Genus Actinobacillus

'Wooden Tongue/ Timber tongue'

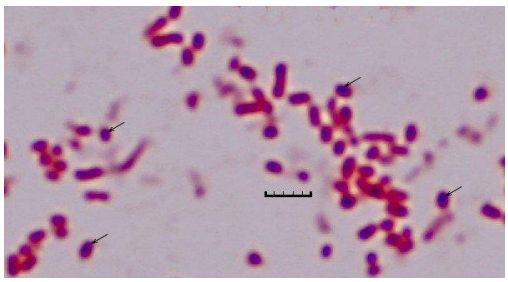
Actinobacillus lignieresii was identified for the first time by **Lignieres and Spitz** in 1902, who listed it as the causative agent in a case of bovine granulomatosis in Argentina. The authors referred to this organism as "l'actinobacille" based on its morphology and its association with the pathological lesion.

In 1910, Brumpt proposed the name of *Actinobacillus lignieresii*.

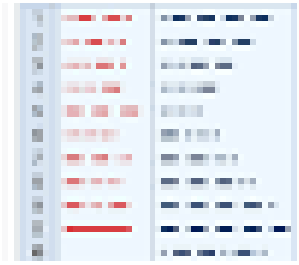
Actinobacillus lignieresii is aerobic, non-motile, nonspore forming, gram-negative coccobacilli that is widespread in soil and manure and is found as **normal flora of the respiratory and upper gastrointestinal tract of ruminants**.
In sheep and cattle, ***A. lignieresii* causes sporadic, noncontagious**, and potentially chronic disease characterized by **diffuse abscess and granuloma formation in tissues** of the head and occasionally other body organs. This disease has not been documented in goats.

Habitat
Actinobacillus species is worldwide in distribution.
A.lignieresii is a commensal of the oral cavity and rumen of cattle and sheep, causes opportunistic infections.
A.equuli occurs as a commensal in the equine intestinal tract and in the mouth.
Actinobacilli *suis* is a commensal of tonsil and upper respiratory tract of healthy pigs.
Actinobacilli cannot survive in the environment, carrier animals play a major role in transmission.

Morphology
Gram Negative, small, rod shaped organism.
Non-motile, Non-sporing and Non-acid fast.
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



Gram staining of *Actinobacillus lignieresii*, strain B10375/10. The arrows indicate some of the granules (www.vetbact.org)



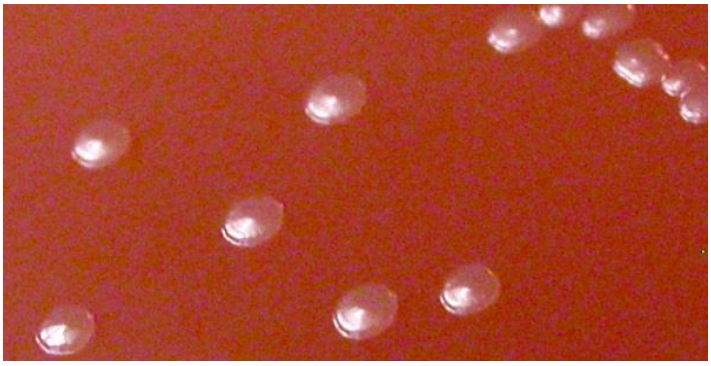
'Morse code'
A system of communication developed by Samuel Morse in which the letters of the alphabet are coded as a combination of dots and dashes so that messages can either be sent using light, sound or wireless

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 *Actinobacillus 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.

Splendore–Hoeppli phenomenon : An intensely eosinophilic, club-shaped material that radiated around the bacteria.

Cultural characteristics

Aerobic, or micro aerophilic, and facultative anaerobe.
The optimum temperature is 37^{O}C on blood agar/Chocolate agar.



Colonies of *Actinobacillus lignieresii*, cultured aerobically and in the presence of 5% CO_2 during 24 h on chocolate agar at 37°C (www.vetbact.org)

Actinobacillus lignieresii

Blood Agar/ Chocolate agar: Small, glistening, non-haemolytic colonies within 24 hrs. Usually slightly sticky (cohesive properties) on primary isolation, but lose this character on subculture.

MacConkey agar: Late lactose fermenter. Colonies are initially pale but become pinkish.

A. equuli* & *A.suis

Blood Agar: Haemolytic, colonies are sticky , looses stickiness on subculture

MacConkey agar: Lactose fermenter

In CAMP test *A.pleuropneumoniae* enhances beta haemolysis of *Staphylococcus aureus*. (i.e. Positive).

Biochemical Properties, Resistance and Toxins

Biochemical properties

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

Resistance

Killed by heating at 60^{0}C for 10 minutes
Culture lose its 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. Exotoxin is not formed in *A.lignieresii*.

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 (hemolytic and cytotoxic) play a major role in pathogenicity.

