

HEMOPHILUS AND ACTINOBACILLUS

Learning objectives

To know in detail about,

- Morphology, cultural and biochemical characters of Haemophilus species
- Collection and transport of specimens for diagnosis of Haemophilus infection
- Wooden tongue and morse biochemical characters of A. ligneresi
- Antigens, toxins and pathogenesis of A.ligneresi in cattle
- Difference between Actinobacillosis and Actinomycosis
- General approaches used to isolate and identify A.ligneresi

HISTORY AND HABIT

Haemophilus

- Species of the genus *Haemophilus* are small, non-motile, non-sporing, Gram-negative rods and filaments.
- They are characterized by their requirement of one or both of two accessory growth factors (X and V) present in blood. (Haemophilus, means blood loving) .

History

- In 1883, Koch had described a bacillus causing conjunctivitis in Egypt , eventually named *H.aegypticus*.
- In 1892, Pfeiffer isolated *H.influenza* from sputum of patients. *H.parasuis* was first described by Glasser in 1910. *H.paragallinarum* was identified by DeBlicke (1932).

Habitat

- *Haemophilus* species are commensals or parasites of the mucous membranes of human and animals, most commonly of the upper respiratory and lower genital tracts.
- *H.somnus* is part of the bacterial flora of the male and female bovine genital tract. *H.paragallinarum* is more closely associated with the upper respiratory tract and sinuses of sick or recovered birds.

MORPHOLOGY AND CULTURAL CHARACTERISTICS

Morphology

- Gram negative, small, medium sized coccobacilli or rods, often markedly pleomorphic, sometimes filamentous, non-motile, non-spore forming and non-acid fast.
- Capsules can be produced. *H.influenzae*, *H.parasuis* and *H.paragallinarum* require one or both of two accessory growth factors X and V.

Cultural characteristics

- They are aerobic and facultatively anaerobic. The opt.temp.is 37°C.
- They are nutritionally fastidious, will not grow on nutrient agar and MacConkey agar.
- The X and V factors must be supplied for all the *Haemophilus* species except *H.somnus*.
- The X factor is a heat labile iron-porphyrin-haematin or other haemins.
- It is necessary for the synthesis of catalase and other enzymes involved in aerobic respiration.

- The V factor is a heat labile factor, present in RBC's and in many other animal and plant cells.
- It is synthesized by fungi and some bacteria (eg: *Staphylococcus aureus*).
- The V factor can be supplied as co-enzyme I, NAD or NADP⁺.
- It appears to act as a hydrogen acceptor in the metabolism of the cell.
- X and V factors requirement of *Haemophilus* species

Species	X factor	V factor
<i>H. influenzae</i>	+	+
<i>H. aegyptius</i>	+	+
<i>H. parasuis</i>	-	+
<i>H. paragallinarum</i>	-	+
<i>H. parainfluenzae</i>	-	+
<i>H. somnus</i>	-	-

- In addition to X and V factors, the growth of the many of the *Haemophilus* species is enhanced by 10% CO₂.
- *Haemophilus* grows on blood agar, but growth is scanty, as the V factor is present mainly intracellularly in red cells.
- The chocolate agar is the most suitable medium for isolation of *Haemophilus*.
- In chocolate agar, the V factor is released from the red cell, and the heat stable X factor is still present.
- On chocolate agar, *H. paragallinarum* produces typical dewdrop like colonies and *H. somnus* shows characteristic yellow tinge colonies.

Sattelitism

- When *Staphylococcus aureus* is streaked across a plate of blood agar on which a specimen containing *Haemophilus* has been inoculated, after 18-24hrs incubation at 37°C under 5-10% CO₂, the colonies of the *Haemophilus* will be large, well developed alongside the streak of *Staphylococcus*, and smaller farther away.
- This phenomenon is called satellitism and demonstrate the dependence of *Haemophilus* on V factor, which is available in high concentration near the staphylococcal growth and only in smaller quantities away from it. [Click here for visual](#)
- Media supplemented with yeast extract, Levinthals medium (clear transparent media may be prepared by boiling and filtering a mixture of blood and nutrient broth) or Filde's agar (by adding a peptic digest of blood to nutrient agar) are also suitable for the primary isolation of *Haemophilus*.

MORPHOLOGY

Biochemical properties

- Biochemical reactions are not helpful in identification.
- The fermentation reactions are irregular. Nitrates are reduced to nitrites.
- *H.parasuis* and *H.paragallinarum* are oxidase negative but *H.somnus* are oxidase positive and catalase negative.

