**Abstract**

In a herd of Holstein Friesian cattle, (n = 98) kept in a loose housing system, we observed an outbreak of an unusual eye condition, which included unilateral or bilateral hyperaemic conjunctiva, lacrimation, photophobia, anterior uveitis, and cloudy cornea in four highly pregnant heifers, housed together with another older dry cow in dry cow group, during winter. The affected heifers were physically examined and conjunctival swabs, blood samples, individual raw milk samples of lactating cows (n = 78), and corn and hay silage samples were collected for laboratory analyses. *Micrococcus* sp. and *Enterococcus faecalis* were isolated from one conjunctival swab and *Listeria monocytogenes* from three others (three heifers). All the affected heifers were serologically negative in complement fixation test for listeriosis. Individual raw milk samples and grass and corn silage samples were collected for laboratory analyses. *Micrococcus* sp. and *Enterococcus faecalis* were isolated from one conjunctival swab and *Listeria monocytogenes* from three others (three heifers). All the affected heifers were serologically negative in complement fixation test for listeriosis. Individual raw milk samples and grass and corn silage samples were negative for *Listeria monocytogenes*. Organoleptic and chemical analysis of wrapped baled grass silage showed that it was of inferior quality. The affected heifers were for 4 d systematically treated with oxytetracycline and ophthalmic ointment with hydrocortisone and oxytetracycline. All the heifers recovered within a fortnight.

Ocular form of listeriosis in heifers was related to feeding of baled grass silage and traumatic injuries were caused by wind carrying particles of sawdust and mineral wool.

**Key words:** cattle, *Listeria monocytogenes*, keratoconjunctivitis, uveitis.

Listeriosis is an infectious disease caused by a Gram-positive asporogenic bacterium, *Listeria monocytogenes*, which is capable of intracellular replication. It affects humans and over 60 species of domestic and wild animals including fish and birds. *Listeria* exists worldwide; furthermore, it has been isolated from soil, silage, faeces, sewage effluent, and stream water. It can survive for years in organic materials and is capable of proliferating at temperatures from 3°C to 45°C. Ruminants are the most frequently affected domestic animals (5). Contaminated silage is a classic source of infection for ruminants. Other sources include particularly organic refuse (e.g., poultry litter).

Stress factors predisposing to clinical disease include nutritional deficiencies, environmental conditions, underlying disease, and pregnancy. Diseases due to listerial infection usually occur in animals in winter and spring (13). Three distinct principal clinical forms of listeriosis exist, which include septicemia, encephalitis, and abortion. (12). Mastitis (4), keratoconjunctivitis, and uveitis (11, 18) have also been associated with *Listeria* infection in ruminants. Ocular signs in the neurological form of listeriosis include facial paralysis with ptosis, medial strabismus (often on the ipsilateral side because of involvement of the abducens nucleus), nystagmus, and amaurosis (5). Uveitis with hypopyon has been described in chronic cases of the disease (15). Ocular pathology in neurological patients is usually due to ocular trauma (predisposed by bulbar exposure due to paralysis of orbicularis oculi muscle) and not primarily due to infection of the eye with *Listeria*. The ocular form in ruminants, without clinical signs of other forms, has been related to feeding contaminated silage in elevated feed bunkers and self-feed baled grass silage in housed cattle and sheep during the winter (1, 9-11, 16, 17, 19). The condition is also known as “silage eye”, anterior uveitis or iritis. It can occur as an outbreak. In a survey performed in England, the condition was the most prevalent among listerial infections (6). The morbidity reached more than 25% and approached 100% in some reported outbreaks in UK (16). Some outbreaks of this condition were not associated with self-feed big bale silage (17).

Although the first cases of the isolation of *L. monocytogenes* from conjunctival swabs taken from sheep and cattle with keratoconjunctivitis, were reported as early as 30 years ago (9, 19), the pathogenesis is still not fully defined. Clinical signs included hyperaemic conjunctiva, lacrimation, photophobia, cloudy cornea, and uveitis (18). Walker and Morgan (17) described two experimental sheep that developed unilateral anterior uveitis in which *L. monocytogenes* was cultured from the conjunctiva of each animal. The animals recovered after treatment with parenteral ampicillin and topical ceprovin. Natural eye infection with *L. monocytogenes*...
has also been documented in a horse, fallow deer, and humans (3, 7, 20).

