Case Report Rapport de cas

An outbreak of *Lawsonia intracellularis* infection in a standardbred herd in Ontario

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Abstract — An outbreak of protein-losing enteropathy associated with *Lawsonia intracelluaris* infection was diagnosed in 6 standardbred foals from a farm in Ontario. Wildlife exposure may have been involved in the perpetuation of disease in this outbreak. The clinical presentation, treatment, outcomes, and pathological findings are described.

Résumé — Éclosion d'infections à *Lawsonia intracellularis* dans un troupeau de Standardbred en Ontario. Une éclosion d'entéropathies exsudatives associée à une infection à *Lawsonia intracellularis* a été diagnostiquée chez 6 poulains Standardbred d'une ferme de l'Ontario. Un contact avec la faune pourrait être impliqué dans la perpétuation de la maladie lors de cette éclosion. La présentation clinique, le traitement, l'évolution et les résultats pathologiques sont décrits.

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In December 2002–January 2003 over a 4-week period, protein-losing enteropathy (PLE) as a result of *Lawsonia intracellularis* infection was diagnosed in 6 standardbred weanlings (2 colts, 4 fillies) on a farm in southwestern Ontario. All foals were between 7 and 8 mo of age when presented to the Ontario Veterinary College Veterinary Teaching Hospital (OVC-VTH) for diarrhea and weight loss. All 6 affected foals had been born on this farm and, following weaning at 5 mo of age, had been housed outside in 2 groups according to sex. A further 18 foals on the farm remained clinically normal. No previous cases of PLE had been reported from this particular owner's herd. However, the farm on which the 6 affected foals had been raised had been purchased 2 y earlier. Six years before, in 1997, while under the management of the previous owner, PLE associated with *Lawsonia intracellularis* had been diagnosed in 3 foals from this farm at the Animal Health Laboratory, University of Guelph. Between 2000 and 2001, no horses had been on the farm for approximately 12 mo. The 6 foals in this 2002–2003 outbreak were of a different breed and were unrelated to the previous cases. The current owner's breeding herd had been moved from a farm where PLE had not been identified.

All foals had a history of diarrhea, ranging from 12 h to 14 d in duration. History and clinical signs at presentation are listed in Table 1. Two foals (Cases 1, 2) were presented with an acute onset of diarrhea. A third foal (Case 3) was treated at the farm for 7 d with supportive therapy (IV and PO fluids), then deteriorated rapidly, and died, and this foal was presented for postmortem examination to the Animal health Laboratory, University of Guelph. The following day, the 3 other sick foals (Cases 4, 5, 6) were presented to the Ontario Veterinary College. No antimicrobials had been administered to any foal prior to presentation.

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Case description

At the time of presentation, all 5 live foals were diarrheic and in poor body condition. Ventral thoracic edema was noted in cases 1, 2, and 4. Cases 1 and 4 were recumbent at the time of presentation and case 2 became recumbent within 2 h of presentation. Generalized weakness was noted in cases 5 and 6. All 5 foals were clinically dehydrated (estimated at 6% to 10%). Tachycardia (60–100 bpm) and hyperemic mucous membranes were present in all 5 foals. Case 1 was hypothermic (34.0°C). Abdominal ultrasonography was performed on cases 2, 4, 5, and 6. Abnormalities detected included increased free abdominal fluid in cases 2 and 4, and thickened small intestinal wall in cases 2, 4, 5, and 6. Small intestinal mural thickness was measured at 5–12 mm (normal < 3 mm). Thickened loops of small intestine were visualized in cases 2, 4, 5, and 6.

The primary differential diagnosis considered at presentation for all 6 cases was PLE due to *Lawsonia intracellularis* infection. This was considered in 2 cases (1 and 3) because of the signalment and clinical signs and in cases 2, 4, 5, and 6 because of the farm history in addition to the clinical signs and abdominal ultrasonographic findings. *Rhodococcus equi* infection was not considered as likely, due to the absence of typical respiratory signs.

