**Listeria monocytogenes-associated meningo-encephalitis in cattle clinically suspected of bovine spongiform encephalopathy in Belgium (1998-2006)**


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**ABSTRACT**

*Listeria monocytogenes* is an important foodborne pathogen both in humans and animals. In order to determine the presence and importance of this zoonotic bacterial disease in a subgroup of the Belgian cattle population, all the brain tissue specimens originating from 2,432 cattle clinically suspected of bovine spongiform encephalopathy (BSE) that had been submitted to the National Reference Laboratory during the period 1998-2006 were examined for the presence of histopathological lesions pathognomonic for *L.* monocytogenes meningoencephalitis. Additional *Listeria*–specific immunohistochemistry was performed in order to confirm the diagnosis of these cases. While in recent years no listeriosis cases have been reported in cattle in Belgium, this study indicates that meningo-encephalitis due to listeriosis is still a non-negligible disease in the Belgian cattle population. The zoonotic character of *L.* monocytogenes justifies maintaining vigilance for this disease.

**SAMENVATTING**

*Listeria monocytogenes* is een belangrijk voedselgeassocieerd pathogene agens bij mens en dier. Om de aanwezigheid en het belang van deze zoönotisch bacteriële ziekte te bepalen in een subgroep van de Belgische rundveebevolking werden alle hersenstalen van de 2.432 rundertjes klinisch verdacht van bovine spongiforme encefalopathie (BSE) die naar het TSE-laboratorium van het CODA werden gestuurd in de periode van 1998 tot 2006, onderzocht op histopathologische letsels pathognomonisch voor *L.* monocytogenes meningo-encefalitis. Bijkomend werd er een *Listeria* specifieke immunohistochemische kleuring uitgevoerd om deze diagnose te bevestigen. Terwijl er de laatste jaren officieel geen listeriosegaven in de Belgische rundveebevolking werden beschreven, toont de voorliggende studie aan dat meningo-encefalitis ten gevolge van listeriose nog steeds een niet te verwaarlozen ziekteagens is in de Belgische rundveebevolking. Het zoönotische karakter van *L.* monocytogenes noopt ertoe om alert te blijven voor deze ziekte.

**INTRODUCTION**

*Listeria monocytogenes* is a gram-positive bacterium that occurs widely in nature. The organism, which is resistant and persists in the environment, may be found in soil, plants, silage and feces. Subclinical infections in animal populations are probably common, as evidenced by the presence of serum agglutinins in normal animals (Borman *et al.*, 1960). On the basis of flagellar and somatic antigens, more than 14 serotypes are recognized, but the vast majority of clinical cases are caused by only three serotypes (1/2a, 1/2b, and 4b) (Summers *et al.*, 1995; Borucki and Call, 2003, Maxie and Youssef, 2007, Laureyns *et al.*, 2008). Listeriosis usually results from infection by *L.* monocytogenes, but *L.* ivanovii has also been associated with abortions in sheep and cows, or septicemia in sheep (Laureys *et al.*, 2008).

There are three syndromes described: 1) septicemic disease with localization in the liver, spleen and other viscera; 2) metritis, placentalitis and abortion; 3) (meningo)encephalitis. These three seldom overlap so that each syndrome probably has a separate pathogenesis (Summers *et al.*, 1995; Maxie and Youssef, 2007). (Meningo)encephalitis is characterized microscopically by a mixed nonsuppurative and suppurative inflammation centered in the pons and medulla oblongata. Inflammation tapers off rostrally and caudally, typically extending from the thalamus to the cervical spinal cord. Additional characteristics are the formation of microabcesses, prominent perivascular cuffs that include lymphocytes, monocytes, plasma
cells and to a lesser extent neutrophils, astrocytosis and microgliosis, as well as secondary areas of malacia and necrosis of individual neurons. In fact, in animals, *Listeria* has a remarkable affinity for the brain stem (summers et al., 1995; Maxie and Youssef, 2007). Listerial meningoencephalitis occurs almost solely in adult ruminants (Maxie and Youssef, 2007). It is a common endemic problem in sheep, cattle and goats. An association with feeding corn or grass silage has been recognized (vasquez-boland et al., 1992; wilesmith and Gitter, 1986). Meningoencephalitis may occur as a flock problem in sheep and goats, whereas single cases are the rule in cattle herds (Rebhun and de Lahunta, 1982).

