

Pustular dermatitis by *Listeria monocytogenes* after the assisted delivery of a dead calf

Pustulaire dermatitis door Listeria monocytogenes na de verlossing van een dood kalf

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ABSTRACT

Two days after the manual delivery of a dead calf, a 55-year-old healthy veterinary practitioner developed widespread pustular rash on both arms, followed by fever, myalgia and headache. Examination of the pustulae revealed *Listeria monocytogenes*. In addition to the case report, this article also briefly discusses the zoonotic aspect of listeriosis.

SAMENVATTING

Twee dagen na de verlossing van een koe met een dood kalf ontwikkelde zich bij een gezonde dierenarts van 55 jaar een pustulaire dermatitis aan beide armen, gevolgd door koorts, myalgie en hoofdpijn. Uit de pustulae werd *Listeria monocytogenes* geïsoleerd. In dit artikel wordt de casus beschreven na een kort overzicht van het zoonotisch aspect van listeriose.

INTRODUCTION

Listeriosis in animals

Listeriosis usually results from infection by *Listeria monocytogenes*, but *L. ivanovii* has also occasionally been associated with abortions in sheep and cows, or septicaemia in sheep.

Listeriosis is an acute, infectious but non-contagious disease of both wild and domestic ruminants, swine, rabbits, chinchillas, dogs, cats, horses, poikilothermic animals and birds.

L. monocytogenes is ubiquitous in the environment and can be isolated from the feces and nasal excretions (Radostits *et al.*, 2007) of healthy animals. The bacterium was isolated from the feces of healthy cattle in 46% of 249 Finnish herds examined (Husu, 1990) and from 82% of samples of cattle feedstuffs on Danish dairy farms (Skovgaard and Morgan, 1988). In the study by Husu 2.9 % of the 314 milk samples collected from the farm bulk tanks on 80 dairy

farms on four different occasions yielded *L. monocytogenes*. A similar prevalence (3.1%) was found in a Japanese study by Yoshida *et al.* in 1998. In New York State *L. monocytogenes* was detected in 12.6% of the sampled milk filters (Hassan *et al.*, 2000). Soil is another reservoir of the bacterium.

As is the case for humans, contamination of food is the most common way of transmission between animals. Ingestion of the organism leads either to an unapparent infection with prolonged fecal excretion, to a bacteremia with localization in various organs, or to development of a fatal septicemia (Radostits *et al.*, 2007). In farm animals symptoms appear typically after consumption of contaminated silage. Clinical disease in animals occurs mainly in ruminants living in temperate climates. It is much less common in tropical and subtropical areas. In bovines and sheep, encephalitis and abortion are the most prevalent manifestations. They may occur sporadically, affecting a single animal in a herd or flock or a few individuals over several weeks, but in sheep and goats more se-

vere abortion outbreaks are possible (Radostits *et al.*, 2007). In the United Kingdom approximately 33,000 bovine abortions occur annually and 0.05 to 0.13 per cent are attributed to listeriosis. This incidence is likely to represent an underestimation because the statutory sampling of aborted material which is required for the detection of *Brucella abortus* is insufficient for the recognition of a listerial infection (McLaughlin and Low, 1994). Septicemia, keratoconjunctivitis, ophthalmitis/uveitis, spinal myelitis and mastitis are less prevalent (Low and Donachie, 1997; Stöber, 2002; Radostits *et al.*, 2007). Gastroenteritis has been reported in Great Britain and New Zealand as a sporadic disease affecting sheep after weaning or sheep on root crops or on pasture where the quality of the pasture is poor and sheep are at high stocking densities (Radostits *et al.*, 2007).

