PARA TUBERCLOSIS AND NOCARDIA

Learning objectives

To know in detail about,

- Johne's disease and bovine farcy
- Pathogenesis and pathogenicity of Johne's disease
- Cultivation and diagnostic methods for Johne's disease
- Johnin test
- Diseases caused by Nocardia in domestic animals

MORPHOLOGY

- General approaches used to isolate and identify Nocardia

- Johne‘s disease or paratuberculosis is caused by *Mycobacterium avium* subsp. paratuberculosis (also referred as *Mycobacterium johnei*).
- The disease causes chronic, contagious fatal enteritis, which can affect cattle, sheep, goats, camels and wild ruminants.
- Note: *Mycobacterium avium* subsp. *paratuberculosis* infection in humans is called as Crohn‘s disease (chronic enteritis in human).
- *Mycobacterium avium* subsp. *paratuberculosis* are acid-fast organisms.
- They are short rods measuring 1-2µm in width with rounded edges. They are motile and does not form spores.
- On artificial media the organism tends to be in shorter club form.

CULTURAL CHARACTERISTICS

- *Mycobacterium avium* subsp *paratuberculosis* requires mycobactin - Killed extract of *M.phelei* or other killed acid-fast organism- enriched media for growth.
- Slants of Herrold‘s egg yolk medium with mycobactin are highly suitable for isolation of organism from specimens.
- The slants are incubated at 37^{o}C for upto 16 weeks and examined weekly for evidence of growth.

- They produce minute grayish white, friable, irregular colonies, less than 1 mm in d.m., in 5-16 weeks. Isolates from sheep may be pigmented.

PATHOGENESIS

- The organism is shed in the faeces, milk and semen of infected animals.
- They remain viable in the environment for up to one year under suitable conditions.
- Calves under one month of age are highly susceptible and they develop clinical disease than animals infected later in life.
- Infection is acquired mainly through ingestion.
- The organism is an intra cellular pathogen and cell mediated reactions are mainly responsible for the enteric lesion.
- Ingested mycobacteria, engulfed by macrophages in which they survive and replicate, are found initially in Peyer‘s patches.
- As the disease progresses, an immune mediated granulomatous reaction develops, with marked lymphocyte and macrophage accumulation in the lamina propria and submucosa.
- The resulting enteropathy leads to loss of plasma proteins and malabsorption of nutrients and water.

PATHOGENICITY

Symptoms

- Clinical signs develop after prolonged subclinical phase of infection. Affected cattle are usually more than 2 years of age when signs are first observed.
- In cattle, the disease is characterized by diarrhoea, initially intermittent, dark and semisolid, but becoming persistent and profuse.
- Progressive weight loss results without loss of appetite, leading to emaciation and eventually death.
- The mortality rate may approach 100%. Asymptomatic carrier cattle have an increased incidence of mastitis and infertility.
- In sheep and goats, the disease is clinically evident only in mature animals. The diarrhoea is less marked and may be absent.

Lesions

- Chronic catarrhal inflammation of the intestine is characteristic.
- In cattle, the mucosa of affected areas of the terminal small intestine and the large intestine is usually thickened and folded into transverse corrugations.
- The mesenteric and ileocaecal lymphnodes are enlarged and oedematous.
- Thickening of the intestinal mucosa is less marked in sheep, and necrosis and caseation may be present in the regional lymphnodes.

DIAGNOSIS

Specimens to be collected

- Specimens for direct microscopy from live animals include scrapings /pinch biopsies from the rectum. Faeces may be submitted for culture.

- In case of dead animals, tissues from affected region of the intestines and from regional lymphnodes are useful for histopathology.

Diagnosis by

- Microscopical examination by staining the faecal smears with acid-fast stain.
- Bacteriological examination
    - Materials decontaminated with 0.3% benzalkonium chloride and concentrated by centrifugation and subsequently cultured in Herrold's egg yolk medium and are incubated at 370C for up to 16 weeks.
- Based on PM lesions
- Serological tests
    - Complement fixation test can be used. But CFT is laborious and relatively insensitive.
    - Agar gel precipitation test has been used for confirming clinical infection. ELISA, using serum absorbed with *M.phlei* may detect subclinically infected animals.
- Johnin test
    - Intra dermal Johnin test
        - Inoculate Johnin PPD into the skin of the neck region.
        - The delayed hypersensitivity reaction is measured after 48 hours.
        - Peak response usually develops a month or so after infection.
    - Intra venous Johnin test
        - The intravenous Johnin test reaction is measured by increase in body temperature following intra venous Johnin PPD injection.

PREVENTION AND CONTROL

- DNA probes, which are highly sensitive, are being used to detect organisms in faeces.

- Animals with clinical signs suggestive of paratuberculosis should be isolated, because they shed large number of bacteria, which can contaminate buildings and pasture.
- Detection and elimination of subclinically infected animals is effective. Inactivated adjuvanated vaccines areavailable .
- A live vaccine consisting of nonpathogenic strain of *Mycobacterium avium* subsp. *paratuberculosis* is inoculated subcutaneously into calves soon after birth and before 4 weeks of age.
- It reduces the incidence of Johne‘s disease in the herd.

Phylum	Actinobacteria
Class	Actinobacteria
Subclass	Actinobacteridae
Order	Actinomycetales
Suborder	Corynebacterineae
Family	Nocardiaceae

Genus	Nocardia

HISTORY AND HABITAT

History

- Nocard described this organism in 1888, following its isolation from a case of bovine farcy , hence the name of the type species is *Nocardia farcinica*.

Habitat

- *Nocardia* species are soil borne saprophytes.

