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Atypical cerebral listeriosis associated with *Listeria innocua* in a beef bull

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ABSTRACT

Natural infections of cattle associated with *Listeria innocua* have not been reported. This report describes the first case of cerebral listeriosis in a bull due to *Listeria innocua*. The animal presented neurological signs characterized by weakness, incoordination and recumbency. Histopathologic evaluation of brain tissue revealed multifocal microabscesses, perivascular lymphocytic cuffing, vasculitis, oedema and haemorrhages. All lesions extended from the medulla oblongata to the basal nuclei/parietal cortex area. Indirect immunohistochemistry labelled for *Listeria* sp. in the brain tissue, but not for *Listeria monocytogenes*, neurotropic Flaviviruses, BVDV, bovine Herpesvirus 1, *Chlamydophila* spp. and *Histophilus somni*. PCR was negative for ovine herpesvirus. *L. innocua* was isolated from brainstem and identified by biochemical tests (Camp and beta-hemolysis negative). Subsequently, the species was confirmed by a duplex PCR and minisequencing assays. *L. innocua* should be histologically considered as a differential diagnosis of thrombotic meningoencephalitis, malignant catarrhal fever and cerebral listeriosis due to *L. monocytogenes* in cattle.

1. Introduction

Listeria spp. are Gram-positive facultative intracellular bacteria ubiquitously distributed in the environment capable of growing at a wide range of pHs and temperatures (Vazquez-Boland et al., 2001). *Listeria* species are divided in two closely related lineages: *Listeria monocytogenes* and *Listeria innocua* form one group, while the second includes *L. welshimeri*, *L. ivanovii*, and *L. seeligeri* (Hain et al., 2006). Recently, two phylogenetically distant species *L. marthii* (Graves et al., 2010) and *L. rocourtiae* (Leclercq et al., 2010) were also described. Almost all cases of human listeriosis are due to *L. monocytogenes*; very rare infections due to *Listeria ivanovii* and *Listeria seeligeri* have been described (McLauchlin and Martin, 2008). *L. monocytogenes* is also the major pathogen for other animals, although approximately 10% of septicaemia in sheep has been reported as due to *L. ivanovii* (McLauchlin and Martin, 2008). Widespread in the environment and in food, *L. innocua* is generally considered nonpathogenic (Vazquez-Boland et al., 2001). On the other hand, *L. innocua* has been associated with a human case of fatal sepsis and identified via blood culture and PCR assay (Perrin et al., 2003) and with a ewe that presented meningoencephalitis by brain bacterial culture and PCR assay (Walker et al., 1994). There are no detailed gross, histopathologic and molecular descriptions of fatal *L. innocua* infection in cattle. The authors describe the neuropathology of one case of *Listeria innocua* meningoencephalitis in a bull in Northwestern Italy.

2. Materials and methods

An 18 months old Blonde Aquitaine bull presented neurological signs characterized by weakness, incoordination and recumbency. The animal died after 1 day and was referred to the Department of Animal Pathology of Turin University for a complete necropsy. During the post mortem, collected tissue samples were fixed in 10% neutral buffered formalin. Obex, pons, mesencephalon, cerebellum, hippocampus, thalamus, basal nuclei area, occipital, parietal and frontal cortex were transversally cut, routinely processed and embedded in paraffin. Five μm serial tissue sections were prepared for histopathologic and immunohistochemical examinations. Deparaffinized sections for histopathology were stained with haematoxylin and eosin (H&E).

Briefly, indirect immunohistochemistry was performed using primary antibodies against *Listeria* sp. (code 43251; Virostat, Portland, USA), *L. monocytogenes* (code 223021; Becton Company, Sparks, USA), Arboflaviviruses (Bioreliance, Rockville, MD, USA), Bovine Viral Diarrhea Virus (BVDV) (provided by Dr. E. J. Dubovi, College of Veterinary Medicine, Cornell University, Ithaca), bovine

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Herpesvirus 1 (VMRD, Pullman, USA), *Chlamydophila* spp. (Chemicon, USA), and *Histophilus somni* (provided by Dr. J. Lopez, Universidad Nacional Autonoma de Mexico, Mexico City). For antigen retrieval, all but one brain tissues were incubated in Proteinase K; citrate buffer (pH 6.0) was used for slides incubated with primary *Listeria* sp. antibody. The secondary antibodies were: immunoperoxidase staining using Vectastain Elite ABC kit (Vector Laboratories, Burlingame, USA) for *Listeria* sp. slides; DAKO LSAB2-HRP (DAKO, Carpinteria, USA) secondary kit detection system (biotin labelled goat anti-rabbit + goat anti-mouse) for *L. monocytogenes*, bovine herpesvirus 1 and *H. somni* slides; and DAKO EnVision™+/HRP kit (DAKO, Carpinteria, USA, code K5361) for Arboflaviviruses, BVDV and *Chlamydophila* spp. Subsequently, the 3,3′-diaminobenzidine chromogen (DAB) was incubated for 1 min at room temperature. Finally, the sections were counterstained using Mayer's haematoxylin. Malignant catarrhal fever (MCF) ovine Herpesvirus polymerase chain reaction (PCR) was performed from paraffin embedded tissues.