Listeriosis in humans

In 2005, the Sentinel Laboratory Network and the National Reference Laboratory reported 62 cases of listeriosis in Belgium. This number is less than in 2004, when 89 cases were recorded. The National Reference Laboratory serotyped 40 *Listeria* strains. The serovars 1/2a and 4b were the most prevalent (40.0% and 42.5%, respectively) (Anonymous, 2007). *L. monocytogenes* is among the four most common causes of bacterial meningitis (Brouwer *et al.*, 2006). The organisms that most frequently cause bacterial meningitis (*Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b) rarely cause parenchymal brain infections such as cerebritis and brain abscesses. In contrast, *L. monocytogenes* has a tropism for the brain itself, particularly the brain stem, as well as for the meninges (Lorber, 2004). Brain infection can also lead to brain abscess (Cone *et al.*, 2003). Endocarditis is another potential symptom. Cases of arthritis, spontaneous bacterial peritonitis, hepatitis, cholecystitis and arteritis, as well as infections of the spinal cord, of aneurysms and of vascular prostheses have been described (Patyn *et al.*, 2001). Sporadic disease may involve healthy humans of any age, but normally speaking the disease is a serious problem only in pregnant women, the very young or unborn, the elderly and otherwise immunocompromised people. Transient bacteremia in healthy persons may go undetected (Lorber, 2004), although a newly recognised syndrome, 'febrile gastroenteritis', also occurs in healthy people: many patients with listerial bacteremia or central nervous system infection report a history of antecedent gastrointestinal symptoms including diarrhea, nausea and vomiting, often accompanied by fever (Ooi and Lorber, 2005).

Listeriosis in humans is mainly known as a foodborne disease. *L. monocytogenes* can be found in milk and dairy products, in various meats and meat products such as beef, pork and fermented sausages, in fresh produce such as radishes and cabbage, and in seafood and fish products (Gandhi and Chikindas, 2006). The major risk of disease results from contamination of foods during processing and from the particular ability of the organism to survive at refrigerator temperature (Kim *et al.*, 2005). Very few re-

ports mention infections by direct contact with infected bovine or ovine fetuses. A cutaneous infection, characterized by papular rash or pustules has been described in people who handle infected newborns, fetuses or cows after an abortion (Cain and McCann, 1986; McLaughlin and Low, 1994; Regan *et al.*, 2005). In some cases the rash may be accompanied by general symptoms such as fever, myalgia and headache. Lohman *et al.* (1999) have reported a case of endophthalmitis by direct inoculation.

CASE HISTORY

On March 27, 2007 the ambulatory service of the Ghent Veterinary Faculty attended to a dystocia in a Belgian Blue White cow. The cow was pregnant for the second time and the first calf had been delivered by Caesarean section. As service happened on pasture by a bull, the owner was not sure about the expected calving date. Upon examination, the veterinary surgeon and two senior year students found the cow to be in fair bodily condition and standing. After thorough washing of the vulva and perivulvar area with disinfectant soap (7.5% polyvinyl pyrrolidone iodine), a vaginal examination without gloves revealed the presence of ruptured fetal membranes and a dead calf in anterior position. The amniotic fluid gave off a foul odor. As the size of the calf was inferior to the normal size of a Belgian Blue White at the end of gestation, there was a suspicion of abortion and the decision had been made to deliver the calf "per vias naturales". Until that moment the students had not been authorized to perform any vaginal examination. Once the diagnosis was made, they were authorized to check the position, condition and size of the calf, their skin protected by long-sleeved disposable gloves. The veterinary surgeon washed his hands and arms with disinfectant soap, followed by isobeta-dine watery solution (50 ml of a 5% chlorhexedin digluconate solution in 5 litres of water). From this moment on, he used disposable gloves that covered both hands and arms entirely and the students were no longer allowed to assist in intravaginal manipulations. After massaging for about 20 minutes to relax the cervix and vagina, an episiotomy was performed and the calf was delivered by extraction. Afterward it was discovered that amniotic fluid appeared to have come in contact with the hands and arms of the veterinary surgeon through minute ruptures in the gloves. Blood and placenta samples were taken for the mandatory *Brucella* examination. Three tetracycline boluses of 2 g tetracycline each were deposited in the uterus and the cow was placed on a regime of procaine penicillin 10,000 Units/kg/day - neomycin 5 mg/kg/day (Neopen®, Intervet Belgium) for five days, in order to prevent infections of the genital tract.

