

CORYNEBACTERIUM AND RHODOCOCCUS

Learning objectives

To know in detail about,

- Ulcerative lymphagitis in horses
- Diseases caused by *Corynebacterium* and *Rhodococcus* species in domestic animals
- Metachromatic granules, cuneiform arrangement, Diphtheroids and Pizzle rot
- Morphology, cultural and biochemical characteristics of *Corynebacterium* and *Rhodococcus* species

MORPHOLOGY

- *Corynebacteria* are Gram positive, non-acid fast, non-motile, non-capsulated, small pleomorphic rods.
- They frequently occur in rods, coccoid, cub and filamentous shape.
- The major pathogen is *Corynebacterium diphtheriae*, which causes diphtheria in children.
- *Corynebacteria* associated with animals are called diphtheroids.

Domain	Bacteria
Phylum	<i>Actinobacteria</i>
Class	<i>Actinobacteria</i>
Subclass	<i>Actinobacteridae</i>
Order	<i>Actinomycetales</i>
Suborder	<i>Corynebacterineae</i>

Family	<i>Corynebacteriaceae</i>
Genus	<i>Corynebacterium</i>
Family	<i>Nocardiaceae</i>
Genus	<i>Rhodococcus</i>

MORPHOLOGY

- They are Gram-positive slender rods with a tendency to clubbing at one or both ends; they are non-sporing, non-motile, non-capsulated and non-acid fast.
- They have granules composed of (high energy phosphate stores) – polymetaphosphate.
- The granules are more strongly Gram positive than the rest of the bacterial cell.
- Stained with Loeffler's methylene blue, the granules take up a reddish purple color and hence they are called metachromatic granules. They are called as volutin or Babes Ernst Granules.
- They are often situated at the poles of the bacilli and are called polar bodies.
- Special stains, such as Albert's, Neisser's and Ponder's have been devised for demonstrating the granules clearly.
- Stained smears from animal tissues often reveal groups of cells in parallel (Palisades) or cells at sharp angles to each other (Chinese letter or Cuneiform arrangement).
- This is due to the incomplete separation of the daughter cells after binary fission.
- *Rhodococcus equi* can appear as a Gram-positive coccus or a rod or club shaped form arranged in clusters. It is capsulated and sometimes weakly acid fast.

CUTURAL CHARACTERISTICS

- Growth is scanty on ordinary media. Enrichment with blood, serum or egg is necessary for good growth. The optimum temperature for growth is 37°C and optimum pH is 7.2. It is an aerobe and facultative anaerobe. Sheep or ox blood agar is used routinely along with MacConkey agar to detect any Gram-negative contaminants that may be present.
- On blood agar, *Corynebacterium ovis* colonies are small, white, dry and non-haemolytic at 24hr incubation.
- A narrow zone of haemolysis occurs at 72 hrs incubation. After several days incubation the colonies can reach 3mm in d.m and appear dry, crumbly and cream in color.
- *Corynebacterium bovis* colonies are small, white, dry and non-haemolytic.
- As it is a lipophilic corynebacterium, they grow very well on media enriched with 0.5 –1% tween 80.
- *Rhodococcus equi* colonies are small, smooth, shiny and non-haemolytic after 24 hrs incubation. But on 4-day culture, the colonies become larger, mucoid and salmon-pink in color.
- The salmon-pink pigmentation is not easily seen against a red background.
- So, the mucoid colonies and salmon-pink pigmentation can easily be demonstrated on nutrient agar (4-day culture).

- Nutrient agar enriched with yeast extract and glucose is useful for enhancing the salmon pink pigmentation.
- The renale groups are non-haemolytic. On nutrient agar, after 48 hrs incubation, *Corynebacterium renale* produces dull yellow colonies.
- The *Corynebacterium pilosum* produces distinct yellow and *Corynebacterium cystitidis* exhibit white colonies.
- On milk agar, *Corynebacterium renale* showing casein digestion. While *Corynebacterium pilosum* and *Corynebacterium cystitidis* do not give this reaction.
- On CAMP tests, the *Corynebacterium ovis*, *Rhodococcus equi* and *Corynebacterium renale* interacting with beta haemolytic strains of *Staphylococcus aureus* gives the following results.