Resistance

- *Haemophilus* species are fragile. They are readily destroyed by heating (55°C for 30mts), refrigeration (0°C to 4°C), drying and disinfectants.
- In cultures the cells die within 2 to 3 days due to autolysis. For long-term preservation the cultures may be lyophilized.

Antigens

- Non-capsulated strains are antigenically heterogenous. Some somatic antigens have been identified.
- The capsular antigens are polysaccharide in nature and they resemble pneumococcal capsular antigens.
- It is immunochemically similar to the K antigens of *E.coli*.
- Based on immunodiffusion test, using heat stable antigens 15 serovars of *H.parasuis*, 9 serotypes of *H.paragallinarum* and 15 serotypes of *H.somnus* have been recognized.

PATHOGENESIS

- Young or previously unexposed animals are highly susceptible. Stress factors contribute to the development of disease.
- The capsule and cytotoxic factor are thought to be virulence factors and endotoxin may play a role in the disease process.
- During stress, the bacteria may invade the mucosal barrier. The invasive mechanism is not known.
- In respiratory tract initially nasopharyngitis. If this infection is not checked, it may lead to sinusitis, otitis media and pneumonia.
- If a bacteraemia develops, joint infections and meningitis may follow.
- Diseases caused by the *Haemophilus* species

Species	Host	Disease
<i>H. somnus</i>	Cattle Sheep	Thrombo embolic meningo encephalitis (TEME) (Sleepers) Pneumonia and pleurisy Arthritis Endometritis and abortion Epididymitis and orchitis in rams
<i>H.parasuis</i>	Pigs	Polyserositis and meningitis in young pigs (Glasser's disease)
<i>H.paragallinarum</i>	Poultry	Infectious coryza
<i>H.influenzae</i>	Human	Variety of diseases ranging from respiratory infections to meningitis

PATHOGENICITY

Symptoms

- *Haemophilus* infection of cattle is manifested by 4 principal syndromes. In sub clinical form animal won't exhibit any clinical signs.
- In acute form if there is respiratory involvement with pneumonia and septicaemia, the symptoms are fever, dry cough and dyspnoea.
- If the bacteria localized in the CNS with TEME, (i.e. sleepers) the signs are lameness and CNS disturbance with high mortality.
- In chronic form, there will be joint infections accompanied by arthritis and reproductive failure.
- In *H.parasuis* infection mostly very young, weaned pigs are highly susceptible.
- The infections occur concurrently with virus infections. In chronic form, the affected pigs exhibit lameness, pyrexia, depression and anorectic.
- In poultry, *H.paragallinarum* causes acute rhinitis, sinusitis with odema and conjunctivitis.
- The disease is characterized by nasal discharge, sneezing and odema of the face. Reduction in growth and egg production also occur.

Lesions

- In Glasser's disease during acute death conditions, the PM reveals large deposition of fibrin in joints and on any or all of the serosal surfaces (poly serositis) of the body.
- In addition there will be fibrinous pericarditis, pleuritis and peritonitis. Catarrhal inflammation of the infra orbital sinus is characteristic of infectious coryza.

DIAGNOSIS AND TREATMENT

Diagnosis

Specimens

- In *H.somnus* infections, the organism can be demonstrated in brain lesions, they can be recovered from semen samples and preputial washings of healthy bulls.
- *Haemophilus* species are highly delicate and do not survive long when removed from the host.
- Clinical material is best frozen (dry ice preferred) and delivered to the laboratory within 24 hrs.
- Refrigeration and transport media may not assure viability.
- A presumptive identification of *Haemophilus* can be made based on the host species, clinical signs and lesions, colony characters, X and V factor requirements, oxidase and catalase reactions and whether or not Co₂ enhances growth.
- Serological tests including agglutination, AGPT, ELISA are used to detect *H.paragallinarum* and *H.somnus* infection.

Treatment

- *Haemophilus* species are susceptible to gentamicin, tetracycline, sulfonamides, chloramphenicol, neomycin and erythromycin.
- Vaccines prepared from *H.paragallinarum* grown in egg yolk, if inoculated intramuscularly reduces the incidence of infectious coryza.

Actinobacillus

HISTORY AND HABITAT

The *Actinobacillus* species are Gram negative, very small, non-motile, non-spore forming and non acid-fast bacilli.

- Small coccal elements are often lying at the pole of a larger form, giving a characteristic ‘Morse-code’ appearance

History

- *Actinobacillus lignieresii* was isolated by Ligniers and Spitz (1902).
- The generic name actinobacillus was first used by Brumpt (1910).

