OVERVIEW OF *LISTERIA MONOCYTOGENES* CAUSED ABORTIONS IN CATTLE IN LATVIA IN 2013

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Abstract
Listeria monocytogenes is a pathogen that causes infectious diseases in animals and foodborne infection in humans. The aim of this retrospective study was to analyse *L. monocytogenes* caused abortions in cattle in Latvia in 2013, and to describe the potential reasons of these abortions. In total, 186 cattle abortion cases were investigated. The pathological material from aborted foetuses included samples of brain, heart, liver, spleen, kidneys and lung, liquid of stomach and liquid from thoracic and abdominal cavities. These samples were tested with bacteriological methods according to ISO 11290-1:2007. *L. monocytogenes* was found in 44 (23.7%) cases of cattle abortions. Positive cases were more distributed in the central and the south-eastern parts of Latvia, because in this territory winters tend to be wetter and colder than in other parts of Latvia. Seasonality was observed of *L. monocytogenes* caused abortions and the majority of cases occurred during spring and autumn, when the cattle were fed with silage. All abortion cases were observed in the second and the third trimester of the gestation. *L. monocytogenes* caused abortions occurred in cattle of different ages, but significantly ($p<0.05$) more often – in 3 years old cattle.
Key words: *Listeria monocytogenes*, cattle, abortions, Latvia.

Introduction

Genus *Listeria* consists of various species, but only two of them are pathogenic to animals – *L. monocytogenes* and *L. ivanovii* (Husu et al., 1990; Frances et al., 1991; Czuprynski et al., 2010) and *L. monocytogenes* also for humans. *L. monocytogenes* is a facultative intracellular pathogen that is associated with severe foodborne infections in humans and may cause infectious disease in many different animal species, especially in farm ruminants – cattle, sheep and goats (Nightingale et al., 2004). Bacteria of genus *Listeria* are widely distributed in different environments – in soil, water, sewage, on the plants, in the intestinal tract of various animal species, and also in farm environments. Listeriosis in animals can be observed in different clinical forms, such as, infections of central nervous system, uterine infection, gastroenteritis (Bartt, 2000; Siegman-Igra et al., 2002; Drevets and Bronze, 2008), and sometimes *L. monocytogenes* can cause keratoconjunctivitis and mastitis (Erdogan et al., 2001). Uterine infection usually results in late-term abortions or stillbirth and septicemia of neonates. The symptoms of neural form of listeriosis include encephalitis, unilateral facial paralysis, circling and excessive salivation. Eye infection is associated with direct inoculation of the eye with *L. monocytogenes* present in feed, especially in silage (Nightingale et al., 2004). Contaminated forage, most often poor quality or damaged silage, can cause outbreaks of listeriosis among the animals (Low and Donachie, 1997). *L. monocytogenes* usually enters the animal through ingestion, then the pathogen is disseminated via haematogenous spread to the viscera, brain, and gravid uterus (Ryser and Marth, 2007). In pregnant animals, listeria can localize in the placentomes and enter the amniotic fluid, subsequently the foetus aspirates the pathogen, which multiples and kills the foetus in the late-term of gestation (Timoney et al., 1988). Different factors can predispose to listeriosis infection – season, immune suppression, for example, pregnancy, and management practices, including feeding silage. Listeriosis in ruminants has a seasonal character, and it mostly occurs in winter or early spring and is associated with feeding of damaged silage (Ryser and Marth, 2007; Hellström, 2011). Faecal shedding by asymptomatically infected livestock poses a risk for contamination of farm environment and raw food at the pre-harvest stages (Zundel and Bernard, 2006). Faecal shedding may reflect levels of *L. monocytogenes* in animal feed. Rodents also are *L. monocytogenes* carriers, therefore can be a potential risk factor for faecal contamination of animal feed (Ryser and Marth, 2007).

