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INFECTIOUS DISEASE

Fatal Peritoneal Migration of *Strongylus edentatus* in a Foal

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Summary

A 7-month-old female mixed breed foal with a 2-day history of recumbency and inability to open its mouth convulsed acutely and died and was submitted for necropsy examination. The foal was thin and large patches of haemorrhage were present throughout the peritoneal wall, the diaphragmatic surfaces and the retroperitoneum. Numerous nematode larvae were visible on the serosal surfaces and penetrated and embedded into the subserosa associated with the haemorrhages. The dorsal portion of the abdominal diaphragm had a partial tear and large numbers of nematodes were within the muscle fibres. Histologically, the larvae had a smooth cuticle, polymyarian/coelomyarian musculature and multinucleated intestinal cells, and were typically surrounded by haemorrhage, neutrophils, dense fibrovascular connective tissue and rare multinucleated giant cells. Parasitological examination identified the larvae as *Strongylus edentatus* based on the morphology of the buccal capsule. Additionally, there was severe muscle necrosis of the tongue and liver tissue analysis detected selenium deficiency. *S. edentatus* infections are uncommon in California, USA, and are typically non-lethal. In this case, the selenium deficiency may have led to immunosuppression, resulting in the hyperinfection with *S. edentatus*, and to the muscle damage and tear of the diaphragm. Although ivermectin treatment was indicated in the history, inadequate deworming or anthelmintic resistance may have played a role in the severity of infection.

Keywords: horse; large strongyles; larval migration; *Strongylus edentatus*

Equine strongyles (family Strongylidae) are the most common and clinically relevant nematodes infecting horses worldwide (McCraw and Slocombe, 1974; Farrar and Klei, 1985; Reinemeyer and Nielsen, 2009; Khan *et al*., 2015; Uzal *et al*., 2016). They are generally classified into two subfamilies: Strongylinae, or large strongyles, of which *Strongylus vulgaris*, *Strongylus equinus* and *Stongylus edentatus* are the most clinically significant, and the subfamily Cyathostominae, or small strongyles, which includes more than 50 different species (Studzińska *et al*., 2012; Khan *et al*., 2015; Uzal *et al*., 2016; Pfister and van Doorn, 2018). Among the large strongyles, *S. vulgaris* has been investigated most extensively and is recognized as the most pathogenic nematode in horses due to its ability to induce verminous thromboembolism and arteritis, primarily in the cranial mesenteric artery (McCraw and Slocombe, 1976; Reinemeyer and Nielsen, 2009). *S. equinus* is less prevalent than the two other large strongyles (Slocombe and McCraw, 1973; Uzal *et al*., 2016). Natural *S. edentatus*-induced pathology is observed rarely (Bell *et al*., 2016), due in part to the extensive use of anthelmintic drugs (Reinemeyer and Nielsen, 2009). Anthelmintic resistance among strongyle nematodes is recognized increasingly, but in horses currently appears to be limited to small strongyles. Resistance to anthelmintics has not been implicated in the large strongyle species and available deworming medications are still considered effective for controlling large strongyle infections (Matthews, 2014).

S. edentatus-related pathology has been described following experimental infections in ponies, yet only

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a few reports of natural infections have been published. McCraw and Slocombe (1974, 1978) described the early and late non-lethal lesions caused by the migration of *S. edentatus* in ponies infected experimentally for 72 weeks. Moreover, three donkeys developed enteritis, inappetence and moribund status after being infected with different larval challenges (Wetzel and Kersten, 1956). Descriptions of pathology associated with natural *S. edentatus* infection include abdominal abscessation from larval migration (Bell *et al.*, 2016), eosinophilic gastroenteritis with colonic intralesional nematodes (Cohen *et al.*, 1992) and periocular cellulitis with subconjunctival phlegmon due to aberrant ocular migration of larvae (Walde and Prosl, 1976). Here we describe a fatal case of *S. edentatus* infection in a foal with poor nutrition and selenium deficiency.