Over the next two days, pustular dermatitis (Figures 1 and 2) developed on the hands and arms of the 55-year-old veterinary surgeon. The pustulae had a diameter of about 3-4 mm. On March 29 general symptoms set in: myalgia, headache, minor axillary lymphadenopathy and 37.5 °C fever. The veterinary surgeon's general practitioner (GP) took samples from the pustulae for bacteriological examination and started an empirical oral therapy with the combinati-



Figure 1. Pustular rash on the arm of a veterinary surgeon with cutaneous listeriosis.

on of amoxicillin 500 mg and clavulanic acid 125 mg (Augmentin®, Glaxo-Smith Kline Belgium), twice a day. On the analysis form, *Listeria dermatitis* was mentioned as being the most likely diagnosis, in order to guide investigations with regard to the most appropriate technique.

The sample collected by the consulting GP before treatment was *L. monocytogenes* positive. A pure culture was obtained after 24 hours incubation on Columbia® agar with 5% sheep blood (Oxoid, Basingstoke, UK). The isolated bacterium was Gram-positive, beta-hemolytic, catalase positive, oxidase negative and showed the typical “salto” movements in physiologic solution. The isolate was identified on Vitek® (bioMérieux, Marcy l’Etoile, France) by means of an ID-GP card. This procedure resulted in the diagnosis “*Listeria monocytogenes* with 99% confidence”. Furthermore, an API Coryne strip® (bioMérieux) was incubated and after 24 hours the reading of the API code 0070164 indicated “95.4% *Listeria monocytogenes/innocua*”. Antibiotic sensitivity was tested by means of Neosensitab® tablets (Rosco, Taastrup, Denmark) on Mueller-Hinton® plate (Oxoid). The strain was susceptible to penicillin, amoxicillin-clavulanic acid, erythromycin and tetracycline and was resistant to co-trimoxazole. The strain was sent to the national reference center for *Listeria* (Belgian Scientific Institute of Public Health, Brussels), where the isolate was identified as a serotype 1/2b *Listeria monocytogenes* strain.

On March 30, one day after the initiation of antimicrobial therapy, pustular material was sampled at the Department of Bacteriology of the Veterinary Faculty, Ghent University. A pustula was disinfected with alcohol and opened by means of a sterile injection needle, whereupon the pustular fluid was absorbed in a cotton-tipped aluminum shafted swab (Copan Diagnostics Inc., Corona, USA) by pressing it against the pustule. Another sample was taken, after disinfection of the skin, by aspiration of pustular fluid by means of a sterile needle and a 2.5 ml syringe. Samples were directly inoculated onto Columbia agar® with 5% sheep blood (Oxoid), *Staphylococcus/Streptococcus* Selective medium (CNA medium®, Oxoid) and *Listeria* selective ALOA medium® (Biolife, Milan, Italy), and were enriched by incuba-



Figure 2. Pustular rash on the arm and hand of a veterinary surgeon with cutaneous listeriosis.

ting at 30°C during 24 hours in Brain Heart Infusion (BHI) broth supplemented with *Listeria* Primary Selective Enrichment Supplement® (SR0142E, Oxoid) and subsequently inoculated onto ALOA medium. Blood agar plates and CNA media were incubated at 37°C with 5% CO₂, while ALOA plates were incubated at 30°C. After 24 hours, a pure culture of small, beta-hemolytic bacteria was obtained on the blood agar and CNA plates, while blue-green colonies, indicative for *Listeria* spp., were seen on ALOA plates. Gram staining revealed Gram-positive rods. A motility test demonstrated markedly more motile bacterial cells on semi-solid medium incubated at 25°C than on this medium incubated at 37°C, consolidating the assumption that the bacterial genus isolated was *Listeria*. The isolate was able to enhance the hemolytic effect of staphylococcal β-haemolysin (CAMP-positive) and in the API Strep test system® (bioMérieux) the isolate was identified as *L. monocytogenes*. With the use of tDNA-PCR (Baele *et al.*, 2000), the organism showed a specific fragment pattern of *L. monocytogenes*.