A fresh sample of the malacic area in the brainstem (Fig. 1a and b) was submitted for bacterial isolation in Blood and MacConkey Agar at 37 °C for 48 h. Subsequently, the same samples were placed in liquid media (Fraser broth and Demi-Fraser broth) and incubated at 37 and 4 °C for 48 h. Cultures were then streaked onto Listeria Oxford Agar (Microbiol, Italy) and incubated at 37 °C for 48 h. To identify the isolate, a miniaturized system was used (colorimetric Vitek 2 identification system, bioMerieux Inc, Bagno a Ripoli, Italy), as well as further CAMP and betahaemolysis tests.

For the confirmation of the species of the isolate, biomolecular assays (Dalmasso et al., 2010) were also performed from the broth cultures and from a frozen sample of the brainstem. DNA was extracted by boiling. A duplex PCR characterized by a specific fragment on phosphatidylinositol-specific phospholipase C (plcA) gene for *L. monocytogenes* (632 bp long) and by a common fragment of 16S rRNA gene for all *Listeria* spp. (437 bp long) was performed. When both bands were detected in the agarose gels, *L. monocytogenes* was immediately identified. When only the 437-bp band was present, it was possible to identify the *Listeria* genus but not to discriminate between the other *Listeria* species (*L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii*, and *L. grayi*). In this case, the amplified product (437-bp band) was purified and submitted to a minisequencing reaction able to detect diagnostic sites that are able to identify five *Listeria* (already mentioned above; according to Dalmasso et al., 2010).

3. Results

At necropsy no significant macroscopic findings were noted in organs or tissues other than the brain. Transversal sections of the brain revealed dark brown areas interpreted as malacic foci involving the obex, pons, mesencephalon, thalamus and basal nuclei. Encephalomalacia was prominent in the obex and the right part of the pons. These lesions continued rostrally involving the right portion of the brain to the basal nuclei area as well as the adjacent parietal cerebral cortex (Fig. 1a–d).

Microscopically, lesions were moderate to severe, including multifocal microabscesses, vasculitis, perivascular lymphocytic cuffing, oedema and haemorrhages (Fig. 2a–d). Microabscesses were characterized by multifocal randomly distributed aggregates of neutrophils, small to coalescing foci of liquefactive necrosis and oedema. In the areas adjacent to the microabscesses there was multifocal gliosis, diffuse infiltration of neutrophils and microglial cells as well as neuronal necrosis. The microabscesses particularly involved the pons, cerebellum, thalamus and adjacent cerebral cortex. Necrotizing vasculitis with frequently associated perivascular haemorrhage was also observed within the brainstem, cerebellum, and white matter of the cerebral cortex. Perivascular cuffing was rarely observed in the obex, pons and midbrain and was composed of one to two layers of lymphocytes and occasional macrophages. Haemorrhages were observed in all areas of the brain, but were more severe in the cerebellum, thalamus and basal nuclei, where coalescing microabscesses were mostly observed. Multifocal moderate mononuclear leptomeningitis adjacent to microabscesses was also observed in the brainstem and cerebellum. Vagus, trigeminal and facial brainstem nuclei were affected by non-coalescing microabscesses and focal perivascular cuffing. Immunohistochemistry for *Listeria* sp. allowed for the identification of the bacteria within the microabscesses, with the highest intensity noted in the cerebellum, thalamus (Fig. 2d) and mesencephalon. *Listeria* antigen was observed in the cytoplasm of mononuclear and

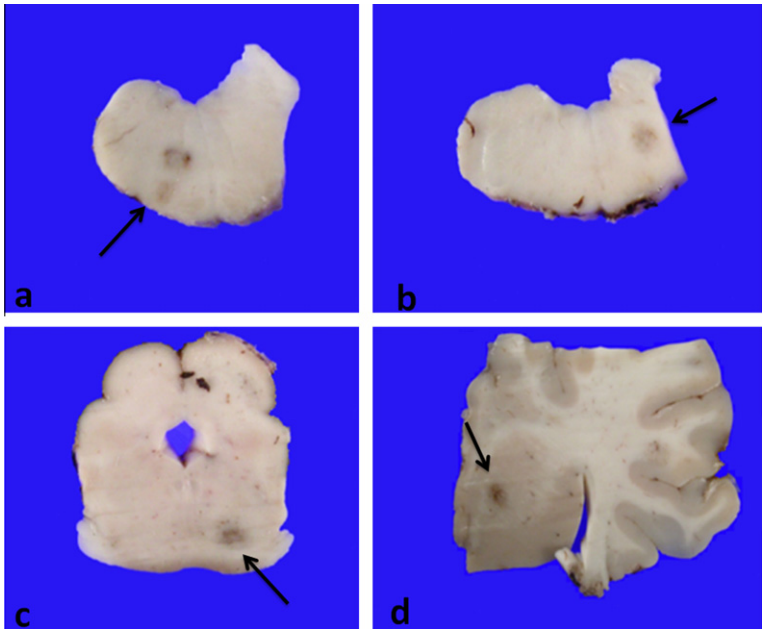

Fig. 1. (a) Brain, obex. (b) Brain, brainstem. (c) Brain, midbrain. (d) Brain, basal nuclei. Note the dark-brown malacic areas (arrows).