Diseases due to *Listeria* in ruminants are of special concern to veterinary science because of its zoonotic potential. Contamination of cattle products with *Listeria* is the most common route of transmission to humans. Animals are unlikely to be direct sources of human infections (5).

This is the first report of “silage eye” cases in Slovenia.

**Material and Methods**

**History.** This communication reports an incident of keratoconjunctivitis and uveitis occurring in a herd of 98 Holstein Friesian cattle in loose housing system on cubicles. Four highly pregnant heifers housed in dry cow group with another cow were affected in February 2006. The owner observed excessive lacrimation and apparent blindness unilaterally in two heifers and bilaterally in other two on the same day. Two days after the initial observation, the condition improved in two heifers, but in the other two remained the same according to owners’ observations. One week prior to the observation of clinical signs in the four heifers, the weather was cold (-10°C) and windy. Strong wind damaged the panel of external stable wall and dispersed the mineral wool insulation mixed with scattered sawdust bedding. Animals were also fed *ad libitum* round baled grass silage, harvested in 2005, presented as whole bales in this group.

**Diagnostic procedures.** Clinical examination was performed in all four affected heifers and a cow that was unaffected. Ocular swabs were taken for bacteriological analyses (isolation and determination of *L. monocytogenes* using a method conforming to international standards), blood samples were taken for complete blood cell count (Automated blood counter ABC vet, France), and differential white blood cell count (cytological examination of blood smears stained with May Grünwald-Giemsa), and serological examination for *L. monocytogenes* antibodies (complement fixation (CF) test) in affected heifers was performed. Two individual milk samples were also obtained from all quarters of 78 lactating cows for bacteriological analyses (according to International standard ISO 11290-1) at two-week intervals. Baled grass silage and corn silage were bacteriologically analysed for presence of *L. monocytogenes* according to International standard ISO 11290-1 and 3 samples of baled grass silage were also analysed for dry matter content, humidity, and ash.

**Treatment.** The affected animals were treated intramuscularly with 10 mg/kg b.w. of oxytetracycline (Egocin LA, Krka, Slovenia) and intraocularly with 4 drops of ophthalmic solution containing hydrocortisone and oxytetracycline every 24 h (Geocorton ophthalmic solution, vials a 5 ml, containing 15 mg of hydrocortisone acetate and 5 mg/mL of oxytetracycline; Pliva, Croatia) every 24 h for 4 d.

**Results**

**Clinical examination.** All the heifers were affected with either unilateral or bilateral ocular lesions. Two animals affected with keratoconjunctivitis displayed excessive lacrimation (epiphora), blepharospasm, photophobia, apparent blindness, varying degrees of corneal clouding and yellowish anterior chamber fluid, scleral injection, and anterior uveitis in one eye (heifer 1 and 4). Other two heifers showed moderate corneal opacity, turbidity of anterior chamber fluid, anterior uveitis, hyperaemic conjunctiva, moderate lacrimation, and photophobia (heifer 2 and 3). Heifer 1, with the most pronounced ocular signs was also febrile (T = 39.5°C), but without any other clinical problems. All vital signs in other three heifers were normal.

![Fig. 1. Heifer 1 – detailed view of the eye (note anterior uveitis, yellowish anterior chamber fluid, opaque cornea, epiphora and hyperaemic conjunctiva).](image1)

![Fig. 2. Heifer 3 – bluish corneal opacity and white focuses on the cornea, floccules of material attached to the endothelium of the cornea, anterior uveitis, and hyperaemic conjunctivae.](image2)

Haematology results presented in Table 1 showed slight leukocytosis in heifers 1, 2, and 3 with
slight lymphocytosis in heifer 2 and neutrophilia in heifer 4. The mentioned changes in leukogram can be attributed to the excitement and anxiety of heifers during blood collection. Too low value of thrombocytes in heifer 3 was probably false and was the consequence of 2 h transportation to the clinical pathology laboratory. All other values were within normal ranges for cattle.

In three heifers, *L. monocytogenes* was isolated from ocular swabs (Table 2). *Micrococcus* sp. and *Enterococcus faecalis* were isolated from one swab in heifer 1.