A serotyping multi-step PCR assay was performed (Borucki and Call, 2003) and the isolate was identified as a serotype 1/2b(3b) strain, corresponding with the result of the (above described) identification on the sample collected by the GP. The presence of virulence genes *sigB*, *inlA*, *inlB*, *actA*, *hly* and *prfA* was demonstrated by conventional PCR as previously described (Marien *et al.*, 2007). Additionally, the DNA sequence of the 3' end of the *inlA* gene of the isolate was determined, revealing the absence of nonsense mutations and thus indicating that this strain expressed functional, full-length internalin.

Forty-eight hours after the start of treatment, no additional skin lesions had appeared. All skin lesions became dry and scabbed, with evidence of healing. The next day the systemic symptoms had disappeared. Treatment with Augmentin was continued for a total of 10 days, resulting in complete resolution of the symptoms. At the end of treatment all skin lesions were reduced to small scars with a diameter not exceeding 2 mm.

Except abortion and retained placenta, the cow did not show any symptoms, either before or after the abortion. A serum sample taken from the cow on

April 10th was antibody positive for *L. monocytogenes*. As it concerned a beef cow with very low milk production and because the duration of pregnancy had been too short, there was no milk in the udder, so it was impossible to take a milk sample for bacteriological examination. A feces sample of the cow taken on May 30th was negative for *L. monocytogenes*.

DISCUSSION

Although human listeriosis is especially known as a foodborne disease, this case shows that infection via direct contact with infected cows is also possible. The case is in agreement with the results of a Dutch inquiry in 1998 by Visser. Three hundred and ten veterinary surgeons were asked whether and how often they had experienced pustular dermatitis after deliveries in cattle and sheep and what course the dermatitis had run. Of the 76 respondents, 62 reported one or more episodes of pustular dermatitis on at least one arm after delivery in cattle or sheep. Sometimes it was associated with secondary symptoms such as headache, fever and lymphadenitis. *Listeria monocytogenes* (7 times out of 13) and *Salmonella enterica* subspecies *enterica* serovar Dublin (*Salmonella* Dublin) (4/13) were the agents cultured most frequently. Pantekoek *et al.* (1974) described a *Salmonella* folliculitis in a veterinary surgeon after a fetotomy of a cow infected with *Salmonella enterica* subspecies *enterica* serovar Saintpaul. Another possible causative agent is *Brucella abortus* (Haxthausen and Thomsen, 1931; Currier, 1989), but as both countries are officially free of brucellosis, contact with the bacterium is unlikely in Belgium and The Netherlands. Other specific zoonotic bacteria that are able to cause pustular dermatitis are *Staphylococcus aureus*, *Streptococcus* species, *Escherichia coli* (Visser, 1991) and *Mycobacterium bovis* (Thomsen, 1937).

Only three of the thirteen serotypes (1/2a, 1/2b and 4b) are responsible for the majority of the clinical cases of listeriosis. Serotype 4b is implicated in most epidemical outbreaks, while serotypes 1/2a and 1/2b are typically identified in sporadic cases of listeriosis (Mc Lauchlin and Low, 1994; Van Kessel *et al.*, 2005). The isolated strain was identified as a serotype 1/2b strain and it contained several important virulence genes already described in the literature. In addition, the respective strain contains an *inlA* gene, encoding functional, full length *InlA*. Full-length *InlA* is far more frequently present in clinical strains than in strains isolated from food, which often contain truncated, non-functional *InlA* (Jacquet *et al.*, 2004; Ducey *et al.*, 2007). As could be expected, we can conclude that the isolated *L. monocytogenes* strain has pathogenic potential.

It is remarkable that the two samples of the pustulae on the veterinary surgeon's arm, both of which were taken after intake of two doses of amoxicillin clavulanic acid, were still positive. Once antibiotic treatment has been initiated, sampling is commonly considered unreliable because of the inhibitory effect of the compound used, resulting in a false negative conclusion, or because of the contamination due to (intrinsically) resistant organisms. Here, 15 hours after the first treatment, the pustulae still contained