Human cases of listeriosis are almost exclusively caused by the species *L. monocytogenes* (one of the six known species). Cooking kills *Listeria*, but the bacteria are known to multiply at chill temperatures down to 2-4°C, which makes its occurrence in ready-to-eat foods with relatively long shelf life particularly problematic. In healthy adult humans, infection does not result in significant disease, but severe illness may occur in the unborn child, infant, the elderly and those with a compromised immune system. Symptoms vary, ranging from mild flu-like symptoms and diarrhea to life-threatening infections characterized by septicemia and meningoencephalitis. Human disease cases are rare, but are important because of the high mortality rate, which often reaches as high as 20-30 % (yde, 2008). In fact, listeriosis is amongst the most important causes of death from foodborne infections in industrialized countries (EFSA, 2007), and recent data suggests that in Europe the incidence of sporadic, non-pregnancy-related cases has increased during the last decades (Antal et al., 2007). Finally, cutaneous lesions are occasionally described (Regan et al., 2005, Laureyns et al., 2008).

In order to evaluate the importance of meningoencephalitis caused by listeriosis in Belgium’s cattle population, the cases sent in as suspected for BSE were screened. Such screening has in fact proven to be a good tool for this purpose, mainly because in several different countries the most frequent differential diagnosis to BSE has been encephalic listeriosis (agerholm et al., 2002; miyashita et al., 2004; McGill and Wells, 1993; saegerman et al., 2004).

**MATERIALS AND METHODS**

As mentioned above, all BSE clinically suspected bovines were screened. The BSE clinically suspected animals included all animals of 24 months and older presenting neurological symptoms at the farm or slaughterhouse, for which BSE could not be excluded, as well as the emergency slaughtered animals. According to the protocol, no necropsy could be performed. After excluding rabies as a routine precaution, using a direct immunofluorescence technique and isolation on cultures of neuroblastoma cells (Vanopdenbosch et al., 1998), samples of the brain stem, the cerebrum and the cerebellum were taken for further BSE testing (using rapid TeSeE Elisa, Western blotting and immunohistochemistry) and histological examination (Vanopdenbosch et al., 1998). For the histological examination, large samples of the three major brain parts mentioned were fixed in a 4% phosphate-buffered formaldehyde solution, processed routinely, paraffin-embedded, and sectioned at 5-µm thickness. The sections were stained with hematoyxlin–eosin staining. The rest of the brain was put in the freezer at –20°C. All samples from cerebrum, cerebellum, midbrain, pons and obex were examined for the typical lesions described above, namely lesions pathognomonic for a *L. monocytogenes* infection.

Additionally, a polyclonal anti-*Listeria monocytogenes* primary antibody (code ATCC 43251; AbD Serotec, Oxford, UK) in 1/300 dilution was used as described by loeb (2004) in order to confirm the

![Figure 1. Yearly percentage of listeriosis cases compared to the total number of BSE suspected cases examined (1998-2006).](image-url)
diagnosis. As negative controls, samples from a case of BHV1 infection (Roels et al., 2000) and a bacterial infection of the 4th ventricle (Roels et al., 2001) were used. This screening, which was performed over a period of 9 years (1998-2006), involved comparing the number of *L. monocytogenes* cases found against the total number of BSE suspect cases examined (Figure 1) and making a monthly breakdown of these numbers and percentages (Figure 2) in order to evaluate the seasonal effect.

The relationship between the monthly and yearly numbers of *L. monocytogenes* meningoencephalitis cases and the total numbers of BSE suspected cases, as well as the percentages of *L. monocytogenes* meningoencephalitis cases and the total numbers of BSE suspected cases as compared to the total cattle population (Figure 3) were controlled using Friedman’s two-way nonparametric analysis of variance and the Pearson correlation test. In order to evaluate the evolution of the numbers of cases, linear regression and the two-sample t-test by category were performed (Statistix 1.0 for windows, 1996).

RESULTS

First of all, the data indicate the presence of *Listeria* meningoencephalitis in the Belgian cattle population, with the highest number in 2001 (60 cases) and lowest number in 2006 (15 cases). In total, 331 cases (13.6%) were found with histopathological lesions pathognomonic for a *L. monocytogenes* infection out of the 2432 clinically suspected BSE cases. None of them had a concurrent BSE infection. The highest percentage of cattle found with *L. monocytogenes*
mengingoencephalitis in proportion to the total number of BSE suspected cases was 21.7% in 1998, and the lowest was 8.1% in 2006. In proportion to the total cattle population, the highest percentage was detected in 2001 (0.0039%), and the lowest in 2006 (0.0011%). The immunohistochemical staining revealed variable degrees of positive rod-shaped dark-stained bacteria mainly in the macrophage cytoplasm of microabscesses, but in some cases the bacteria were lying free in the neuropil and only associated with some glial reaction. Occasionally, the bacteria were fragmented. The presence of positive staining bacteria was sometimes very limited and localized. This could be due to the lack of the limited number of microabscesses. In fact, Loeb (2004) mentions that microabscesses are necessary for a reliable immunostain. However, it turned out that all the cases could be confirmed using this method.