A 7-month-old female foal raised on pasture presented with weight loss and a 2-day history of recumbency, inappetence and inability to open its mouth or chew. The foal remained able to drink water. Haematological examination did not show any abnormalities; the foal was normothermic and did not respond to treatment with non-steroidal anti-inflammatory drugs (Banamine®, Schering-Plough Animal Health, Kenilworth, New Jersey, USA; 50 mg/ml for 2 days, intravenously). Vaccinations for eastern and western encephalomyelitis viruses, equine influenza virus and tetanus had been administered 3 months previously, and the foal was reportedly previously dewormed with ivermectin (unknown doses, manufacturer and administration details), but the date was not noted. On the day of presentation, the foal suddenly seizured and died and the carcass was submitted for post-mortem examination to the California Animal Health and Food Safety Laboratory System, Davis, California, USA.

On necropsy examination, the foal had a very thin body condition with a small amount of fat stores in the coronary groove and the retroperitoneal region. In the abdomen, extensive haemorrhages were observed throughout the peritoneal wall, the retroperitoneum and the dorsal diaphragm. The dorsal abdominal diaphragm was stretched with an approximately 9 cm long tear in the serosa and a partial tear of the muscle fibres with haemorrhage and oedema. Numerous 1–1.5 cm long and 0.2 cm wide pale brown–yellow nematode larvae were observed on the torn surface of the diaphragm (Fig. 1), on serosal surfaces and many were embedded in the subserosa of the peritoneal wall and retroperitoneal regions where haemorrhages were also present. Larvae were often superficially or deeply penetrated and embedded in the exposed diaphragmatic muscles (Supplementary Figs. 1 and 2) and the renal fascia within the perirenal

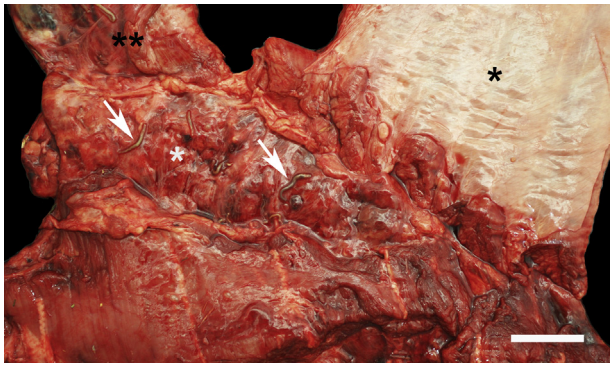

Fig. 1. Tear of the dorsal portion of the abdominal diaphragm (white asterisk) with multiple *Strongylus edentatus* larvae migrating (white arrows). Note the intact central portion of the diaphragm (black asterisk) and the damaged renal fascia and perirenal fat (double black asterisk). Bar, 2 cm.

fat and renal capsule (Supplementary Fig. 3), as well as within the peritoneal haemorrhages at the level of the abdominal (Supplementary Fig. 4) and hypaxial (Fig. 2) muscles.

The large colon and caecum contained small amounts of dark green, thick digesta. Two morphologically distinct nematodes, both thinner and shorter compared with the migrating larvae, were noted within the pasty green contents of the caecum and large colon. The dorsum of the tongue was covered by a diffuse white and gritty plaque and, on cross-section, the muscle was diffusely tan–white in colour. The right caudal lung lobe was focally extensively consolidated. Samples of areas with haemorrhages, including the diaphragm and the parietal peritoneum, as well as the major organs, were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Several nematodes embedded in the diaphragm and peritoneum were

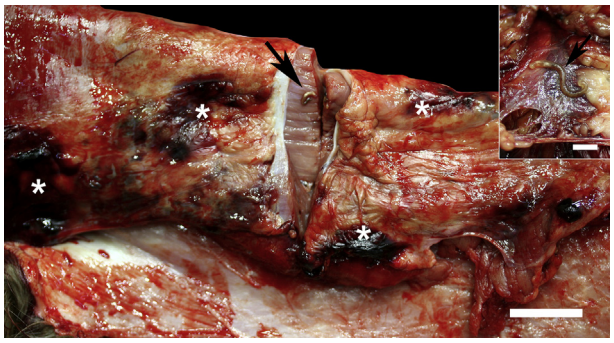

Fig. 2. Multifocal subperitoneal haemorrhages (white asterisks) and a *S. edentatus* larva penetrating the hypaxial muscle (black arrow); the cut section was intentionally made to show the larva. Bar, 4 cm. Inset: magnification of parasitic larva disrupting the serosa and peritoneal fat (back arrow). Bar, 0.5 cm.

preserved in 70% ethanol and cleared with lactophenol for morphological identification. Swabs of the pulmonary consolidation were collected aseptically and submitted for aerobic culture. Fresh liver tissue was sampled to determine the heavy metal and selenium levels.

