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SPONTANEOUSLY ARISING DISEASE

A Histopathological Study of Bovine Ganglia

H. Jahns* **and C. Fast†**

** Pathobiology Section, UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland and † Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institute, Greifswald-Isle of Riems, Germany*

Summary

One hundred and sixty-eight ganglia from 54 cattle aged 10 days to 10 years were examined microscopically. Samples from six autonomic ganglia and one sensory ganglion were represented. Thirteen animals were clinically normal and 41 were submitted for post-mortem examination. Neuronal vacuolation, spheroid formation, lipofuscin accumulation and central chromatolysis were observed sporadically and were of varying magnitude. Neuronal vacuolation and spheroid formation were not age-related changes, while lipofuscin accumulation was more common in older animals and central chromatolysis was more common in younger cattle. Non-suppurative inflammation and neuronophagia were also common findings (23 out of 54 animals, 42.6%) in autonomic ganglia that did not contain herpesvirus DNA as determined by polymerase chain reaction. Renaut bodies, features of peripheral nerves, were most commonly noted in the vagus. None of the histopathological findings were related to any particular disease in which loss of autonomic nervous system function might be expected. Furthermore, all changes were as common in clinically normal animals as in animals with disease.

Keywords: autonomic ganglia; bovine; herpesvirus

Introduction

Peripheral nerve diseases involving the ganglia are uncommon in domestic animals (Summers *et al.*, 1995). Dysautonomia is the most frequently diagnosed condition affecting autonomic ganglia and the disease has been reported in horses (equine grass sickness), cats (Key-Gaskell syndrome), dogs, rabbits, hares and sheep with common clinical signs of oesophageal dysfunction, inappetence and gut stasis (Key and Gaskell, 1982; Pollin and Griffiths, 1992; Griffiths and Whitwell, 1993; Pruden *et al.*, 2004; Hahn *et al.*, 2005; Niessen *et al.*, 2007; Wylie and Proudman, 2009). Histopathological changes associated with dysautonomia are central chromatolysis and fine vacuolation of neuronal cytoplasm, variable numbers of dead neurons, marked neuronal loss and the presence of spheroids (Mahaffey, 1959; Howell *et al.*, 1974; Pogson *et al.*, 1992; Uzal *et al.*, 1992).

The other main group of diseases of ganglia are primary inflammatory processes such as sensory neuronopathies or ganglioradiculitis that mainly involve sensory ganglia and peripheral nerves, but also a variety of different autonomic ganglia (Panciera *et al.*, 2002; Foss *et al.*, 2011). Clinical presentations vary with the location of the lesions, but often result in cranial nerve deficits and gait abnormalities (Wouda *et al.*, 1983; Steiss *et al.*, 1987; Panciera *et al.*, 2002; Foss *et al.*, 2011).

Bovine ganglia are rarely examined histopathologically, with the exception of studies of bovine spongiform encephalopathy (BSE) (Hoffmann *et al.*, 2007) and bovine herpesvirus (BHV) 1 infection (Nandi *et al.*, 2009). Dysautonomia and other specific diseases of ganglia have not, as yet, been reported in cattle. In contrast, a few studies examining small numbers of ganglia reported incidental changes in horses (Brownlee, 1959; Uzal *et al.*, 1992), sheep (Pruden *et al.*, 2004) and cattle (Guderjahn, 1961; Rech *et al.*, 2006). Lipofuscin accumulation, vacuolation, chromatolysis and mild inflammation were the features reported.

Correspondence to: H. Jahns (e-mail: hanne.jahns@ucd.ie).

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Megaoesophagus was diagnosed *post mortem* in a 2-year-old severely dehydrated heifer that presented to the University Veterinary Hospital (UVH), Dublin, with a 5-day history of spontaneous reflux of saliva, water and food material. When the only histopathological change observed was chromatolysis of neurons in the cranial cervical ganglion, the question of the significance of this feature and its role in the disease arose. A review of the literature revealed a lack of information on common background changes in bovine ganglia. The aim of the present study was to fill this knowledge deficit by describing the spectrum of histopathological changes in a survey of ganglia from 54 cattle.