Table 1. Clinical history of 6 standardbred foals diagnosed with protein-losing enteropathy (PLE) associated with *Lawsonia intracelluaris* infection

Case	Age (months)	Sex	Time elapsed since first case presented	Duration of signs prior to presentation	Prior treatments	Status at presentation
1	7	F	0	12 h	flunixin meglumine	recumbent, severe (10%) dehydration
2	7	F	14 d	6 h	none	weak, severe (10%) dehydration
3	8	M	28 d	7 d	electrolytes PO and IV	dead
4	8	F	28 d	2 d	none	recumbent, moderate (6–8%) dehydration
5	7	M	28 d	14 d	oral electrolytes	weak, moderate (7%) dehydration
6	8	F	28 d	14 d	oral electrolytes	weak, moderate (8%) dehydration

Table 2. Treatment summary of 6 standardbred foals diagnosed with protein-losing enteropathy (PLE) associated with *Lawsonia intracelluaris* infection

Case	Duration of hospitalization	Antimicrobial therapy	Fluid therapy	Plasma therapy	Additional therapy	Outcome
1	2 h	None	Hypertonic saline LRS	None	None	Euthanized
2	6 h	E/R	LRS	1000 mL	Flunixin meglumine	Euthanized
3	N/A	N/A	N/A	N/A	N/A	Dead on arrival
4	18 d	E/R	LRS	None	Prednisolone	Alive
5	18 d	E/R	LRS	None	Prednisolone rantidine	Alive
6	45 d	E/R chloramphenicol ceftiofur	LRS Hypertonic saline	1200 mL	Prednisolone ranitidine flunixin meglumine	Alive

N/A = not available
E/R = erythromycin phosphate (37.5 mg/kg BW, PO q12h) in combination with rifampin (10 mg/kg BW, PO q24h)
LRS = lactated Ringer's solution

Venous blood samples were collected at admission for blood-gas analysis, hematocrit, total plasma protein, and plasma electrolyte analysis, as well as for a complete blood (cell) count (CBC) and serum biochemical profile. Serum samples were submitted from cases 4, 5, and 6 for *Lawsonia intracellularis* serologic testing. On admission, fecal samples were collected for the polymerase chain reaction (PCR) for *Lawsonia intracellularis* DNA. To rule out gastrointestinal parasites feces were submitted for fecal floatation. Feces were submitted for bacteriological culture for *Salmonella* spp. and *Clostridium* spp., and for ELISA testing for *Clostridium difficile* toxins A and B.

The abnormalities in the CBC included leucocytosis (values ranged from 14.8 to 15.9 $\times$ 10^9/L; reference range, 5.1 to 11.0 $\times$ 10^9/L) in 4 cases and an elevated hematocrit (0.46; reference range, 0.28 to 0.44 L/L) in case 1. Abnormalities in the serum biochemical profile were as follows: hypoproteinemia (18 to 50 g/L; reference range, 58 to 75 g/L) and hypoalbuminemia (6 to 17 g/L; reference range, 30 to 42 g/L) in cases 2, 3, 4, 5, and 6. Hyponatremia (109 to 132 mmol/L; reference range, 136 to 144 mmol/L) was present in cases 1 and 2 and, hypochloremia (84 mmol/L; reference range 95 to 104 mmol/L) and a mild hypokalemia (3.2 mmol/L; reference range, 3.4 to 4.3 mmol/L) and hypocalcemia (1.72 to 2.3 mmol/L; reference range, 2.75 to 3.25 mmol/L). In cases 2 and 3, serum creatine kinase (CK) and aspartate aminotransferase (AST) were also elevated [5930 and 5227 U/L; (reference range, 108 to 430 U/L), 1490 and 602 U/L; (reference range, 259 to 595 U/L), respectively]. Plasma fibrinogen was elevated in all 5 foals (3.2 to 9.6 g/L; reference range, 0 to 2.5 g/L). The polymerase chain reaction (PCR) for *Lawsonia intracellularis* DNA on the fecal sample was positive in cases 1, 2, 3, and 5. The fecal flotation tests were negative and no *Clostridium difficile* toxins A and B were detected in any foal tested. Serum samples from cases 4 and 6 of the samples from cases 4, 5, and 6 submitted for *Lawsonia intracellularis* serologic testing were positive.

Blood samples were collected from all 18 other clinically normal foals on the farm. Total serum protein, serum albumin, and serum globulin levels were analyzed in all 18 foals. Hypoproteinemia was detected in 2 foals and hypoalbuminemia was present in 5 foals. None of these animals developed any clinical signs of PLE.

