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Borna Disease in Naturally Infected Cattle

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Summary

Based on the immunohistochemical demonstration of viral antigen and on the histological brain lesions, Borna disease was diagnosed in a cow and a bull which had suffered from a severe, subacute progressive disorder of the central nervous system. Virus-specific antigen was characteristically localized in neurons, predominantly in the perikaryon and dendrites. In a serum sample available from one of the animals a Borna disease virus antibody titre of 1 in 80 was demonstrated. This is the first report of the natural disease in cattle.

Introduction

Borna disease (BD), characterized by a non-purulent meningoencephalitis, occurs naturally in rabbits, sheep and horses as a result of infection with the causative virus (Metzler *et al.*, 1978; Waelchli *et al.*, 1985; Ludwig *et al.*, 1988). Borna disease virus (BDV) has recently been characterized as an enveloped single-stranded RNA virus (Duchala *et al.*, 1989; De La Torre *et al.*, 1990) with evidence for negative-sense polarity (Briese *et al.*, 1992). The course of the disease in spontaneous infection is usually subacute and fatal (Ludwig *et al.*, 1988).

Sporadic outbreaks have been reported in Germany (Lange *et al.*, 1987) and Switzerland (Metzler *et al.*, 1976; Waelchli *et al.*, 1985; Rohner-Cotti, 1992). However, there is increasing evidence from serological surveys that BDV infections occur in horses without giving rise to clinical signs, suggesting that the virus is more widely distributed than previously thought. These findings include seropositive results from horses in the Netherlands and other European countries (Lange *et al.*, 1987), the USA, Africa and India (Stitz and Rott, unpublished).

Experimentally, a wide spectrum of species is susceptible to BDV. The clinical signs vary from severe central nervous system (CNS) symptoms to merely abnormal behaviour (Ludwig *et al.*, 1988; Richt *et al.*, 1992). Pathological changes, which are limited to the CNS, typically consist of a marked perivascular mononuclear infiltration in the affected areas, with a predilection for the grey matter. The meninges and white matter are only mildly involved. The pattern of inflammation reflects an immunopathological T-cell reaction rather than direct virus–host cell interaction, and the immunopathological

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nature of the disease has been well established in the rat (Stitz *et al.*, 1993). Neurons do not show any obvious changes, but may contain intranuclear eosinophilic (Joest-Degen) inclusion bodies, which are considered to be pathognomonic for BD (Ludwig *et al.*, 1988; Deschl *et al.*, 1990). In the absence of inclusion bodies, BD may be difficult to diagnose histologically unless antigen is demonstrated immunohistochemically.

This report describes the clinical signs, the histological brain lesions and the immunohistological demonstration of viral antigen in two cases of spontaneous BD in cattle, thereby extending the number of species known to be susceptible to natural BDV infection.

Materials and Methods

Animals (Cases 1 and 2)

Case 1, a 14-year-old brown Swiss cow with a history of circling and tremor for 8 days, was presented at the Clinic of Veterinary Internal Medicine, University of Zurich. The cow originated from a dairy herd kept near an area in the eastern part of Switzerland where BD is known to be endemic. Because of poor prognosis the cow was slaughtered and its head submitted for further pathological investigation.

Case 2 was a 3-year-old bull from the same endemic area. It had shown a progressive CNS disorder for about 3 weeks. After slaughter its brain was submitted for histological examination, in particular for the exclusion of bovine spongiform encephalopathy (BSE).

Histological Examination

The brains were fixed by immersion in 4% buffered formaldehyde. Samples from the rostral cortex, basal ganglia, thalamus, hippocampus, occipital cortex, midbrain, pontine region, cerebellum and medulla oblongata were paraffin wax-embedded and sections were routinely stained with haematoxylin and eosin (HE).

Immunohistochemical Examination

Paraffin wax sections from the brain were examined with a BDV monoclonal antibody (38/17 C1), specifically recognizing the p38 of BDV (Thiedemann *et al.*, 1992) and two rabbit BDV antisera in a labelled streptavidin-biotin method (LSAB Kit, Dako A/S, Glostrup, Denmark).

Serological Examination

A serum sample from case 1, taken some days before slaughter, was tested by an indirect immunofluorescence assay (Danner and Mayr, 1973; Rohner-Cotti, 1992) for the presence of BDV antibodies. No serum was available from case 2.

