

# ACTINOMYCETES

## Learning objectives


To know in detail about,

- Principal host and diseases caused by Actinomyces

- Ray fungus and summer mastitis
- Morphology and cultural characteristics of Actinomyces
- Pathogenesis of lumpy jaw
- Specimens to be collected and the general approaches used to diagnose the lumpy jaw in cattle

## SYSTEMATICS

- The actinomycetes comprise a heterologous group of prokaryotes that have the ability to form Gram positive, branching filaments of less than 1µm in diameter.
- The main animal pathogens in the actinomycetes are in the genera *Actinomyces*, *Arcanobacterium*, *Actinobaculum*, *Nocardia* and *Dermatophilus*.
- Non-pathogenic, prolific producers of antimicrobial substances – streptomycetes are also included in Actinomycetes

<b>Domain</b>	Bacteria
<b>Phylum</b>	<i>Actinobacteria</i>
<b>Class</b>	<i>Actinobacteria</i>
<b>Subclass</b>	<i>Actinobacteridae</i>
<b>Order</b> 	<i>Actinomycetales</i>
<b>Suborder</b>	<i>Actinomyceneae</i>
<b>Family</b>	<i>Actinomycetacea</i>
<b>Genus</b>	<i>Actinomyces, Arcanobacterium</i>

## HABITAT AND HISTORY

- The *Actinomyces* species are present on mucous membrane of the host animal, often in the oral cavity, tonsils, and nasopharynx.
- The soil is the natural habitat of many *Actinomyces* species.
- The generic name *Actinomyces* was first used by Harz (1879). Boestrom (1891) isolated *Actinomyces bovis*.
- Cummins (1962) clearly demonstrated *Actinomyces* were bacteria and they were distinct from other branching genera.

## MORPHOLOGY

- The organisms show considerable pleomorphism. *Actinomyces* species are usually long and filamentous although short V, Y, and T configuration also occur.
- In lesions of actinomycosis, the pus contains small pale yellow granules referred as sulfur granules .

- The sulphur granule is composed of bacterial filaments and mineralized calcium phosphate of host origin.
- When the granules are crushed and Gram stained, a mass of Gram-positive branching filaments about 1µm in width, short rods, and cocci are evident.
- Around this mass, a circle of club shaped bodies with their narrow ends pointing towards the centre-staining Gram negative. Hence, called ray fungus .
- They are non-acid fast, non-spore forming, nonmotile, non-capsulated and do not form endospores or conidia.
- In case of *Arcanobacterium pyogenes* infections the pus or mastitic milk does not contain any granules.
- Gram stained smears reveal large numbers of small, highly pleomorphic, Gram-positive rods, cocci and pear shaped cells.
- Occasionally short branching typical Chinese letter appearances are also seen.

### CULTURAL CHARACTERISTICS

- They cannot grow on Sabouraud dextrose agar. *Actinomyces* require enriched media for growth. They grow well on sheep or ox blood agar.
- *Actinomyces bovis* is capnophilic (i.e. required 5-10% CO<sub>2</sub> for its growth).
- *Arcanobacterium pyogenes* and *Actinomyces viscosus* will grow aerobically but 5-10% CO<sub>2</sub> will enhance their growth.
- *Actinomyces bovis* and *Actinomyces viscosus* usually require 2-4 days but the growth of *Arcanobacterium pyogenes* can usually be seen in 24 hrs.
- *Actinomyces bovis* colonies are non-haemolytic, very small (< 1mm), white, rough or smooth and adhere tenaciously to solid medium.
- Gram stained smears show Gram positive, slightly branched filaments or short forms. On subculture, the bacterium may become diphtheroidal or coccobacillary.
- *Actinomyces bovis* grows well in thioglycollate medium, giving a characteristic diffuse growth in about 7-10 days.
- In broth cultures, it grows in coarse aggregates, which in some cases may result in a granular deposit with a completely clear supernatant.
- *Arcanobacterium pyogenes* produce a hazy- haemolysis after 24hrs incubation along the streak lines.
- At 48 hrs incubation, the colonies are surrounded by a narrow zone of complete haemolysis.
- *Arcanobacterium pyogenes* has the ability to pit a loeffler serum slope in 24-48 hrs. (i.e. A loopful of pure culture of the medium is taken and a heavy inoculum is made in a small area in the center of the slope, taking care not to break the surface of the medium. The medium is incubated at 37°C for 24 –48 hrs).
- *Arcanobacterium pyogenes* will give positive CAMP test with *Staphylococcus aureus* (i.e. **enhancement** of staphylococcal haemolysis).
- In litmus milk, the organism produces acid and clot after 3 days of growth.
- *Actinomyces viscosus* commonly produces two colonial forms, one being smooth, entire, convex and glistening and the other is smaller, rough dry and irregular.
- Neither is haemolytic. The larger colonial type yields Gram-positive diphtheroid forms and the smaller colony has short branching filaments.

### BIOCHEMICAL PROPERTIES, RESISTANCE, ANTIGENS AND TOXINS

#### Biochemical tests

- Both *Arcanobacterium pyogenes* and *Actinomyces bovis* are catalase negative, ferments several sugars and produce acid.

