Genus Listeria and Erysepelothrix

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Listeria

- Kingdom Bacteria
- Division Firmicutes
- Class Bacilli
- Order Bacillales
- Family *Listeriaceae*
- Genus *Listeria*

History:

- *L. monocytogenes* first described by Murray (1926) who named it as Bacterium monocytogenes because of characteristic monocytosis infection in laboratory animals (rabbits).
- 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.

Introduction

- Gram positive rods/ coccobacillary up to 0.4-0.5 µm in length, old cultures stain gram negative
- From rapidly growing cultures or tissues the cells can appear coccal
- Motile by few (1-5) peritrichous flagella-end - over - end tumbling motility, Flagella are produced at room temperatures, but not at 37°C
- Beta hemolytic - CAMP Test positive with *Staphylococcus aureus* (*L. monocytogens* and *L. seeligerii*) or *Rhodococcus equi* (*L. ivanovii*).
- Psychrophilic- Grows well at refrigerated temperature - Basis of a cold- enrichment technique.
- Facultative intracellular bacterium of the reticulo endothelial system that causes listeriosis (circling disease, silage disease)

- Small, Gram-positive rods
- Grow on non-enriched media
- Catalase Positive, Oxidase-negative, Facultative anaerobes
- Non-acid fast, Non-spore forming
- Tolerates wide temperature and pH ranges
- Small haemolytic colonies on blood agar
- Tumbling motility at 25°C (non motile at 37°C)
- Aesculin hydrolysed
- Environmental saprophytes
- Outbreaks of listeriosis often related to silage feeding

- The genus is composed of six species, three of which are pathogenic.
- *Listeria monocytogenes*, the most important of these pathogens, has been implicated world- wide in diseases of many animal species and humans.
- It was first isolated from laboratory rabbits with septicaernia and monocytosis (Murray et al., 1926).
- The organism can grow over a wide temperature range from 4°C to 45°C and can tolerate pH values between 5.5 and 9.6.
- The other two pathogens, *L. ivanovii* and *L. innocua*, are less frequently implicated in diseases of animals.

Habitat and Ecology:

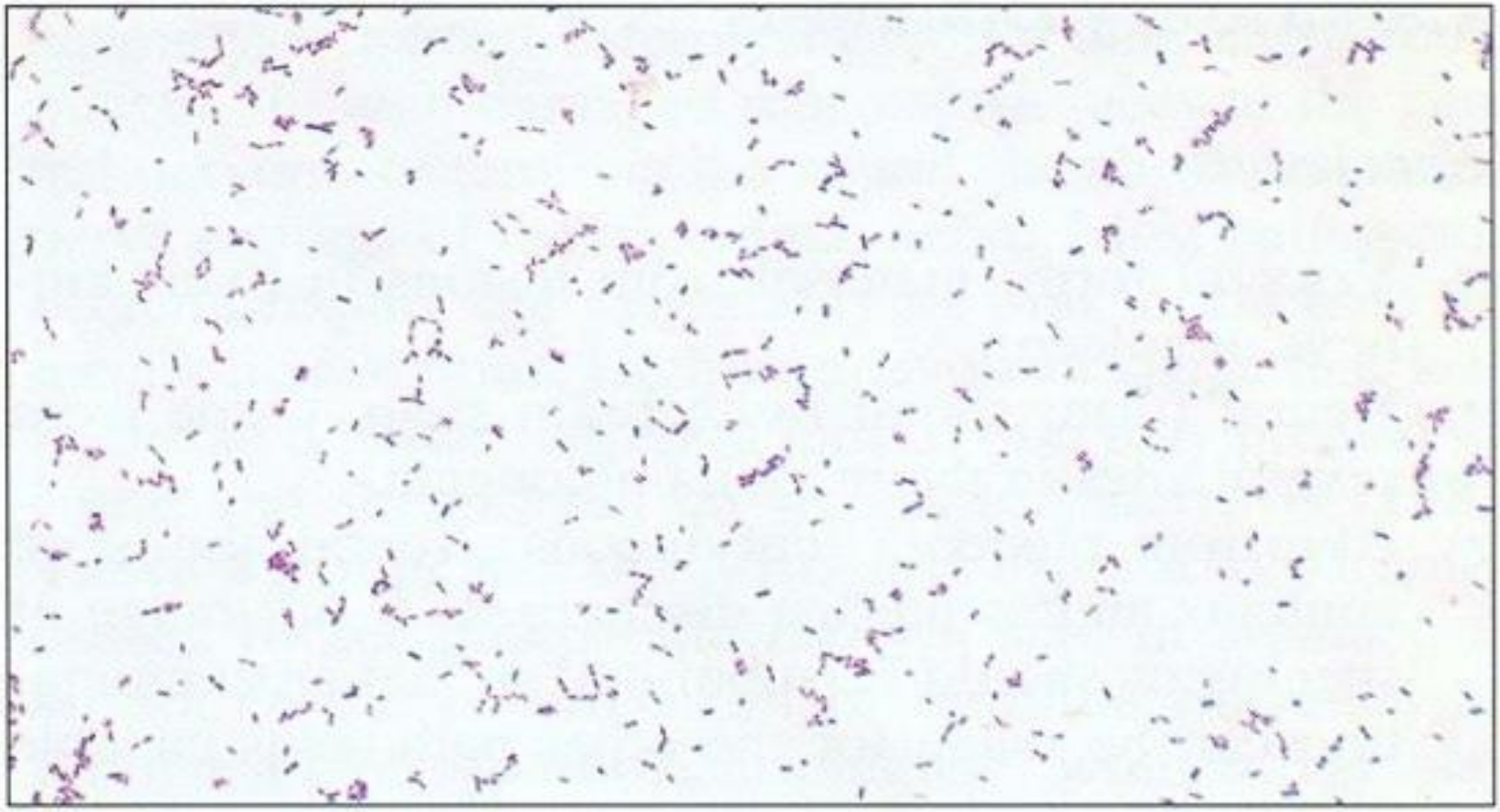
- *Listeria monocytogenes*, is wide spread in the environment throughout the world.
- Found in soil, dust, mud, vegetation, silage, sewage and most of the animals that have been tested.
- Grow well under a wide temperature from 4 - 44°C, relatively resistant to high salt concentrations (10% NaCl) and can grow from pH 5-9.
- Over growth of the bacterium in improperly prepared silage (poorly fermented silage with pH>5) / vegetation is the source of infection to animals.
- Found in the faeces of both clinically ill and sub clinically infected ruminants & humans (Healthy carriers).

