Case Report Rapport de cas

Enteropathogenic *Escherichia coli* (EPEC) infection in association with acute gastroenteritis in 7 dogs from Saskatchewan

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Abstract — Seven dogs diagnosed with enteropathogenic *Escherichia coli* (EPEC) infection in association with acute gastroenteritis are described. Disease severity ranged from mild in adults to fatal disease in young dogs. Enteropathogenic *E. coli* infection should be considered as a possible differential diagnosis in dogs with diarrhea.

Résumé — Infection par *Escherichia coli* entéropathogène (ECEP) en association avec une gastro-entérite aiguë chez 7 chiens de la Saskatchewan. On décrit sept chiens diagnostiqués avec une infection par *Escherichia coli* entéropathogène (ECEP) en association avec une gastro-entérite aigüe. La gravité de la maladie allait de légère chez les adultes à une maladie mortelle chez les jeunes chiens. *E. coli* entéropathogène devrait être considéré comme un diagnostic différentiel chez les chiens souffrant de la diarrhée.

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Based on virulence factors, pathogenic *E. coli* are classified into 6 major pathotypes: enteropathogenic (EPEC), enterotoxigenic (ETEC), Shiga toxin-producing (STEC) [including enterohemorrhagic (EHEC)], diffusely adherent (DAEC), enteroinvasive (EIEC), and enteroaggregative *E. coli* (EAEC) (1). The hallmark of EPEC infection is the attaching-and-effacing lesion identified on histopathological examination. These lesions are characterized by intimate attachment of the bacterium to the intestinal epithelial cell membrane and effacement of microvilli. The *eae* gene, present in all EPEC and most EHEC and absent from nonpathogenic *E. coli,* codes for intimin, one of several factors required for adhesion and virulence (2). The presence of this gene provides sufficient evidence to document potential virulence (1,3). The EPEC pathotypes are further differentiated from EHEC by inability to produce Shiga toxin, most often identified by the absence of specific genes *stx1, stx2* (1,4). These EPECs are among the most important causes of diarrhea in children less than 2 y of age, whereas older individuals seem resistant to developing disease (1). As a zoonotic potential for EPEC has been suggested (5–7), EPEC in dogs could be important pathogens related to public health concerns.

The EPECs have been described in diarrheic dogs that had classical small and large intestinal attaching-and-effacing lesions on postmortem examination (4,8–11). Additionally, epidemiological data suggest equivalent prevalence of EPEC in dogs with acute and chronic diarrhea, though causation was not thoroughly investigated (12). Experimental infection with EPEC has been shown to cause diarrhea (13), but EPEC pathotypes have also been isolated from healthy dogs (12,14). These pathotypes were identified in 5% to 9% of healthy household controls (12) and in 17% to 46% of healthy kenneled controls. In a retrospective study of 122 dogs that died with diarrhea, 44 dogs were found to have EPEC (15), with 34% of these dogs having EPEC as the only pathogen detected. Many of the studies on EPEC in dogs described only patients with diarrhea and were based on necropsy data or were carried out in healthy animals, so that accurate clinical descriptions of affected individuals are missing. Interpretation of test results is thus still troublesome (16). This report describes the clinical course of 7 dogs with acute gastroenteritis associated with EPEC.

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Case description

Retrospective review of electronic medical records of dogs with acute gastroenteritis presented to the Western College of Veterinary Medicine, Veterinary Medical Centre between August 2010 and August 2013, identified 7 dogs positive for EPEC.

Fecal diagnostics including parvovirus testing, fecal flotation, routine aerobic and anaerobic fecal culture including special agar media for each of *Salmonella* (Brilliant Green and XLT4, Oxoid, Nepean, Ontario) and *Campylobacter* spp. (Preston's campy agar, GMP, University of Saskatchewan, Saskatoon, Saskatchewan), as well as polymerase chain reaction (PCR) analysis for *E. coli* genes to identify EPEC and differentiate from EHEC *(stx1, stx2, eae)* are summarized in Table 1. Polymerase chain reaction analysis was performed on DNA extracted from *E. coli* colony isolates from feces. All *E. coli* isolates were confirmed as EPEC based on the presence of the *eae* gene and were not EHEC (absence of *stx1* and *stx2*) or ETEC (absence of *estA* gene). Most dogs also had blood analysis.