viable *L. monocytogenes* bacteria. Guidelines for suspected bacterial meningitis recommend amoxicillin-based empirical antimicrobial therapy for patients aged more than 50 years or with risk factors to cover *L. monocytogenes*, because cephalosporins demonstrate poor activity against this bacterium (Brouwer *et al.*, 2006). Brouwer *et al.* (2006) also recommend amoxicillin, administered at a dosage of 2 g every 4 hours for at least 21 days, whenever meningitis due to *L. monocytogenes* is proven, either by Gram staining or CSF culture. Although an antibiogram based on the disk-diffusion method has been implemented in the examinations of this case, it must be emphasized that this method is not really reliable for *L. monocytogenes*. Due to the slow growth of the organism, this technique is dissuaded by the Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Committee for Clinical Laboratory Standards (NCCLS, 2002). To obtain a reliable antimicrobial susceptibility pattern, Minimal Inhibitory Concentrations (MIC) should be determined by quantitative dilution tests.

Veterinary surgeons, veterinary students, farmers and workers on cattle or sheep farms should be considered at risk when the anamnesis reveals contact with dead bovine fetuses. Although listeric infection is more common in sheep, it is remarkable that human cutaneous infection is only described in association with bovine abortions and stillbirths. It has been suggested that the duration of the manipulation is of importance. Furthermore, skin exposure will certainly be greater when dealing with cattle because of the physical limitations of intrauterine manipulations during abortions in sheep. Long-sleeved disposable gloves offer poor protection as they easily tear during assisted bovine deliveries (McLauchlin and Low, 1994).

The prevalence of *Listeria* infections in cows is probably higher than has been reported, as ingestion of the organism can lead to unapparent infection with prolonged fecal excretion. Investigation for *L. monocytogenes* infection is not at all a routine procedure, particularly in abortion cases. For economic reasons, only the obligatory *B. abortus* samples are taken in sporadic abortion (McLauchlin and Low, 1994). And even in many cases of 'abortion storms', for the same cost-cutting reasons, only the most common abortive organisms are tested, such as *Neospora caninum*, Infectious Bovine Rhinotracheitis virus and Bovine Viral Diarrhoea virus. In this sporadic case of abortion a placenta sample and a blood sample were collected for routine *B. abortus* examination. After samples of the veterinarian's skin revealed *L. monocytogenes* infection, a blood sample from the cow was examined for serology. Although it was seropositive for *L. monocytogenes*, this is not clear evidence of a causative relationship between delivery of the dead fetus and the pustular rash in the veterinarian. Paired samples would be more reliable, but are not useful in an abortion case. In the majority of infectious abortion cases, seroconversion has already happened at the moment of abortion, as several weeks pass by between infection and birth (Anderson, 2007).

Although cows shed *L. monocytogenes* over several months after initial infection, this shedding occurs

intermittently and continuous daily sampling is necessary to reveal the patterns of positive samples (Ho *et al.*, 2007). The fact that the single feces sample in this case was negative does not exclude infection of the cow involved.

CONCLUSION

Whenever farm workers or veterinarians present rash on the arms or general symptoms shortly after manual delivery of ovine or bovine dead fetuses, listeriosis is to be included in the differential diagnosis. As a general rule veterinary surgeons should at all times wear disposable gloves during assisted delivery. However, manipulation during prolonged delivery can cause minute ruptures, which means that even this protection measure may not be sufficient in all cases.

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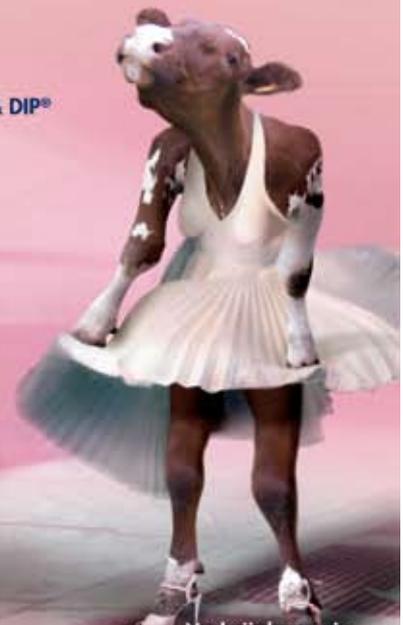
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