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Listeriosis

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INTRODUCTION

Listeria monocytogenes, an ubiquitous saprophytic bacterium, is widely distributed in nature and may cause disease in humans and several different animal species [2,6]. Disease is usually sporadic and occurs most commonly in sheep, goats and cattle[2,6]. In these species infection classically gives rise to three well recognized syndromes characterized by meningo-encephalitis, abortion or stillbirth and neonatal septicaemia[2,6]. Gastroenteritis has also been reported recently as a clinical presentation in sheep[4]. Other less common clinical manifestations include mastitis and keratoconjunctivitis[2,6]. Septicaemia is more frequently found in monogastric animals and its prevalence is low and clinical disease in humans, although rare, is often fatal[2,6]. In southern Africa listeriosis has been reported in sheep, goats and chinchillas but in general is not considered to be economically significant[2]. However, during 2011, which year was characterized by an unusually long cold and wet winter following a wet summer, Vetdiagnostix isolated this bacterium from four different bovine abortion outbreaks and one outbreak of caprine meningoencephalitis in the province of KwaZulu-Natal.

The importance of this organism as a food borne zoonosis has prompted intense investigations into the pathogenesis of clinical disease in humans, and into the mechanisms of survival and replication of the organism in different foods and processed food products. With mice as a model this bacterium has been used extensively not only to study the survival and immunology of listeriosis, but also intracellular bacteria in general[6].

There is an abundance of in depth articles in this regard and these outnumber the publications on primary animal related disease by far. A few articles are listed below in the references which may make for very interesting reading.

AETIOLOGY

Listeria sp. is a gram-positive, non-acid fast, non-spore forming, short, rod-shaped bacterium[2,11]. Two pathogenic species have been identified, namely *L. monocytogenes,* an important animal and human pathogen, and *L. ivanovii* which predominantly infects ruminants[2,11]. *Listeria ivanovii* is commonly isolated abroad from cases of abortion in sheep, but is of low pathogenicity[2,11]. Non-pathogenic members of the genus are *L. innocua, L. seeligeri, L. welshimeri* and *L. grayi*[11]. The non-pathogenic *L. innocua* seems to be without the specific genes associated with infection of the host. These main virulence gene clusters include the genes encoding InlA and InlB and the bile salt hydrolase (bsh) gene[11]. Three distinct phylogenetic lineages of *L. monocytogenes* are recognized, which lineage I strains containing isolates that have caused epidemics in humans (including serovars 1/2b and 4b), lineage II strains containing isolates responsible for sporadic human disease (including serovars 1/2a and 1/2c) and lineage III strains comprising mostly animal pathogens[11].

L. monocytogenes is very tolerant to high osmolarity and low pH[9]. Cold stress tolerance mechanisms described for *L. monocytogenes* survival includes production of cold shock proteins, maintenance of cell membrane, intracellular uptake of compatible solutes and oligopeptides, and production of several other proteins[9]. A contributory role of the general stress sigma factor σ^B, alternative sigma factors, and two component regularity systems in the survival of *L. monocytogenes* at low temperature has been documented[9]. *L. monocytogenes* is virtually non-motile at temperatures of 37 ^{0}C and the motility genes are down-regulated. However, at or below 30 ^{0}C the strains are highly flagellated and motile[9]. Motility genes fhA and motA play a role in the cold tolerance of *L. monocytogenes* at low temperature[9].

EPIDEMIOLOGY

Listeriosis is seen throughout the world, although more frequently diagnosed in regions with cold temperate climates such as New Zealand, Australia, North America, Europe and UK compared to regions with a tropical or subtropical climate[2]. Clinical disease is more often observed during late winter and early spring[2].
Listeria sp. has been reported to grow at temperatures ranging from 0-45 ^{0}C and pH ranges of 4,1 – 9,6[7]. It has been isolated from many different mammals and bird species, both healthy and diseased[2,11]. It can also

be isolated from soil, groundwater, water sewage, mud and silage[2]. Listeriosis is often diagnosed in livestock fed large quantities of poor quality silage with a pH in excess of 5.5[2]. A high pH and spoilage of silage, as a consequence of poor preparation and maintenance, may lead to the growth of bacteria particularly in the top and side layers of the silage and the use of trench silos has been associated with increased incidences in cattle[2]. In animals it is suspected that nutritional stress and reduced resistance of animals exposed to adverse environmental factors may also predispose to disease[2]. Outbreaks have also been associated with feeding animals raw vegetables and fruit[2]. Sheep, goats and cattle are the species most frequently affected and although the morbidity is usually less than 10% the mortality rate in those suffering from meningo-encephalitis is usually 100%[2]. The higher prevalence of listerial meningoencephalitis in certain age groups of sheep has been linked to the changing of teeth in early spring[2]. A large percentage of healthy sheep and goats may carry *L. monocytogenes* subclinically and excrete organisms in their faeces and milk under conditions of stress[2].