Habitat

- *Actinobacillus* species is worldwide in distribution. They are commensals of the respiratory, alimentary or genital mucosa.
- *A. lignieresii* is a commensal in the oral and rumen of cattle and sheep.
- *A. equuli* occurs as a commensal in the equine intestinal tract and in the mouth.
- *A. suis* is present in the tonsil and upper respiratory tract of healthy pigs.
- Actinobacilli cannot survive in the environment, carrier animals play a major role in transmission

MORPHOLOGY AND CULTURAL CHARACTERISTICS

Morphology

- Gram –ve, small, rod shaped organism. They are non-motile, non-spore forming and non-acid fast.
- They are non capsulated (except *A. pleuropneumoniae*) but extracellular slime is present in three major species (*A. lignieresii*, *A. equuli* and *A. suis*)
- In media containing fermentable carbohydrates, the occurrence of rather long, almost filamentous forms is seen.
- Small granules are found scattered along the bacilli, often lying at the pole of a bacillary or filamentous form, giving a characteristic ‘Morse code’ form.
- In lesion in the animal body small grayish white granules are present.
- If these granules are crushed on a slide and stained, club colonies are seen consisting of club-like processes of calcium phosphate, with Gram-negative rods of *A. lignieresii* in the center.
- Both bacilli and club forms are Gram negative.
- They can be distinguished with ZN stain in which the club appears red and the bacilli blue.

Cultural characteristics

- They are aerobic, or micro aerophilic, and facultative anaerobe.
- The optimum temperature is 37°C on blood agar, *A. lignieresii* develops small, glistening, non-haemolytic colonies within 24 hrs.
- They are usually slightly sticky (cohesive properties) on primary isolation, but lose this character on subculture.
- The organism grows well on MacConkey agar and it is a late lactose fermenter. The colonies are first pale but become pinkish.
- *A. equuli* strains are haemolytic. The colonies are sticky with this feature remaining on subculture. It is lactose fermenter on MacConkey agar.
- *A. suis* are also haemolytic. Colonies are stickier. It grows well on MacConkey agar.
- In CAMP test *A. pleuropneumoniae* enhances beta haemolysis of *Staphylococcus aureus*. (i.e. Positive).

BIOCHEMICAL PROPERTIES, RESISTANCE, PATHOGENS AND TOXINS

Biochemical properties

- They are catalase positive (except *A.pleuropneumoniae*). Oxidase and urease positive.
- Ferment several sugars, produce acid and gas. IMViC negative, H₂S positive.

Resistance

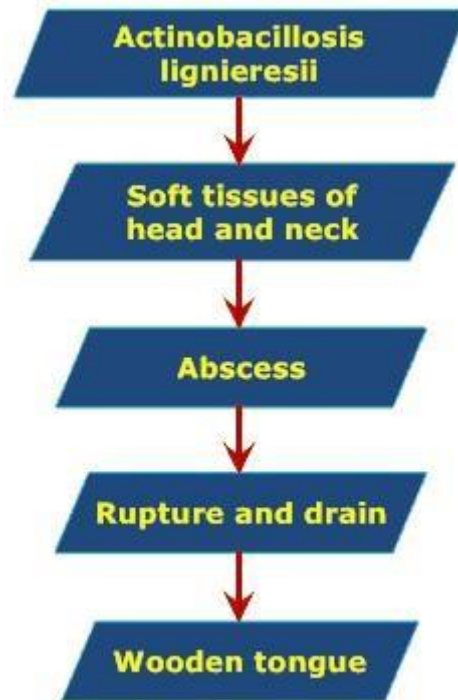
- They are rapidly killed by heating at 62°C for 10 mins and by drying.
- Culture lose their viability rapidly and should be subcultured every 5-7 days.

Antigens and Toxins

- In *A.lignieresii*, heat stable somatic and heat labile surface antigens are described.
- Six antigenic types (1-6) and two subtypes (1a and 1c) have been demonstrated.
- In *A.equuli*, as in *A.lignieresii*, both heat labile and heat stable antigens can be demonstrated.
- In *A.suis*, the antigens have not been studied in any detail.
- In *A.pleuropneumoniae*, 12 serotypes and 2 biotypes have been described,.
- Biotype 1 requires NAD for its growth while biotype 2 is NAD independent.
- In *A.pleuropneumoniae*, capsule, LPS, outer membrane proteins and toxins (haemolytic and cytotoxic) play a major role in pathogenicity.
- Exotoxin is not formed in *A.lignieresii*.