Materials and Methods

Ganglia from 54 cattle aged from 10 days to 10 years (mean 38.54 ± 27.48 months) were examined. Ganglia were harvested from two sources, healthy control animals ($n = 13$), ranging in age from 5 to 58 months, that had been examined *post mortem* and represented negative control animals in a German BSE pathogenicity study (Hoffmann *et al.*, 2007) and cattle ($n = 41$) submitted to the UVH Dublin for post-mortem examination following a variety of illnesses. All cattle over the age of 48 months from the latter source underwent mandatory BSE testing (HerdChek® BSE-Scrapie Ag Test, Idexx at IdentiGEN, Dublin, Ireland) and no evidence of prion proteins was found. The post-mortem diagnosis was recorded for each case.

Collection of Ganglia

In total, 168 ganglia from various locations were examined (Table 1). The following autonomic ganglia were collected: cervical cranial ganglion ($n = 53$), distal ganglion of the vagus nerve (ganglion nodosum) ($n = 42$), middle cervical ganglion ($n = 8$), cervicothoracic ganglion (ganglion stellatum) ($n = 19$), cranial mesenteric ganglion ($n = 21$) and caudal mesenteric ganglion ($n = 12$). One sensory ganglion, the trigeminal ganglion ($n = 13$), was also sampled. Tissues were fixed in 10% neutral buffered formalin for 3–7 days before processing.

Histopathology

Coronal sections (3 mm) of each ganglion were processed routinely and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE) and examined microscopically. The number of neurons affected by a change was expressed as a percentage of the total number of neurons. However, lesions that were present in very low number, such as neuronal and perineuronal vacuolation, satellitosis and spheroids, were enumerated in each section examined. Gliosis was assessed as diffuse, mild, moderate or severe or nodular and the numbers of nodules were recorded. Inflammation was graded arbitrarily as: absent or mild, representing up to two small foci; moderate, representing up to seven small foci; and severe, representing up to eight or more foci or a focally dense infiltrate. Additional findings such as neuronophagia, changes of the interstitial tissue or peripheral nerve were recorded. Where appropriate, sections were stained with cresyl violet for Nissl substance, von Kossa's stain for calcium, periodic acid–Schiff (PAS) for lipofuscin and toluidine blue for Renaut bodies (Bancroft and Gamble, 2007).

Immunohistochemistry

Inflammatory cell infiltrates were labelled by immunohistochemistry (IHC) with primary antibodies specific for the T-cell marker CD3 (AbD Serotec, Oxford, UK; monoclonal rat antibody; citrate buffer antigen retrieval; dilution 1 in 100) and the B-cell marker CD79a (AbD Serotec; monoclonal mouse antibody; citrate buffer antigen retrieval; dilution 1 in 400).

Virology

Ganglia with mild to severe inflammatory changes from various locations in nine different cattle were analysed by polymerase chain reaction (PCR) for BHV1, bovine parainfluenza virus (BPIV) 3, bovine respiratory syncytial virus (BRSV) nucleic acids and pan-herpesviral DNA. These included the cranial cervical ganglion ($n = 3$), cervicothoracic ganglion ($n = 3$) and cranial mesenteric ganglion ($n = 3$). Nine ganglia matching the above locations from another nine cases without inflammatory changes were also evaluated.

For the PCR analysis 30 μm sections were taken aseptically from each block and placed in a 1 ml PCR Eppendorf tube. DNA was extracted from the paraffin wax-embedded sections using a commercial kit (RecoverAll™ total nucleic acid isolation kit for FFPE [Ambion]; Applied Biosystems, Warrington, Cheshire, UK). A real time multiplex reverse transcriptase (RT) PCR, which detects BHV1, BPIV3 and BRSV nucleic acids, was performed (Thonur *et al.*, 2012). IBR 6660, PI3Euro L3380, PI3NonEuro A2112 and BRSV vaccine strain (Rispoval) were used as positive controls, respectively. In addition, a pan-herpesvirus nested PCR was conducted using degenerate deoxyinosine-substituted primers specific for the herpesvirus DNA polymerase gene (Ehlers *et al.*,