Isolation of *Listeria monocytogenes* from the gallbladder of a dog with liver insufficiency

Isolatie van Listeria monocytogenes *uit de galblaas van een hond met leverinsufficiëntie*

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ABSTRACT

***Listeria monocytogenes* can cause disease and death in humans and a large variety of animal species. Canine listeriosis seems, however, to be rare. In the present report, *L. monocytogenes* was isolated as a pure culture from the gallbladder of a dog with liver insufficiency. The animal suffered from auto-immune-mediated polyarthritis and had therefore been treated with prednisolone for several months. The *L. monocytogenes* isolate tested positive in PCR for the virulence genes *inlA, inlB, hly, actA, sigB* and *prfA* and expressed functional, full-length internalin. This is the first reported case of a dog with a *L. monocytogenes* infection of the gallbladder. Bacterial persistence in the gallbladder of pet animals may constitute a health hazard for their owner.**

SAMENVATTING

Listeria monocytogenes kan ziekte en sterfte veroorzaken bij de mens en verschillende diersoorten. Listeriose komt echter zelden voor bij honden. In deze casuïstiek werd een reincultuur van *L. monocytogenes* geïsoleerd uit de galblaas van een hond met leverinsufficiëntie. Het dier leed aan auto-immuungemedieerde polyartritis waarvoor het gedurende meerdere maanden behandeld werd met prednisolone. In het *L. monocytogenes*-isolaat werden via PCR de virulentiegenen *inlA, inlB, hly, actA, sigB* en *prfA* aangetoond en het isolaat bracht een functioneel internaline tot expressie. De persistentie van *L. monocytogenes* in de galblaas van gezelschapsdieren kan een gezondheidsrisico inhouden voor de mens.

INTRODUCTION

Listeria monocytogenes is a Gram-positive, rod-shaped, non-spore-forming bacterium that can cause disease and death in humans and a large variety of animal species. Canine listeriosis seems, however, to be rare and only a few reports have been dedicated to the presence of *L. monocytogenes* in this species (Chapman, 1947; Weber and Plagemann, 1991; Schroeder and Van Rensburg, 1993; Loncarevic *et al.,* 1999; Läikkö *et al.,* 2004).

The present report describes a case in which *L. monocytogenes* was isolated as a pure culture from the gallbladder of an immunocompromised dog with liver insufficiency.

CASE HISTORY

In December 2004, a 16-month-old female cross between a Labrador Retriever and a Berner Sennen dog was presented at the Faculty of Veterinary Medicine, Ghent University, because of locomotory problems which had been present for two months. Physical examination revealed distention of multiple joints. Analyses of joint fluid, including cytology and cell counts, were compatible with a polyarthritis. Routine blood examination showed a mild non-regenerative anemia and leucocytosis. Further workup, to rule out underlying diseases, included bacteriological culture of synovial fluid and urine, chest and joint radiographs, and abdominal ultrasonography. These

were unremarkable. Rheumatoid factors were negative. The dog was diagnosed with a non-erosive idiopathic auto-immune-mediated polyarthritis and was prescribed an oral immunosuppressive therapy with prednisolone (1.5 mg/kg bid). Initially, the clinical condition of the patient improved dramatically and slowly tapering of the dosage of prednisolone was started. Three months later, the dog relapsed and the prednisolone dosage had to be increased again. In April 2005, the condition of the dog seriously deteriorated. Besides very painful joints, the dog was now also suffering from diarrhea and hepatomegaly. Blood analysis revealed neutrophilia with left shift and an increase in serum concentrations of bilirubin, liver enzyme activity (aspartate transaminase, alanine transaminase, gamma glutamyl transpeptidase, alkaline phosphatase) and pre- and post-prandial bile acids. Cytology of a fine needle aspirate of the liver showed anisocytosis, swollen hepatocytes, hydropic degeneration and rarely found inflammatory cells. Cholecystocentesis was performed and this sample was inoculated onto Columbia agar with 5% sheep blood (Oxoid, Basingstoke, UK), *Staphylococcus/Streptococcus* Selective medium (CNA medium, Oxoid) and McConkey agar (Oxoid) and incubated at 37°C. After 24 hours, a pure culture of small, beta-hemolytic bacteria was obtained on the sheep blood agar and CNA medium. Gram staining revealed Gram-positive rods and, after purification onto selective ALOA medium (Biolife, Milan, Italy) incubated for 24 hours at 30°C, blue-green colonies, indicative of *Listeria* spp., were seen. 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*. In the API-Strep test system (bioMérieux, Marcy l'Etoile, France) the isolate was identified as a *Listeria* species with 99% confidence. Combined with the result of a positive CAMP test with *Staphylococcus aureus*, according to the API-Strep test system, the isolate was *L. monocytogenes* or *L. seeligeri*. Intergenic spacer polymerase chain reaction (tDNA-PCR) identified the isolate as *L. monocytogenes* (Vaneechoutte *et al.,* 1998; Baele *et al.,* 2000).