All the heifers were serologically negative for *L. monocytogenes* in CF test. Milk samples from cows, taken two weeks apart, were bacteriologically negative for *L. monocytogenes*. Grass silage and corn silage samples were also negative for *L. monocytogenes*.

**Treatment results.** Heifers completely recovered after systemic and local antibiotic treatment. A slightly elevated body temperature in one heifer ceased next day after the therapy.

**Discussion**

Inflammatory ocular diseases in cattle are predominantly caused by Gram-negative bacteria, *Moraxella bovis*. Infections associated with *M. bovis* usually occur in the warmer part of the year and it is unusual to encounter the infections in March, although reports of outbreaks of the disease during winter do exist (8). *M. bovis* was not isolated in the cases reported here and the disease occurred in March. Other differential diagnoses with similar ocular signs include infections caused by *Mycoplasma, Chlamydia, Rickettsia, Theelazia* and viruses (disease is usually associated with clinical signs in other systems, most often respiratory) (2) but these agents were not detected in bacteriological and serological examinations. We were able to isolate *Listeria* from three heifers and only from one eye in each case. It is not uncommon for the disease to appear unilaterally (9-11). Ocular infections with *Listeria* appear predominantly during winter and spring (10, 11), which is in accordance with our cases. The isolation of *Enterococcus faecalis* from one eye indicates the faecal contamination of the eye.

**Table 2**

<table>
<thead>
<tr>
<th>Bacteriological analysis of ocular swabs</th>
<th>L eye</th>
<th>R eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifer 1</td>
<td><em>L. monocytogenes</em></td>
<td>Micrococcus sp., <em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td>Heifer 2</td>
<td>Neg. for <em>L. monocytogenes</em></td>
<td>Neg. for <em>L. monocytogenes</em></td>
</tr>
<tr>
<td>Heifer 3</td>
<td>Neg. for <em>L. monocytogenes</em></td>
<td><em>L. monocytogenes</em></td>
</tr>
<tr>
<td>Heifer 4</td>
<td>Neg. for <em>L. monocytogenes</em></td>
<td><em>L. monocytogenes</em></td>
</tr>
</tbody>
</table>

**Table 1**

<table>
<thead>
<tr>
<th>Results of haematological examination</th>
<th>Heifer 1</th>
<th>Heifer 2</th>
<th>Heifer 3</th>
<th>Heifer 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (10^12/L)</td>
<td>8.21</td>
<td>7.29</td>
<td>7.14</td>
<td>6.69</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>124</td>
<td>127</td>
<td>113</td>
<td>110</td>
</tr>
<tr>
<td>Haematocrit (L/L)</td>
<td>0.364</td>
<td>0.365</td>
<td>0.336</td>
<td>0.314</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>44</td>
<td>50</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>15.1</td>
<td>17.4</td>
<td>15.9</td>
<td>16.4</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>340</td>
<td>348</td>
<td>337</td>
<td>349</td>
</tr>
<tr>
<td>Thrombocytes (10^9/L)</td>
<td>273</td>
<td>284</td>
<td>119</td>
<td>288</td>
</tr>
<tr>
<td>Leukocytes (10^9/L)</td>
<td>11.8</td>
<td>10.4</td>
<td>8.4</td>
<td>11.2</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>27</td>
<td>26</td>
<td>30</td>
<td>47</td>
</tr>
<tr>
<td>Band neutrophils (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>64</td>
<td>70</td>
<td>62</td>
<td>47</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>1</td>
<td>0</td>
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</table>
The widespread distribution of environmental and animal-associated occurrence of *Listeria* makes localising the source of a particular outbreak difficult. In the study performed by Evans *et al.*, (7), they discovered that the *Listeria* present in the eyes of cattle affected with silage eye, were genetically different from the *Listeria* in silage, which was fed to the animals. They also found out that *Listeria* isolated from eyes was genetically the same as that shed by faeces of a healthy animal and that isolated from a soil sample. When they compared molecular subtypes of *Listeria* from ocular listeriosis with those isolated from animals with systemic infections, they discovered that they were the same. They concluded that there are no specific strains of *L. monocytogenes* that have special ocular tissue tropism. It is solely environmental and host factors that facilitate infection and the observed clinical signs. In light of these findings, there is a possibility that *Listeria* could enter the brain via nerves innervating the infected eye and produce the meningoencephalic form of the disease (20).

