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Matrix metalloproteinase expression in sheep with listerial meningoencephalitis

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ABSTRACT

Matrix metalloproteinases (MMPs) have been implicated in the pathogenesis of several central nervous system (CNS) diseases. In this study, we investigated the presence of *Listeria monocytogenes* antigens and detected the expression of MMP-9 and MMP-7 in the brains of 22 sheep with clinical signs and histopathological findings characteristic of listerial meningoencephalitis. Archived sections from the brainstem, cerebrum, and cerebellum were stained for immunohistochemistry. *L. monocytogenes* antigens were located mainly in the cytoplasm of neutrophils and some macrophages and/or extracellularly within microabscesses of the brainstem. MMP-9 was mainly immunolocalised in the endothelial cells, microglial cells, and neurons especially in inflammatory areas. MMP-7 immunoreactivity was detected in perivascular cuffs, microglial cells, and only a few neurons. Overall, immunohistochemistry in formalin-fixed, paraffin-embedded tissues is a useful tool for the diagnosis of encephalitic listeriosis caused by *L. monocytogenes*, and MMP-9 and MMP-7 may contribute to the pathogenesis of listerial meningoencephalitis.

1. Introduction

Listeria monocytogenes is the causative agent of listeriosis, a severe disease associated with a high mortality rate. Nearly all domestic animals are susceptible to *L. monocytogenes* infection, but animal listeriosis most commonly occurs in ruminants. Listeriosis is also an important food-borne zoonosis. It usually presents with three distinct clinical syndromes, septicemia, encephalitis (meningoencephalitis), and abortion. Listeriosis has also been associated with mastitis and some ophthalmic lesions, such as keratoconjunctivitis (Jubb and Huxtable, 1993; OIE, 2009; Picoux, 2008).

The detection of *L. monocytogenes* is very important with regard to food hygiene and safety. Conventional bacteriological testing and histological examination are the classic methods used for the laboratory diagnosis of listeriosis in animal specimens. Immunohistochemistry is a much more efficient tool compared with other methods and can be very helpful for confirming listerial encephalitis (Campero et al., 2002; Loeb, 2004; OIE, 2009; Picoux, 2008).

One of the important clinical signs of listeriosis is infection of the central nervous system (CNS). *L. monocytogenes* has affinity for the brain stem. But, usually there are no gross lesions seen in the brain of ruminants. The characteristic histopathological lesions of listerial encephalitis are microabscesses that commonly include neutrophils mixed with varying amounts of macrophages and microglial cells (Jubb and Huxtable, 1993; OIE, 2009; Oevermann et al., 2010). Inflammatory cells stimulate the secretion of proinflammatory cytokines, and the microglial cells activated by these cytokines may impair neuronal function by producing matrix metalloproteinases (MMPs) such as MMP-9 (Berman et al., 1999). An emerging body of evidence suggests that MMPs are important factors in the pathogenesis of meningitis. Gelatinase B (MMP-9) and matrilysin (MMP-7) have been shown to be increased during inflammatory diseases of the CNS (Berman et al., 1999; Kieseier et al., 1999).

To the best of our knowledge, however, neither study detected MMP expression in the brains of sheep naturally infected with *L. monocytogenes*. We therefore investigated the expression of MMP-9 and MMP-7, and evidence of *L. monocytogenes* antigens in the brains of naturally infected sheep with *L. monocytogenes* by using the avidin–biotin-peroxidase complex (ABC) immunohistochemistry staining method.

This retrospective study was based on archived tissue sections selected from 22 sheep with a diagnosis of listeriosis based on clinical signs, bacteriological culture (nine cases) and histopathological examinations. Sections of normal brain tissue, obtained from five healthy sheep without any clinical symptoms or histopathological findings of CNS abnormalities, were used as a negative control.

Brainstem, cerebrum, and cerebellum samples were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 4 μm sections, and stained with hematoxylin and eosin (HE) using routine protocols, and selected sections were stained with