CULTURAL CHARACTERISTICS:

- Can grow on ordinary medium.
- Blood agar plates -produce small bluish white colonies with or without hemolysis.
- Commercial selective and indicator media include – PALCAM agar (polymixin-acriflavin-lithium chloride -ceftazidime-esculin-mannitol agar) to isolate from foods.
- On brain heart infusion agar-blue-green sheen when light is reflected obliquely at 45°C.
- Grow between 4 and 45°C (heat tolerant).
- Grows well at refrigerated temperature.

Bio-chemical properties:

- Catalase positive
- motility in tube media at 25^0C
- H_2S negative, Indole negative.
- Bile Esculin Agar (*BEA*) positive
- Glucose, trehalose, and salicin positive
- Growth at 4ºC
- Nitrate and urease negative
- Resistant to drying and can survive up to two years in dry soil and feces.
- Anton Test: Broth culture in eye sac of rabbit or guinea pig produces conjunctivitis



203 *L. monocytogenes* in a stained smear from a culture with both Gram-positive rods and cocci present. (Gram stain, ×1000)

Virulence factor

- **Internalins (InlA)–** are surface associated proteins that act to facilitate the uptake of the bacterium into epithelial cells
- **Listeriolysin O (LLO) -** is a cholesterol-binding haemolysin. It is a pore forming toxin that facilitates the escape of the organism from the endosome to the cytosol. Inhibits phagolysosome formation.
- **Phospholipase C-** lyse the phagosome membrane and escapes the organism into cytosol. It is phosphotidyl inositol independent.
- **ActA** – a surface protein (transmembrane protein) that facilitates the rearrangement of actin to propel the organism through the cell and into an adjacent cell

Differentiation of Listeria species

- The pattern of haemolysis on sheep blood agar, CAMP tests and acid production from a short range of sugars are useful differentiating laboratory methods for Listeria species.
- Sixteen serotypes, based on cell wall and flagellar antigens, are recognized.
- Phage typing is reproducible and discriminating but its diagnostic applications are limited.
- A chemiluminescent DNA probe assay is available for rapid and specific identification of *L. Monocytogenes* from colonies on primary isolation plates.
- DNA fingerprinting methods are currently used in reference laboratories.

