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

Listeria monocytogenes *geassocieerde meningo-encefalitis bij runderen klinisch verdacht van boviene spongiforme encefalopathie in België (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 pathogeen agens bij mens en dier. Om de aanwezigheid en het belang van deze zoönotisch bacteriële ziekte te bepalen in een subgroep van de Belgische rundveepopulatie werden alle hersenstalen van de 2.432 runderen klinisch verdacht van boviene 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 confirmeren. Terwijl er de laatste jaren officieel geen listeriosegevallen in de Belgische rundveepopulatie 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 rundveepopulatie. 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 (Laureyns *et al*, 2008).

There are three syndromes described: 1) septicemic disease with localization in the liver, spleen and other viscera; 2) metritis, placentitis 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 exa- mination, 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 hematoxylin-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

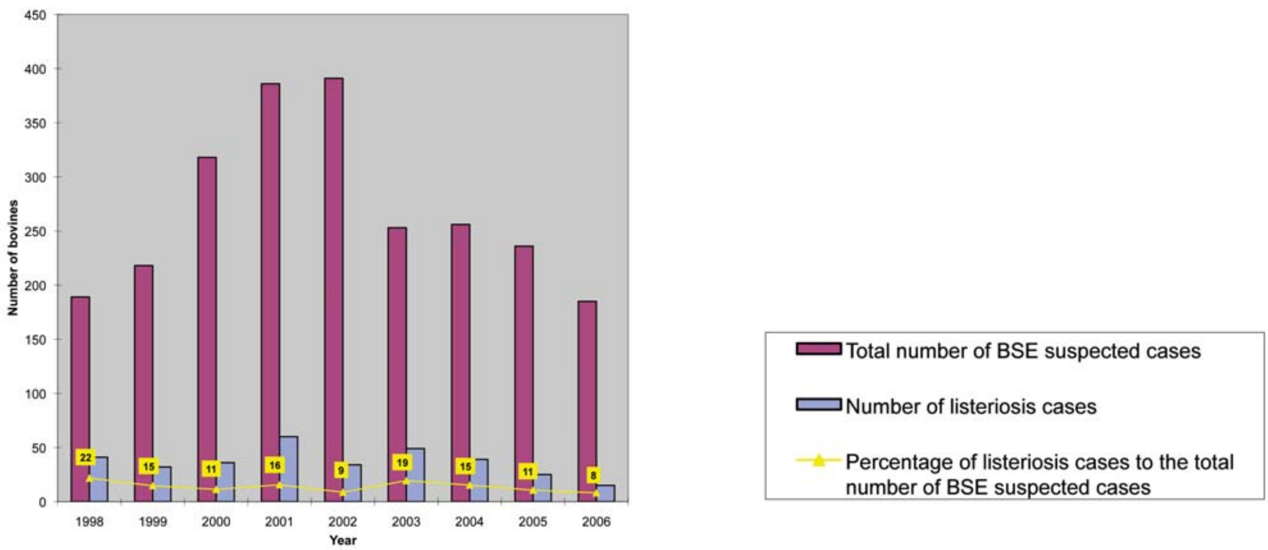

Figure 1. Yearly percentage of listeriosis cases compared to the total number of BSE suspected cases examined (1998-2006).