*Association of ocular irritation with mineral wool, sawdust, and contamination with faecal bacteria in association with the harsh weather and *L. Monocytogenes*. Probably from poor quality grass silage fed as unwrapped big bales, resulted in *L. monocytogenes* ocular infection. When animals eat from big bales, they undermine the upper layers of a bale, which results in constant falling of silage on their heads and enables ocular infection (11). There is also a possibility that harsh and windy weather transmits contaminated particles of silage dust to heifer’s eyes (19). All three samples of grass silage show poor conservation quality due to high dry matter and ash content. Silages with high dry matter content (over 40%) cannot be consolidated adequately, which results that anaerobic conditions are not achieved and fermentation cannot be consolidated adequately, which results that *L. monocytogenes* were favourable in silages that we examined, we were not able to isolate *Listeria* from them. The reason for this might be that samples were not taken from the bales at the beginning of infection, but some time later from other bales. It is also possible that the number of *Listeria* in the silage was too low (14). *L. monocytogenes* present in poorly prepared silage multiplies and is the most common source of infection for cattle (16).

It would appear that the clinical presentation of the disease was of a localised infection as only one heifer was febrile but we could not with no doubt assume the source of fever were the eye, but no other systemic signs of infection were evident in this case. The local nature of silage eye was present also in clinical studies presented by some other researchers, as they also did not observe any other systemic signs of the disease (7, 10, 11). Unremarkable haematology results and negative serology (all the heifers were negative for antibodies against *L. monocytogenes*) additionally confirmed that there was no systemic involvement of the infection in our case.

Similar clinical signs, as described in our cases, were also reported by other authors (7, 19). Bluish corneal clouding is due to corneal oedema, immune reaction, and leukocyte infiltration of cornea. There was no visible interruption of corneal integrity in all the heifers, consistently with the description of another author (18). The yellowish anterior chamber fluid is due to an accumulation of blood serum and white blood cells (18). Interestingly, nobody described any purulent ocular discharge, which would be expected after such a serious eye irritation, neither was it present in our cases.

If the disease is left untreated, it can run up to three weeks and with systemic and local antibiotic treatment up to 10 d (11). However, in our case, signs disappeared within two weeks after the onset of the treatment in severely affected animals and in mildly affected heifers in 4 d without therapy, just by discontinuing feeding from big bales. Weber *et al.*, (19) also reported that cattle with ocular listeriosis spontaneously healed within a week after they removed the possible source of infection. In two of the cases described here, the condition started improving before the therapy was initiated, which is consistent with their findings. Watson (18) suggested the treatment with atropine as a mydriatic and local application of corticosteroids to reduce inflammation. Local treatment with hydrocortisone in combination with oxytetracycline proved beneficial in our cases also and this therapy did not appear to have negative effects on the cornea. According to Watson (18), local antibiotic treatment alone is ineffective.

In our case as well as in reports of other authors, the disease did not progress to any other form of listeriosis and animals completely recovered without any visible ocular lesions (10, 11). Watson (18) also reported that mild corneal opacity, posterior and anterior synchia, secondary ulceration of the cornea; keratocoele, and panophthalmitis could be sequelae following infection in some cases.

Interestingly, these were the first and the only listeriosis cases diagnosed at this farm so far. Transmission of the disease to humans was unlikely in the described cases, since heifers were free of clinical signs before the lactation started. *Listeria* was not isolated from milk when heifers started lactating and they were also serologically negative for antibodies against *L. monocytogenes*, which suggests that systemic spread of bacteria did not occur.

On the basis of all obtained data, we concluded that ocular infection with opportunistic bacteria *L. monocytogenes* developed after a significant physical irritation of the eyes with mineral wool and sawdust during harsh cold and windy weather period. The source of infection was most probably low quality of baled grass silage fed *at libitum* as unwrapped big bales. Feeding whole bales is not recommended, especially if they are not of good quality. An outbreak of ocular listeriosis should be a serious sign for the farm manager that preventive measures for listerial infections should be carried out.
References