Listeria species	Haemolysis on sheep blood agar	CAMP test		Acid production from sugars		
		S. aureus	*R. equi*	D-mannitol	L-rhamnose	D-xylose
L. monocytogenes	+	+	–	–	+	–
L. ivanovii	++	–	+	–	–	+
L. innocua	–	–	–	–	v	–
L. seeligeri	+	+	–	–	–	+
L. welshimeri	–	–	–	–	v	+
L. grayi	–	–	–	+	v	–

v variable reactions

Clinical manifestations of infections of Listeria spp.

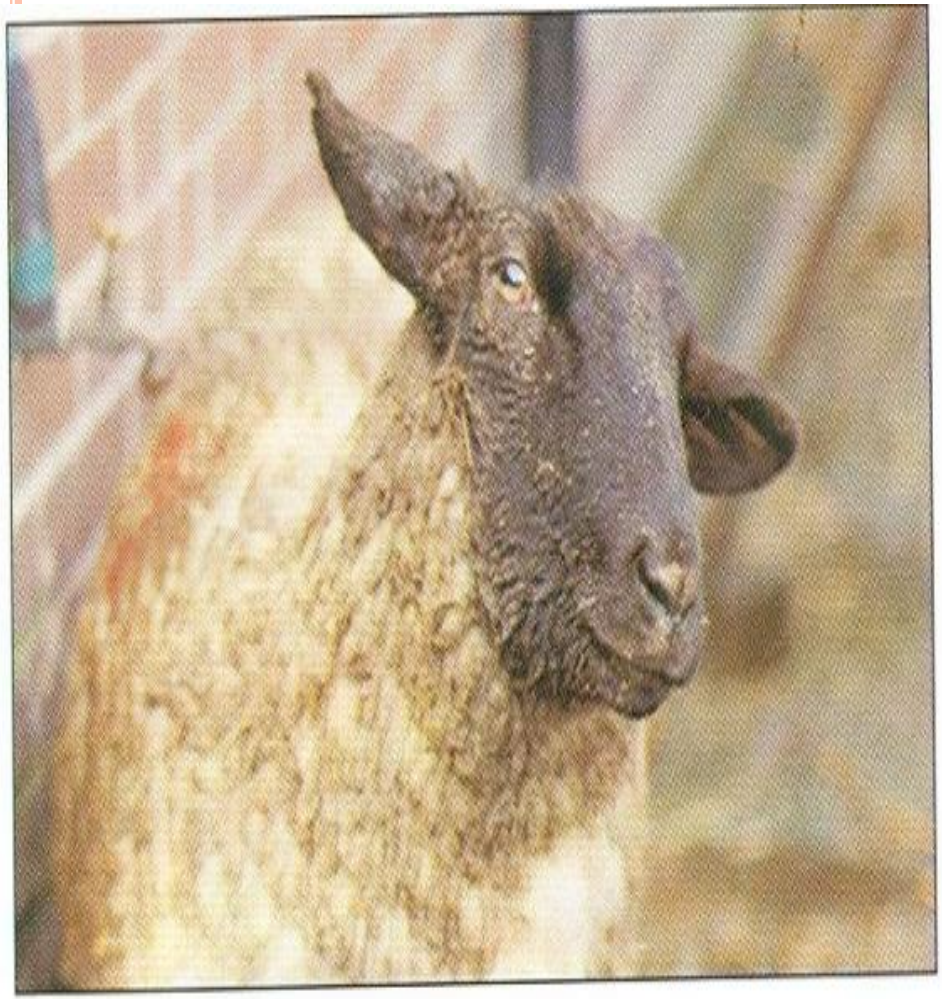
Species	Hosts	Forms of disease
Listeria monocytogenes	Sheep, cattle, goats	Encephalitis (neural form) Abortion Septicaemia Endophthalmitis (ocular form)
	Cattle	Mastitis (rare)
	Dogs, cats, horses	Abortion, encephalitis (rare)
	Pig	Abortion, septicaemia, encephalitis
	Birds	Septicaemia
L. ivanovii	Sheep, cattle	Abortion
L. innocua	Sheep	Meningoencephalitis (rare)

- Encephalitis (infection of the central nervous system).
  - **"Circling disease"** -- caused by brain involvement
  - Unilateral facial paralysis results in drooling of saliva and drooping of the eyelid and ear

