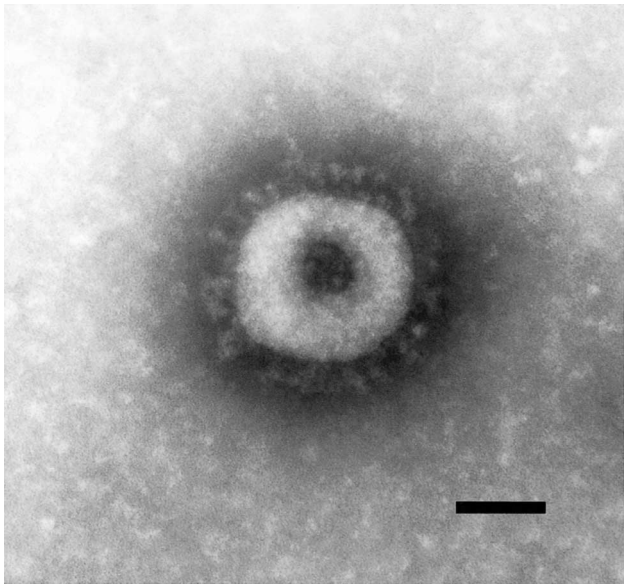

Figure 4. Direct negative-contrast transmission electronmicroscopic demonstration of viral particles in the feces. The viral particles were approximately 140 nm in diameter and had club-like surface projections characteristic of coronaviruses. Bar = 50 nm.

ronavirus. Furthermore, coronavirus infection was diagnosed in sitatunga and waterbucks in the United Kingdom and elk in the USA.[1,12] The elk coronavirus had 99% homology of the nucleocapsid gene sequence with bovine coronavirus.[12] Bovine coronavirus has been shown to infect epithelial cells of the small intestinal villi, colonic crypts, and the upper and lower respiratory tract in calves and adult cattle,[2] whereas transmissible gastroenteritis virus infection appears to be restricted to epithelial cells of the small intestinal villi and respiratory tract in pigs.[14]

Although coronavirus infection has been included as differential diagnosis for diarrhea in neonatal camels in textbooks on camel diseases, no report has been made in the literature of coronavirus-induced disease in camels[18]. In the present camel, the infection was restricted to the colon. Among the different regions of the bovine intestine, the spiral colon is most frequently involved and stays positive for the longest time by immunohistochemistry.[10]

The coronavirus infection may have predisposed the animal to the putative fatal clostridial infection. Digestive disorders, such as dietetic diarrhea and bacterial diseases of the alimentary system including colibacillosis, *Clostridium perfringens* infection, salmonellosis, and rotavirus infection are the most common causes of mortality in camel calves.[11,17,18] Clostridial enterotoxemia has been described in dromedaries in Asia and Africa as a common cause of neonatal diarrhea, and *C. perfringens* types A, C, and D have been isolated.[5,17,18] The mucosal and submucosal emphysematous colonic lesions and the presence of gram-positive bacilli seem to be consistent with infection with *C. perfringens.* The failure to isolate *Clostridium* sp. in the present case may be related to antibiotic treatment. Association of infection with *Clostridium* sp. with enteric viruses has not been described in camels in previous reports.

The possible interspecies coronavirus infection may have implication for the cohusbandry of individuals of the family Camelidae with individuals of other families such as Bovidae, Cervidae, and Equidae. Serologic data as well as electron microscopic and virologic examination of fecal samples are needed to study the epidemiology of coronavirus infections in camels.

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b. Torbugesic, Fort Dodge Animal Health, Ames, IA.
c. Novin, Miles, West Haven, CT.
d. Banamine, Schering-Plough Animal Health Corp., Union, NJ.
e. Provided by S. Kapil, Kansas State University, Manhattan, KS.
f. Provided by S. Kapil, Kansas State University, Manhattan, KS.
g. Provided by L. Saif, Ohio State University, Columbus, OH.
h. Provided by S. Srikumaran, University of Nebraska, Lincoln, NE.

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Resistance of domestic cats to a US sheep scrapie agent by intracerebral route

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Abstract. Feline spongiform encephalopathy (FSE) is thought to have resulted from consumption of food contaminated with bovine spongiform encephalopathy and the latter is believed to result from the consumption of food contaminated with scrapie. However, no direct experimental documentation exists to indicate that the scrapie agent is capable of amplifying in cats, and, therefore, crossing the species barrier. During 1979, 6 cats ranging in age from 3.5 to 18 months were intracerebrally inoculated with sheep scrapie (inoculum G-639-PP) and were observed for an extended period. Inoculated cats did not develop neurologic disease, and microscopic lesions of spongiform encephalopathy were not evident. Immunohistochemistry and Western blot techniques failed to detect the abnormal form of prion protein (PrP^{res}). These results indicate that the sheep scrapie agent (G-639-PP) used in this study was not capable of amplifying in cats and therefore was unable to cross the species barrier to produce FSE.

Feline spongiform encephalopathy (FSE) is a fatal neurologic disease that is classified as a transmissible spongiform encephalopathy (TSE) or a prion disease. Feline spongiform encephalopathy first appeared in the United Kingdom (UK) in the 1990s during the epizootic of bovine spongiform encephalopathy (BSE), and since then approximately 90 cases of FSE have been seen in the UK and in Europe.[7] Feline spongiform encephalopathy is thought to have been transmitted by consumption of food contaminated with BSE, which is suspected to have resulted from consumption of food contaminated with scrapie.[1] In the United States, at least 2 experiments have been conducted to show that intracerebral inoculations of sheep scrapie into cattle produces a form of TSE, but the experimental disease is clinicopathologically different from BSE.[2,4] Also, a second passage of 1 of the scrapie inocula failed to induce BSE in the intracerebrally inoculated cattle.[3] Experimental infection of cats by the scrapie agent has not been reported, and, therefore, no evidence exists to suggest that the abnormal form of prion protein (PrP^{res}) in scrapie can amplify in cats and thereby cross the species barrier. One such experiment was done in 1979 but results of the study were not reported. Furthermore, at that time no in vitro laboratory tests were available to confirm the presence of PrP^{res} in tissues of these cats. This communication documents results (histopathology, immunohistochemistry, and Western blot) of the 1979 experiment that involved intracerebral inoculations of domestic cats with sheep scrapie agent.

In 1979, 2 18-month-old queens (of unknown relationship) and their progeny (2 kittens each, all 3.5 months of age) were obtained from a local farmer at Mission, Texas, for this study. All were intracerebrally inoculated with 0.5 ml of a 10% solution (wt/vol) of a scrapie-positive sheep brain (Suffolk G-639-PP) that was obtained at death from a 32-month-old Suffolk ewe with clinical signs of scrapie (Table 1). The brain of this ewe was histopathologically positive for scrapie and when it was inoculated intracerebrally into mice, it produced scrapie in 9 of 10 animals at 352–460 days after inoculation.

During the observation period, none of the inoculated cats developed neurologic signs. The cats were euthanized when in extremis (because of unrelated medical conditions; Table 1), and a detailed necropsy was conducted. Representative tissue samples were immersion-fixed in formalin, and sam-

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