	Staphylococcal beta haemolysis
<i>Corynebacterium ovis</i>	Inhibition
<i>Rhodococcus equi</i>	Enhancement
<i>Corynebacterium renale</i>	Enhancement

BIOCHEMICAL CHARACTERS, RESISTANCE AND ANTIGENS AND TOXINS

Biochemical characters

- They are catalase positive, oxidase negative. Except *Corynebacterium bovis* others are urease positive.
- The renale group is very strong urease positive (less than one hour).
- All diphtheroids ferments sugar except *Rhodococcus equi*. *Corynebacterium bovis* and *Corynebacterium renale* ferments both glucose and maltose.
- Two biotypes of *Corynebacterium ovis* are recognized.
- The ovine/caprine strains lack nitrate-reducing capacity, while the equine/bovine strains usually reduce nitrate.

Resistance

- Diphtheroids are readily destroyed by heat, 60°C for one hour.
- They are highly susceptible to disinfectants.
- It is more resistant to the action of light, dessication and freezing.
- *Rhodococcus equi* is resistant to 2.5% oxalic acid for one hour.

Antigens and toxins

- The diphtheroid antigen and toxins are not well documented.
- *Corynebacterium ovis* produces a filterable toxin similar to that produced by *Corynebacterium diphtheriae*.
- It is a haemolytic toxin, which has phospholipase activity to the cell wall lipids.
- In *Corynebacterium renale*, the pili is antigenic, Renalin- a *Corynebacterium renale* extracellular protein may play a role in lysis of cell membranes.

- *Rhodococcus equi* produces diffusible *Rhodococcus equi* factors (Phospholipase C and Cholesterol oxidase) and these as well as the capsule and cell wall constituents probably play a major role.

PATHOGENESIS

- The diphtheroids infections are characterized by the development of suppurative lesions and clinical manifestations do not develop in the absence of predisposing factors.

Corynebacterium ovis

- The prevalence of caseous lymphadenitis may be as high as 50% in adult sheep.
- Some clinically normal sheep may carry the organism in the digestive tract, excrete in the faeces and contaminate the environment.
- When bacteria enter the host via skin wounds (or tick bite), multiply and are phagocytosed.
- Phagosome-lysosome fusion takes place. But *Corynebacterium ovis* multiplies in the phagolysosome and phagocytic cells die.
- Permeability of local blood vessels increases, encouraging the spread of infection from the initial site to other locations, often-regional lymphnodes.
- Produces toxins – Phospholipase-D. Abscesses may develop at either primary or secondary sites, eventually rupturing and discharging a thick, caseous pus containing large numbers of viable bacteria. In some instances, lesions become metastatic and, as they increase in number, the thin ewe syndrome develops, resulting in progressive debilitation and death.

Corynebacterium renale

- *Corynebacterium renale* is a normal flora in the lower urogenital tract. This group possess fimbriae which allow attachment to the urogenital mucosa.
- The major predisposing factors that put a cattle at risk are the shortness of the female urethra and the effects of pregnancy and parturition, thus, disease occurs most frequently in mature cows.
- The vulva may be an important portal entry for *Corynebacterium renale* into the urinary tract.
- Bacteria grow readily in urine and ascend (through vesiculo urethral reflex) to the kidney.
- *Corynebacterium renale* has high urease activity. The urease is nephrotoxic and produces pyelonephritis.

Rhodococcus equi

- *Rhodococcus equi* may be a commensal in the intestine of horses and it is largely a soil organism.
- The soil enriched with equine faeces and summer temperatures are favours the rapid multiplication of this bacterium.
- The disease is usually seen in 2-4 month old foals, possibly due to the decline in maternal antibody at about 6 weeks of age.
- The main route of infection is by inhalation. *Rhodococcus equi* is a facultative intracellular pathogen.
- Its ability to survive, persist in, and eventually to destroy alveolar macrophages is the basis of its pathogenicity.
- It causes granulomatous inflammation and abscesses in the lung tissue.