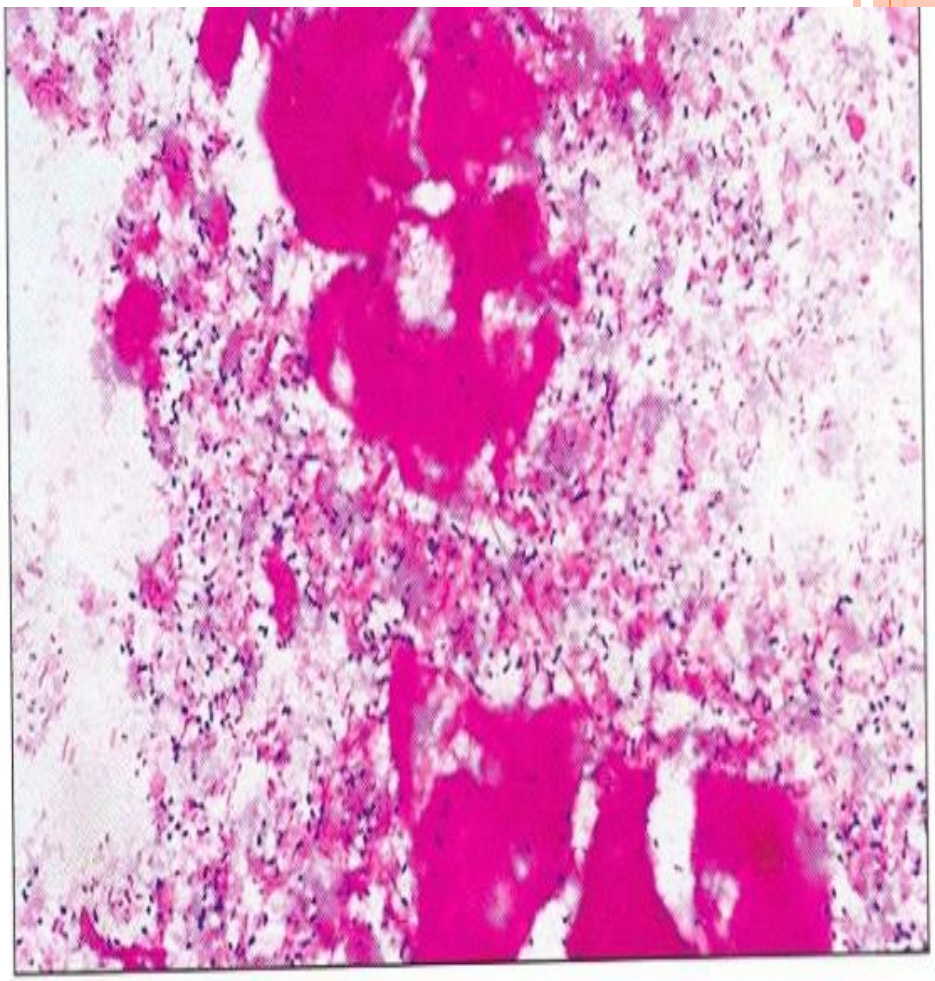
- Abortion (infection of the uterus or the fetus).
  - Generally during the **last two months of gestation** because of production practices
  - Occurs in winter
  - Associated with consumption of spoiled silage
  - Animals that abort are resistant to re-infection

- Septicemia (infection of the blood).
  - Primarily occurs in young animals
  - Can be exhibited by sudden death
  - Kerato-conjunctivitis

- Mastitis (infection of the mammary gland).
  - Bacteria can be shed in milk
  - Shedding can persist for more than 3 years



198 *Listeria monocytogenes:* neural form of listeriosis in a silage-fed sheep showing unilateral facial paralysis.



199 *L. monocytogenes* in a Gram-stained smear of material from a placenta (bovine abortion). (×1000)

Diagnosis

- Characteristic neurological signs or abortion in association with silage feeding may suggest listeriosis.
- Cerebrospinal fluid {CSF) and tissue from the medulla and pons of animals with neurological signs should be sampled.
- Fresh tissue is required for isolation of organisms and fixed tissue for histopathological examination.
- Specimens from cases of abortion should include cotyledons, foetal abomasal contents and uterine discharges.
- Smears from cotyledons or from liver lesions may reveal Gram-positive coccobacillary bacteria.

- Histological examination of brain tissue reveals microabscesses and heavy perivascular mononuclear cuffing in the medulla and elsewhere in the brain stem.
- White cell numbers exceeding 1.2 x 10^7 /L and a protein concentration of greater than 0.4g/L in CSF are found in neural listeriosis.
- Inoculation in developing chicken embryos causes development of focal necrotic lesions on the chorio allantoic membrane (CAM).