Results

Clinical Observations

After its arrival at the clinic, case 1 showed non-specific neurological signs such as head tilt and deviation to the right, occasional circling to the right, refusal or inability to pick up food from the left, mild proprioceptive deficits and reduced response to the menace test. The most striking feature was a unique type of

circling, like that of the hands of a clock, with the hindlimbs standing still in the centre. The signs could not be attributed to any known bovine CNS disorder. As stated above, the animal was slaughtered.

Case 2 was at first anorectic and refused to drink from the automated water supplies. Later hypermetria and struggling became evident and an increasing tremor was noted. Finally, the bull fell and was slaughtered.

Histological Examination

The results were basically identical in both animals, neither of which had gross brain lesions. Microscopical examination revealed a moderate to severe, non-purulent meningoencephalitis in all sections examined (Fig. 1).

Perivascular cuffs contained primarily lymphocytes and cells morphologically consistent with macrophages. Plasma cells were present in some infiltrates, especially in cuffs of more than three layers. Infiltration often seemed to extend to the adjacent parenchyma.

Degenerative changes in the neuropil were not obvious and only a moderate, diffuse gliosis was noted. In case 1, but not in case 2, small intranuclear, eosinophilic inclusion bodies were detected in many neurons, especially in the hippocampus (Figs 2 and 3). The meningeal reaction was minimal in case 1 but moderate to severe in case 2.

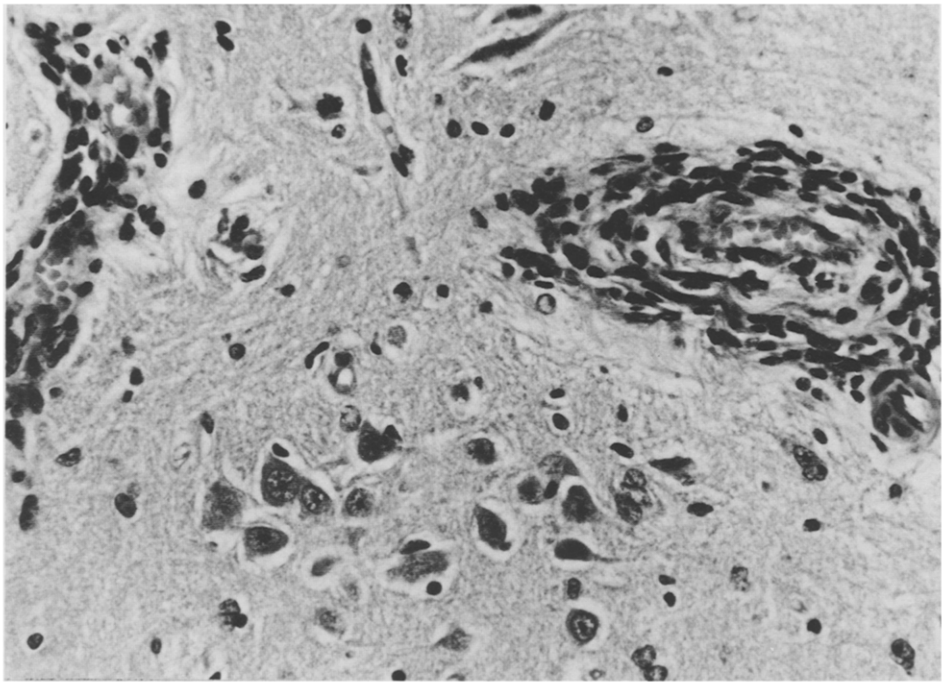

Fig. 1. Case 1. Cortex. Mononuclear perivascular infiltrates. HE. × 300.