Following admission, IV fluid therapy was initiated in cases 1, 2, 4, 5, and 6. The course of treatment in all foals is outlined

Table 3. Results of diagnostic testing for 6 standardbred foals diagnosed with protein-losing enteropathy (PLE) associated with *Lawsonia intracelluaris* infection

Case	PCR	Serology	Gross pathology	Histopathology
1	Positive	N/A	Thickening duodenum to proximal 2/3 ileum (5–12 mm)	Argentophilic organisms in cytoplasm of enterocytes
2	Positive	N/A	Thickening bands of small intestine. Duodenum to ileum (10–14 mm)	Argentophilic organisms in cytoplasm of enterocytes
3	Positive	N/A	Thickened small intestine (up to 14 mm)	Argentophilic organisms in cytoplasm of enterocytes
4	Negative	Positive	N/A	N/A
5	Positive	Negative	N/A	N/A
6	Negative	Positive	N/A	N/A

N/A = not available

in Table 2. Due to severe hypovolemia, 1 L of intravenous hypertonic saline was administered to case 1. One liter of equine plasma was administered, IV, on admission to case 2. Additional therapy for cases 2, 4, 5, and 6 included erythromycin phosphate (Gallimycin; Bimeda-MTC, Cambridge, Ontario), 37.5 mg/kg bodyweight (BW), PO, q12h, rifampin (Rofact; Valleant Canada, Saint Laurent, Quebec), 10 mg/kg BW, PO, q12h, and flunixin meglumine (Flunazine; Vetquinol, Lavaltrie, Quebec), 1.1 mg/kg BW, IV, q12–24h. Ranitidine (Apo-ranitidine; Apotex, Etobicoke, Ontario), 6.6 mg/kg BW, PO, q8h, was administered to cases 5 and 6 as gastric ulcer prophylaxis. Prednisolone (Prednisolone suspension; The Veterinary Pharmacy, Guelph, Ontario), 30 mg, PO, q12h, was administered to cases 4, 5, and 6 in an effort to decrease intestinal inflammation. Cases 1 and 2 deteriorated rapidly and were euthanized. Ventral edema initially progressed, becoming evident in all 3 surviving foals (cases 4, 5, 6) by day 4 of hospitalization. Cases 4 and 5 gradually became stronger, and their attitude, appetite, and clinical status steadily improved during hospitalization. The vital parameters were within normal limits and total serum protein and serum albumin levels increased. These foals were discharged 18 d after admission with normal vital parameters and normal feces. Continuation of antimicrobial therapy was recommended for a further 21 d.

Five days after admission, case 6 became pyrexic (40.3°C), tachypneic (72 bpm), tachycardic (76 bpm), dehydrated, and showed moderate signs of colic. Therapy with flunixin meglumine, 1.1 mg/kg BW, IV, q12h, was repeated and butorphanol (Torbugesic; Fort Dodge, Iowa, USA), 5 mg, IV, q9h, was administered. One liter hypertonic saline and 1200 mL of hyperimmune plasma were administered, IV. Corticosteroid therapy was discontinued. A venous blood-gas analysis performed at this time showed metabolic acidosis (pH 7.2), and marked hemoconcentration (packed cell volume PCV 56 L/L). The foal improved clinically and no further signs of colic were observed. *Salmonella typhimurium* was isolated on bacteriological culture of the feces.

Antibiotic therapy was changed on day 6 from erythromycin to chloramphenicol 20 mg/kg BW, PO, q6h for 21 days, due to the persistence of pyrexia. Flunixin meglumine, 0.25 mg/kg IV, q8h, was also added and continued until day 9. During hospitalization a jugular abscess was detected on palpation and confirmed ultrasonographically. Results of microbial culture of catheter tip and fine-needle aspirate revealed an infection with *Citrobacter freundii* and *Salmonella typhimurium,* both susceptible to ceftiofur. The filly was prescribed ceftiofur, 2 mg/kg IV, q12h for 8d. The clinical status of the filly gradually improved, hematological and serum biochemistry values normalized and the filly was discharged on day 46.

Cases 1, 2, and 3 were submitted for postmortem examination. Ventral edema and ascites was noted in all 3 cases. Summary of the postmortem results are presented in Table 3. Significant abnormalities were limited to the small intestine. The mucosa of the duodenum and the ileum was greatly thickened and folded, with a nodular or ridge-like appearance. Mural thickness was measured at 5–14 mm and was similar in sections of duodenum and ileum. The mucosa of the distal part of the ileum was diffusely granular, had linear erosions, and ulcerated Peyer's patches.