In good-quality silage, prepared from grass, whole crop cereals, maize, or leguminous plants, which may or may not be wilted (dried to optimum moisture content) in the field before ensiling, the onset of anaerobic conditions stimulates the indigenous or added lactic acid bacteria to multiply quickly. As these bacteria expand to populations as high as 10^9 CFU g^{-1} within 48 h, they convert the plant sugar to lactic acid, causing conditions for rapid decrease in pH of silage (McDonald et al., 1991). Commonly, a pH of well-preserved and well-prepared silage is lower than 4.5 and such acidic conditions inhibit the growth of microorganisms that reduce the quality of silage, including *Listeria*. Higher dry-matter silage usually tends to have a higher pH, but in these silages the level of available water is lower and this may help to minimize the growth of *Listeria*. Grass silage in

cooler, wetter climate countries tends to have lower sugar levels and higher moisture contents and this results in a poorer, slower fermentation. Therefore in countries with warmer climates, these silages may be more susceptible to *L. monocytogenes* contamination and growth than grass and maize silage. Although *Listeria* dies in a well-fermented silage, if the pH increases before all bacterial cells are killed, the surviving *Listeria* cells will multiply (Ryser and Marth, 2007). D.R. Fenlon et al. (1996) showed that *L. monocytogenes* could survive over one year in bags that were used to wrap big bales and stored for reuse. This indicates that *L. monocytogenes* may survive in silage and can be a potential source of infection in cattle.

The aim of the present study was to analyse *L. monocytogenes* caused abortions in cattle in Latvia in 2013, and to describe the potential reasons of these abortions.

Materials and Methods

This was a retrospective study, which was done on the basis of investigation results of the Institute of Food Safety, Animal Health and Environment 'BIOR', obtained after the bacteriological tests of pathological material from aborted foetuses. Cattle abortion cases were investigated within the annual plan of Food and Veterinary Service of Latvia.

The pathological material from aborted foetuses included samples of brain, heart, liver, spleen, kidneys and lung, liquid of stomach and liquid from thoracic and abdominal cavities. The bacteriological investigation was done according to the ISO 11290-1:2007 with some modifications. For organ samples, 10 – 25 g of organ tissue were aseptically transferred into sterile stomacher closure bags and ½ Frazer broth (Biolife, Italy) was added for enrichment to achieve a 1:10 dilution, then the sample was homogenized in ½ Frazer broth by stomaching (BagMixer® 400, Interscience, France) at a normal speed for 60 s. After incubation at 30 °C for 24 h, 1.0 mL of enrichments in ½ Frazer broth were transferred into Frazer broth (Biolife, Italy) and 0.1 mL of enriched broth were plated onto ALOA medium (Biolife, Italy). Frazer broth and ALOA medium were incubated for 24 h at 30 °C and 37 °C, respectively. After 24 h incubation, 0.1 mL of enriched Frazer broth were plated onto ALOA medium and incubated for 24 h at 37 °C. For liquid samples direct plating onto ALOA medium were performed with subsequent incubation for 24 h at 37 °C. Small and round (0.5 – 1 mm in the diameter) colonies in blue-green colour, surrounded by a characteristic opaque halo on ALOA medium were considered as *L. monocytogenes* and were plated onto sheep blood agar (Biolife, Italy) and incubated for 24 h at 37 °C. Small, round colonies (0.5 – 1 mm

in the diameter) of grey or greyish white colour, which displayed haemolysis on the sheep blood agar were considered as *L. monocytogenes*. Gram staining, catalase tests and commercial identification system Crystal GP (Becton, Dickinson and Company, USA) were performed for the identification and confirmation of *L. monocytogenes*.

According to the Latvian Environment, Geology and Meteorology Centre (LEGMC), the meteorological data – average air temperature and average rainfall – were used to analyse the seasonal influence on *L. monocytogenes* caused abortions.

In order to calculate the age significance in cattle *L. monocytogenes* caused abortion cases, t-test (Microsoft Excel 2007) at the confidence level 95% was performed.

Results and Discussion

In 2013 *L. monocytogenes* were isolated in 44 cases (23.7%) out of 186 investigated aborted foetuses. This is the highest rate of *L. monocytogenes* caused abortions in cattle during the last three years in Latvia. *L. monocytogenes* caused abortions were detected in 22.9% and 16.1% of bacteriologically investigated cattle abortions in 2011 and 2012, respectively (Šteingolde et al., 2013).

Cattle abortion cases due to *L. monocytogenes* were observed mostly in spring (Fig. 1). This could be explained by the fact that listeriosis cases have a seasonal character and more often are observed during indoor season – when animals are fed with silage (Ryser and Marth, 2007). Switching cattle from grazing to a diet of silage, mostly during the indoor season, leads to increased faecal shedding of *L. monocytogenes* (Husu, 1990). Therefore, also in the autumn were observed more abortion cases due to *L. monocytogenes*, than in the summer months. According to the LEGMC data, the April of 2013 was colder and wetter comparing with the previous years, and this could be a potential reason why that pasture season started later than in other years. Therefore, the relatively high number of listeric abortion cases in May 2013 was associated with a more prolonged silage feeding period than in 2011 and 2012. Only two cases of *L. monocytogenes* caused abortions in cattle were observed during the May 2011 and 2012 (Šteingolde et al., 2013).