Table 1. Results[a] of fecal tests for 7 dogs with enteropathogenic *Escherichia coli* infection

Dog	Parvovirus test	Fecal culture
1	Negative ELISA (× 2) Negative IHC	*E. coli* 4+ *C. perfringens* 1+
2	Negative ELISA (× 2)	*E. coli* 2+ *C. perfringens* 1+
3	Negative ELISA	*E. coli* 1+ *C. perfringens* 1+
4	ND	*E. coli* 4+
5	ND	*E. coli* 3+ *C. perfringens* 4+
6	ND	*E. coli* 3+ *C. perfringens* 2+
7	ND	*E. coli* 4+ *Streptococcus* spp. 4+ *Campylobacter* spp. — few

[a] PCR showed that all *E. coli* were positive for the *eae* gene and negative for *estA, stx1* and *stx 2* genes. ND — not done.

Dogs ranged in age from 5 mo to 8 y. There was no gender or breed predilection identified. Three dogs were appropriately vaccinated against canine distemper virus, canine adenovirus type 2, and canine parvovirus type 2, whereas the remaining dogs did not receive a complete vaccination series. Fecal parvovirus tests (ParvoCITE fecal ELISA; IDEXX, Westbrook, Maine, USA) were negative for all incompletely vaccinated dogs.

At presentation, 2 dogs had signs of dehydration and systemic inflammatory response syndrome (fever, tachycardia, prolonged capillary refill time, poor peripheral pulses, and pale mucous membranes). Three dogs exhibited pain on abdominal palpation.

Dogs in this study were divided into 2 groups based on the severity of their clinical signs; those under 1 y of age appeared to have severe or potentially fatal disease, and those over 1 y appeared to have a more benign clinical course of disease. Clinical signs for all dogs are summarized in Table 2.

Two dogs were under 1 y of age at the time of presentation. The first dog was a 6-month-old female Siberian husky cross living at a local shelter. She exhibited hematochezia and large volume, infrequent (mixed bowel) diarrhea for 4 d and vomiting for 1 d prior to presentation. Upon arrival at the hospital she was febrile (39.6°C) and in hypovolemic shock. A complete blood (cell) count (CBC) identified profound neutropenia. Serum biochemical profile revealed mild decreases in serum urea nitrogen and creatinine attributed to aggressive IV fluid therapy and mild increase in serum alkaline phosphatase attributed to the bone isoform in a growing puppy. Total protein was decreased, characterized by profound hypoalbuminemia, likely related to gastrointestinal loss and negative acute phase reaction. Fecal diagnostics are summarized in Table 1.

This dog was treated with IV fluid to correct shock and electrolyte abnormalities. A synthetic colloid (Pentospan; Bristol Myers, Montreal, Quebec) was administered at 20 mL/kg body weight (BW) per day as a constant rate infusion (CRI) to maintain colloid osmotic pressure. Antimicrobial therapy, including ampicillin (Teva, Toronto, Ontario), 22 mg/kg BW, IV, q6h, and gentamicin (Gentocin; Merck, Kirkland, Quebec), 8 mg/kg BW, IV, q24h, was added after 24 h of IV fluid therapy. Despite antiemetic therapy with metoclopramide (Sandoz, Quebec City, Quebec), 2 mg/kg BW, IV, q24h, as a CRI and maropitant (Cerenia; Pfizer, Kirkland), 1 mg/kg BW, SQ, q24h, vomiting and diarrhea continued. Two days into treatment the dog developed epistaxis. Evaluation of a blood smear suggested moderate thrombocytopenia. A fresh frozen plasma transfusion (WCVM, Saskatoon), 10 mL/kg BW, IV, was administered to treat suspected disseminated intravascular coagulation. Due to the lack of clinical improvement, euthanasia was elected on day 5 of therapy.