The majority of cases of gastroenteritis reported in sheep in New Zealand were seen between May and August, and peaked in July[1]. In outbreaks where the known ages of affected animals were recorded, 63% of cases occurred in mixed-age ewes, 17% in two-tooth ewes and 7% in unshorn yearlings[1]. In some instances the disease was associated with a high stocking rate and reduced feed intake[1].

L. monocytogenes can be isolated from milk of mastitic, aborting, and apparently healthy cows. Excretion in milk is usually intermittent but may persist for many months. Infected milk is a hazard because the organism may survive certain forms of pasteurization[6]. *Listeria sp*. have also been isolated from milk of sheep, goats and women.

Asymptomatic carrier animals may contaminate food items and *L. monocytogenes* has been isolated from a variety of raw foods such as uncooked meats and vegetables[6]. Vegetables most likely become contaminated from the soil or when manure is used as fertilizer and food items such as hot dogs, soft cheeses and cold cuts at deli counters may become contaminated during processing, after cooking and before or during packaging[6]. This organism can survive in, or on, foodstuffs for very long periods of time however, these products may not be associated with illness[7]. The organism may also be present in unpasteurized milk or foods made from unpasteurized milk[6].

ZOONOTIC RISK

It is not entirely certain that animals serve as a reservoir of infection for humans because *Listeria* organisms have been isolated from faeces of a significant number of apparently healthy people as well as other animals. However, despite this and the apparently low invasiveness of *L. monocytogenes*, all suspected material should be handled with caution. Aborted foetuses and necropsy of septicaemic animals is potentially dangerous, but in cases with encephalitis, *L. monocytogenes* is usually confined to the brain and presents less risk of transmission unless the brain is removed. While human listeriosis is rare (upper estimate of 250 cases per 7 million population per year) mortality can reach 33%[3, 6]. Most cases involve elderly patients, pregnant women, or immunocompromised people[6].

Highly immunosuppressive regimens for organ or bone marrow transplantations, human immunodeficiency virus (HIV) infection, and immunocompromising diseases such as cancer, autoimmune diseases, alcoholism and diabetes mellitus have all been identified as major risk factors for the development of clinical listeriosis in humans[3]. In healthy individuals the disease caused by *L. monocytogenes* is usually restricted to a self-limiting gastroenteritis; however, in immunocompromised individuals and pregnant woman, the bacterium is capable of causing systemic infections that lead to meningitis, encephalitis and, in the case of pregnant women, infection of the developing foetus, which can lead to abortion, stillbirth or neonatal infections[2,5].

PATHOGENESIS

L. monocytogenes is an intracellular pathogen and its pathogenicity is related to its ability to invade, survive and grow within host cells, such as endothelial cells, enterocytes, fibroblasts or within macrophages in the gastrointestinal tract[5,11]. Studies suggest that *L. monocytogenes* seems to mediate its saprophyte-to-cytosolic parasitic transition through the modulation of the activity of a virulence regulatory protein designated PrfA[5]. This is stimulated by a range of environmental triggers that include available carbon sources[5].

Research has highlighted the importance of surface invasion proteins such as internalin A (In1A) and internalin B (In1B)[3]. Following entry into a host cell *L. monocytogenes* manages to escape from phagosomes through the combined actions of a pore-forming hemolysin, listeriolysin O, and two phospholipases, P1cA and PlcB[3]. This in turn allows the bacterium to replicate in the cytosol[3]. Cytosolic protein and the growing F-actin filaments form a molecular motor that propels bacteria through the cytosol and into adjacent cells, thus enabling the bacteria to spread cell-to-cell[3]. Ingestion of the organism with penetration of the intestinal mucosa may result in a clinically inapparent infection with localization of bacteria in various organs, or a fatal septicaemia[2]. In pregnant animals organisms

localize in the uterus and usually cause abortion if infection takes place early in pregnancy[2]. In late pregnancy infection may result in stillbirth or, in lambs, fatal septicaemia soon after birth[2].

It is not known precisely how bacteria reach the brain in animals developing meningoencephalitis, however, they probably gain entrance through wounds in the mucosa of the oral cavity[2].