- Heavily infected sputum may be swallowed by the affected foal leading to ulcerative colitis and mesentric lymphadenitis.
- Corynebacteria are pyogenic bacteria causing a variety of suppurative conditions in animals.
- The main diseases, hosts and natural habitats of the Corynebacteria are

Species	Main host (s)	Diseases	Natural habitat
<i>C. pseudotuberculosis</i> (<i>Corynebacterium ovis</i> or Preisz Nocard Bacillus)	Sheep and Goats (Non-nitrate reducing biotype)	Caseous lymphadenitis	Skin, mucous membrane and G.I. tract
	Horses and Cattle (Nitrate reducing biotype)	Ulcerative lymphangitis	
<i>Corynebacterium renale</i>	Cattle	Pyelonephritis and cystitis	Prepuce and semen of asymptomatic bulls, vaginal mucous membrane of heathy cows
	Pigs	Kidney abscess	
	Male sheep	Balanoposthitis (Pizzle rot)	
<i>Corynebacterium cystitidis</i>	Cattle	Severe cystitis, rarely Pyelonephritis	Male genital tract
<i>Corynebacterium pilosum</i>	Cattle	Pyelonephritis	Male genital tract and urine
<i>Corynebacterium bovis</i>	Cattle	Subclinical mastitis	Udder and teat canal of cows
<i>Rhodococcus equi</i> (<i>Corynebacterium equi</i>)	Foals (2-4 months)	Suppurative bronchonephritis	Soil and Faeces of foals and other herbivores
	Pigs	Cervical	Soil

		lymphadenitis	
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PATHOGENICITY

Symptoms

Corynebacterium ovis

- Caseous lymphadenitis in sheep is characterized by skin wounds, enlarged lymph glands and abscesses distributed throughout the body.
- But the disease can be a mild infection and it is unnoticed until postmortem is done.
- Ulcerative lymphangitis in horses are similar to glanders.
- Initially there is a pain and swelling of the hind limbs and enlargement of lymphatic vessels.
- Ulcers develop, discharging purulent material. In severe cases it spreads to abdomen, forelegs and neck, leading to death.

Corynebacterium renale

- In pyelonephritis, characteristically frequent passage of turbid or blood stained urine by animals, which are pregnant or recently calved.
- Restlessness and kicking at the abdomen may indicate renal pain. The urine contains red blood cells, pus cells and albumin.
- Ulcerative balanoposthitis (Pizzle rot), particularly common in Merino sheep and Angora goats, is characterized by ulceration around the preputial orifice, with a brownish crust developing over the lesion

Rhodococcus equi

- In suppurative bronchopneumonia, the foals develop cough, fever and increased respiratory rate.
- In severe cases purulent discharge from nose just before death.

Lesions

Corynebacterium ovis

- In caseous lymphadenitis the superficial lymphnode contains a mass of greenish yellow caseation in concentric layers, which have an onion ring appearance in, cross section (hence, named it as *Corynebacterium pseudotuberculosis*). In advanced cases, similar lesions are seen in lungs, kidney, liver and spleen.

Corynebacterium renale

- The kidneys are enlarged, necrosis and suppuration in the medulla. Wedge shaped suppurative foci in the cortex.
- The kidney pelvis contains blood and pus
- The bladder contains blood and pus with petechial haemorrhages and ulceration of the tract.

Rhodococcus equi

- Characterized by pyogranulomatous lesions with abscesses in the lung, associated lymphnodes and pus in the bronchi.

DIAGNOSIS, CONTROL AND PREVENTION

Diagnosis

- Specimens
 - Pus or exudates are collected from suppurative conditions and mid stream urine for isolation of the *Corynebacterium renale*.
 - A tracheal wash technique with infusion of saline, can be used for the recovery of *Rhodococcus equi* from affected foals.
- Diagnosis is mainly based on history, symptoms and lesions
- Isolation and identification of the organism
 - Based on microscopical appearance, colonial morphology on blood agar, CAMP tests and Biochemical tests.

Control and prevention

- Diphtheroids are susceptible to penicillin, tetracycline, erythromycin, lincomycin, neomycin and gentamicin.