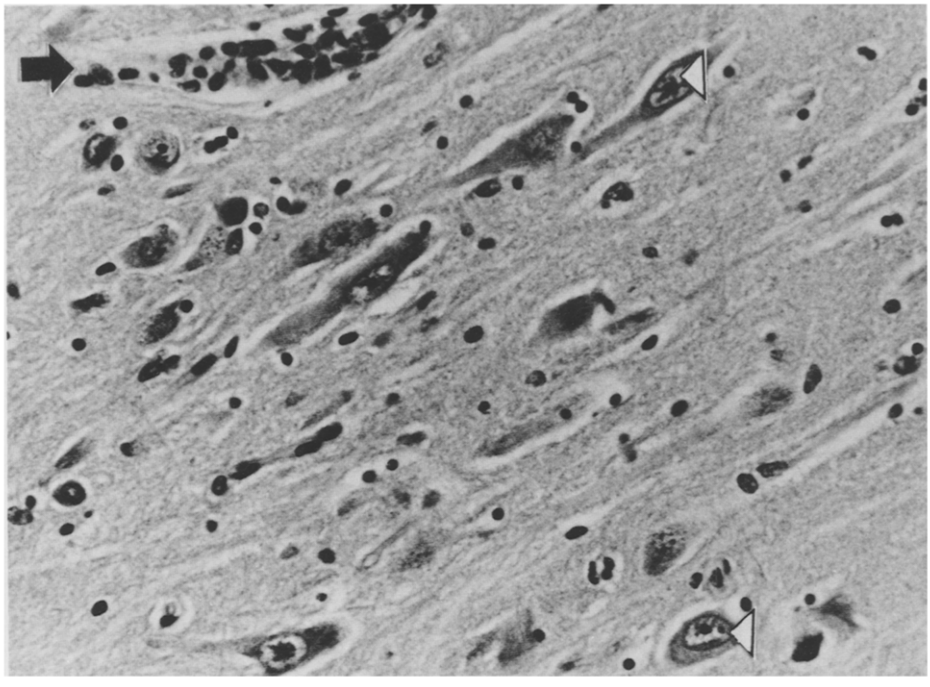

Fig. 2. Case 1. Cortex. Perivascular infiltrate (arrow) and two neurons containing intranuclear (Joest-Degen) inclusion bodies (arrowheads). HE. × 475.

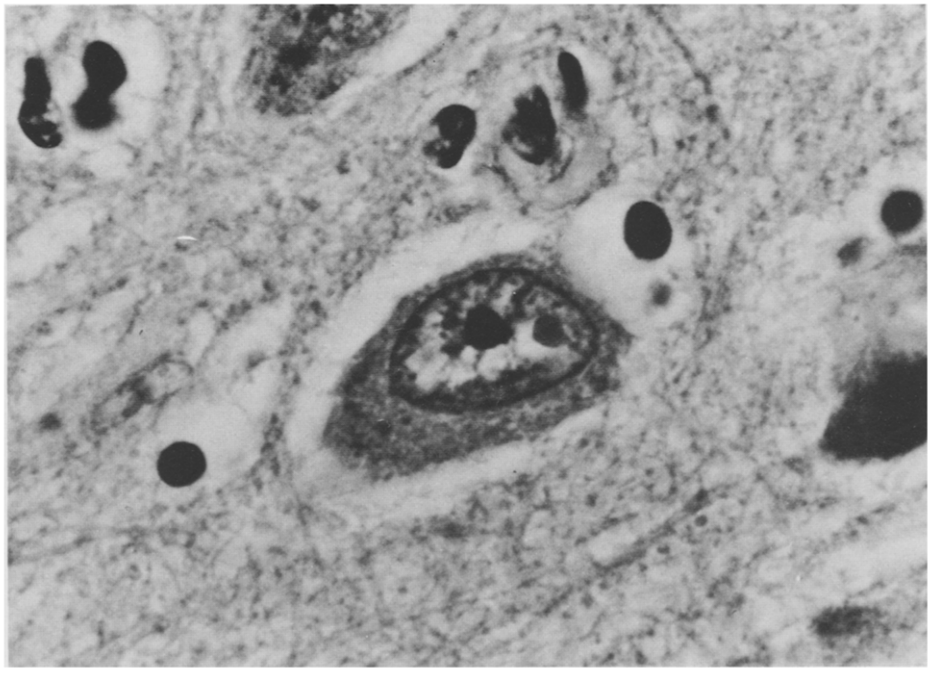

Fig. 3. High power magnification of neuron containing inclusion body. HE. × 1500.

Immunohistochemical Examination

Numerous neurons within all sections examined showed a strongly positive immunohistochemical reaction. There was diffuse staining of the perikaryon and of dendrites and, less often, of small intranuclear aggregates (Fig. 4). Less intensive staining was observed within the cytoplasm and nucleus of astrocytes.

The staining pattern depended to some extent on the antibody used. The most intense staining was given by the monoclonal antibody 38/17 CI in the nucleus as well as in the cytoplasm, whereas the rabbit antisera detected predominantly intranuclear antigens, corresponding at least partly to inclusion bodies.

Serological Examination

Serum antibodies to BDV were detected by indirect immunofluorescence at a titre of 1 in 80 in case 1.