Mechanisms proposed by which organisms may reach the brain are[3]:

- Transport across the blood-brain or blood-choroid barriers within parasitized leukocytes. Recent studies in mouse models revealed that a specific subset of monocytes, partly characterized by high expression of the Ly-6C antigen, which become parasitized in the bone marrow may play a key role in transporting intracellular bacteria across the blood-brain barrier and into the central nervous system[3].
- Direct invasion of endothelial cells by extracellular blood-borne bacteria[3].
- Retrograde migration into the brain within the axons of cranial nerves (e.g. trigeminal nerve)[2,3]. The development of unilateral micro-abscesses and the perivascular infiltration of lymphocytes largely restricted to the brain stem (pons and medulla oblongata) are typically seen which seems to support this assumption[2]. Recent studies in different models indicated non-neuronal cells take up bacteria more avidly than neurons, with microglia being the most easily infected[3]. A more reliable means for *L. monocytogenes* to infect neurons seems to be through cell-to-cell spread from an infected macrophage[3].

CLINICAL SIGNS

A wide variety of different animal species may be affected by *L. monocytogenes* but it has been found to exhibit distinct species specificities with regards to the characteristics and outcome of infection[6]. In a recent study in France these differences seen in different animals are summarised in table 1[6]. In general it is far more important as an animal pathogen and only accidentally seen as clinical disease in humans following ingestion of contaminated food[6].

In outbreaks of disease in domestic animals, typically, only one of the three syndromes including meningoencephalitis, abortion or septicaemia will be observed[2]. In livestock, meningoencephalitis is most commonly seen in ruminants and the course of the disease is usually two to three days in goats, sheep and calves while, in contrast, it may be one to two weeks in adult cattle[2]. Rectal temperatures are elevated during the early stages of disease but may be normal to subnormal in the later stages[2]. Location and severity of the lesions in the brain stem will determine the severity and expression of neurological signs. Affected animals may be depressed, walk in circles, exhibit incoordination and show tilting of the head[2]. Unilateral facial nerve paralysis may result in drooping of the lips, ears and eyelids and also paralysis of the muscles of the jaw and pharynx hindering normal mastication and swallowing[2]. Affected animals may be observed with food hanging from their mouths and drooling saliva[2]. They often stand like this for long periods and lethargy frequently progresses to somnolence, ultimately followed by generalized paralysis and death[2]. Convulsions and paddling movements may occasionally be present[2]. Paralysis of one limb as consequence of localized myelitis has been recorded in lambs, but more commonly in adult ruminants[2].

Acute diarrhoea in sheep, which may appear very similar to salmonellosis, has been described particularly in the south island of New Zealand[4]. Enteric listeriosis may occur within 2 days of feeding poor-quality silage and baleage[1,4], in contrast to encephalitic listeriosis where the incubation period after experimental inoculation into tooth pulp was at least 3 weeks[4]. Clinical signs reported include lethargy, anorexia, green and khaki-coloured diarrhoea, and sudden death[1]. The mortality rates from 21 recorded cases varied from 0.16% to 3.3%[1].

Sporadic abortions are more common in cattle than in sheep and goats, although it rarely reaches a rate of 15%[2]. Abortion during advanced pregnancy is often associated with retained placentas, clinical illness and fever in affected cows and ewes[2]. Cows and ewes may die due to septicaemia developing as consequence of retention of the foetus[2]. Ewes are usually asymptomatic before they abort 7 to 11 days after infection and the aborted foetuses are usually decomposed[2]. Individual animals may abort again during subsequent pregnancies. Septicaemic listeriosis is the most common form encountered in foetuses and neonates of ruminants, and is sometimes found in monogastric animals[2]. In these cases many organs and tissues are affected[2]. The occurrence of keratoconjunctivitis has been described in cattle[2].

PATHOLOGY

Macroscopic lesions are usually not seen in the central nervous system of animals which succumbed to meningoencephalitis[2,8]. Occasionally the meninges over the ventral brain stem may be thickened and slightly yellowish or opaque due to mononuclear cellular infiltrate, or may be congested and oedematous[2,8]. On the cover page a brain from an adult Boergoat which succumbed to meningoencepahlitis shows mild lesions of congestion. Microscopic lesions are mostly confined to the white and/or grey matter of the brain stem, particularly the pons and the medulla oblongata, the spinal cord and meninges[2,8]. Early stage lesions consist

of small accumulations of lymphocytes and monocytes and a few neutrophils, around or in close proximity to blood vessels. In more advanced stages of infection extensive perivascular infiltrations of mononuclear cells and micro-abscesses may be seen in the periventricular grey and/or white matter[2,8].