The gestation period when cattle aborted was known in 30 cases out of total 44 cases. Our study proved the viewpoint that abortions caused by *L. monocytogenes* usually occur in the late-term of gestation (Timoney et al., 1988; OIE manual, 2008). Abortion cases during the first trimester of gestation were not observed in this study. Most often (63%) *L. monocytogenes* caused abortions were observed in the third trimester of gestation: in 11 cases abortions
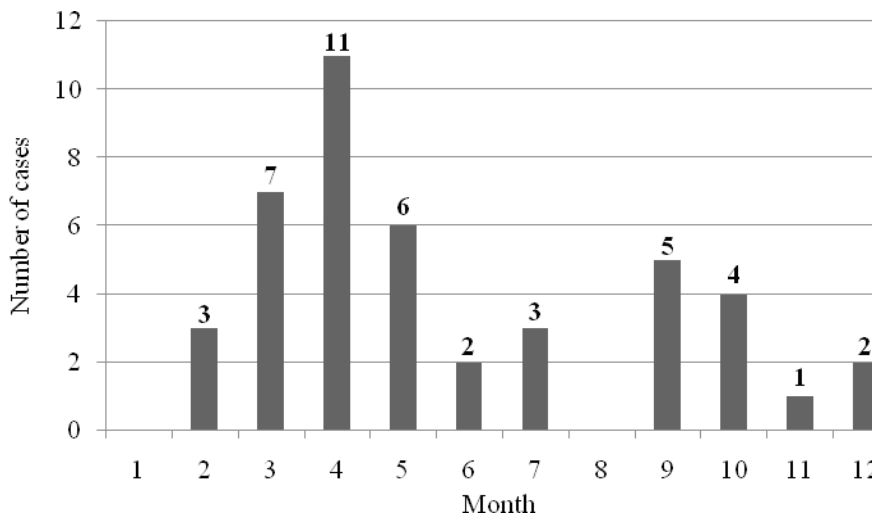
Figure 1. Occurrence of *L. monocytogenes* caused abortions in cattle in 2013: 1 – January;
2 – February; 3 – March; 4 – April; 5 – May; 6 – June; 7 – July; 8 – August; 9 – September;
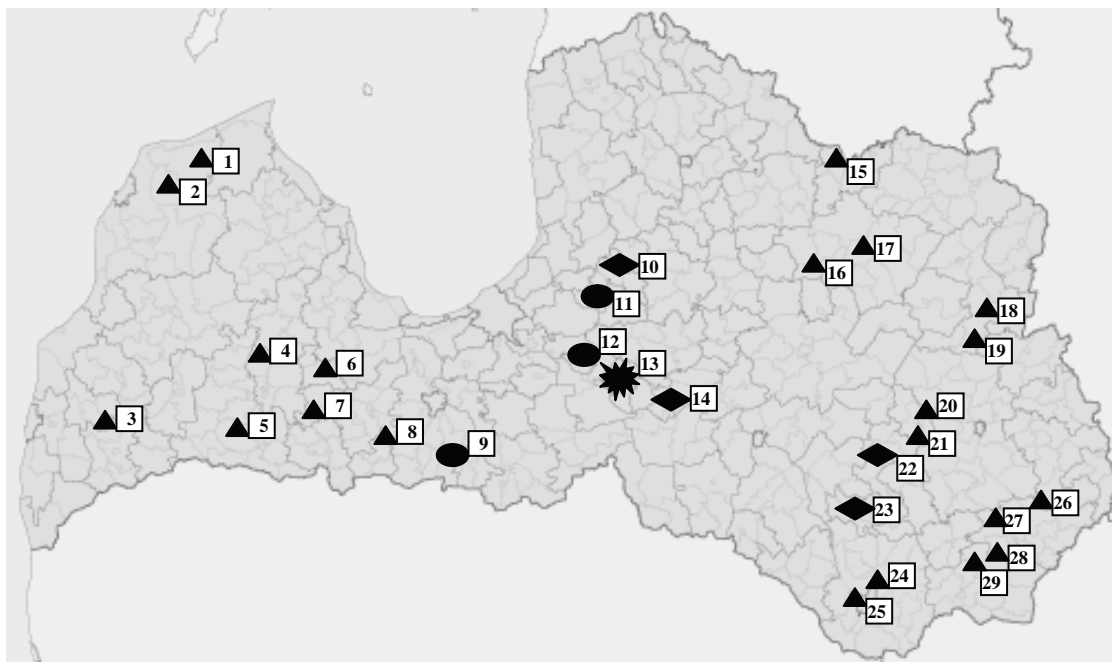10 – October; 11 – November; 12 – December.

