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SPONTANEOUSLY ARISING DISEASE

Recurrent Outbreaks of Myelodysplasia in Newborn Calves

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Summary

The present study records recurrent outbreaks of myelodysplasia of unknown origin occurring in a specific geographical location in the north of Spain, and involving up to 30% of the calves born in affected herds. The affected calves were of different breeds and displayed non-progressive signs of spinal cord dysfunction. The disease has occurred annually in February–March over a period of at least 15 years. Only calves born to cattle grazed on mountainside pastures and under high grazing pressure were affected. Seven calves were subjected to necropsy examination. Myelodysplasia was not associated with vertebral defects or arthrogryposis and involved the entire length of the spinal cord. Microscopically, there was abnormal distribution of the grey matter, aberrations of the central canal and failure of formation of the ventral median fissure. Infectious, nutritional and physical disorders were ruled out as possible aetiologies. A critical period of embryonic susceptibility to the causal agent was identified. This was during the time of secondary neurulation when cows in the early stages of gestation were grazed on mountainside pastures. Consequently, the presence of neuroteratogenic plants in these pastures is proposed as a likely cause. Two plants, *Carex brevicollis* and *Erythronium dens-canis*, which contain alkaloids, were identified on the mountainsides where affected cattle were grazed and not in other pastures, and are proposed as the possible aetiology of the disease.

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Introduction

Myelodysplasia is a spinal cord malformation resulting primarily from a segmental disorder of the neural tube, whereby a part of the spinal cord fails to develop properly. There are few reports of this pathological change in cattle and these were isolated cases that affected only a small number of animals and were often associated with skeletal deformities, cleft palate and arthrogryposis (Leipold *et al.*, 1977, 1993; Hiraga and Abe, 1987; Doige *et al.*, 1990; Ohfuji, 1999; Imai and Moritomo, 2009; Hill, 2010). Myelodysplasia has been related to genetic factors in Charolais and Hereford calves, although the mode of inheritance is unknown (Leipold *et al.*, 1969). The origin of the disease in most cases is unknown and several causal agents have been proposed, including: vitamin A (van der Lugt and Prozesky, 1989; Hill *et al.*, 2009) and copper deficiencies (Suttle, 1988), viral infection (Done *et al.*, 1980; Kono *et al.*, 2008) or toxic causes such as consumption of neuroteratogenic plants (Leipold *et al.*, 1969, 1977; Keeler, 1978; Washburn and Streeter, 2004).

Similar lesions of myelodysplasia confined to the spinal cord have been reproduced in rats by subjecting embryos to a teratogenic compound (e.g. trypan blue or thiadiazole) at the time of closure of the caudal neuropore (Warkany *et al.*, 1958; Beaudoin, 1974).

The present study documents recurrent outbreaks of non-progressive ataxia affecting newborn calves of different breeds in a specific geographical location (Puerto de Áliva, Cantabria, Spain).

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Materials and Methods

Animals

Annually, over a period of at least 15 years, veterinary clinicians from the region of Puerto de Áliva (UTM coordinates: X: 353000-359000; Y: 4783000-4779000, Cantabria, Spain) reported in February and March outbreaks of congenital abnormalities of the nervous system in newborn calves. In 2002 (archival material) and in 2011, three (calves number 1, 2 and 3) and four (calves number 4, 5, 6 and 7) of these calves, respectively, aged 20–30 days and with a history of ataxia, were submitted to the Veterinary Pathology Service of the University of Leon for clinical and pathological examination. The calves had been born between February and March and were referred alive in March. The affected calves included animals of the Charolais, brown Swiss and Tudanca breeds from different farms with similar management practices (animals are kept housed from November to late May and the grazing season begins in June). The affected region was visited in June 2011 and an epidemiological survey was carried out among the farmers. The aim of this survey was to characterize the presentation of the condition, the management practices, the environmental conditions and the epidemiology related to the affected calves.

Pathological Examination

Following clinical examination, seven calves were humanely destroyed for necropsy examination. Tissue samples were collected from the central nervous system (CNS) (including the cortex, diencephalon, corpus callosum, hippocampus, midbrain, cerebellar cortex, pons and cerebellar peduncles, medulla oblongata and cervical, thoracic and lumbar spinal cord), skeletal muscles (tongue, oesophagus, diaphragm and intercostal muscles, psoas, abductor, longissimus dorsi, semitendinosus and semimembranosus muscles) and joints. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (3 μm) were stained with haematoxylin and eosin (HE).

Immunohistochemistry (IHC) for detection of bovine viral diarrhoea virus (BVDV) antigen was performed on paraffin wax embedded tissue samples from the thymus, spleen, ileocaecal valve, lymph nodes and skin from the ear. Sections were labelled with primary monoclonal antibody specific for NS3 (p80/125) of BVDV and a secondary polymer-based detection system (EnVision + System Labelled Polymer-horseradish peroxidase anti-mouse; Dako, Glostrup, Denmark). The chromogen was 3,3′diaminobenzidine (DAB; Vector Laboratories, Burlingame, California, USA) and sections were counterstained with haematoxylin. The specificity of the technique was evaluated by inclusion of negative (tissue bovine samples previously tested as BVDV negative) and positive controls (positive samples previously tested as BVDV positive).

Laboratory Analysis

Serum samples from the four calves (4, 5, 6 and 7) submitted in 2011, their dams and other adult animals from affected farms were taken for laboratory analysis. Complete haematological and biochemical profiles were performed, including determination of serum concentrations of copper and iron. Analysis of copper concentration in dry weight of liver from the affected calves was conducted by atomic absorption spectrophotometry.

In order to identify BVDV infection the antibody response against BVDV NS3 (p80/125) was determined in serum samples from four affected calves (4, 5, 6 and 7) and their dams using a commercial enzyme-linked immunosorbent assay (ELISA; Ingezim BVD, Ingenasa, Spain). Another ELISA was used for the detection of BVDV antigen in serum (HerdChek™; IDEXX Laboratories, Hoofddorp, The Netherlands).

Results

Epidemiology

Calves with clinical signs of spinal cord dysfunction were reported annually over a 15-year period. Affected animals were born from cows that had been inseminated in May–June and had given birth in February–March. No cases were reported outside this time frame and two farms with a delayed mating season (July–August) never had affected animals. The outbreaks of disease occurred only in herds that graze on highland summer pastures in the Puerto de Áliva (Picos de Europa, Cantabria, Spain) region without supplementary feeding. No herds were affected in nearby regions. A total population of 2,500 adult cattle from 50 to 55 farms, mixed with a large population of horses and sheep, is distributed throughout these pastures in June–November, representing high grazing pressure. However, herds grazed on the rocky mountainsides (1,300–1,700 m in altitude) were affected, with morbidity during the critical period ranging from 20% to 30%. No cases have been observed in lower pastures. No known neuroteratogenic plants were found on the above-mentioned pastures, but other plants that contain alkaloids, such as *Carex brevicollis* and *Erythronium dens-canis*, were identified exclusively in the affected areas.

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