

# Case Report

## **Lawsonia intracellularis proliferative enteropathy in a weanling foal, with a tentative histological diagnosis of lymphocytic plasmacytic enteritis**

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### Summary

**This Case Report describes a weanling filly with protein-losing enteropathy associated with *Lawsonia intracellularis* infection. This was diagnosed on the basis of a significant antibody response and a positive faecal PCR result. The histopathological lesion observed in proliferative enteropathy is mucosal hyperplasia, commonly affecting the ileum and colon in foals. Duodenal biopsies obtained from this filly revealed a lymphocytic plasmacytic infiltrate. The filly recovered completely following treatment with erythromycin and no additional medication was administered to treat the lymphocytic plasmacytic infiltrate. This Case Report suggests that lymphocytic plasmacytic infiltrates observed on duodenal biopsies may represent a nonspecific intestinal immune response.**

### Introduction

Proliferative enteropathy is a transmissible enteric disease caused by *Lawsonia intracellularis*, an intracellular bacterium that localises in epithelial cells of the intestinal crypts (Lawson and Gebhart 2000). Proliferative enteropathy has been reported in numerous species, but is clinically most prevalent in the pig, and it is in this species that most research has been performed (Lawson and Gebhart 2000). Most reports of *L. intracellularis* infection in foals originate from North America and Canada (Williams *et al.* 1996; Frank *et al.* 1998; Brees *et al.* 1999; Lavoie *et al.* 2000; Schumacher *et al.* 2000a; Bihl 2003; Dauvillier *et al.* 2006; Sampieri *et al.* 2006), although isolated cases have been reported in Australia (McClintock and Collins 2004),

England (Sainty 2002), Belgium (Deprez *et al.* 2005) and Switzerland (Wuersch *et al.* 2006). Equine proliferative enteropathy most commonly causes weight loss, diarrhoea, colic and hypoproteinaemia in weanling foals (Lavoie *et al.* 2000). This report describes a filly with protein-losing enteropathy associated with *L. intracellularis*, in which 'lymphocytic-plasmacytic enteritis' was observed on duodenal biopsies.

### Case history and clinical findings

An 8-month-old Thoroughbred-cross filly was referred because of a sudden onset of inappetence and depression of one week's duration. The filly had been weaned 4 weeks prior to the onset of clinical signs. Clinical examination revealed her to be in reasonable body condition, weighing 299 kg. She was quiet, spent increased time in recumbency and bruxism was heard. The filly showed complete disinterest in feed and had negligible faecal output. Temperature, pulse and respiratory rate were within normal limits. Mucous membranes appeared slightly congested and capillary refill time was 3 s. A plaque of nonpainful pitting oedema was present on the ventral thorax.

Haematological examination revealed a mild inflammatory response (white blood cell count  $12.9 \times 10^9/l$ , reference range [rr]  $5.00\text{--}7.80 \times 10^9/l$ , and an elevated fibrinogen 5.0 g/l, rr 1.0–4.0 g/l). Biochemistry revealed severe panhypoproteinaemia; total serum protein 21.2 g/l (rr 70.0–80.0 g/l), albumin 12.2 g/l (rr 35.0–42.0 g/l) and globulin 9.0 g/l (rr 25.0–50.0 g/l). Serum urea concentration was increased (8.4 mmol/l, rr 3.2–5.2 mmol/l), whilst creatinine was normal (130  $\mu\text{mol/l}$ , rr 125–190  $\mu\text{mol/l}$ ). All other clinico-pathological parameters examined were within reference ranges.

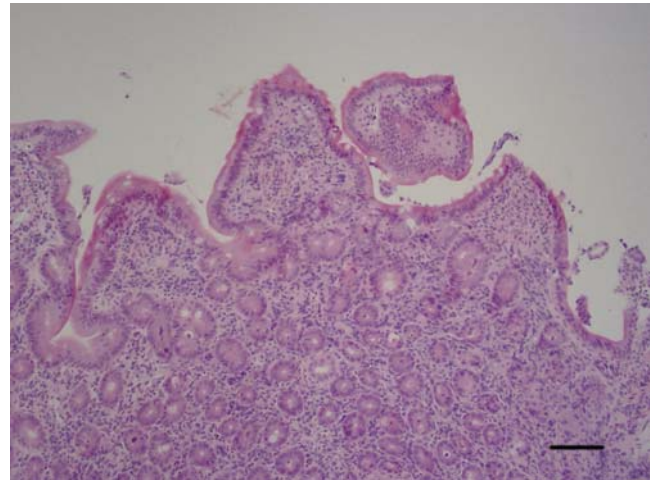
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Thoracic ultrasonography revealed no abnormality. Transabdominal ultrasonographic examination revealed multiple small intestinal segments with significant thickening of the intestinal wall, with some sections measuring up to 0.55 cm (normal range  $\leq 0.3$  cm; Reef 1998) (MyLab 30)<sup>1</sup>. A normal volume of anechoic peritoneal fluid was present and analysis of a sample revealed a normal total nucleated cell count, cytological profile and protein level. Rectal examination was unremarkable and urine analysis was within normal limits.

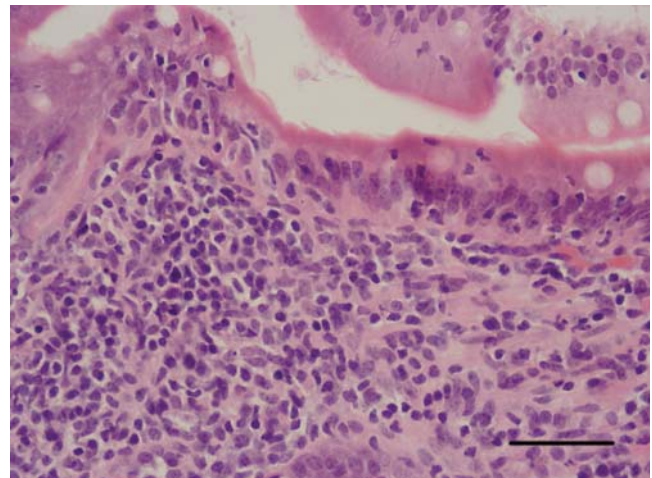
A tentative diagnosis of protein-losing enteropathy was made. Differential diagnoses included parasitism, inflammatory or neoplastic intestinal disease, proliferative enteropathy and gastrointestinal ulceration. Other infectious causes of enteritis were considered, but since the foal did not suffer from diarrhoea, these were not pursued. A faecal worm egg count was negative, and cyathostome larvae were not detected on a wet faecal smear. Gastroscopy<sup>2</sup> was performed and no evidence of ulceration was observed in the squamous or glandular mucosae, and normal pyloric motility was apparent. A faecal sample was submitted for routine extraction of DNA and reaction with *L. intracellularis*-specific oligo-primers in a polymerase chain reaction (PCR) analysis as described by Knittel *et al.* (1998) and performed commercially<sup>3</sup>. A serum sample was tested in serial dilutions in the indirect immunofluorescence assay (IFAT) incorporating whole *L. intracellularis* antigen, as described by Knittel *et al.* (1998), excepting that anti-equine IgG fluorescein conjugate was used as the second stage antibody. Known negative and positive equine serum samples were included in each batch of assays. In this assay, serum dilutions showing reactions to *L. intracellularis* antigen at dilutions above 1:30 are considered positive in all affected species (Knittel *et al.* 1998; Lavoie *et al.* 2000).

The filly was initially treated with 2 l of hyperimmune plasma<sup>4</sup> and 3 l of hetastarch<sup>5</sup> by slow i.v. infusion. Total protein levels increased to 24.8 g/l the following day. Feed was withheld overnight and an oral glucose tolerance test (OGTT) was performed the following day (Taylor *et al.* 1997). Glucose<sup>6</sup> was administered at 1 g/kg bwt in a 20% solution by nasogastric tube. The glucose value at 120 min showed only a 2.3% increase over the basal level. The peak glucose value occurred at 180 min and showed a 14.2% increase over the basal level.

A rectal biopsy was obtained (Traver and Thacker 1979), under light, standing sedation (0.5 mg/kg bwt xylazine i.v.). Subsequently, 3 transendoscopic<sup>2</sup> duodenal mucosal pinch biopsies were collected from different areas of the proximal duodenum (Brown *et al.* 1985) and submitted for histopathological examination. Biopsies were fixed immediately in 10% neutral buffered formalin, processed routinely, sectioned at 4  $\mu$ m and stained with haematoxylin and eosin. The duodenal biopsy was also stained by the Warthin-Starry technique. Whilst awaiting the results of the biopsy samples, faecal PCR and serology, treatment was commenced with oral erythromycin



**Fig 1:** Duodenal biopsy, weanling foal. Villous stunting and distortion is seen in a slightly tangential section. H&E, x10 magnification, bar 100  $\mu$ m.



**Fig 2:** Duodenal biopsy, weanling foal. Increased numbers of mixed inflammatory cells are present within the lamina propria. H&E, x20 magnification, bar 100  $\mu$ m.

ethylsuccinate at 25 mg/kg bwt 3 times daily. After 4 days of treatment the filly responded to therapy, on the basis of a markedly improved appetite and demeanour.

Microscopic examination of the rectal mucosa was unremarkable, but the duodenal biopsies revealed some villous stunting (**Fig 1**) and a moderate cellular infiltrate of the mucosa. At higher magnification the infiltrate was comprised of mixed mononuclear inflammatory cells, mainly lymphocytes and plasma cells (**Fig 2**), and a tentative morphological diagnosis of 'lymphocytic plasmacytic enteritis' was made. Organisms were not identified in sections stained by Warthin-Starry.

A positive result for faecal *L. intracellularis* DNA was obtained by PCR. The serum assay for the presence of equine IgG antibodies to *L. intracellularis* antigens was positive at a dilution up to 1:400.

The ventral oedema resolved within 7 days of initiation of antibiotic treatment and the filly was discharged after

14 days. At that time the total protein had increased to 33.8 g/l (albumin 19.1 g/l, globulin 14.7 g/l), but the fibrinogen and white blood cell count had only decreased marginally to 4.8 g/l and  $11.5 \times 10^9/l$ , respectively. Oral erythromycin ethylsuccinate was continued for a further 3 weeks. The white blood cell count continued to fall concurrently with a rise in total protein. A subsequent indirect fluorescent antibody assay for *L. intracellularis* was performed 3 weeks after the first determination and was positive at a dilution up to 1:200. Two weeks following cessation of therapy the weanling was reported to be bright, with a good appetite. At this time haematological and serum biochemical parameters were within reference ranges. One year following cessation of treatment, the owner reported that the filly was in good bodily condition with a normal weight.

## Discussion

This report describes a case of protein-losing enteropathy associated with *L. intracellularis* infection, identified on the basis of a significant antibody response and a positive faecal PCR result. The specificity and sensitivity for the serum IFAT assay used here are both considered over 90% (Knittel *et al.* 1998; Dauvillier *et al.* 2006). Proliferative enteropathy has been diagnosed using this assay in suspected cases in foals with titres of 1:30 (Lavoie *et al.* 2000) to 1:960 (McClintock and Collins 2004). The initial titre in this case was 1:400 indicating a strong positive reaction. As with many enteric infections, the sensitivity of faecal PCR for equine *L. intracellularis* is affected by variability in shedding and components of faeces that are inhibitory for PCR (Frank *et al.* 1998; Wuersch *et al.* 2006). PCR testing of faeces for *L. intracellularis* has a very high specificity (Lawson and Gebhart 2000), therefore the positive result in this case, further confirms the diagnosis.

