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INFECTIOUS DISEASE

Mannheimia haemolytica A1-induced Fibrinosuppurative Meningoencephalitis in a Naturally-infected Holstein–Friesian Calf

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Summary

Mannheimia haemolytica is an opportunistic bacterium that is widely recognized among the bovine respiratory disease complex as the predominant pathogen causing broncho- and pleuropneumonia in cattle. Among the characterized *M. haemolytica* serotypes, A1 is the major cause of severe pulmonary lesions in cattle. This report describes post-mortem findings in a Holstein–Friesian calf with fibrinosuppurative meningoencephalitis and fibrinonecrotizing, haemorrhagic broncho- and pleuropneumonia, from which *M. haemolytica* and bovine viral diarrhoea virus (BVDV) were isolated. Microscopical evaluation showed expansion of the brainstem and cerebellar leptomeninges by neutrophils and fibrin, associated with gram-negative coccobacilli. Occasional blood vessels within the midbrain and cerebellum contained fibrin thrombi. Bacterial culture of cerebellum and lung yielded *M. haemolytica* with unusually high haemolytic activity. The isolates were confirmed as serotype A1 by rapid plate agglutination. Lung tissue was positive for BVDV by polymerase chain reaction. The broncho- and pleuropneumonia in this calf were consistent with typical mannheimiosis due to serotype A1; however, extrapulmonary infections due to *M. haemolytica*, as seen in this case, are rarely reported. To our knowledge, this is the first documentation of a natural BVDV and *M. haemolytica* co-infection associated with fibrinosuppurative meningoencephalitis in a calf.

Keywords: bovine viral diarrhoea virus; calf; *Mannheimia haemolytica*; meningoencephalitis

Mannheimia haemolytica is a commensal of the upper respiratory tract of healthy ruminants, but can become a significant cause of pneumonia in dairy and feedlot cattle. Disruption of the commensal relationship can lead to the establishment of *M. haemolytica* A1 as the dominant serotype (Al-Ghamdi *et al.*, 2000; Zecchinon *et al.*, 2005; Griffin *et al.*, 2010). Immunomodulatory factors, such as weaning, shipping, commingling, changes in climatic conditions and bacterial and viral co-infections, may affect the bovine host and facilitate the development of serotype A1-induced fibrinosuppurative and necrotizing bronchopneumonia (Griffin, 2000; Zecchinon *et al.*, 2005; Rice *et al.*, 2007; Singh *et al.*, 2011).

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A 5-month-old neutered male Holstein–Friesian calf was submitted for necropsy examination to the Athens Veterinary Diagnostic Laboratory, Athens, Georgia, with a history of weight loss, lethargy, hind-limb ataxia and rare crackles in the left cranial lung quadrant. Following treatment with florfenicol (single dose 6 ml; Nuflor™; Merck Animal Health Summit, New Jersey, USA) and daily oral electrolytes, the calf's body condition improved and clinical signs appeared to resolve. However, improvement was short-lived, with the calf becoming laterally recumbent prior to its demise. The bovine viral diarrhoea virus (BVDV)-exposure status of the calf prior to vaccination with Bovishield Gold 4 (modified live virus strains of infectious bovine rhinotracheitis virus), BVDV types 1 and 2 and parainfluenza 3 virus

(Pfizer Animal Health, New York, USA) was unknown.

The calf had stunted growth, was dehydrated and in poor nutritional condition. The surfaces of the occipital lobe of the cerebrum, brainstem and cranial cerebellum were covered by multifocal, petechial to ecchymotic haemorrhages (Fig. 1). The leptomeninges of the brainstem and cerebellum were cloudy and had numerous, scattered fibrin strands. There was caudal herniation of the cerebellum (Fig. 1). The cranioventral aspect of the lungs was bilaterally mottled dark red, black and tan, and both cranial lobes were adhered to the parietal pleura by multiple fibrin strands. On cut section, the pulmonary parenchyma was disrupted by multifocal, variably sized, firm nodules. Multifocal petechiae were present throughout the myocardium, abomasal mucosa, pharyngeal tonsils and peripheral lymph nodes, while the pulmonary and renal parenchyma had more widespread ecchymotic haemorrhages. Both renal capsules contained multiple, pale white foci, and the cortices had multifocal acute infarcts. Pharyngeal tonsils and peripheral lymph nodes were markedly enlarged. There was serous atrophy of epicardial fat. The abomasal mucosa was multifocally ulcerated and numerous whipworms (*Trichuris* spp.) were embedded in the caecal mucosa. Other organs, including joints and spleen, had no gross evidence of disease.

