Lawsonia intracellularis proliferative enteropathy in a foal

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Summary

A weanling foal was diagnosed with proliferative enteropathy caused by *Lawsonia intracellularis* based on history, clinical findings of depression, anorexia, weight loss, colic, diarrhea, and ventral edema, and a combination of serology and fecal PCR. An epidemiological investigation on the premises revealed that many of the other foals and adult horses were seropositive for *L. intracellularis,* despite being clinically normal, and identified a dog as a potential carrier and source of infection for the foal. The foal was successfully treated with a combination of azithromycin and rifampin.

Keywords: equine proliferative enteropathy, *Lawsonia intracellularis,* foal, epidemiology

Equine proliferative Enteropathie durch *Lawsonia intracellularis*

In einem Fohlen wurde aufgrund der Vorgeschichte, der klinischen Befunde (Apathie, Anorexie, Abmagerung, Kolik, Diarrhoe und Unterbauchödem) und der diagnostischen Ergebnissen (Serologie und PCR) eine proliferative Enteropathie durch *Lawsonia intracellularis* diagnostiziert. Um den möglichen Erregerkontakt zu identifizieren, wurden Kot- und Blutproben von gesunden Tieren (Pferde, Hunde) aus der Umgebung mittels Serologie und PCR untersucht. Die Ergebnisse zeigen, dass zahlreiche Fohlen, adulte Pferde und ein Hund seropositiv für *L. intracellularis* waren. Die Bedeutung des Hundes als Übertraeger wird diskutiert. Das erkrankte Fohlen wurde mit einer Kombination von Azithromycin und Rifampin erfolgreich behandelt.

Schlüsselwörter: Equine proliferative Enteropathie, *Lawsonia intracellularis,* Fohlen, Epidemiologie

Case history

A 6-month-old Quarter Horse filly was admitted to the Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California in Davis with a 7 day history of lethargy, anorexia, mild weight loss, fever, ventral edema, mild colic, weakness, and intermittent diarrhea. The foal was observed to drink and urinate excessively during this time. Prior treatment with trimethoprim-sulfamethoxazole and long-acting ampicillin by the referring veterinarian did not result in clinical improvement. The foal had been growing well and was active, bright, and vigorous until recent illness. The foal had been administered ivermectin at 4-weeks interval since birth and had not been vaccinated. The foal was in contact with 3 other foals between 6 and 10 months old, and 13 adult horses between 3 and 26 years old. There had been no recent history of movement of horses on or off the premises and no other foals or adult horses had shown any signs of illness in the past 6 months. There were 2 pigs between 4 and 12 months of age on the premises for 10 months prior to illness in the foal. All four foals were housed together in a foaling stall with a run for 4 weeks prior to development of clinically apparent disease in the aforementioned foal. Both pigs had been previously housed in this stall for an 8 day period just 7 days prior to introduction of the foals into the stall. There were 3 cows, 2 dogs, 3 zebra and various wildlife (rabbit, deer, raccoon, skunk) on the premises.

Clinical and laboratory findings

At the time of admission the foal was lethargic, depressed, and reluctant to move. The foal was well grown with a moderate body condition score of 5/9, and weighed 203 kg. Mild pectoral, ventral abdominal, and distal limb edema was present. Physical examination revealed mild tachycardia (60 beats/min), normal rectal temperature (37.9°C) and respiratory rate (18 breaths/min). Jugular refill was normal and peripheral pulses were strong. Oral mucous membranes

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and sclera were injected, mucous membranes were dry and capillary refill time was prolonged (2–3 sec). Nasal discharge was not evident, and lung auscultation and rebreathing examination did not reveal any abnormalities. Abdominal auscultation revealed slightly increased borborygmy. A small amount of fecal staining of the tail was observed, consistent with reported historical diarrhea, although feces were formed upon presentation. Initial diagnostic investigation included hematology, serum biochemistry analysis, urinalysis, abdominocentesis, and thoracic and abdominal ultrasonography.

Hematology results revealed an inflammatory leukogram; moderate leucocytosis (26.5×10^3 WBC/μL; reference range 5.0–11.5×10^3 WBC/μL), due to mature neutrophilia (17.6×10^3 cells/μL; 2.6–6.8×10^3 cells/μL), lymphocytosis (7.9×10^3 cells/μL; 1.6-5.8×10^3 cells/μL) and monocytosis (0.7×10^3 cells/μL; 0–0.5×10^3 cells/μL). Serum biochemical analysis revealed mild hyperglycemia (121 mg/dl; 50–107 mg/dl), hyponatremia (124 mmol/L; 125–137 mmol/L), hypochloremia (84 mmol/L; 88–101 mmol/L), hypokalemia (2.1 mmol/L; 3.0–5.6 mmol/L), hypocalcemia (8.0 mg/dl; 11.9–14.7 mg/dl), hypoproteinemia (2.2 g/dl; 5.8–7.7 g/dl), hypoalbuminemia (0.8 g/dl; 2.3–3.6 g/dl), hypoglobulinemia (1.4 g/dl; 1.7–4.7 g/dl), and mild elevation in creatine kinase (440 IU/L; 119-287 IU/L). Results for ionized calcium was 0.93 mmol/L (1.44–1.77 mmol/L), confirming the presence of hypocalcemia despite hypoalbuminemia. Results of urinalysis performed from a voided sample at the time of admission, prior to initiation of fluid therapy revealed aciduria (pH 6.0; 7.0–9.0), glucosuria (2+ glucose) and isosthenuria (specific gravity 1.012). Abdominal fluid analysis revealed a transudate (protein 0.4 g/dl, total nucleated cells 600/μl).

Abdominal ultrasonographic examination revealed segmental, thickened loops of small intestine (>10mm wall thickness). Small intestinal motility was subjectively increased, and there was an excessive amount of peritoneal fluid within the abdominal cavity. Renal and hepatic ultrasound did not reveal any abnormal findings. Thoracic ultrasound was normal.

Diagnosis and treatment

Based on historical, clinical, and diagnostic findings, a diagnosis of proliferative enteropathy (PLE) was suspected. Infectious causes of PLE were further investigated. Fecal flotation, bacterial culture for *Salmonella* spp. and *Clostridium* spp., PCR for *Rhodococcus equi* and immunoassay for *Clostridium difficile* antigen and toxins A were negative. A diagnosis of *Lawsonia intracellularis* PLE was established by fecal real-time TaqMan PCR according to Herbst et al. (2003). Serologic analysis for the presence of mondayer antibody to *L. intracellularis* using immunoperoxidase assay (IMPA; Guedes et al. 2002) was negative at the time of admission.

Treatment for *L. intracellularis* PLE was initiated immediately and consisted of supportive care aimed at correction of fluid deficits, electrolyte abnormalities and hypoproteinemia, antimicrobial therapy, anti-inflammatory, and antiulcer medication. Isotonic intravenous fluid therapy was initiated using Normasol (Normasol-R; Abbott Laboratories, North Chicago, IL, USA) supplemented with 0.1 mL/kg of 23% calcium gluconate/L, 0.1 mEq/kg potassium chloride/L, and 1 mL vitamin B complex/L given at a rate of 2.5 ml/kg/hr. A total of 3 liters of hyperimmune J5 plasma (Polymune-Plus; Veterinary Dynamics, Templeton, CA, USA) were administered IV at 5 ml/kg/hr. Antimicrobial therapy was initiated using rifampin (Rifampin; Eon Labs, Lake Success, NY, USA) (5 mg/kg PO BID). Flunixin meglumine (Banamine; Schering-Plough, Union, NJ, USA) (0.5 mg/kg IV BID) and omeprazole (GastroGard; Merial, Duluth, GA, USA) (4 mg/kg PO SID) were also administered. The foal was offered free access to good quality alfalfa and grass hay, and 2 kg of complete feed twice daily. Over the following 12 hours the foal was quiet and alert, had normal vital signs, ate well and passed normal amounts of formed feces, but lay in sternal and lateral recumbency for extended periods of time. It also developed submandibular and retropharyngeal edema so feed were subsequently elevated above ground level.

Results of biochemical analysis performed on day 2 of hospitalization revealed that electrolyte abnormalities had resolved and total protein concentration had slightly increased to 2.6 g/dl. Combination antimicrobial treatment with azithromycin (Zithromax; Pfizer, New York, NY, USA) (10 mg/kg PO SID) was initiated on day 3, following observation period to assess gastrointestinal tolerance to treatment with rifampin, and continued at this dose for 5 consecutive days, then every other day for 21 days. Over the 7 day period of hospitalization the foal's appetite and attitude continued to improve. Feces remained formed and urination was normal in frequency and amount. Ventral, distal limb, and submandibular edema improved daily. Intravenous fluids and flunixin meglumine were discontinued on day 4. The foal's body weight remained stable between 200 and 203 kg during hospitalization. A CBC and serum biochemistry analysis performed on day 4 revealed persistent leucocytosis (20.6×10^3 WBC/μL), due to neutrophilia (13.1×10^3 cells/μL) and lymphocytosis (6.8×10^3 cells/μL), and mild thrombocytosis (2.6×10^3 platelets/μL; 100–225×10^3 platelets/μL). Total protein had increased (2.6 g/dl) due to increased albumin (1.0 g/dl) and globulin (1.6 g/dl) levels, although panhypoproteinemia persisted. All other biochemical parameters

had normalized. Results of a CBC performed on day 7 revealed persistent leucocytosis (20.5 $\times 10^3$ WBC/μL), neutrophilia (11.7$\times 10^3$ cells/μL), lymphocytosis (8.0$\times 10^3$ cells/μL), and monocytosis (0.6$\times 10^3$ cells/μL). Plasma protein had increased to 3.1 g/dl.
The foal was discharged on day 8 with instructions to continue antimicrobial therapy with rifampin (5 mg/kg PO BID) for a further 14 days, and azithromycin (10 mg/kg PO EID) for an additional 16 days. Recommendations for diet included free choice good quality hay high in protein and nutrients (alfalfa and alfalfa mix), complete peletted feed, and access to fresh water at all times. Additional recommendations included housing the foal in isolation from other foals and adult horses. At the time of discharge the foal was bright and alert, had an excellent appetite and attitude, was urinating and defecating normally, and peripheral edema had completely resolved. Seroconversion to *L. intracellularis* was detected on day 8 with a positive titer of 30. At 7 days following discharge from the VMTH the foal was reportedly doing well, eating well, defecating normally, gaining weight, and had a normal rectal temperature. Results of CBC and biochemical panel performed on day 14 revealed normal white cell count, and improving hypoproteinemia (4.1 g/dl). Biochemistry panel revealed persistent hypoalbuminemia (1.6 g/dl). Follow-up serologic analysis on day 58 detected a positive titer of 120.