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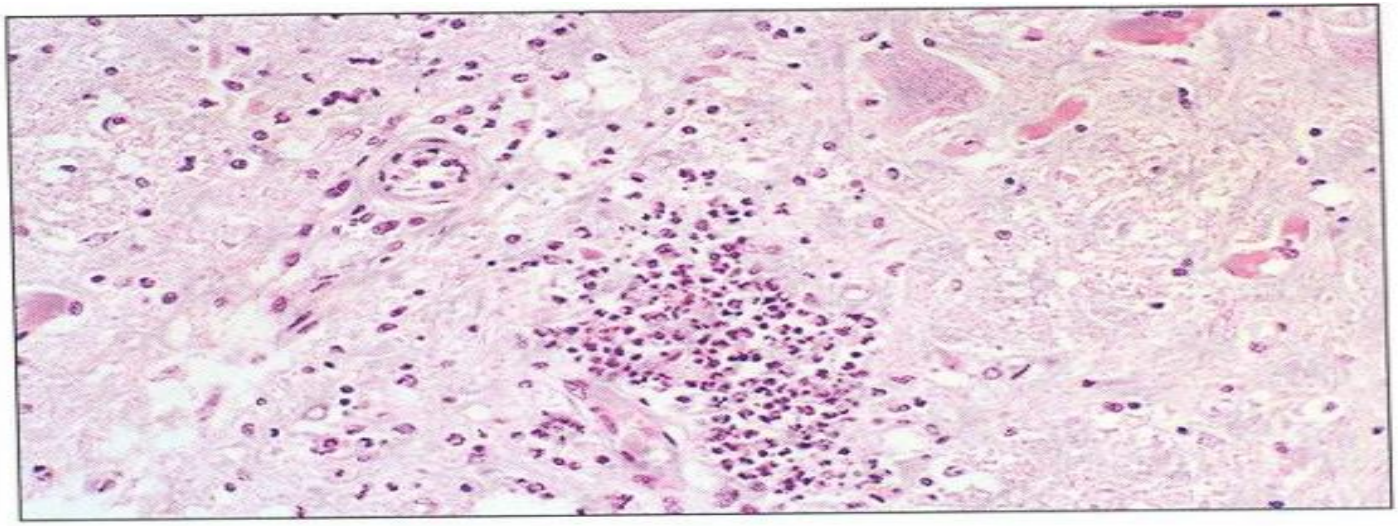

200 A microabscess in the medulla of a sheep with listeriosis. (H&E stain, ×400)

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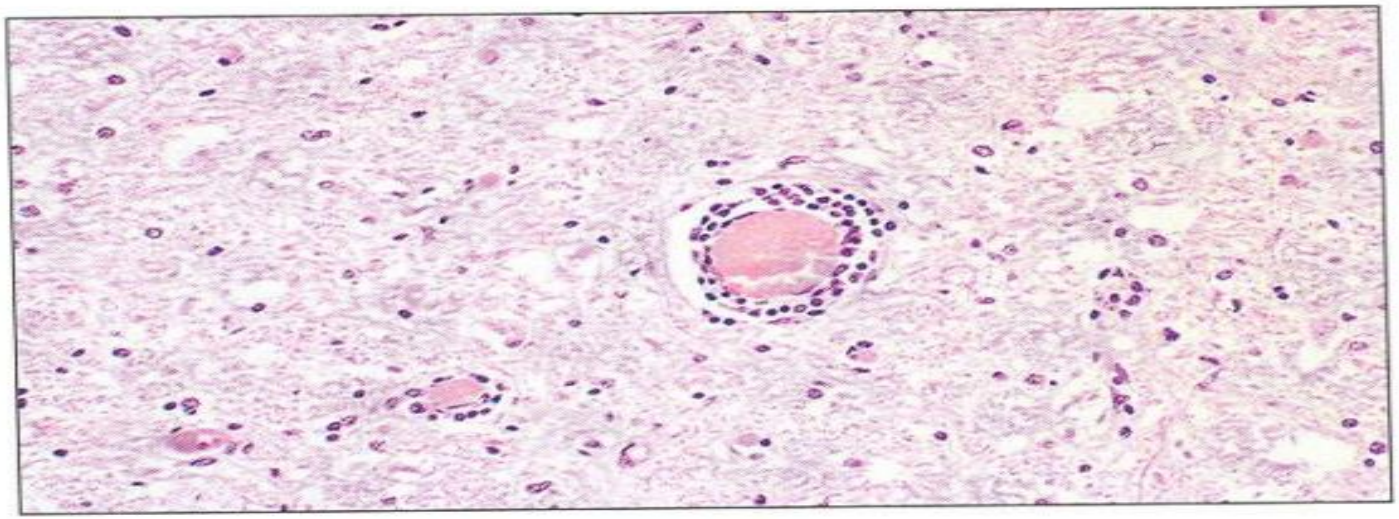