Tissue sections (4 µm) were stained with haematoxylin and eosin (HE). The site of the diaphragmatic tear, the mesentery and intraperitoneal subserosal regions had severe suppurative exudation mixed with haemorrhage and dissecting bands of fibroplasia with fibroblasts, macrophages and intralesional nematode larvae characterized by a body cavity, smooth cuticle, polymyarian/coelomyarian musculature and multinucleated intestinal cells (Fig. 3). Within the caecal wall, and occasionally in the diaphragm, there were degenerate nematode larvae surrounded by multinucleated giant cells, macrophages and fibroplasia. The nematode larvae were identified as *S. edentatus* based on the morphological characteristics of the head and buccal capsule (Fig. 4).

The tongue muscle was markedly necrotic, with severe mineral deposition and fibrous tissue replacement. The mucosa was hyperplastic and hyperkeratotic, mixed with coccobacilli. The alveoli and airways of the regionally consolidated pulmonary parenchyma were filled with neutrophils, fibrin, necrotic debris and coccobacilli, and *Actinobacillus* spp., *Streptococcus* spp. and *Aspergillus niger* were isolated on cultures. The liver selenium concentration was 0.098 ppm, significantly lower than the suggested reference range (0.3–1.0 ppm).

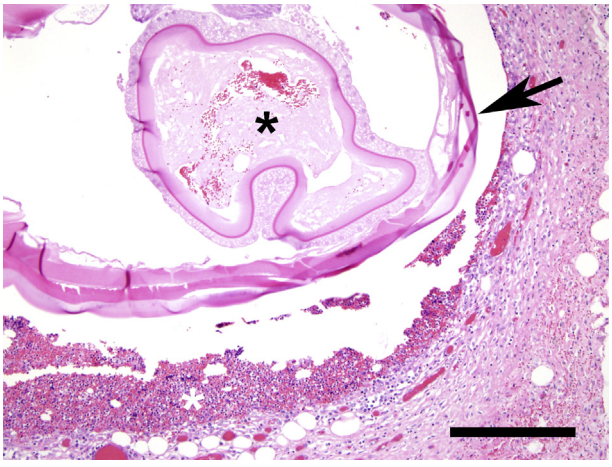

Fig. 3. Cross-section of a *S. edentatus* larva in the diaphragm (arrow). Note the intestine of the parasite containing host erythrocytes (black asterisk). The larva is surrounded by neutrophils, haemorrhage and fibrovascular connective tissue (white asterisk). HE. Bar, 100 µm.

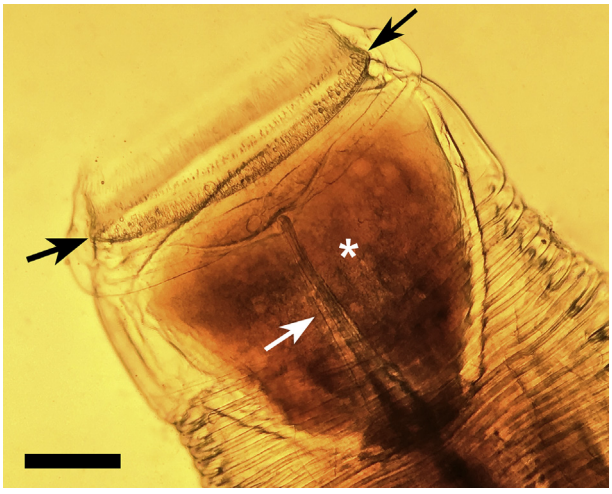

Fig. 4. Cleared *S. edentatus* specimen with characteristic leaf crown (black arrows), buccal capsule (white asterisk), dorsal gutter (white arrow) and absence of a tooth. Lactophenol clearing. Bar, 200 µm.