The brain in the photograph appearing on the cover page is that of an adult Boergoat. It exhibits a mild diffuse congestion of the meninges with, histologically, typical lesions of meringo-encephalitis.

Aborted foetuses and neonates, especially lambs, which have died as consequence of septicaemic disease may consistently develop lesions which can be seen as small, yellowish, pinpoint, necrotic foci in the liver or small (1 to 3mm in diameter) erosions in the abomasums[2,8]. The coats may be meconium-stained in aborted lambs[2,8]. Foetuses are usually severely autolysed when expelled[2,8]. Lesions of subcutaneous oedema, hydrothorax, ascites and an enlarged pale or bronze-red, friable liver are commonly present in new born lambs that have died as consequence of congenital infection[2,8]. Various degrees of oedema, sometimes greyish white and leathery areas of necrosis, 100 to 120mm in diameter, are evident around the cotyledons[2,8]. Affected cotyledons are paler than normal and may vary from a mottled orange to a pale greyish-pink in colour[2,8].

Post mortem findings of sheep dying in New Zealand due to gastroenteritis exhibited red discolouration and sometimes haemorrhage, ulceration, or erosion of the abomasal mucosa[1]. The small intestine, especially the duodenum, also often showed red discolouration[1]. In a small number of affected animals the caecum and colon appeared more prominently affected[1]. A distended gallbladder was noted, probably as consequence of anorexia in a few cases[1].

A recent retrospective study described the histological lesion seen in the enteric form of the disease in sheep[4]. Multifocal to extensive, mainly neutrophilic infiltrates were evident in the lamina propria, muscularis mucosa and superficial submucosa, being the most prominent in the muscularis mucosa[4]. The mesenteric lymph nodes were also affected and in some animals the liver as well[4]. Large numbers of gram-positive rods were demonstrated within these areas of inflammation and could be identified immunohistochemically[4]. Bacteria were found free within the cytoplasm of myofibres of the muscularis mucosa by electron microscopy[4].

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

A diagnosis can be made on the presence of typical clinical signs, epidemiological findings and histopathology. Confirmation of the diagnosis can be achieved by isolation of *L. monocytogenes* from affected tissues. Cultures should be attempted from the brain stem in cases of meningoencephalitis syndrome and from all organs and including the placenta, faeces, urine, blood and milk in animals which developed the septicaemic syndrome (organisms can be very irregularly distributed within the body)[2]. Exposing tissues to temperatures of 4 ^{0}C for up to two months, described as "cold enrichment", may enhance isolation of *L. monocytogenes* from tissue specimens[2].

Differentials for meningoencephalitis would include rabies, brain abscesses, *Coenuris cerebralis* infestation, lead poisoning, heartwater and cerebrocortical necrosis (thiamine deficiency)[2].

Clinically animals suffering from listeriosis may be differentiated from rabies by the circling and unilateral facial nerve paralysis not usually present in rabies, and the absence of bellowing and aggression that is usually present in rabies[2]. In thromboembolic meningoencephalitis caused by *Histophilus somnus*, the course of the disease is short and often characterised by alert downer animals or sudden death[2]. The abortion and septicaemia syndromes should be differentiated from the other well-known causes of foetal loss and neonatal mortality in the respective species[2].

TREATMENT AND CONTROL

L. monocytogenes is susceptible to the penicillins, ceftiofur, erythromycin, and trimethoprim/sulfonamide[2]. High doses are required because of the difficulty in achieving minimum bacteriocidal concentrations in the brain and fluoroquinolones may be considered due to their excellent activity in vitro against a wide spectrum of organisms and their good penetration into the cerebrospinal fluid (CSF)[12]. Recovery depends on early, aggressive antibiotic treatment. If signs of encephalitis are severe, death usually occurs despite treatment[2]. Supportive therapy, including fluids and electrolytes, is required for animals having difficulty eating and drinking[2].

Recent vaccine research has been promising, but previously results with vaccines have been equivocal, which together with the sporadic nature of the disease, lead to questions about the cost-benefit of vaccination. In an outbreak, affected animals should be segregated. If silage is being fed, use of the particular silage should be discontinued on a trial basis. Spoiled silage should be avoided. Corn ensiled before being too mature and grass silage containing additives are likely to have a more acid pH, which discourages multiplication of *L. monocytogenes.* Prevention of the disease includes provision of good quality baleage, silage and elimination of stressful conditions such as dense stocking rates and minimising ingestion of soil-contaminated pastures[1].

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QUESTIONS FOR CPD

1. Which one of the following statements is correct?
a. *L.ivanovii* is an animal pathogen.
b. *L.monocytogenes* has only one phylogenetic lineage.
c. *L.innocua* is pathogenic in mice.
d. *L.grayi* is a highly pathogenic strain.
e. *L. seeligeri* is the most important human pathogen.

2. Listeriosis is more commonly seen in:
a. Regions with cold temperate climates.
b. Regions with a tropical climate.
c. During the summer months.
d. During periods of high environmental temperatures.
e. The North West Province.

3. Clinical disease is most rarely seen in:
a. Cattle
b. Humans
c. Horses
d. Dogs
e. Sheep

4. *Listeria monocytogenes* is:
a. An extracellular pathogen.
b. Able to escape intracellular phagocytosis.
c. Unable to invade mammalian cells.
d. Unable to produce listeriolysin O
e. Unable to spread cell to cell.

5. Clinical disease is known to:
a. Cause unilateral facial nerve paralysis.
b. Have no adverse effects on reproduction.
c. Be associated with congenital malformations.
d. Be the most common cause of keratoconjunctivitis.
e. Be unable to cause septicaemia.

6. The pathology in animals exhibiting meningoencephalitis is characterized by:
a. Striking macroscopic lesions of malacia.
b. The absence of microscopic lesions of perivasculitis.
c. The presence of typical intranuclear inclusions.
d. The presence of microscopic brain lesions.
e. Lesions being confined to the cerebellum.

7. In aborted foetuses/neonates the pathology seen may include:
a. A normal appearing placenta.
b. Very fresh foetuses being expelled.
c. The presence of lesions of abomasal ulceration.
d. Lesions typical of cerebellar atrophy.
e. Typical lesions of congenital articular rigidity.

8. Clinical disease in sheep is characterised by:
a. High morbidity rate, >40%.
b. Low mortality rate in affected animals.
c. High incidence in yearlings.
d. Abortion, diarrhoea, septicaemic and neurological symptoms occurring concurrently.
e. None of the above.

9. The greatest risk of zoonotic infections with *Listeria* sp may occur when:
a. Post mortem is carried out using protective clothing.
b. Ingesting unpasteurised dairy products only.
c. Exposure to infected silage.
d. Handling lambs.
e. Ingesting contaminated raw and/or cooked foods.

10. Confirmation of diagnosis of listeriosis requires:
a. History of exposure to silage.
b. Symptoms of typical neurological disease and abortions.
c. Macroscopic post mortem lesions including severe meningoencephalitis.
d. Isolation of *Listeria sp.* from affected tissues.
e. History of cold weather conditions.

Table 1: Clinical manifestation of infection in different animal species.

SPECIES	SYNDROMES	REMARKS
Bovine, Ovine, Caprine	Abortion Nervous Septicaemia Ocular Mammary Digestive	Most frequent Most frequent Neonatal forms Conjunctivitis, keratitis, uveitis, associated or not with other manifestations. Rare, frequently carrier state. Reported
Equine	Nervous Abortion Septicaemia Digestive	Very rare but reported 1 % of abortion Reported in foal Reported in foal
Porcine	Nervous Healthy carrier	Very rare, reported in young animals, may resolve spontaneously Frequent
Cat, Dog	Nervous Septiceamia Cutaneous	Very rare, mostly in young animals, may be associated with viral and parasitic infections. Very rare. -
Domestic Rabbits, Hare	Abortion and genital Nervous Septiceamic Mammary Respiratory Cutaneous or subcutaneous Conjunctival	Pyometritis - Reported in association with abortive and various forms, and neonates - - - - -
Mouse Rat Guinea pig, Chinchilla, Squirrel, Gerbil, Muskrat, Lemming, etc.	Carriage Carriage Septicaemia Conjunctival & respiratory	Used in experimental infection (mostly iv), but little evidence for infection in the wild - Frequently “epidemic” in captive animals and wild animal colonies -
Bird Game and Captive Animals	Septicaemia Digestive Nervous Nervous and/or septicemia	Most frequent, young more susceptible,sporadic cases and outbreaks in breeding plants Rare Sporadic cases
Fish	Septicaemia Cutaneous	Sporadic cases in breeding plants Associated or not with septicaemia
Batracian	Healthy carrier	-
Reptile	Healthy carrier	-