201 Perivascular cuffing in an ovine medulla indicative of the neural form of listeriosis. (H&E stain, ×400)

Isolation Methods

- Specimens from cases of abortion and septicaemia can be inoculated directly onto blood, selective blood (PALCAM) and MacConkey agars. The plates are incubated aerobically at 37'C for 24 to 48 hours.

Cold-enrichment

- Small pieces of spinal cord and medulla are homogenized and a 10% suspension is made in a nutrient broth.
- Broth suspension is placed in the refrigerator at 4^0C and sub cultured into blood agar once weekly for up to 12 weeks.

Identification criteria for *L. monocytogenes* isolates:

- Colonies are small, smooth and flat with a blue-green colour when illuminated obliquely.
- Individual colonies are usually surrounded by a narrow zone of complete haemolysis.
- CAMP test is positive with *Staphylococcus aureus* but not with *Rhododoccur equi*
- Aesculin is hydrolysed.
- Isolates incubated in broth at 25'C for 2 to 4 hours exhibit a characteristic tumbling motility.
- Most isolates of animal origin are virulent, a characteristic which can be confirmed by animal inoculation (Anton test).

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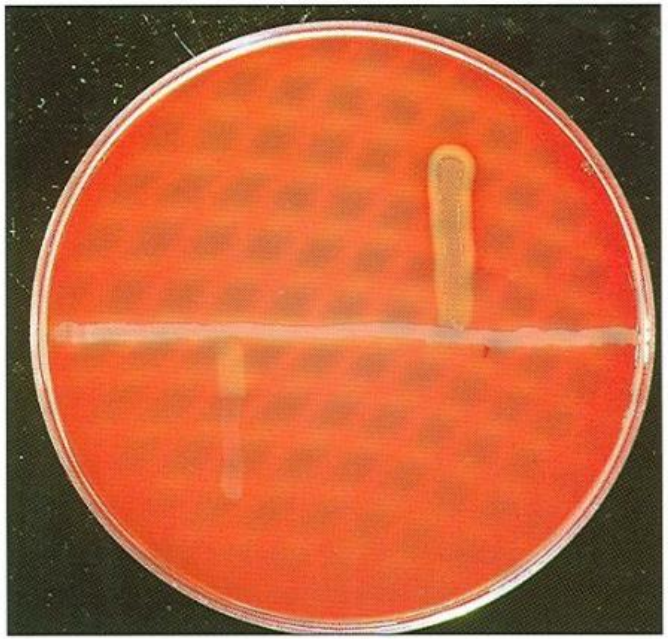

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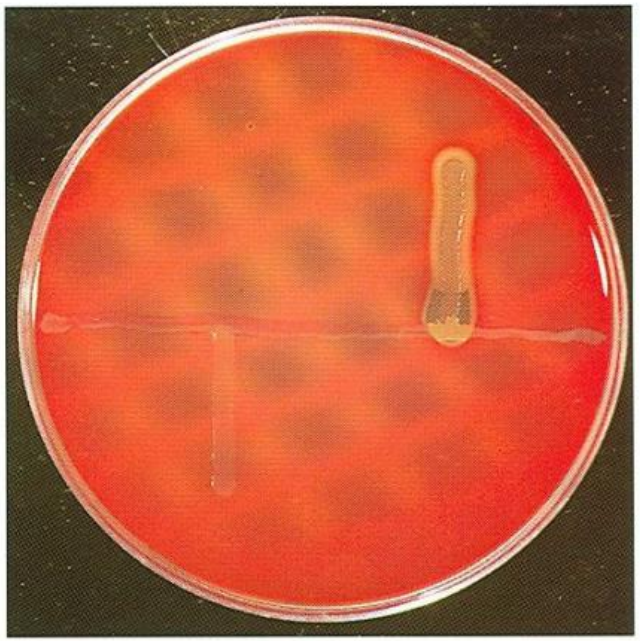

205 CAMP test with *Staphylococcus aureus* (horizontal) showing enhancement of the effect of the staphylococcal beta-haemolysin by *L. monocytogenes* (left) but not by *L. ivanovii* (right).