PATHOGENESIS

- *A.lignieresii* , is a commensal of oral cavity and the intestinal tract.
- It can survive for upto five days in hay or straw.
- The actual mechanisms of actinobacilli are unknown.



- Bovine actinobacillosis is spread by the lymphatics. The specific disease is wooden (timber) tongue, but granulomatous lesions can also involve the head, neck and limbs.
- Less commonly, the lungs and other internal organs are also affected.
- *A.suis* : Infection occurs via the aerosal route by close contact or through skin.
- Once the organism has entered the blood stream it spreads rapidly throughout the body.
- Several factors, LPS, cytotoxin etc are responsible for gross lesions and they are usually seen in the lungs, kidney, heart, spleen, intestines and skin.
- The lungs may also be filled with serous or serofibrinous exudates with pleuropneumonia.
- *A.pleuropneumoniae* : The organism enters the lungs, multiplies rapidly.
- During growth the organism releases a large quantity of OMP, LPS, cytokines and other factors which causes destruction of neutrophils that is likely to be responsible for the massive and tissue damage.
- Diseases caused by the pathogenic actinobacilli

Species	Host	Disease
<i>A. lignieresi</i>	Cattle	Bovine actinobacillosis (Wooden (timber) tongue) Polygranulomatous lesions around head, neck and limb
<i>A.equuli</i>	Neonatal foals Mares	Sleepy foal disease or Joint ill Abortion./septicaemia
<i>A.pleuropneumoniae</i> (<i>Haemophilus pleuropneumoniae</i>)	Pigs	Arthritis, nephritis and endocarditis

<i>A.suis</i>	Pigs under 3 months of age Older pigs	Acute fatal septicaemia Arthritis, pneumonia and pericarditis
<i>A.seminis</i>	Rams	Epididymitis

PATHOGENICITY

Symptoms

- **Cattle**
 - Chronic pyogranulomatous lesions occur on tongue and other soft tissues.
 - Enlargement and protrusion of the tongue that interferes feeding. The pus contains soft grayish white granules.
- **Pigs**
 - *A.suis* can infect pigs of all ages. But infection is most serious in very young animals.
 - In neonates and suckling pigs, *A.suis* can cause an acute and rapidly fatal septicaemia.
 - Death occurs within 15hrs. Affected animal may show signs of cyanosis, petechial haemorrhages, fever, respiratory distress, neurologic disturbances and arthritis.
 - In older animals, the disease is less severe and may be characterized by fever, anorexia and persistent cough. The mortality is also much lower.
 - *A.pleuropneumoniae* can cause acute and rapidly fatal pleuropneumonia.
 - The acute form is characterized by extensive haemorrhage and fibrin deposit in the lungs.
 - Affected animals show signs of severe respiratory distress, cyanosis, fever and vomiting.
 - In chronically infected animals the organism may be sequestered in the lungs in the necrotic lesions, tonsils and URT and may responsible for spread of infection.
- **Sheep**
 - *Actinobacillus seminis* is a common cause of epididymitis in young rams.
 - The organism is found in the prepuce. The infection occurs probably following an ascending opportunistic infection.
 - Abscesses and purulent discharge through fistulae on the scrotal skin are most commonly seen.
- **Horse**
 - Sleepy foal disease is an acute, potentially fatal septicaemia of newborn foals caused by *Actinobacillus equuli*.
 - Occasionally it causes abortion and peritonitis in adult horses.
 - The organism is found in the reproductive and intestinal tracts of mares.
 - Foals can be infected *in utero* and after birth via the umbilicus.
 - Affected foals are febrile and recumbent. Death usually occurs in 1 to 2 days.

DIAGNOSIS, TREATMENT, PREVENTION AND CONTROL

Diagnosis

- **Specimens to be collected**
 - Pus, biopsy material and tissues in case of wooden tongue.
 - Tracheal washings or affected portions of lung in pleuropneumonia cases.
 - Based on history and symptoms

- Isolation and identification of organism
- Club colonies in tissue sections, growth pattern on blood and MacConkey agar and biochemical test are highly useful.

Treatment

- In wooden tongue, sodium iodide parentally or potassium orally is effective.
- Sulphonamides or combination of penicillin and streptomycin are usually effective.
- Oral isoniazid for 30 days has been used in animals with refractory lesions.
- Ampicillin, carbenicillin, potentiated sulphonamides and tetracyclines are effective against *A.suis* infection.

Control and prevention

- Polyvalent bacterins may induce protective immunity but fail to prevent transmission or the development of a carrier state.