Discussion

These are, to our knowledge, the first confirmed natural cases of BD in cattle. There is, however, some evidence suggesting that cattle are susceptible to infection with BDV. Thus, experimental infection of two calves was reported by Matthias (1954). The animals were inoculated intracerebrally (IC), but

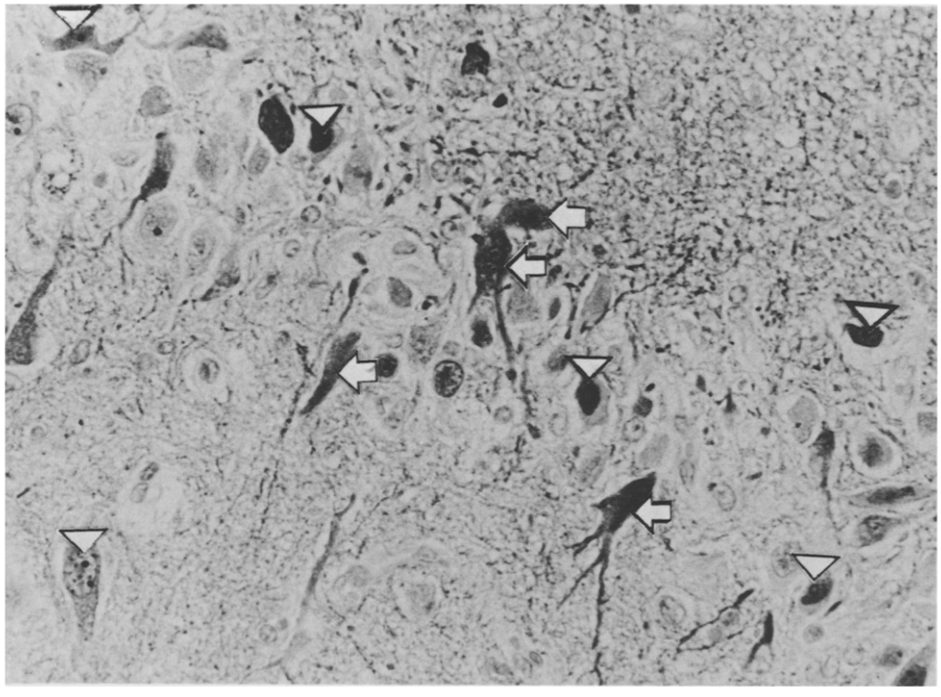

Fig. 4. Case 1. Hippocampus. Immunohistochemistry (mAb 38/17 CI). Intracytoplasmic (arrows) and intranuclear (arrowheads) reaction. LSAB. ×600.

neither developed signs of BD within the observation periods of 40 and 139 days. However, histologically, both calves showed the typical pattern of inflammation within the brain, as well as Joest-Degen inclusion bodies. IC inoculation of rabbits with brain material from the encephalitic calves resulted in BD and death.

Reports of naturally occurring BD in cattle are rare and rather controversial as they were published decades ago and were not confirmed by antigen demonstration (Nicolau and Galloway, 1928). In addition, a certain confusion exists in the older literature due to the opinion that the agent of BD might be aetiologically related to that of malignant catarrhal fever, a disease sharing some histological features with BD (Beller and Zeller, 1951). BD in cattle has remained a matter of doubt up to the present.

Nothing is known about the prevalence of BDV antibodies in cattle. Screening by the complement fixation test of 146 bovine and 115 porcine serum samples from a BD endemic area in Germany failed to reveal seropositive animals (Wagner, 1970). It must be emphasized, however, that serology is not a sensitive epidemiological tool for detecting BDV carriers because there is no close relation between serological status and presence of virus within the host (Metzler *et al.*, 1979). This view is supported by the finding that neonatally infected rats which become virus carriers are immunologically tolerant at both the B- and T-cell levels (Hirano *et al.*, 1983; Narayan *et al.*, 1983).

The two cases described in this report suggest a complex neurological pattern, as might be expected in a disease with disseminated brain lesions. In cattle originating from an endemic area, BD should be considered in the differential diagnosis of any slowly progressive neurological disorder, especially when the symptoms of more common diseases are missing.

Together with the experimental results mentioned above, these two confirmed cases of bovine BD prove that cattle are susceptible to BDV and that BD occurs naturally in this species. Retrospective studies, particularly those in which immunohistochemical techniques are applied to cases previously diagnosed as sporadic bovine meningo-encephalomyelitis (Fankhauser, 1961), which indeed was the preliminary diagnosis in case 1, might reveal further information on the prevalence and distribution of BDV in the bovine population.

This report supports the notion that BDV is more widely distributed than previously thought and it remains to be determined whether immunohistological methods will identify other host species in endemic areas. This approach might also lead to the detection of the agent in areas with no history of BD and assist in elucidating the aetiology of as yet ill-defined diseases of the CNS.

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