Using statistical tools, it was found that there were significant differences between the different months and between the different years concerning both the numbers of listeriosis cases and the total numbers of clinically suspected BSE cases examined. Additionally, there was also a correlation between the number of listeriosis cases per month and the total number of BSE suspected cases per month ($r = 0.95; P < 0.001$) and per year ($r = 0.84; P = 0.009$). No significant correlation could be found either between the number or the percentage (Figure 3) of listeriosis cases per year and the total number of BSE suspected cases per year. Finally, no statistically significant decline could be noted.

Comparing the number of cases per month in this 9-year period, this data confirms the cyclic, seasonal pattern (Summers et al., 1995), with the highest prevalence being from December to June and the lowest from July to November (Figure 2).

DISCUSSION

Eighty years ago L. monocytogenes was first recognized as an animal pathogen (Murray et al., 1926) and as a predominantly foodborne disease (Pirie, 1927).

Diagnosis of Listeria meningoencephalitis can be done using bacterial isolation, but this technique is not very sensitive. In fact, isolation of the organism by direct plating is relatively easy when the numbers are large in a normally sterile site, as in the case of the septicemic form of the disease, but isolation is difficult when the organism is present in low numbers, as in the case of the encephalitic form or when samples are heavily contaminated (OIE, 2004). Another restriction to bacterial testing was the fact that for a majority of the samples, only formalin-fixed material was available. For this reason, and because of the CODA TSE Laboratory’s experience in histopathology, the histopathological evaluation was preferred over bacterial isolation. In fact, the OIE describes histopathology as characteristic of the disease (OIE, 2004). Studies have shown that the application of immunohistochemistry in the diagnosis is more reliable in confirming L. monocytogenes infection than bacteriological culture and the finding of gram-positive bacteria (Parkash et al., 1998; Campero et al., 2002; Loeb, 2004).

Previous studies suggest that the prevalence of L. monocytogenes on cattle farms is seasonal, with most cases occurring in late winter and early spring. In part, this may reflect the fact that animals housed through the winter are fed silage, but cases also occur frequently at pasture (Husu, 1990; Nightingale et al., 2005; Summers et al., 1995). Similar findings were seen in the present study, with the majority of the cases occurring between December and June.

In cattle, the number of positive cases is generally low, with percentages varying from 4.6 to 0.7% of the clinically suspected cattle (EFSA, 2007). In Belgium, the only data available is on the bacterial isolation of L. monocytogenes in humans and food. Except for a recent case report (Laureyns et al., 2008), no other data on the presence of L. monocytogenes in animal species in Belgium has been reported (EFSA, 2007). Even though the significance of this syndrome in the global picture of Listeria infections in cattle is a matter of discussion, the detection of L. monocytogenes meningoencephalitis in the Belgian cattle population may constitute a valuable piece of information. The present survey also clearly shows that, at least until 2006, L. monocytogenes has always been present in cattle in Belgium. How closely the number of detected cases reflects the true prevalence of L. monocytogenes meningoencephalitis among the Belgian cattle population is difficult to establish for certain. The number of cases detected is probably appreciably lower than the true prevalence, due to the fact that only cattle that are 24 months of age and older are included in the group of clinically suspected BSE cases examined (Vanopdenbosch et al., 1998).

In any case, L. monocytogenes remains a major concern to the food industry and public health authorities. Listeria is ubiquitous and widely distributed in the environment (soil, vegetables, meat, milk, fish) and is mostly transmitted to humans via the consumption of contaminated food. Unfortunately, the specific source of contamination is rarely demonstrated, even though a monitoring program is in place to control more than 100 meat cutting plants and more than 200 retail trades representative of the Belgian food production industry (Yde, 2008). In view of the likely role of ruminants as a reservoir for human infections (Boerrlin and Piffaretti, 1991; Borucki et al., 2004, Nightingale et al., 2004), the present study demonstrates that the surveillance of L. monocytogenes cases in ruminants can add to the global surveillance of Listeria prevalence in food and humans, with the aim of evaluating the public and animal health threat.

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