A complete necropsy identified marked generalized edema including marked ascites. Tarry content was present in the small intestine and frank blood was present in the large intestine, which was consistent with the clinical picture of both small and large bowel diarrhea in this dog. Histopathology of the small intestines revealed diffuse crypt necrosis with incomplete epithelial lining and partial regeneration of the small intestinal mucosa. Due to marked autolysis, attaching-and-effacing lesions could not be assessed. Immunohistochemical testing on small intestinal sections was negative for canine parvovirus type 2. Acute, extensive erosive to ulcerative esophagitis was present and attributed to protracted vomiting. The bone marrow was hypocellular (estimate 30%) with adequate megakaryocytes present. The myeloid line predominated with a left shift in maturation suggesting early regeneration.

The second dog was a Labrador retriever cross less than 1 y of age which was presented for evaluation of 2 d of vomiting and mild small bowel diarrhea. The dog had been hospitalized at the WCVM 10 d earlier for management of a hip luxation. He was treated with carprofen and tramadol, both of which were stopped when his vomiting and diarrhea began. On physical examination, the dog was quiet and responsive with vital parameters within normal limits. At presentation this dog had signs of dehydration (estimated fluid loss of 7% of body weight), hypersalivation, and mild discomfort on abdominal palpation.

The CBC showed a normal leukocyte count with a moderate left shift and lymphopenia, with slight toxic changes present in the neutrophils. A mild microcytosis was present which likely represents acute inflammation associated with iron deficiency related to sequestration. Serum biochemical profile identified a mild hypochloremia, mild hypercholesterolemia, and mild increase in alanine aminotransferase representing mild hepatocellular injury.

The dog was treated with IV fluid therapy to correct dehydration and electrolyte derangements. Maropitant (1 mg/kg BW, SC q24h) and metoclopramide (2 mg/kg BW, IV, q24h, CRI) were utilized as antiemetic agents, though nausea and vomiting persisted. Ondansetron (Sandoz, Boucherville, Quebec), 0.1 mg/kg BW, IV, q12h, was added after 24 h to aid control of nausea. Antimicrobial therapy consisted of ampicillin, 22 mg/kg BW, IV, q6h, for 24 h during rehydration therapy, with gentamicin, 8 mg/kg BW, IV, q24h, added after that. Two days after initiating supportive therapy, a recheck of the CBC found a severe leukopenia characterized by neutropenia and lymphopenia. A mild, normocytic, normochromic nonregenerative anemia

Table 2. Clincal signs for 7 dogs with enteropathogenic *Escherichia coli* infection

Dog	Signalment	DA2PP vaccinations	Diarrhea	Vomiting	Temperature	Physical examination
1[a]	5 mo F Siberian husky cross	2	4 d, hematochezia, increased volume	1 d	39.6°C	Abdominal pain
2[b]	9 mo M Labrador cross	1	2 d, soft feces, increased volume	2 d	NR	Mild abdominal pain
3	1 y M Maltese cross	2	1 d, hematochezia	NR	NR	NR
4	2 y FS miniature dachsund	3 as puppy, 1 y booster	2 d, increased frequency, mild hematochezia	NR	39.3°C	Abdominal pain
5[c]	4 y F English springer spaniel	3 as puppy, 1 y booster	2 d, increased frequency, mild hematochezia	2 d, hematemesis	NR	NR
6	3 y FS German shepherd cross	3 as puppy, 1 y booster	1 d, hematochezia, mucus	NR	NR	NR
7	8 y MN German short-haired pointer	Up-to-date, no details available	1 mo, hematochezia	None	38.2°C	Mild peripheral lymphadenomegaly

[a] Dog 1 was > 12% dehydrated and in hypovolemic shock.
[b] Dog 2 was 7% dehydrated.
[c] Dog 5 was tachycardic.
NR — not recorded.

was also noted. Total protein level was also decreased. Recheck of the fecal parvovirus test was negative.

After 5 d of supportive care, there was a normal leukocyte count characterized by a slight left shift. The previously identified anemia had resolved. The dog was discharged with no further vomiting and diarrhea.