Herein, we report a case of fatal peritoneal migration with diaphragmatic tear due to *S. edentatus* migration in a foal with selenium deficiency. The gross lesions observed in this foal were similar to those described in horses infected naturally with *S. edentatus*, in which the larvae migrate in the subserosa, inducing large haemorrhages, and occasionally penetrate the underlying muscle layers (Malan *et al.*, 1981; Cohen *et al.*, 1992; Uzal *et al.*, 2016). The most striking lesion, which caused the death of the foal, was the diaphragmatic tear associated with numerous *S. edentatus* larvae. In experimental infections of ponies with *S. edentatus*, the larvae caused multifocal necrotic nodules primarily in the abdominal portion of the diaphragm at 20 weeks post infection (McCraw and Slocombe, 1978). Histologically, we observed granulomatous inflammation surrounding the degenerate larvae, but when viable, the larvae were surrounded by neutrophils, haemorrhage and fibroplasia. Similar lesions have been documented in experimental infections of ponies with *S. edentatus* (McCraw and Slocombe, 1978).

Interestingly, in this case the liver was not significantly affected and mostly had portal lymphoplasmacytic infiltration with fibrosis and bile duct hyperplasia. McCraw and Slocombe (1974, 1978) documented severe hepatic lesions, primarily in the early phase of infection (10 weeks post infection); however, these were rare in the later stage of infection (36 weeks post infection), likely due to the rapid repair after the nematodes leave the liver to continue their migration throughout the peritoneum. The presented foal may have been either in the late stage of infection so that hepatic

lesions had already resolved or in an early acute stage where there had not yet been extensive hepatic migration before the diaphragm acutely ruptured, resulting in death. Absence of appreciable adult worms or strongyle-type eggs on faecal flotation may support the latter possibility.

The extremely low selenium concentration in this foal was the likely cause of the glossal muscular necrosis and consequent impediment of opening the mouth on clinical presentation (Miller and Zachary, 2017). The fact that the animal was only grazing without reported mineral/vitamin supplementation indicates that the pasture was deficient in selenium. Californian livestock are particularly at risk for selenium deficiencies due to the low concentrations of this mineral in the soil and grass (Dunbar *et al*., 1988). Low selenium levels in animals negatively alters the immune response toward nematode infections (Smith *et al*., 2013), and immunodeficiency is a known trigger of nematode hyperinfection in human and animal hosts (Evering and Weiss, 2006). Therefore, it is likely that the selenium deficiency-induced immunocompromised status contributed to the fatal migration of *S. edentatus* in this foal as well as the skeletal muscle fragility leading to the rupture of the diaphragm.

Exposure to large numbers of infective larvae on pasture, inappropriate deworming and possible anthelmintic resistance may have also contributed to the fatal outcome in this case. The life cycle of *S. edentatus* involves the ingestion of infective L3 larvae on pasture, larval penetration of the intestinal mucosa and migration to the liver parenchyma where they moult to L4 larvae by 6–8 weeks post infection; the larvae then travel under the peritoneal serosa to many sites with predilection for the flanks and hepatic ligaments (Shite *et al*., 2015). Since no large strongyles multiply within the host or reproduce to shed eggs until L5 is formed, the large numbers of L4 larvae in the subperitoneum are directly related to the numbers of ingested L3 from grazing areas and indicate significant pasture contamination with L3. The owner reported deworming the foal with ivermectin, but the date and dose administered were unknown. It may be that the infection was acquired subsequent to treatment or the drug was not effective either because of lack of sensitivity to anthelmintics of migratory stages, anthelmintic resistance (Tzelos and Matthews, 2017) or because of inadequate dosing or poor drug absorption if given orally.

Clinical cases of *S. edentatus* infection have been observed rarely in horses, mostly due to the practice of routine deworming and the efficacy of anthelmintic drugs in preventing infection. Nevertheless, fatal peritoneal migration of the larval stages resulting in compromise to the diaphragm poses a potential risk for death in grazing foals that are susceptible to infection, such as those with nutritional deficiencies or when there is inadequate use, failure of absorption or resistance to the anthelmintic drugs.

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Conflict of Interest Statement

The authors declare no conflict of interest with respect to the publication of this manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2019.09.004.

References

Bell RP, Reed SK, Messer IVNT (2016) Successful surgical management of abdominal abscessation secondary to *Strongylus edentatus* migration. *Equine Veterinary Education*, **28**, 505–509.