- Reduction of nitrate is negative. *Actinomyces viscosus* is catalase positive.

### Resistance

- *Actinomyces* are killed at a moist heat temperature of 60°C for 20 mins and they are susceptible to various disinfectants.

### Antigens and toxins

- With the exception of *Arcanobacterium pyogenes*, *Actinomyces* species have not been shown to produce any toxin.
- *Arcanobacterium pyogenes* produces a haemolytic exotoxin, which is dermonecrotic and lethal and it also produces a protease and an extracellular neuraminidase.

## PATHOGENESIS

- *Actinomyces bovis*, present as part of the normal flora of the mouth.
- Trauma to the tissues is the initiating event in disease and may occur as a result of shedding of teeth or as a result of coarse feed.
- Whenever there is a trauma, the organism invades a variety of tissues and often produces lesions in bone.
- Growth of the organism may involve maxillary bone, tongue, pharynx, lungs, lymph nodes and S/c tissues of the head and neck.
- It initiates rarefying osteomyelitis and soft tissue reaction, the condition being referred to as lumpy jaw.
- Granulation, mononuclear infiltration and fibrosis occur in the lesions with sinus tracts leading to the outside.
- Exudate from the tracts contains pus with sulphur granules.
- *Arcanobacterium pyogenes* is a commensal on the exposed mucosal surfaces of cattle, sheep and swine
- *Arcanobacterium pyogenes* infection is often a sequel to earlier tissue injury or to infection with other bacteria. (i.e. *Fusobacterium necrophorum*, *Peptostreptococcus indolicus*). It produces toxins and established mastitis with abscess formation. The acute bovine mastitis is referred to as summer mastitis.
- *Actinomyces viscosus* serotype 1 appears to be responsible for disease in dogs.
- Two syndromes can occur, either separately or together.
- One is a localized granulomatous lesion involving skin and subcutis; the other is a pyothorax, with granulomas in the thoracic cavity and often a large accumulation of sanguinopurulent pleural fluid containing soft white granules.
- Diseases caused by the pathogenic actinomycetes

Actinomycete	Host (s)	Disease
<i>Actinomyces bovis</i> (Syn: Ray fungus)	Cattle	Bovine actinomycosis (Lumpy jaw)
	Horses	Poll evil/Fistulous withers (occur as a mixed infection with <i>Brucella</i> species)
<i>Arcanobacterium pyogenes</i> ( <i>Actinomyces</i> )	Cattle, Sheep and Pigs	Chronic or acute suppurative mastitis, suppurative pneumonia, septic arthritis, vegetative endocarditis (Cattle), endometritis, umbilical infections, wound

<i>pyogenes</i> )	mainly	infections and Seminal vesiculitis (Bulls and Boars). Summer mastitis – a mixed infection with <i>Peptostreptococcus indolicus</i>
<i>Actinomyces viscosus</i>	Dogs	Canine actinomycosis <ul style="list-style-type: none"> <li>• Localised cutaneous granulomatous abscess and/or</li> <li>• Pyothorax and granulomas in the thoracic cavity.</li> </ul>
<i>Actinomyces israelii</i>	Human	Human actinomycosis
<i>Actinobaculum suis</i> ( <i>Actinomyces suis</i> )	Pigs	Pyogranulomatous mastitis, ascending pyelonephritis, cystitis.

## PATHOGENICITY

### Symptoms

- In case of lumpy jaw in cattle there is marked swelling associated with suppurative and proliferative osteomyelitis in the region.
- Lumpy jaw produces ill health by interfering with mastication.
- *Arcanobacterium pyogenes* infection occurs most frequently in heifer and dry cows during summer months.
- Hence, it is named as summer mastitis.
- The affected quarter become enlarged and firm.
- Animals have fever with general toxæmia.
- The mortality and morbidity rate may be high.

### Lesions

- In lumpy jaw, area of suppuration accompanied by the granulation tissues, erosion of old bones and formation of new bones.
- The pus characteristically contains small sulphur granules.
- In summer mastitis, abscess develops at any site containing greenish yellow foul smelling pus.

## DIAGNOSIS, CONTROL AND PREVENTION

### Diagnosis

- Specimens to be collected
  - It includes pus, exudates, aspirates, tissue and scrapings from the wall of abscesses.
  - If they have been incised. A volume of fluid or pus should be collected and submitted, if possible, rather than just a small amount on a swab.
  - Thin sections of granulomas in 10% formalin are useful for histopathology.
- Direct microscopy

- The pus or exudate is placed in a Petridish and washed carefully with a little distilled water to expose the yellowish sulphur granules of *Actinomyces bovis* or the softer greyish white granules of *Actinomyces viscosus*.
- A granule is placed on a microscopic slide in a drop of 10% KOH and gently crushed by applying pressure on the cover slip.
- The characteristic clubs can be examined under the low power microscope.
- If it is stained with Gram's, the ray fungus can be demonstrated.
- Isolation and Identification of organism
- Fat
- Pitting of loeffler serum slope and CAMP test in case of *Arcanobacterium pyogenes*

### **Control and Prevention**

- Actinomycetes are highly sensitive to tetracycline, chloramphenicol and penicillin including benzyl penicillin and ampicillin.