Dogs in the second group were all more than 1 y of age. Three dogs were presented for evaluation of 1 to 2 d of hemorrhagic diarrhea, as well as vomiting. Dog 3 had been treated 2 wk earlier with metronidazole for episodes of hematochezia and large bowel diarrhea which resolved during treatment, but returned when therapy was completed. Dog 4 had been seen by her family veterinarian the day before presentation and treated with sucralfate, with no improvement in clinical signs. Dog 7 had a month-long history of episodic bloody diarrhea. No dog had been seen or suspected to ingest a foreign body or toxin, nor did any of the dogs have a history of dietary indiscretion.

Three dogs (numbers 3, 6, and 7) were managed as outpatients and were fed easily digestible diets for 1 to 2 d. Dogs 3 and 6 had predominately large bowel diarrhea, characterized by increased frequency, mucus, and frank blood. Dogs 4 and 5 were also presented with primarily large bowel diarrhea, with dog 4 having small flecks of fresh blood in the feces, while dog 5 had hemorrhagic staining around the perineum. Both were treated with IV fluid therapy, ampicillin, 22 mg/kg BW, IV, q6h, and bland diets for 12 to 24 h. They were then discharged from the hospital with no further clinical signs reported by owners. Dog 4 was discharged with instructions for the owner to administer amoxicillin, 100 mg PO, q12h, for 7 d.

The CBC of dog 3 was entirely within the reference interval. The serum biochemistry profile identified a moderate hypercholesterolemia; however, this was not a fasted sample. Urinalysis noted well-concentrated urine (UGS: 1.060) with a mild increase in WBC/hpf of 6 to 8. Urine culture was negative. A resting cortisol level was determined to rule out hypoadrenocorticism and was found to be normal, eliminating hypoadrenocorticism as a differential diagnosis.

Dog 4 was presented with mild electrolyte and acid-base derangements that were readily corrected with 24 h of IV fluid therapy. Dog 5 had no electrolyte abnormalities noted, though a complete serum biochemistry profile was not performed. Resting cortisol was mildly elevated, eliminating hypoadrenocorticism as a differential diagnosis. Dog 6 had no CBC or biochemistry profile performed. Dog 7 had a CBC that was within reference intervals. Liver enzyme activity was markedly increased [alanine aminotransferase (ALT) 1824 U/L; reference interval (RI): 19 to 59 U/L, alkaline phosphatase (ALP) 1024 U/L; RI: 9 to 90 U/L]. Six months after this presentation, dog 7 was diagnosed with hepatic insufficiency of unknown etiology.

Fecal diagnostics are summarized in Table 1. The flotations available for 3/7 dogs were negative. Fluorescent antibody testing for *Giardia* spp. and *Cryptosporidium canis* was available for 1 dog and both were negative. Fecal culture identified *E. coli* in all 7 dogs, with 1 found to be 1+ growth, 1 with 2+ growth, 2 with 3+ growth and 3 with 4+ growth. The number of colonies tested varied and neither the number tested nor results for each colony were reported by the diagnostic laboratory. Following DNA extraction, all samples were PCR positive for *eae* utilizing previously published primers (17). *Clostridium perfringens* was identified in 5/6 dogs, with 1+ growth in 3 dogs, 2+ growth in 1 and 4+ growth in 1. Dog 7 also had 4+ *Streptococcus* spp. and a small number of *Campylobacter* present. *Salmonella* spp. were not identified in any dog.

Discussion

We have described 7 dogs with gastrointestinal signs that also had EPEC isolated from fecal samples. Clinical signs could not readily be attributed to other extra- or intra-gastrointestinal diseases with the exception of dog 7 which concurrently had *Campylobacter* spp. and hepatic disease; however, an extensive