Cohen ND, Loy JK, Lay JC, Craig TM, McMullan WC (1992) Eosinophilic gastroenteritis with encapsulated nematodes in a horse. *Journal of the American Veterinary Medical Association*, **200**, 1518–1520.

Dunbar JR, Norman BB, Oliver MN (1988) Preliminary report on the survey of selenium whole blood values of beef herds in twelve central and coastal California counties. In: *Selenium Contents in Animal and Human Food Crops Grown in California*, UC Cooperative Extension, UC Division of Agriculture and Natural Resources, Oakland, pp. 81–83. Publication 3330.

Evering T, Weiss LM (2006) The immunology of parasite infections in immunocompromised hosts. *Parasite Immunology*, **28**, 549–565.

Farrar RG, Klei TR (1985) *In vitro* development of *Strongylus edentatus* to the fourth larval stage with notes on *Strongylus vulgaris* and *Strongylus equinus*. *The Journal of Parasitology*, **71**, 489–499.

Khan MA, Roohi N, Rana MAA (2015) Strongylosis in equines: a review. *Journal of Animal and Plant Science*, **25**, 1–9.

Malan FS, Reineck RK, Scialdo RC (1981) Recovery of helminths postmortem from equines. 1 Parasites in arteries, subperitoneum, liver and lungs. *Onderstepoort Journal of Veterinary Research*, **48**, 141–143.

Matthews JB (2014) Anthelmintic resistance in equine nematodes. *International Journal for Parasitology*, **4**, 310–315.

McCraw BM, Slocombe OD (1974) Early development of and pathology associated with *Strongylus edentatus*. *Canadian Journal of Comparative Medicine*, **38**, 124–138.

McCraw BM, Slocombe OD (1976) *Strongylus vulgaris* in the horse: a review. *Canadian Veterinary Journal*, **17**, 150–157.

McCraw BM, Slocombe OD (1978) *Strongylus edentatus*: development and lesions from ten weeks post infection to patency. *Canadian Journal of Comparative Medicine*, **42**, 340–356.

Miller MA, Zachary JF (2017) Mechanisms and morphology of cellular injury, adaptation and death. In: *Pathologic Basis of Veterinary Diseases*, 6th Edit., JF Zachary, Ed., Elsevier, Missouri, pp. 2–43.

Pfister K, van Doorn D (2018) New perspectives in equine intestinal parasitic disease: insights in monitoring helminth infections. *Veterinary Clinics of North America: Equine Practice*, **34**, 141–153.

Reinemeyer CR, Nielsen MK (2009) Parasitism and colic. *Veterinary Clinics of North America: Equine Practice*, **25**, 233–245.

Shite A, Admassu B, Abere A (2015) Large strongyle parasites in equines: a review. *Advances in Biological Research*, **9**, 247–252.

Slocombe JOD, McCraw BM (1973) Gastrointestinal nematodes in horses in Ontario. *Canadian Veterinary Journal*, **14**, 101–105.

Smith AD, Cheung L, Beshah E, Shea-Donohue T, Urban JF (2013) Selenium status alters the immune response and expulsion of adult *Heligmosomoides bakeri* worms in mice. *Infection and Immunity*, **81**, 2546–2553.

Studzińska MB, Tomczuk K, Demkowska-Kutrzepa M, Szczepaniak K (2012) The Strongylidae belonging to *Strongylus* genus in horse from south-eastern Poland. *Parasitological Research*, **111**, 1417–1421.

Tzelos T, Matthews J (2017) Anthelmintic resistance in equine helminths and mitigating its effect. *Practice*, **38**, 489–499.

Uzal FA, Plattner BL, Hostetter JM (2016) Alimentary system. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, 6th Edit., Vol. 2, MG Maxie, Ed., Elsevier, St. Louis, pp. 1–257.

Walde I, Prosl H (1976) *Strongylus edentatus* as the reason for subconjunctival phlegmon and granuloma formation in a horse. *Tierarztliche Praxis*, **4**, 493–496.

Wetzel R, Kersten W (1956) Die Leberphase der Entwicklung von *Strongylus edentatus*. *Wiener Tierärtzliche Monatsschrift*, **43**, 664–673.

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