Epidemiology

An investigation of the premises where the foal was located at the time of illness was conducted soon after hospitalization of the foal. A single, fresh fecal sample was collected from each of the 4 foals (including feces from the sick foal prior to admission to the VMTH), 10 adult horses, and 3 pregnant broodmares and analyzed by PCR for *L. intracellularis.* A single whole blood sample was collected from all 16 equids and submitted for serological analysis for detection of antibody to *L. intracellularis* by IMPA. Blood samples were also collected in EDTA tubes from the 3 foals for CBC, as well as in serum tubes for total protein, albumin and globulin determination. Fecal samples were collected from 4 cows, 3 zebra's, 2 dogs, and from the pen where the pigs were housed. Sequential fecal samples were also obtained from the 3 other foals, and two dogs, in addition to those collected from the sick foal, every 7 days for one month following return of the hospitalized foal to the premises.

All fecal samples from all other equids on the premises were negative on PCR testing for *L. intracellularis,* as were the cow, zebra, one dog, and pig fecal samples. However, feces from another dog sampled initially on day 8 were PCR positive for *L. intracellularis*. This dog was in contact with all animals on the premises and housed adjacent the foaling stall which housed the foals and pigs. Results of serology using IMP assay of all other equids on the premises revealed that 12 of the 16 samples were positive with titers ranging from 30 to 60. Two of these were from foals. Complete blood count, total protein concentration, albumin and globulin for the 3 foals on the premises were within reference ranges and foals were healthy in appearance.

Discussion

The foal described in this report demonstrated characteristic clinical findings supportive of equine proliferative enteropathy (EPE); a disease that has been previously reported in equids as sporadic, isolated cases (Duhamel and Wheeldon, 1982; Williams et al., 1996; Frank et al., 1998; Brees et al., 1999; Schumacher et al., 2000; Bihr, 2003; McClintock and Collins, 2004; Deprez et al., 2005; Sampieri et al., 2006; Wuersch et al., 2006) and in an endemic outbreak (Lavoie et al., 2000). EPE is a well described, transmissible enteric disease caused by the bacterium *Lawsonia intracellularis* (Lawson and Gebhart, 2000; Smith and Lawson, 2001). The disease primarily affects weanling foals between the ages of 3–6 months (average 5½ months) and is characterized by profound depression, fever, weight loss, colic, diarrhea and hypoproteinemia causing ventral edema. Diagnosis is based on clinical signs, the presence of leucocytosis, mild anemia, and hypoproteinemia on routine CBC and biochemistry panel, and typical abdominal ultrasound findings of loops of thickened small intestine, as was identified in the foal in this report. Definitive antemortem diagnosis requires pathogen detection from feces via PCR or biopsy specimens, or serologic testing for the presence of antibody to *L. intracellularis*. Isolation of *L. intracellularis* requires cell culture medium due to the organism's obligatory intracellular nature, and is not routinely performed.
Prevalence and method of infection of *L. intracellularis* have not been determined in horses. Of the reports of individual cases and outbreaks of PLE caused by *L. intracellularis* in foals, two describe the recent presence of pigs on the premises (Williams et al., 1996; Brees et al., 1999) and one identifies the presence of a pig farm in close proximity to foals (Lavoie et al., 2000). In the majority of reported clinical cases a direct source of infection has not been identified. A possible direct association between pigs and disease in foals caused by *L. intracellularis* has been suggested, but has not been established. The role of wild and domestic animals as reservoir hosts of *L. intracellularis* or in natural transmission to equids has not been determined. Based on findings of the investigation of the premises in this re-

port, there is evidence for exposure of horses and other animals to the organism. However, the source of infection for the clinically affected foal cannot be determined from these results.

Prognosis for recovery from EPE is good with appropriate antimicrobial therapy directed against intracellular bacteria. The majority of cases of successful treatment of affected foals report the use of erythromycin alone or in combination with rifampin for a period of 21 days (Lavoie et al., 2000). Other effective agents include penicillin, ampicillin, chloramphenicol, and doxycycline. The foal in this report did not respond to initial therapy with long-acting ampicillin, although dose and duration of therapy may not have been adequate. Azithromycin is a macrolide that has been shown to be more effective in treatment of *R. equi* in foals, has fewer side effects, and reduced frequency of administration compared with erythromycin (Giguere et al., 2004). Successful treatment of the foal in this report with the combination of azithromycin and rifampin suggests that this combination, or the use of either drug alone, can be alternative antimicrobial choices.

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