search for possible causes was not always undertaken. It is thus possible that EPEC was a complicating or concurrent infection rather than the primary cause of clinical signs. Unfortunately fecal parvovirus test results were not available for 4 dogs; however, they were well-vaccinated and more than 2 y of age, making parvoviral enteritis unlikely. The sensitivity of the ParvoCITE fecal ELISA test is highest during maximal excretion (4 to 7 d after infection), and thus false negatives may be seen before or after this interval (18). Dog 1 had 2 negative fecal parvovirus test results as well as negative postmortem immunohistochemistry analysis. Considering that there were 9 d from the onset of clinical symptoms until necropsy was performed, it cannot be ruled out that the IHC result was a false negative. Autolysis during necropsy examination prevented us from determining whether attaching and effacing lesions were present. Dog 2 also had 2 negative fecal parvovirus test results; however, given the young age of the dog and lack of confirmatory IHC analysis parvoviral enteritis could remain a possible differential diagnosis for this dog as well. Dog 3 was incompletely vaccinated; however, a negative fecal parvovirus test and a completely normal CBC make parvovirus infection unlikely. Dogs 4 to 7 were all older than 2 y and all had been appropriately vaccinated. These dogs experienced mild to moderate clinical signs and no significant inflammatory responses were identified on CBC analysis. This clinical picture makes parvoviral infection a less likely cause of their gastrointestinal disease. Unfortunately no dogs were tested for canine coronavirus, which has been associated with both mild (19) and severe (20,21) diarrhea in young dogs. Although typically associated with self-limiting disease, coronavirus infection cannot be excluded as an inciting or contributing factor in these dogs.

Results of fecal flotations were not available for 4 dogs and thus intestinal parasitism such as trichuriasis could not be ruled out in these cases. Based on previous regional analysis, only 4.4% of healthy dogs in the Saskatoon, Saskatchewan region have gastrointestinal parasitism, with round worms being the most commonly identified species and *Trichuris* being identified in only 0.7% of the population (22). While it is unlikely that primary intestinal parasitism would cause signs of neutropenia (dog 2), co-infections have been reported and may be important in pathogenesis (4,8–10).

In the present study, 6 of 7 dogs were reported to have bloody diarrhea, while 1 dog also had hematemesis. Five of 6 dogs exhibited predominantly large bowel signs with hematochezia and 2 dogs had mixed bowel signs. Sancak et al (12) observed blood in 28% of dogs with acute diarrhea and 24% of dogs with chronic diarrhea, but they did not correlate this to patients found to have EPEC. To the authors' knowledge, this is the first report of EPEC being associated with hemorrhagic diarrhea in naturally infected dogs. *Escherichia coli* was identified on fecal culture in all cases; however, the amount of growth varied substantially among dogs and does not appear related to the magnitude of clinical disease. Polymerase chain reaction for presence of *eae* and absence of heat-stable toxin and Shiga toxin is necessary to diagnose EPEC infection. Postmortem confirmation of attaching-and-effacing lesions in dog 1 could not be obtained. Additionally, *Clostridium* ELISA toxin testing could have been used to further evaluate the significance of this organism in dogs with positive culture results (16).

Severe neutropenia was identified in the 2 most severely affected dogs. While parvoviral enteritis is a common cause for vomiting, diarrhea, and bone marrow suppression in incompletely vaccinated, young dogs, parvovirus was not identified in dog 1 or dog 2. Enteropathogenic *E. coli* has not previously been described as a cause of bone marrow suppression in humans or animals. It is a possibility that the left shift and regenerative response seen on histopathology in dog 1 was due to regeneration of the myeloid lineage after overwhelming sepsis. Adult animals seem to develop disease from EPEC as well, although the course of disease is milder and potentially self-limiting (1,4,8–10). The clinicopathological findings, duration of hospitalization, and clinical outcome in these dogs are consistent with these reports.

Although an exhaustive diagnostic workup was not undertaken in all cases, EPEC remains a differential for clinical disease in the cases discussed. The clinical spectrum of disease potentially caused by EPEC in this case series is broad, though there was a trend towards more severe disease in animals less than 1 y of age, which is consistent with previous findings (4,8–11). We recommend including EPEC as a differential diagnosis in young animals with gastrointestinal symptoms and/or neutropenia as well as in older animals with signs of acute non-specific gastroenteritis. Polymerase chain reaction analysis for the presence of EPEC would be a useful diagnostic test in this situation. Due to the potential for zoonotic disease (5–7), diagnosis and treatment are recommended especially in households with children less than 2 y old. CVJ

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