

LISTERIA

Learning objectives

To know in detail about,

- Disease syndromes of pathogenic *Listeria* sp in animals
- Morphology and cultural characteristics of *Listeria* sp
- Pathogenesis of circling disease in cattle
- Different forms of listeriosis in cattle
- Tumbling motility, Anton's test and cold enrichment

SYSTEMATICS

	Domain	<i>Bacteria</i>	
	Phylum	<i>Firmicutes</i>	
	Class	<i>Bacilli</i>	

Order	<i>Bacillales</i>
Family	<i>Listeriaceae</i>
Genus	<i>Listeria</i>

- *Listeria* has been divided into seven species with two distinct groups.
- Among which the *Listeria monocytogenes* and *Listeria ivanovii* are haemolytic and pathogenic for animals.
- The *Listeria murrayi* and *Listeria grayi* are nonhaemolytic, rarely isolated and considered to be non pathogenic.
- Among which the genus *L. monocytogenes*, is the cause of septicaemia, abortion and CNS infections in a wide range of animal species including humans.

HISTORY

- *L.monocytogenes* first described by Murray (1926) who named it as bacterium monocytogenes because of characteristic monocytosis infection in laboratory animals.
- It was renamed *Listerella hepatolytica* by Pirie (1927) and the present name given by him in 1940.
- The *Listeria monocytogenes* was first isolated by Gill (1929) from sheep.

HABITAT

- *Listeria* species are widely distributed in the environment and can be isolated from soil, faeces, plants, decaying vegetation and silage (pH 5.5) in which the bacteria can multiply.
- Silage is commonly implicated in outbreaks of listeriosis in cattle and sheep.
- In poor quality sailage the listerial numbers may reach 10⁷ cfu/kg of silage.
- Asymptomatic faecal carriers occur in man and many animal species.
- *L.monocytogenes* can be excreted in bovine milk.
- Human foods associated with listeriosis in man include soft cheeses, milk and poultry meat.

MORPHOLOGY AND CULTURAL CHARACTERISTICS

Morphology

- *L.monocytogenes* are medium sized, Gram+ve rods, non-spore forming and non-acid fast.
- Old cultures stain Gram –ve. From rapidly growing cultures or animal tissues the cells can appear coccal.
- They are motile by few (1-5) peritrichous flagella. They are motile at room temperature, but not at 37°C.

Cultural characteristics

- *L.monocytogenes* is able to grow at temperature ranges from 4 to 45°C and grow at pH range of 5.5 to 9.6.

- It is relatively resistant to high salt (10%) concentrations. They are facultative anaerobes.
- The growth is **enhanced** by agar enriched with glucose, blood, liver extract and by 10% CO₂.
- They grow on nutrient agar, blood agar but not on MacConkey agar.
- Small transparent colonies with smooth borders appear on blood agar in 24hrs, becoming grayish white in 48hrs.
- *L.monocytogenes* and other non-pathogenic listeria produce narrow zones of beta haemolysis, often only under the colony itself.
- *L.ivanovii* produces a comparatively wide zone of haemolysis and is very similar in appearance to beta haemolytic streptococci.
- *L.monocytogenes* produces a CAMP reaction with the haemolysis of *S.aureus*.
- In contrast, *Listeria ivanovii* is negative in the CAMP reaction with *S.aureus*.
- On TSA or BHI agar, these colonies have a characteristic blue-green sheen when light is reflected obliquely at a 45° angle off their surface.
- In fluid medium, slimy tenacious precipitate forms after incubation for several days.
- *L.monocytogenes*, particularly shows the characteristic tumbling motility when a 2-4 hr broth culture, incubated at 25°C, is examined by the hanging drop method.
- This motility is an end-over-end tumbling of individual cells with periods of quiescence.
- When grown in semisolid motility media the *Listeria* spp. give an unusual umbrella shaped growth in the subsurface.

BIOCHEMICAL PROPERTIES, RESISTANCE, ANTIGENS AND TOXINS

Biochemical properties

- All the *Listeria* species hydrolyse aesculin, Catalase +ve, Oxidase –ve, Indole –ve.
- They produced acid from glucose and rhamnose, but not from xylose and mannitol. Nitrates not reduced.

Resistance

- It is killed by moist heat at 55°C for 40 minutes and is readily susceptible to the lethal effects of disinfectants.
- Under natural conditions, in summer they survive for 1 month and in winter for 3-4 months.

Antigens and toxins

- Based on somatic and flagellar antigens, so far 16 serovars have been identified.
- Of these 16 serovars, all cases of animal and human infections are caused by 3 serotypes. 1/2 a, 1/2 b and 4 b.
- Numbers indicate O antigen and alphabet indicate H antigens.

PATHOGENESIS

- In both cattle and sheep, listeriosis can manifest itself in four ways
 - as a CNS infection (meningo encephalitis in adults and meningitis in the young)
 - as abortion
 - as a generalized septicaemia with involvement of the liver and other organs

- as mastitis in dairy cattle.