MORPHOLOGY

- *Nocardia* has the ability to form Gram-positive, branching filaments of less than 1um in d.m.
- It is closely related to *Corynebacterium*, *Mycobacterium* and *Rhodococcus* species.
- They are obligate aerobes. Some produce true mycelia and some strains are acid-fast. All species are non-motile.
- Gram stained smears from lesions revealed Gram-positive branching filaments that often showed fragmentation into coccobacillary elements.
- The modified ZN stained smears exhibit a similar morphology but most of the filaments retain the carbol-fuchsin dye and stain red.

CULTURAL AND BIOCHEMICAL PROPERTIES

- The *Nocardia* species grow very well in blood agar incubated aerobically at 370C for up to 7 days.The colonies on blood agar are often vivid white and powdery if aerial filaments and spores are formed. Occasionally the colonies are smooth, heaped and variably pigmented.
- Inoculate the suspected colonies from blood agar into Sabouraud dextrose agar (SDA) and incubate at 370C for up to 10 days.
- Both types of colonies firmly adherents to the agar surface. The colonies on SDA are dry, wrinkled and yellow, becoming deep orange color with age.
- Gram-stained smears from colonies show Gram-positive branching filaments that characteristically break up into rods or coccobacillary elements with age.
- An MZN –stained smear from young culture reveals red staining, branching filaments.

There are three morphological forms

- Group I strains have limited mycelia development due to early fragmentation of hyphae into coccoid forms within 2 to 14 hours of incubation.
- Group II strains produce mycelia, which fragment in about 18 to 20 hours after incubation, though these mycelia break up into mycelial fragments within two days of growth.
- The pathogenic *Nocardia* species belong to Group III. The colonies are usually leathery in appearance and pigmented . Extensive mycelium produced because fragmentation does not begin until after 5 days incubation.

Biochemical Properties

- To differentiate *Nocardia* species tests such as decomposition of casein, hypoxanthine, tyrosine, urea and xanthine are useful.
- They are oxidase and Catalase positive. Reduce nitrates to nitrites. Gelatin not hydrolyzed.

PATHOGENESIS

- Nocardia are aerobic and essentially saprophytic.
- They cause suppurative and pyogranulomatous reactions in immunocompromised hosts or animals that have been exposed to large doses of the bacterium.
- The pathogenic Nocardia survive within phagocytic vacuoles by preventing phagolysosome formation.
- This is probably due to the surface lipids as Nocardia species have a cell wall similar to the mycobacteria.
- Other cell wall lipids may provoke the characteristic granulomatous reaction.
- Exudates are sanguinopurulent and can sometimes contain soft granules consisting of bacteria, neutrophils and debris.
- They lack the microstructure of the sulphur granules produced by some of the Actinomyces species.
- Diseases caused by the pathogenic Nocardia are

Species	Host (s)	Disease
Nocardia asteroides *Nocardia farcinica*	Dogs/ Cats Cattle	Localised cutaneous granulomatous abscesses nocardiosis Pyothorax and granulomas in the thoracic cavity Chronic granulomatous mastitis Bovine farcy in tropical regions

PATHOGENICITY

- *Nocardia* species have been isolated from a variety of clinical situations, though the genus is in general an opportunistic pathogen.
- In primary nocardiosis, severe, suppurative or cavitary pulmonary infection simulating tuberculosis is often observed and in some cases, show cutaneous and subcutaneous abscesses which are diagnostic of *N. asteroids*.
- Blood stream invasion with secondary, often fatal, involvement of the internal organs and the central nervous system are seen in *N. asteroids*.
- In the bovine species the most important disease condition is mastitis whose presentation is in the form of extensive fibrosis. *N. asteroids* is the most often isolated species.

DIAGNOSIS

- Specimens to be collected
  - Specimens should include exudates, aspirates, mastitic milk samples, tissue from granulomas and thin sections from granulomas in 10% formalin for histopathology.
- Based on direct microscopy

    - Soft granules are not common in exudates from *N. asteroides* infections. Smears made from exudates, aspirates, granulomatous tissue and from centrifuged deposits of bovine mastitic milk are stained by Gram's and MZN stain.
    - Gram-stained smears reveal Gram-positive branching filaments that oftenshow some fragmentation into coccobacillary elements.
    - The MZN –stained smears exhibit a similar morphology but most of the filaments retain the carbol fuchsin dye and stain red.
- Based on isolation and identification
    - Characteristic colonial morphology on blood and SDA agar and microscopic appearance.
- Based on biochemical reactions
- Differential diagnosis with Actinomyces
- Actinomyces infections respond well to penicillin and other commonly used antibiotics, nocardial infections are often refractory to treatment and *N. asteroides* issusceptible only to limited range of antimicrobial agents such as Trimethoprim-sulphamethoxazole or erythromycin.

Characters	Nocardiosis	Actinomycosis
Granules in exudates	Not common	Usually present
Filaments MZN positive	+	-
Fragmentation of filaments	+	-
Growth on SDA	+	-
Powdery, white colonies (aerial hyphae)	+	-
Susceptibility to penicillin	-	+

Note

- Infection with Nocardia is by inhalation from the environment, while Actinomycesinfection begins in the host as a normal flora invading damaged tissues.
- Disseminated disease caused by Nocardia is more common in the dogs, while granuloma formation is the rule with Actinomyces infection and spreads by localextension.
- When there is a doubt as to whether an animal has actinomycosis or nocardiosis, precautionary measures to preserve the anaerobic Actinomyces should be instituted,including

prompt delivery to the laboratory under anaerobic condition, culturing on brain heart infusion agar, blood plates and incubating under anaerobic, microaerophilic, and aerobic conditions.

- While Actinomyces fails to grow on Sabouraud agar, Nocardia grows uninhibited.
- Acid-fast staining procedure of the sample exudate before culturing will also behelpful in the presumptive identification of the infecting agent.