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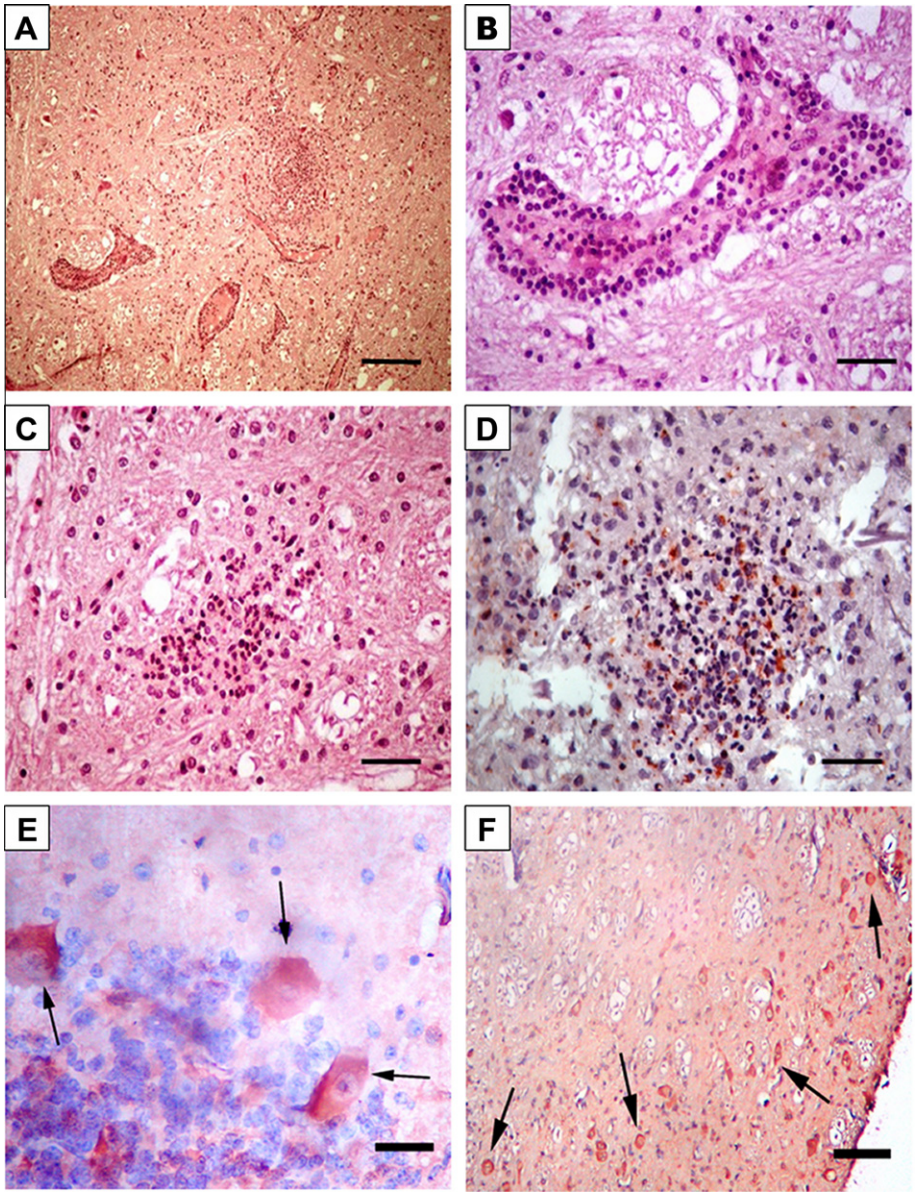

Fig. 1. Listerial meningoencephalitis. (A) Perivascular cuffing with lymphocytes and microabscesses. HE stain, Bar: 100 μm. (B) Perivascular cuffing composed of lymphocytes with few plasma cells, macrophages and neutrophils. HE stain, Bar: 30 μm. (C) Microabscess composed mainly of neutrophils. HE stain, Bar: 30 μm. (D) İmmunohistochemical demonstration of *Listeria monocytogenes* antigens in microabsceses. Mayer's hematoxylin counterstain, Bar: 30 μm. (E) A few MMP-7 positive in neurons. Mayer's hematoxylin counterstain, IHC stain, Bar: 10 μm.(F) MMP-9 immunoreactivity is seen numerous neurons (thin arrows), Mayer's hematoxylin counterstain, IHC stain, Bar: 30 μm.

Brown-Hopps Gram stain. Immunohistochemistry was performed using the avidin–biotin immunoperoxidase complex (ABC) method. The Universal LSAB 2 Kit (DAKO Corporation, USA) was used as the commercial secondary system, and the primary antibodies used were a rabbit polyclonal antiserum against *L. monocytogenes* (*Listeria* O antiserum poly, serotypes 1 and 4) (Difco, Detroit, USA), anti-MMP-9 (Collagenase IV, Ab-9, rabbit polyclonal antibody, Thermo scientific, California), and anti-MMP-7 (Matrilysin, Ab-1, monoclonal antibody, Thermo scientific, California). All tissue sections were deparaffinized in xylene and rehydrated in graded alcohol. During the antigen retrieval step, the tissue sections were heated in a microwave oven in 0.01 mol/L citric acid for 5 min, followed by cooling for 20 min. Endogenous peroxidase was blocked by immersing the sections in 0.3% hydrogen peroxide in absolute methanol for 30 min. The sections were washed with phosphate-buffered saline (PBS; pH 7.2) and pretreated for 5 min with a protein blocker. All sections were incubated with the primary antibody (MMP-9 and MMP-7 1:200, *Listeria* O antiserum 1:1000) for 60 min at room temperature. After washing with PBS, the sections were incubated for 20 min with a biotinylated goat anti-rabbit antibody at room temperature. After other PBS rinse, the sections were treated with horseradish peroxidase–conjugated streptavidin for 20 min. After washing in PBS, amino-ethyl carbazole (AEC) was used as a chromogen, and Mayer's hematoxylin was used for counterstaining. As a positive control, sections from FFPE encephalitic listeriosis with confirmed bacterial isolation of *L. monocytogenes* were used. The negative control replaced the primary antibody with PBS and was included for each slide run. Additionally, brain samples obtained from five clinically healthy sheep and samples negative for bacteria were used as negative controls.

In this study, 22 cases with clinical signs and histopathological findings characteristic of listerial meningoencephalitis were