206 CAMP test with *Rhodococcus equi* (horizontal): no reaction by *L. monocytogenes* (left) and enhancement of haemolysis by *L. ivanovii* (right).

207 *Rhodococcus equi* streaked across (left to right) a vertical streak of *L. ivanovii* giving an enhanced haemolytic effect.

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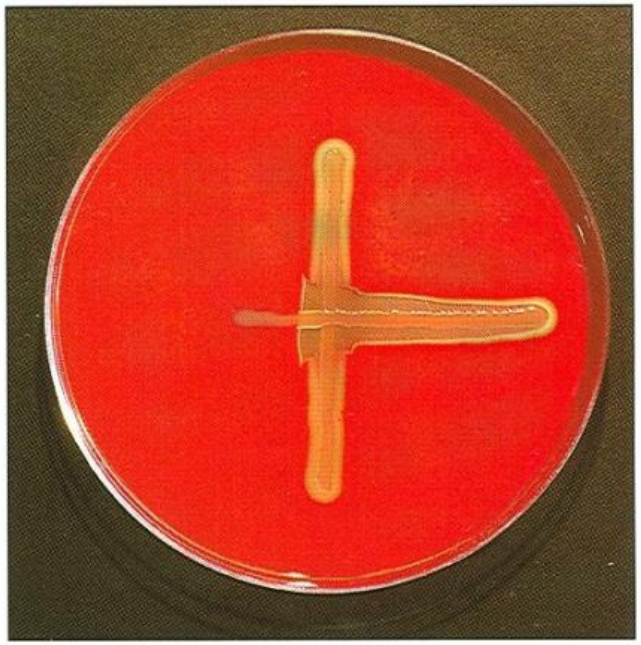


ANTON'S TEST:

- Inoculation of live bacterial suspension into the conjunctiva of a rabbit or guinea pig only
- *L. monocytogenes* causes a purulent kerato-conjuctivitis within 24-36 hrs of inoculation.

Erysipelothrix

Classification

- Kingdom Bacteria
- Phylum Firmicutes
- Class Erysipelotrichi
- Order Erysipelotrichales
- Family Erysipelotrichidae
- Genus *Erysipelothrix*

Introduction

- *Erysipelothrix rhusiopathiae (*previously named *Erysipelothrix insidiosa)* is, a non-motile, non-spore forming, Gram- positive, facultative anaerobe.
- It is catalase-negative, oxidase-negative, resistant to high salt concentrations (8.5% NaCl) and grows in the temperature range 5°C to 42°C and in the pH range of 6.7 to 9.2.
- Gram-positive, small rods (smooth form) or filaments (rough form) Growth on non-enriched media Small colonies, Exhibit alpha haemolysis or non haemolytic.
- Causes swine erysipelas and turkey erysipelas

Usual habitat

- It is claimed that up to 50% of healthy pigs harbour *E. rhusiopathiae* in tonsillar tissues.
- Carrier pigs excrete the organism in faeces and in oro-nasal secretions.
- The bacterium has also been isolated from sheep, cattle, horses, dogs, cats, poultry.

Colonial morphology and haemolytic activity

- Non-haemolytic, pin-point colonies (0.05 mm) appear after incubation for 24 hours and, after 48 hours, a narrow zone of greenish, incomplete haemolysis develops around the colonies.
- Smooth colonies are up to 1.5 mm in diameter, convex and circular with even edges while rough colonies are slightly larger, flat and opaque with irregular edges.
- A 'bottle-brush' type of growth is characteristic of rough isolates when they are stab- inoculated into nutrient gelatin and incubated at room temperature for up to 5 days.

Biochemical reactions

- Catalase negative, Oxidase negative
- Coagulase-positive, few pathogens produce this enzyme apart from some staphylococci.
- H_2S production is detected by a thin, black central line in triple sugar iron (TSI) agar when this medium is stab-inoculated at 37ºC for 24 hours.

VIRULENCE FACTORS

- A capsule which protects against phagocytosis, ability to adhere to endothelial cells and the production of neuraminidase, an enzyme which enhance cell penetration.
- In septicaemic form, vascular damage is characterized by swelling of endothelial cells, adherence of monocytes to vascular walls and widespread micro-thrombus formation.
- Localization of the bacteria in joint synovia and on heart valves via haematogenous spread leads to chronic lesions at these sites.
- Long term articular damage may result from an immune response to persistent bacterial antigens. Viable organisms are rarely isolated from chronically affected joints.