Flow Chart

- Silage is commonly implicated in outbreaks of Listeriosis in cattle and sheep. Most pathogenic bacteria require the availability of iron in the host for metabolic activities.
- High iron levels in silage that lead to elevated tissue concentrations of iron may predispose cattle and sheep fed on silage to Listeriosis.
- The pathogenic listeria can penetrate the epithelial barrier in the intestine and multiply in hepatic and splenic macrophages aided by the haemolysin named listeriolysin O. It leads to septicaemia.
- If the pathogen penetrates through damaged mucosal surfaces to the CNS, via the trigeminal nerve or an alternate route it may penetrate through the dental pulp (when sheep are cutting or losing teeth) to the CNS, resulting in neural form.

PATHOGENECITY

Symptoms

- Four syndromes
 - **Subclinical**
 - Infections are the most common form of infection.
 - Usually outbreak occurs when fed with poor quality, high pH silage, particularly during cold weather.
 - **Neonatal infections**
 - Characterised as visceral infections with a septicemia.
 - Often gastroenteritis and bilateral meningitis.
 - Deaths are frequent in neonatal animals.
 - **Listerial abortion**
 - It is a sporadic condition in cattle and sheep.
 - It occurs after 4-8 months pregnancy. Retained placenta is common.
 - **Neural Listeriosis (circling disease):**
 - The incubation period ranges from 14 to 40 days.
 - The disease is more common in winter or early spring.
 - The clinical presentation of meningoencephalitis in adult ruminants may begin with signs of depression and confusion.
 - The ears droop; animal holds its head to one side.
 - Protrusion of the tongue and salivation are common and twitching or paralysis of the facial and throat muscles may occur.
 - When the animal moves, it tends to be in a single direction, giving rise to the common name of circling disease.
 - In the terminal stages, the animal may fall and will be unable to rise.
 - In poultry, there are signs of
 - Torticollis
 - Weakness
 - Incoordination of legs and
 - Sudden death in young birds
- The disease is usually fatal in sheep, pigs and horses.

Lesions

- Micro-abscess in the brain stem, usually unilateral, together with perivascular cuffing is very characteristic of listeriosis.

- The lesions are most common in the mid brain, pons and medulla oblongata.
- In addition to this there will be generalized septicaemia, focal necrosis of the liver and spleen will be seen.

DIAGNOSIS

- **Stained smears** from lesions may reveal Gram +ve rods (often coccobacillary)
- **Histopathological examination** of brain tissue can often give a presumptive diagnosis of neural listeriosis
- **Isolation and Identification**
 - Inoculation of specimens on selective media include blood agar with an antibiotic supplement or blood agar containing 0.05% potassium tellurite (inhibitory to Gram –ve).
 - Specimens from the visceral form of the disease or from abortion cases are inoculated directly onto the laboratory media.
 - A cold-enrichment procedure is necessary for brain tissue from neural listeriosis.
 - Small pieces of spinal cord and medulla are homogenized and a 10% suspension is made in a nutrient broth.
 - The broth suspension is placed in the refrigerator at 4°C and sub cultured on to blood agar once weekly for upto 12 weeks.
- **Inoculation in developing chicken embryos** causes development of focal necrotic lesions on the chorio allantoic membrane.(CAM)
- **Anton's test**
 - Inoculation of live bacterial suspension into the conjunctiva of a rabbit or guinea pig only *L.monocytogenes* causes a purulent keratoconjunctivitis within 24-36hrs of inoculation.
- **Intra peritoneal inoculation** of mice with a 24hr broth culture.
 - Both *L.monocytogenes* and *L.ivanovii* are pathogenic for mice.
 - They die within in 5 days with necrotic lesions present in the liver.

CONTROL, PREVENTION AND PUBLIC HEALTH SIGNIFICANCE

Specimens to be collected

- **Visceral form**
 - Material from lesions in liver, kidneys or spleen
- **Neural form**
 - Spinal fluid, brain stem, and tissue from several sites in the medulla oblongata
- **Abortion**
 - Placenta (cotyledon), foetal abomasal contents and/or uterine discharges.
- Ruminants in early stages of septicaemic listeriosis respond to systemic therapy with ampicillin or amoxicillin.
- Response to antibiotic therapy may be poor in neural listeriosis although prolonged higher doses of ampicillin or amoxicillin combined with an aminoglycoside may be effective.
- Ocular listeriosis requires treatment with antibiotics and corticosteroids injected subconjunctivally.
- Poor quality silage should be avoided. Vaccination with killed vaccines, which do not induce effective cell-mediated immune response, is not protective because *L.monocytogenes* is an intracellular pathogen. Live, attenuated vaccines,

which contain serovars 1/2a, 1/2b, and 4b are reported to reduce the prevalence of listeriosis in sheep.

Public health significance

- *L.monocytogenes* causes meningoencephalitis, meningitis, encephalitis and uterine infection with abortion, stillbirths, granulomatosis and valvular endocarditis in humans.
- The source could be soil, contaminated milk, cheese, meat, vegetables and humancarriers.

Infection is frequently associated with immuno-compromised persons.