The results of a multi-step polymerase chain reaction (PCR) method, as previously described (Borucki and Call, 2003), revealed that the isolate belonged to serotype group 1/2b(3b). The presence of virulence genes *inlA, inlB, hly, actA, sigB* and *prfA* was demonstrated by conventional PCR using the primers mentioned in Table 1 and an annealing temperature of 53°C. Additionally, the DNA sequence of the 3' end of the *inlA* gene of the isolate was determined and revealed the absence of nonsense mutations, thus indicating that this strain expressed functional, full-length internalin.

The combined results of the blood, the cytological and the bacteriological investigations resulted in a diagnosis of liver insufficiency and bacterial cholecystitis. Treatment was started with amoxicillin-clavulanic acid, enrofloxacin and choleretics (ursodeoxycholic acid). A few days later, the owners decided to have the dog euthanized because of its deteriorating condition.

DISCUSSION

Isolation of *L. monocytogenes* from the gallbladder has already been described in humans (Gahan and Hill, 2005). In humans, the incidence of clinical listeriosis is the highest in neonates, pregnant women, the elderly and immunosuppressed persons, since in the majority of healthy adults, the infection remains subclinical (Quinn *et al.,* 1999). In the case described, the dog had been treated with corticosteroids for several months, which, most probably, acted as a predisposing factor for clinical listeriosis.

Table 1. PCR primer sequences used to demonstrate virulence genes in *Listeria monocytogenes*.

Gene	Primer	Sequence (5' → 3')	Amplicon length
inlA	F[1]	AATCTAGCACCACTGTCGGG	1774 bp
	R	TTCTGCAAAAGCATCATCTG	
inlB	F	TTCTTTGGAGCATAATGGTATAAGTGA	514 bp
	R	ATTACCGTTCCATCAACATC	
actA	F	CAACAGAAGAAGAGTTGAACG	632 bp
	R	GTCTTCTGCACTTTTAGCAAT	
hly	F	TGATGACGAAATGGCTTACA	656 bp
	R	TGAATTGAGCAACGTATCCTC	
sigB	F	CCGTAGAAGAGCTIACGAGAGAA	375 bp
	R	CAACGCCTCTCGAAGTTTTTTAA	
prfA	F	CAATGGGATCCACAAGAATATTGTAT	368 bp
	R	TGCCATCAGGAGTTTCTTTACCA	

[1]F, forward; R, reverse

It is generally very difficult to locate the origin of a case of listeriosis. Dogs might get infected by eating contaminated food or through contact with other infected animals. Another possible route of infection is from bacteria that otherwise are a part of the normal intestinal microbiota in the case of decreased host resistance (Loncarevic *et al.,* 1999). Asymptomatic fecal carriers do indeed occur in man and many animal species (Quinn *et al.,* 1999), including dogs and cats (Weber *et al.,* 1995).

PCR revealed the presence of several important virulence genes in the *L. monocytogenes* isolate. Additionally, the isolate expressed functional, full-length internalin, as opposed to the truncated, non-functional form of internalin that has been described in the literature (Jacquet *et al.,* 2004). Therefore, we may conclude that the isolated *L. monocytogenes* strain has pathogenic potential.

Although outbreaks of listeriosis in humans are usually of food-borne origin, direct contact with infected animals can also lead to human infection (Quinn *et al.,* 1999; Gahan and Hill, 2005). Therefore, dogs with a *L. monocytogenes* infection of the gallbladder may be a source of infection for their owners. Indeed, Hardy *et al.* demonstrated extracellular replication of *L. monocytogenes* in the lumen of the gallbladder of intravenously and orally infected mice. The bacteria were released into the intestinal tract and shed in the environment (Hardy *et al.,* 2006). Bacterial persistence in the gallbladder may represent a reservoir of infection since this organ is known to be recalcitrant to antimicrobial therapy, and in the cell-free concentrated bile of the gallbladder the bacteria are protected from cellular host immune responses (Hardy *et al.,* 2006).

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