Box 14.1 Clinical manifestations of *Erysipelothrix rhusiopathiae* infection in domestic animals.

- Pigs (swine erysipelas)
  - — septicaemia
  - — 'diamond skin' lesions
  - — chronic arthritis
  - — chronic valvular endocarditis
  - — abortion
- Sheep
  - — polyarthritis in lambs
  - — post-dipping lameness
  - — pneumonia
  - — valvular endocarditis
- Turkeys (turkey erysipelas)
  - — septicaemia
  - — arthritis
  - — valvular endocarditis

Swine erysipelas

- Sub-clinically-infected carrier pigs are the main reservoir of infection.
- Pigs with acute disease excrete large numbers of organisms in faeces.
- Infection is usually acquired through ingestion of contaminated food or water and less commonly through minor skin abrasions.
- The frequency of disease outbreaks in free-range pigs may be reduced by keeping them on concrete.
- Pigs under 3 months of age are normally protected by maternally-derived antibodies while animals over 3 years of age usually have acquired a protective active immunity through exposure to strains of low virulence.
- Factors which may predispose to disease development include changes in diet, extreme ambient temperatures and fatigue.

Clinical manifestations:

- **Pigs (swine erysipelas) –** It occur in 4 forms. There are two acute and two chronic forms. Chronic arthritis has the most significant negative impact on productivity.
- **Septicaemic form –** It occurs after incubation period of 2-3 days. During an outbreak, some pigs will be found dead and others are febrile, depressed and walk with a stiff, stiled gait or remain recumbent. Mortality may be high and pregnant sow with septicaemic form may abort.

- **Diamond skin form –** Systemic signs are less severe and mortality rates are much lower than septicaemic form. Pigs are febrile and cutaneous lesions are seen as small, light pink or purple, raised areas to more extensive and characteristic diamond-shaped erythematous plaques. Some of these lesions resolve within one week; others become necrotic and may slough.
- **Chronic arthritis –** It is commonly seen in older pigs. There is stiffness, lameness or reluctance to bear weight on affected limbs. Joint lesions, which may be initially mild, can lead to erosion of articular cartilage with eventual fibrosis and ankylosis
- **Chronic valvular endocarditis –** It is the least common form. In this form, wart like thrombotic masses are present, usually on the mittral valves.


Swine Disease
Eradication Center
University of Minnesota

- **Sheep** - polyarthrjtis in lambs - post-dipping lameness - pneumonia - valvular endocarditis
- **Turkeys (turkey erysipelas)** - septicaemia - arthritis -valvular endocarditis

DIAGNOSIS:

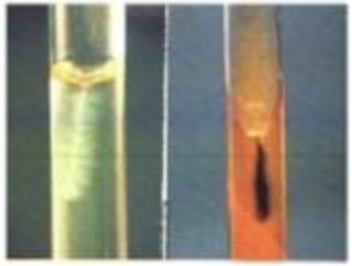
- Diamond-shaped skin lesions are pathognomonic.
- Specimens for laboratory examination include blood for haemoculture and postmortem specimens of liver, spleen, heart valves or synovial tissues. Organisms are rarely recovered from skin lesions or chronically affected joints.
- Microscopic examination of specimens from acutely affected animals may reveal slender Gram-positive rods. Filamentous forms may be demonstrable in smears from chronic valvular lesions.
- Blood and MacConkey agar plates, inoculated with specimen material are incubated aerobically at 37˚C for 24-48 hours. Selective media, containing either sodium azide (0.1%) or crystal violet (0.001%), may be used for contaminated samples.

Identification criteria for isolates

- Colonial morphology after incubation for 48 hours.
- Absence of growth on MacConkey agar.
- Appearance in Gram-stained smears from colonies
- Negative catalase test
- Coagulase production
- H_2S production in TSI agar slants
- Biochemical test profile.
- Serological tests are not applicable for diagnosis.

Erysipelothrix rhusiopathiae: Laboratory Diagnosis

- Alpha hemolytic on SBA
- Non-motile
- Catalase negative
- Hydrogen sulfide production on TSIA
- VP negative
- Gelatin stab culture yields a test tube brush-like pattern at 22°C



Further Readings

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease