Short communication

Typhlocolitis in a horse infected with *Listeria monocytogenes*

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INTRODUCTION

*Listeria monocytogenes* (*L. monocytogenes*) causes clinical disease primarily in ruminants, although disease also occurs in monogastric animals, birds and humans (Clark et al. 2004, Holzer et al. 2012).

The intestine is the primary port of entry for the bacterium, but the clinical manifestation of the infection is variable, including septicaemia, meningitis/meningoencephalitis and abortion (Rütten et al. 2006, Hoelzer et al. 2012). *L. monocytogenes* is widely distributed in the environment and has been isolated from clinically healthy monogastric mammals (Clark et al. 2004, Hoelzer et al. 2012). Large numbers of the bacteria, sometimes exceeding 10⁶ colony forming units/g faeces, were however found in the faeces of severely ill horses in Iceland (Gudmundsdóttir et al. 2004). In horses, as in other animals, *L. monocytogenes* causes mainly abortion, septicaemia and less frequently encephalitis (Rütten et al. 2006, Warner et al. 2012). Although a disease ascribed to alimentary listeriosis has long been recognized in horses in Iceland, little study has been done on the disease and a description of the post-mortem findings has been lacking. This paper gives an account of alimentary listeriosis in a herd of horses and describes the post-mortem findings in one of the affected individuals, a 6-month-old filly with acute typhlocolitis.

MATERIALS AND METHODS

Case history

Early in January 2012, three 6-month-old foals and a 20-year-old mare were found dead in a paddock. In addition, a 6-month-old filly was found off feed and depressed, but the filly died within one hour, before treatment could be initiated. Later that same day a three-year-old horse was found to be severely ill; he was depressed, and had tachycardia, fever and hyperaemic conjunctiva. There were no signs of diarrhoea or colic. The animal was transported to a stable where it was attended to by a veterinarian. It died in the night despite treatment.

These horses belonged to a group of 30 that had been in a small paddock for two days. The weather was unusually cold (-15°C) with snow covering the ground, limiting access to pasture and water. The day before the onset of illness, the horses had received two big bales of grass haylage for the first time that winter.

Post mortem examination

The untreated 6-month-old filly, which died 30 hours after receiving the grass haylage, underwent a full post-mortem examination at the Institute for Experimental Pathology, Keldur. The post-mortem interval was approximately 36 hours. Samples of the lungs, heart, liver, kidney, spleen, lymph nodes, a. mesenterica cranialis, ileum, caecum and colon were fixed, routinely processed and sections stained with H&E.
Immunohistochemistry for *L. monocytogenes* was performed on sections of the intestine, lung and spleen, using a commercial anti-*Listeria* antibody (*Listeria* O antiseraum poly serotype 1 and 4, Difco Laboratories) (Fairley et al. 2013).

**Bacteriological examination**

Samples were taken for routine diagnostic bacteriology and Salmonella culture. Routine diagnostic bacteriology was performed on samples from the small intestine, colon and brain by direct plating onto sheep blood agar plates and SSI enteric medium and incubation under aerobic and anaerobic conditions at 37°C (Quinn et al. 1994).

Salmonella culture was performed on samples from the small intestine, colon and spleen by pre-enrichment in Buffered Peptone Water at 37°C, selective enrichment in Rappaport Vassiliadis Soya Broth at 42°C, plating onto selective agar plates and incubation under aerobic conditions at 37°C (NMKL 71, 5th ed. 1999).

**RESULTS**

Post-mortem examination showed a filly in good body condition. A few dL of serosanguinous fluid were found in the thoracic and abdominal cavities and a few mL of similar fluid in the pericardial sac. The trachea contained froth and the lungs were oedematous. The stomach content was watery. In the small intestine and caecum, the content was watery and greenish, whereas it became slightly more consolidated in the proximal colon. The contents were normal in the distal colon and rectum. The mucosa of the caecum and the right ventral colon was matted and greenish-grey. There were small areas of haemorrhage in the serosa and mucosa, and moderate oedema in the submucosa (Figure 1). A few *Parascaris equorum* worms were found in the stomach and in the small intestine. There was inflammation of the cranial mesenteric artery with one visible *Strongylus vulgaris* larva.

Histological examination showed moderate to severe autolysis, with proliferation of large bacterial rods in the internal organs. The histopathological changes were primarily in the caecum, right ventral colon, and mesenteric lymph nodes, and in the cranial mesenteric artery. Thrombi were seen in vessels of the intestinal mucosa. There was acute inflammation and necrosis of the mucosa and upper submucosa, including the muscularis mucosa, with numerous intralresional small bacterial rods. The inflammatory response was moderate with infiltration of mainly neutrophils and macrophages, and a few eosinophils. The inflammation was often associated with the isolated lymphoid follicles, both in the domes and in the interfollicular areas (Figures 2 and 3). A few nematode larvae, compatible with cyathostomes, were present in the superficial mucosa of the caecum. The mesenteric lymph nodes had scattered, subcapsular inflammatory foci. There was an extensive inflammation in the wall of the cranial mesenteric artery, with a thrombus containing a *strongylus vulgaris* larva. In the lungs, liver and spleen, there were clusters of small bacterial rods, but no inflammatory reaction. No bacteria or inflammatory reactions were seen in the brain.

Immunohistochemical staining of the caecum, proximal colon and mesenteric lymph nodes with *Listeria* antibody gave positive results (Figure 4). The clusters of small bacterial rods in the liver and spleen were also positive for *Listeria*. Immunohistochemical staining was not performed on the brain.

Bacteriological culture resulted in isolation of almost pure culture of *L. monocytogenes* from the spleen, sparse growth of mixed bacteria, including *L. monocytogenes* from the brain, and mixed bacterial culture, primarily *E. coli*, from the intestine. Salmonella culture was negative.

The post-mortem diagnosis was a necrotizing typhlocolitis and a terminal septicaemia caused by *L. monocytogenes* infection.

**DISCUSSION**

This paper describes a case of alimentary listeriosis in a foal, with a detailed description of the pathological findings. Histopathological examination revealed a severe, necrotizing typhlocol-
itis with intralesional *L. monocytogenes* bacteria. The location and type of inflammation in the present case resembled lesions associated with enteric salmonellosis. *Salmonella* infection was first suspected in the present case, and the small and large intestine were therefore specifically cultured for that bacterium and not for *Listeria*, hence the negative microbiological results for *Listeria*.

Although a gastroenteric disease ascribed to *L. monocytogenes* infection has been recognized for many years in Iceland, earlier descriptions of the pathological findings are lacking. The histopathological lesions in the large intestine in the present case were similar to the findings in a young foal with neonatal *Listeria* septicaemia (Warner et al. 2012), but unlike another recently described case of alimentary listeriosis in a 15-year-old American Quarter horse (Nemeth et al. 2013). In the present case, typhlocolitis was the primary lesion with a peracute septicaemia being a secondary event as there were no inflammatory reactions to the *Listeria* bacteria in other internal organs. The recent case in the American Quarter horse was multifactorial, including infection with both *Listeria* and *Salmonella*, and the inflammatory response was granulomatous (Nemeth et al. 2013), a finding not seen in the present case.

While it is possible that the verminous arteritis of the cranial mesenteric artery was a con-
tributing factor in the typhlocolitis in the filly, the histopathological findings were notable in that the inflammation and the necrosis involved the muscularis mucosa as well as the mucosa and submucosa, and that the bacteria were associated with the inflammatory lesions, as demonstrated with immunohistochemistry (Figures 3 and 4). Similar orientation of the inflammatory response to the muscularis mucosae of the gastrointestinal tract has been described in sheep with alimentary listeriosis (Clark et al. 2004, Otter et al. 2004, Fairley et al. 2012) and recently also in a calf (Fairley et al. 2013). But as in the calf, the involvement of the muscularis mucosa was not as conspicuous in the horse in the present paper as in the case in sheep.

A common practice in Iceland is to feed horses with grass haylage during the winter and Listeria infections in horses most likely stem from this source (Gudmundsdottir et al. 2004, Rütten et al. 2006). The rest of the bales of grass haylage in the present case were removed and destroyed, and no further evidence of disease was seen in the herd.

In Iceland, alimentary listeriosis has been described to affect only one or a few horses in a herd and the disease has often been more severe in foals and young horses. It has been associated with febrile gastroenteritis and septicaemia, often with inappetence, depression and high mortality (Gudmundsdottir et al. 2004). While diarrhoea has been a common clinical sign of Icelandic horses with alimentary listeriosis (Gudmundsdottir et al. 2004), none of the six horses that died in the current outbreak showed evidence of this, possibly due to the peracute nature of the infection.

The disease ascribed to alimentary listeriosis has long been recognized in horses in Iceland, presumably due to the practice of feeding grass haylage during the winter months. With refinement in the production of haylage, reports on suspected listeriosis in horses have become rare, but not extinct. This paper gives the first description of the pathological findings in an Icelandic horse with a peracute form of alimentary listeriosis.

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REFERENCES
Fairley RA, Pesavento PA, Clark RG 2012. Listeria monocytogenes infection of the alimentary tract (enteric listeriosis) of sheep in New Zealand. Journal of Comparative Pathology 146, 308-313.

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