The most common clinical signs reported with *L. intracellularis* infection are weight loss, diarrhoea, colic and hypoproteinaemia (Lavoie *et al.* 2000). As in the case presented here, Bihl (2003) described a filly with lethargy, anorexia and ventral oedema. The dramatic weight loss and diarrhoea common in other reports (Lavoie *et al.* 2000) was not observed in the case described above (Bihl 2003), or the present report. The diagnosis of small intestinal malabsorption was suggested by the OGTT. Proliferative enterocytes are immature (Lavoie and Drolet 2007) and therefore are unlikely to have normal function. However, to the authors' knowledge, only 3 reports of oral glucose absorption tests (Lavoie *et al.* 2000) and one report of a xylose absorption test (Bihl 2003) in cases of proliferative enteropathy are reported and normal absorption curves were present in all 4 cases. It is unclear why the present case had profound malabsorption, which has not been described previously. However, if the lymphocytic-plasmacytic infiltrate observed in the duodenal biopsies was present

diffusely along the small intestine, this may have accounted for the malabsorption.

Consistent with a previous report, transendoscopic biopsy of the small intestine offers a relatively noninvasive method of evaluating horses for intestinal inflammation (Divers *et al.* 2006). Although *L. intracellularis* infections more commonly occur in the ileum and colon in foals, duodenal biopsies have been suggested, as some reports indicate that the proximal small intestine can be involved (Lavoie and Drolet 2003). The histopathological lesions observed in proliferative enteropathy range from multifocal to confluent regions of mucosal hyperplasia (Bryant 2006). The affected crypts are elongated, the epithelial cells may be immature and there is often an absence of goblet cells (Lavoie and Drolet 2007). Inflammatory cell infiltrates are not typical of proliferative enteropathy in pigs (McOrist and Gebhart 2006); however, mononuclear inflammation may be present secondarily in some equine cases (Williams *et al.* 1996; Brees *et al.* 1999; Lavoie and Drolet 2007). It is likely that the available sampling sites (duodenum and rectum) may have missed the specific histological lesions associated with *L. intracellularis* in this case. It is also unknown whether the mononuclear inflammatory infiltrate was localised to the duodenum or may have been present diffusely through the intestinal tract.

The finding of a mononuclear inflammatory cell infiltrate, which was predominantly lymphoplasmacytic led to the tentative morphological diagnosis of 'lymphocytic plasmacytic enteritis'. Lymphocytic-plasmacytic enteritis is characterised by mucosal infiltration of lymphocytes and plasma cells in the absence of granulomatous change (MacAllister *et al.* 1990; Kemper *et al.* 2000). In dogs, the presence of lymphocytes and plasma cells probably represents a common immunological response to a variety of antigens present in the gut lumen (Fogle and Bissett 2007). This Case Report suggests that the same may be true for equine species, whereby a lymphocytic plasmacytic infiltrate represents a nonspecific intestinal immune response. Such an inflammatory infiltrate is a common finding in duodenal biopsies from horses with a variety of manifestations of chronic enteropathies (Brazil 2009).

Diffuse lymphocytic plasmacytic enteritis has been reported to carry a poor prognosis in the horse and all horses in 2 published case series died or were subjected to euthanasia (Kemper *et al.* 2000; Schumacher *et al.* 2000b). However, the present case and that reported by Divers *et al.* (2006) both had a mononuclear cell infiltrate observed in duodenal biopsies and both responded to different medical treatments. Erythromycin, with or without rifampicin, is the antimicrobial most frequently described in the treatment of *L. intracellularis* (Lavoie *et al.* 2000; Bihl 2003; McClintock and Collins 2004), and a rapid response to treatment is often observed (Lavoie *et al.* 2000), as in the present case. No additional medication was administered to treat the lymphocytic

plasmacytic enteritis, which was probably secondary to the *L. intracellularis* infection although typical histological changes or organisms were not identified at this site. Therefore, horses should not necessarily be subjected to euthanasia on the basis of lymphocytic plasmacytic enteritis identified in duodenal biopsies.