Tissues were collected for bacterial culture and histopathology. Fresh tissues were stored at −20°C for further diagnostic testing. For histopathology, tissues were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE).

Microscopically, leptomeningitis was most severe in the brainstem and cerebellum, but also affected the cerebrum. The leptomeninges overlying the brainstem and cerebellum were multifocally expanded by abundant neutrophils, admixed with fibrin, haemorrhage and occasional clusters of coccobacilli (Fig. 2), which were characterized as gram-negative by Lillie Twort stain. Microhaemorrhages and multifocal, perivascular neutrophilic inflammation were present within the parenchyma of the brainstem, cerebrum and cerebellum (Fig. 2). Rare, dilated blood vessels within the cerebellar leptomeninges contained scant to moderate amounts of fibrin, while vasculature within the parenchyma of the midbrain contained multiple fibrin thrombi (Fig. 3). The choroid plexus was infiltrated by numerous neutrophils and plasma cells, with abundant haemorrhage and fibrin.

The pulmonary parenchyma had multiple, extensive, foci of infarction. Pulmonary vessels contained fibrin thrombi and numerous alveoli were filled with fibrin. There was interlobular oedema and the pulmonary pleura was expanded by oedema and fibrin. The renal medulla had multiple infarcts and the cortical interstitium contained multiple, scattered aggregates of macrophages, with fewer lymphocytes and plasma cells. The liver exhibited mild to moderate periportal hepatitis with predominantly plasma cells and macrophages, accompanied by generalized Kupffer cell hyperplasia. The spleen had marked lymphoid hypoplasia and necrosis, with lymphoid depletion of germinal centres. No significant microscopical lesions were observed in skeletal muscle, pancreas, thyroid, heart, rumen, small intestine and large intestine.

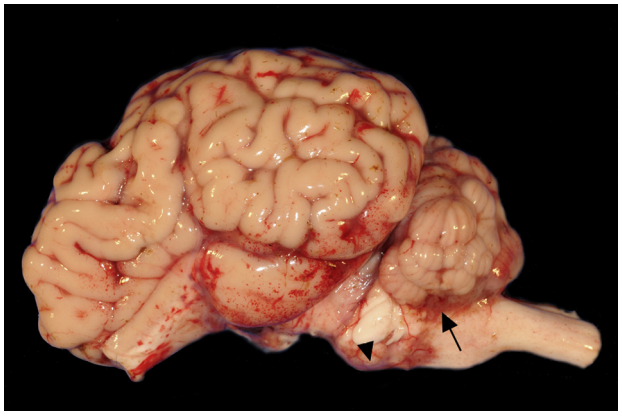

Fig. 1. Multifocal, petechial to ecchymotic haemorrhages on the surface of the cerebrum, brainstem and cranial cerebellum. The leptomeninges of the brainstem and cerebellum are cloudy (arrowhead) and scattered, but frequent, fibrin strands are present at the cerebellopontine angle (arrow).

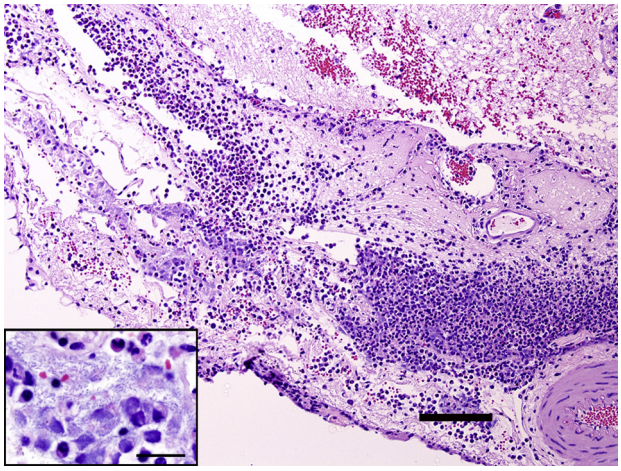

Fig. 2. Severe cerebellar leptomeningitis characterized by an abundance of neutrophils with associated fibrin, haemorrhage and occasional clusters of coccobacilli. HE. Bar, 100 μm. Inset. HE. Bar, 50 μm.