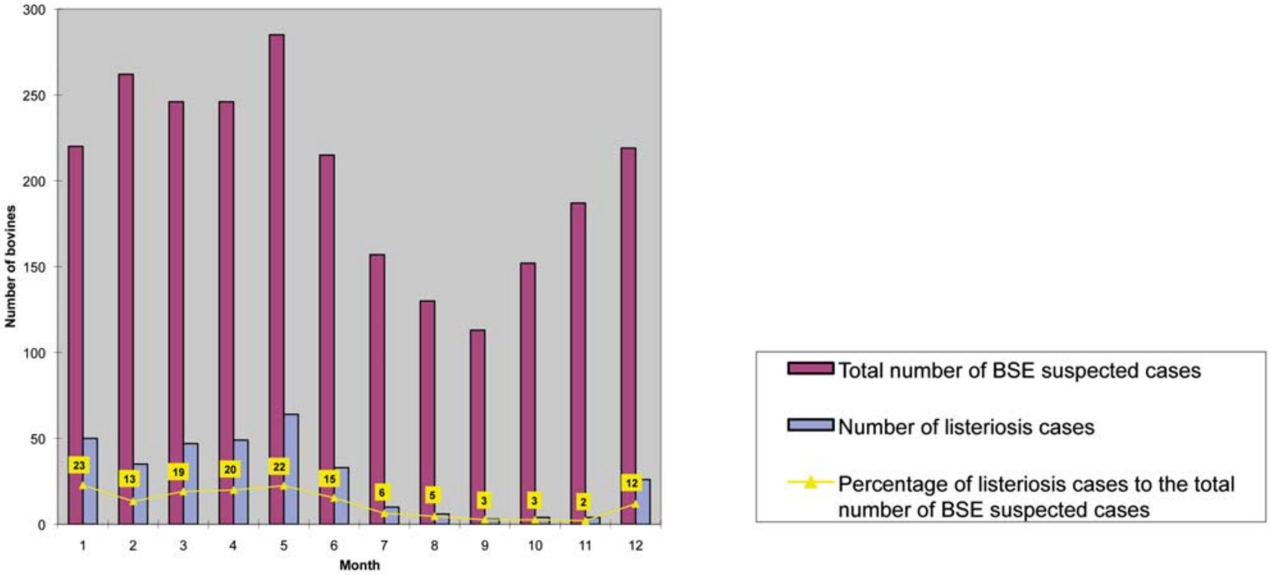

Figure 2. Monthly percentage of listeriosis cases compared to the total number of BSE suspected cases examined (1998-2006).

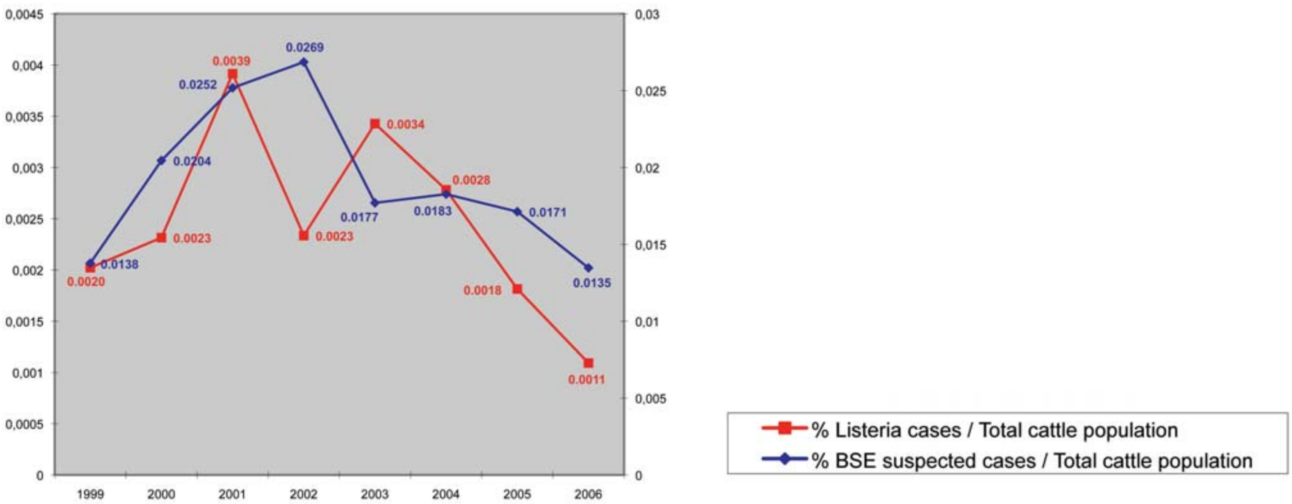

Figure 3. **Yearly percentage of Listeria and BSE suspected cases compared to the total cattle population of that year (1999-2006).**

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* meningoencephalitis 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 micro-abscesses, 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 or the limited number of microabcesses. In fact, Loeb (2004) mentions that microabcesses 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 (Boerlin 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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