In conclusion, the finding of lymphocytic plasmacytic enteritis in duodenal biopsies in the present case was associated with evidence of *L. intracellularis* infection. Lymphoplasmacytic infiltration is a description of changes present in the mucosa and in the present case, at least, may have been a secondary process. Further work to characterise inflammatory infiltrates in the horse intestine are required to more fully predict the possible clinical outcomes.

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## Manufacturers' addresses

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<sup>3</sup>SAC Veterinary Services, Penicuik, Midlothian, UK.

<sup>4</sup>Veterinary Immunogenics, Penrith, Cumbria, UK.

<sup>5</sup>Baxter, Thetford, Norfolk, UK.

<sup>6</sup>Battle, Hayward and Bower Ltd, Lincoln, UK.

## References

- Buhr, T.P. (2003) Protein-losing enteropathy caused by *Lawsonia intracellularis* in a weanling foal. *Can. vet. J.* **44**, 65-66.
- Brazil, T.J. (2009) Value of duodenal biopsies in the investigation of weight loss and chronic enteropathy in the horse. Abstract presentation. In: *Proceedings of the Third European College of Equine Internal Medicine*, *J. vet. intern. Med.* **23**, 420-439.
- Brees, D.J., Sondhoff, A.H., Kluge, J.P., Andreasen, C.B. and Brown, C.M. (1999) *Lawsonia intracellularis*-like organism infection in a miniature foal. *J. Am. vet. med. Ass.* **215**, 511-514.
- Brown, C.M., Slocombe, R.F. and Derksen, F.J. (1985) Fiberoptic gastroduodenoscopy in the horse. *J. Am. vet. med. Ass.* **186**, 965-968.
- Bryant, U.K. (2006) Proliferative enteropathy in horses. In: *Equine Disease Quarterly*, University of Kentucky. **15**, 5-6.
- Dauvillier, J., Picandet, V., Harel, J., Gottschalk, M., Desrosiers, R., Jean, D. and Lavoie, J.P. (2006) Diagnostic and epidemiological features of *Lawsonia intracellularis* enteropathy in 2 foals. *Can. vet. J.* **47**, 689-691.
- Deprez, P., Chiers, K., Gebhart, C.J., Ducatelle, R., Lefère, L., Vanschandevijl, K. and van Loon, G. (2005) *Lawsonia intracellularis* infection in a 12-month-old colt in Belgium. *Vet. Rec.* **157**, 774-776.
- Divers, T.J., Pelligrini-Masini, A. and McDonough, S. (2006) Diagnosis of inflammatory bowel disease in a Hackney pony by gastroduodenal endoscopy and biopsy and successful treatment with corticosteroids. *Equine vet. Educ.* **18**, 284-287.
- Fogle, J.E. and Bissett, S.A. (2007) Mucosal immunity and chronic idiopathic enteropathies in dogs. *Comp. cont. Educ. pract. Vet.* **29**, 290-301.
- Frank, N., Fishman, C.E., Gebhart, C.J. and Levy, M. (1998) *Lawsonia intracellularis* proliferative enteropathy in a weanling foal. *Equine vet. J.* **30**, 549-552.
- Kemper, D.L., Perkins, G.A., Schumacher, J., Edwards, J.F., Valentine, B.A., Divers, T.J. and Cohen, N.D. (2000) Equine lymphocytic-plasmacytic enterocolitis: a retrospective study of 14 cases. *Equine vet. J., Suppl.* **32**, 108-112.