Figure 2. Illustrative distribution of *L. monocytogenes* caused abortions in cattle during the year 2013:
✸ - 5 cases in region; ◆ - 3 cases in region; ● - 2 cases in region; ▲ - 1 case in region.

1 – Ances region	9 – Sesavas region	17 – Galgauskas region	25 – Kalkūnes region
2 – Popes region	10 – Siguldas region	18 – Tilžas region	26 – Ezernieku region
3 – Bunkas region	11 – Allažu region	19 – Krišjāņu region	27 – Andrupenes region
4 – Gaiķu region	12 – Ogresgala region	20 – Viļānu region	28 – Skaistas region
5 – Kursīšu region	13 – Lielvārdes region	21 – Galēnu region	29 – Kombuļu region
6 – Jaunpils region	14 – Aizkraukles region	22 – Saunas region	
7 – Zebrenes region	15 – Gaujienas region	23 – Rožkalnu region	
8 – Tērvetes region	16 – Druvienas region	24 – Daugavpils region	

were observed in the 7th month of gestation, in 7 cases abortions occurred in the 8th month of gestation and in 1 case – in the 9th month of gestation. The remaining 37% of *L. monocytogenes* caused abortions were observed during the second trimester of gestation: in 3 cases abortions occurred in the 5th month of gestation and in 8 cases – in the 6th month of gestation. This could be associated with a tendency that in small herds cattle parturitions are planned mostly in spring. Late-term gestation, changes in farming and feeding methods – pasture season changes to indoor season, movement of pregnant cattle to parturition premises in larger herds are stress factors that also can predispose to *L. monocytogenes* caused abortions.

The age of aborted cattle was also analysed. The age was known in 43 of total 44 aborted cattle cases. Significantly the most frequent age of aborted cattle was 3 years – in 35% of cases. Out of a total of 43 cases, cattle were 4 years old in 26% of cases, 2 years old – in 14%, 5 years old in 9%, 6 years old in 9%, and in a very few cases – 5% and 2% – cattle were 8 and 7 years old, respectively. This observation indicated that younger cattle are less resistant to *L. monocytogenes* than older, but there is a need for further studies to confirm this observation.

L. monocytogenes caused abortions were observed in the whole territory of Latvia (Fig. 2). These abortion cases were more distributed in the central and the south-eastern parts of Latvia. These regions of Latvia tend to have colder winters than other parts of the country, but the highest rainfall rate tends to be at the northern and the south-western seacoasts and in the eastern part

of central Latvia. According to the LEGMC data, the lowest rainfall rate is observed in the southern part of Latvia. Moist weather in the central part and colder weather in the south-eastern part of Latvia could be contributing factors for *L. monocytogenes* presence in silage and subsequent abortion cases, because silage in cooler, wetter climate countries tends to have lower sugar levels and higher moisture contents and that may promote the growth of *L.monocytogenes* in the silage (Ryser and Marth, 2007).

Conclusions

1. *L. monocytogenes* caused abortions in Latvia in 2013 were observed in 23.7% of bacteriologically investigated abortion cases, and these cases were more distributed in the central and south-eastern parts of Latvia.

2. *L. monocytogenes* caused abortions occurred more often in spring and autumn that was associated with the indoor season, when the cattle were fed with silage. Higher rate of *L. monocytogenes* abortion cases in May 2013, possibly, was associated with a prolonged silage feeding period due to colder and wetter spring, especially in April, than in previous years.

3. All abortion cases in cattle due to *L. monocytogenes* were observed in the late-term of gestation – in the second and third trimester of gestation.

4. *L. monocytogenes* caused abortions occurred in cattle of different ages, but significantly more often – in 3 years old cattle (p<0.05).

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