On histopathologic examination the small intestinal mucosa was markedly hyperplastic with some areas of ulceration. The ileal crypts were highly prolific and comprised 2–5 layers of cells with a high rate of mitotic figures. Marked lymphoplasmacytic infiltration was present in the lamina propria. The submucosa was edematous. Numerous argentophilic organisms were detected with silver staining in all 3 cases.

The necropsy diagnosis was of acute hemorrhagic enteritis and PLE. The findings at postmortem in all 3 cases were compatible with a diagnosis of *Lawsonia intracellularis* infection. The PCR for *Lawsonia intracellularis* DNA was positive on small intestinal content in all 3 cases.

Discussion

Proliferative enteropathy (PE), an intestinal disease characterized by thickening of the small intestinal mucosa has been identified in a number of animal species, including the pig, horse, dog, ostrich, and deer (1–3). Initially identified as a cause of PE in swine (2), *Lawsonia intracellularis* has been identified as a cause of severe PLE in the foal (4–12). Most of the previous reports document individual cases (5,6–12). The prevalence of this disease within the equine population is unknown. An association with the presence of swine has not been consistently identified (4–12).

Lawsonia intracellularis is an obligate intracellular bacterium (2). In the pig, an incubation period of 2–3 wk has been estimated (13). Fecal shedding has been identified as a possible source for maintenance of the disease within the herd and the organism is capable of survival in the environment for as long as 2 wk (13). The disease is poorly understood in the horse; however, self-perpetuation within breeding farms has been proposed (6).

The typical presentation in the horse is a weanling, 4–7 mo old, with depression, weight loss, subcutaneous edema, and poor body condition (4,6). Diarrhea is a common but inconsistent finding. The disease appears to be a chronic, progressive disorder where animals are not often presented until the clinical signs are advanced. The most common clinical biochemical abnormalities are severe hypoproteinemia and hypoalbuminemia. Leucocytosis is common, but inconsistent (4,6). Elevated serum CK and AST have also been reported (6).

No sex predisposition was noted in the current group of foals. Although cases of PLE had previously been diagnosed among a group of thoroughbred foals housed on the same facilities (6), the magnitude of the present outbreak appears unique. No horses had been present on the farm for 12 mo prior to the purchase by the present owner. No relationship or contact existed between the 2 groups of animals. If the organism is capable of survival in the environment for only 2 wk, the outbreaks are unrelated or an intermediary carrier of the organism exists. Rodents and birds are considered to play only a minor role in PE transmission (10). Proliferative enteropathy attributed to *Lawsonia intracellularis* has been reported in white-tailed deer (4,6). Deer were reported to be present in moderate numbers in the area surrounding the farm presented in this report.

Antemortem diagnosis remains difficult in the horse; however, in this outbreak, 2 foals were identified by serologic testing and 3 of 5 with fecal PCR. The PCR has been proposed as a potentially sensitive and specific method for the antemortem diagnosis of PE (15,16). In experimental porcine models, however, serologic testing has proved to have higher sensitivity (19). In this report, neither test was highly sensitive. Intermittent shedding or collection of samples after treatment had been initiated may have influenced the PCR results, and the timing of sampling, immune function, or antimicrobial treatment may have influenced serology results. The clinical diagnosis was confirmed at postmortem in 3 of 5 foals tested in this current report. Serological analysis would facilitate the determination of prevalence within a herd (17). Definitive diagnosis of PE is based on the detection of the characteristic bacterium in the lamina propria (16,18–20). Special staining techniques, such as argentine (Silver) stain, are necessary.

Since all cases were presented with advanced clinical signs, blood samples were collected from all foals remaining on the farm in an attempt to identify subclinical or mild disease. A slight decrease in total serum protein was identified in 2 foals (55, 57 g/L) and mild hypoalbuminemia (25–28 g/L) in 5 foals. These animals were identified for close monitoring and were not treated. Clinical signs did not develop in any of the foals remaining on the farm.

The mode of transmission of *Lawsonia intracellularis* remains elusive, not just in horses, but also in other susceptible species. As the survival of the organism in nature is limited (13), continuous self-perpetuating infection through fecal shedding seems most likely. However, based on our findings, it is possible that wildlife have a significant role in the transmission of the disease. To define the epidemiology of the disease further, in-depth investigations of outbreaks such as this are needed. Herd dispersal precluded further investigations on the reported premises. CVJ

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