- Knittel, J.P., Jordan, D.M., Schwartz, K.J., Janke, B.H., Roof, M.B., McOrist, S. and Harris, D.L. (1998) Evaluation of *antemortem* polymerase chain reaction and serologic methods for detection of *Lawsonia intracellularis*-exposed pigs. *Am. J. vet. Res.* **59**, 722-726.
- Lavoie, J.P., Drolet, R., Parsons, D., Leguillette, R., Sauvageau, R., Shapiro, J., Houle, L. and Gebhart, C.J. (2000) Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinaemia in foals on three breeding farms in Canada. *Equine vet. J.* **32**, 418-425.
- Lavoie, J.P. and Drolet, R. (2003) *Lawsonia intracellularis* proliferative enteropathy. In: *Current Therapy in Equine Medicine 5*, Ed: N.E. Robinson, W.B. Saunders, Philadelphia. pp 164-166.
- Lavoie, J.P. and Drolet, R. (2007) *Lawsonia intracellularis*. In: *Equine Infectious Diseases*, Eds: D.C. Sellon and M.T. Long, W.B. Saunders, St Louis. pp 313-316.
- Lawson, G.H.K. and Gebhart, C.J. (2000) Proliferative enteropathy. *J. Comp. Path.* **122**, 77-100.
- MacAllister, C.G., Mosier, D., Qualls, C.W. and Cowell, R.L. (1990) Lymphocytic/plasmacytic enteritis in two horses. *J. Am. vet. med. Ass.* **196**, 1995-1998.
- McClintock, S.A. and Collins, A.M. (2004) *Lawsonia intracellularis* proliferative enteropathy in a weanling foal in Australia. *Aust. vet. J.* **82**, 750-752.
- McOrist, S. and Gebhart, C.J. (2006) Proliferative enteropathies. In: *Diseases of Swine*, 9th edn., Eds: B.E. Straw, J. Zimmerman, S. D'Allaire and D.J. Taylor, Iowa State University Press, Ames. pp 727-737.
- Reef, V. (1998) Adult abdominal ultrasonography. In: *Equine Diagnostic Ultrasound*, Ed: V. Reef, W.B. Saunders, Philadelphia. pp 273-363.
- Sainty, T.J. (2002) Proliferative enteritis caused by *Lawsonia intracellularis* infection. In: *Proceedings of the 41st British Equine Veterinary Association Congress*, Equine Veterinary Journal Ltd, Newmarket. p 48.
- Sampieri, F., Hinchcliff, K.W. and Toribio, R.E. (2006) Tetracycline therapy of *Lawsonia intracellularis* enteropathy in foals. *Equine vet. J.* **38**, 89-92.
- Schumacher, J., Schumacher, J., Rolsma, M., Brock, K.V. and Gebhart, C.J. (2000a) Surgical and medical treatment of an Arabian filly with proliferative enteropathy caused by *Lawsonia intracellularis*. *J. vet. intern. Med.* **14**, 630-632.
- Schumacher, J., Edwards, J.F. and Cohen, N.D. (2000b) Chronic inflammatory bowel diseases of the horse. *J. vet. intern. Med.* **14**, 258-265.
- Taylor, F.G.R., Hillyer, M.H. and Edwards, G.B. (1997) Alimentary diseases. In: *Diagnostic Techniques in Equine Medicine*, Eds: F.G.R. Taylor and M.H. Hillyer, W.B. Saunders, London. pp 19-64.
- Traver, D.S. and Thacker, H.L. (1979) Malabsorption syndromes in the horse: Use of a rectal biopsy in differential diagnosis. *Proc. Am. Ass. equine Practns.* **24**, 487-498.
- Williams, N.M., Harrison, L.R. and Gebhart, C.J. (1996) Proliferative enteropathy in a foal caused by *Lawsonia intracellularis*-like bacterium. *J. vet. diag. Invest.* **8**, 254-256.
- Wuersch, K., Huessy, D., Koch, C. and Oevermann, A. (2006) *Lawsonia intracellularis* proliferative enteropathy in a filly. *J. vet. Med. A* **53**, 17-21.