Pathogenesis

Bovine Actinobacillosis 'Wooden Tongue' or **Timber tongue**

Wooden tongue is a well-defined disease of the **soft tissues of the mouth region** in adult cattle. It is caused by *Actinobacillus lignieresii*, part of the normal bacterial flora of oral cavity and the intestinal tract. The bacteria usually invade the skin through a wound or minor trauma caused by sticks or straw or barley awns.

Bovine actinobacillosis is spread by the lymphatics.

Actinobacillus has a tendency to invade soft tissues, such as lungs, abdominal viscera and regional lymph nodes. (Actinomycetes, attack skeletal tissues)

Why name wooden tongue?

The tongue often protrudes between the lips. Nodules and ulcers may be visible on the sides of the tongue. **As the disease becomes chronic, fibrous tissue is deposited in the tongue, which becomes hard, shrunken and immobile**, hence the name 'wooden tongue'

Clinical Signs

Skin lesions are common, with abscesses of the tongue (cattle) and lip lesions (sheep). **Soft-tissue or lymph node** swelling accompanied by draining tracts are observed also in the head and neck regions, as well as other areas. The swollen **tongue may protrude from the mouth** causing difficulty prehending food, anorexia, and excessive salivation.

Epizootiology and Transmission

The organism penetrates wounds of the skin, mouth, nose, gastrointestinal tract, testicles, and mammary gland causing chronic inflammation and abscess formation. Rough feed material and foreign bodies may play a role in causing abrasions.

Necropsy Findings

Purulent discharges of white–green exudate containing **small white–gray granules** drain from the tracts that often extend from the area of colonization to the skin surface.

Diagnosis:

1. **Isolation and identification**
   Material to be collected: Purulent discharges of white–green exudate

   Blood Agar/ Chocolate agar: Small, glistening, non-haemolytic colonies within 24 hrs. Usually slightly sticky (cohesive properties) on primary isolation, but lose this character on subculture.

   Morphology
   Gram Negative, small, rod shaped organism, Non-motile, Non-sporing and Non-acid fast. Non capsulated (except *A.pleuropneumoniae*) but extracellular slime is present in three major species (*A.lignieresii, A.equuli* and *A.suis*)
   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.

2. **Molecular techniques such as PCR and ELISA have also been developed that detect the presence of the organism in tissue samples**.
   PCR: Primers for amplification of a 180 base pair (bp) fragment of *Actinobacillus lignieresii* 16S ribosomal RNA can be used.

Prevention and Control

Avoid poor quality, coarse feed. Isolation or disposal of animals with disease is recommended. No vaccine is available.

Treatment

IV administration of sodium iodide is the treatment of choice; oral potassium iodide also may be used. Clinical response is generally seen within 48 h of starting IV treatment. Systemic antibiotics such as ceftiofur, ampicillin, or florfenicol may be effective. Treatment can include surgical debridement and flushing with iodine.

Actinobacillus equuli* subsp *equuli

Neonatal septicemia (sleepy foal disease)

In foals, predisposing factors to the development of disease are failure of passive transfer of maternal antibodies, unsanitary conditions in the foaling area and concurrent immunosuppressive conditions.
Infection in foals is acquired in utero, during parturition, or shortly after birth as an umbilical infection or less commonly from gastrointestinal (ingestion) or respiratory (inhalation) infections.
Disease presentation is often peracute with death at birth or within the 1st few days due to fulminant septicemia.

Foals afflicted by the peracute disease are born weak, refuse to suck, or do so sluggishly, and are feverish, dull, and lethargic. They stand with heads hanging and ears drooping before they become recumbent and die. They seldom live for more than 24 hours.

In adult horses, actinobacillosis is rare and usually acts as a secondary bacterial infection complicating some other primary condition, usually some other concurrent pathogenic bacteria or viral infection, but under the right circumstances, Actinobacillus spp can be primary pathogens.
Infection in adult horses is generally more localized and includes cutaneous abscesses, guttural pouch infections, endometritis, pericarditis, endocarditis, peritonitis, encephalitis, arthritis, orchitis and abortion. The syndromes of acute peritonitis, encephalitis and fatal pulmonary hemorrhage with pneumonia due to endothelial damage caused by bacterial toxins, have been more recently described. In addition, it has been documented that the same mare may abort in successive pregnancies. Rare cases of septicemia associated with both *Actinobacillus equuli* subsp *equuli* and *Actinobacillus equuli* subsp *hemolyticus*, have been documented

Control:
Neonatal septicemia (sleepy foal disease) is best prevented through employing a combination of strict environmental hygiene, practicing of foaling camp rotation, ensuring intake of colostrum as soon after birth as possible, umbilical disinfection and immediate removal of the expelled placenta and soiled bedding post foaling. Remember, the mare's reproductive tract and fetal membranes are the primary source of bacteria for the foal.

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.

Reference:

Caffarena, R. D., Rabaza, A., Casaux, L., Rioseco, M. M., Schild, C. O., Monesiglio, C., ... & Riet-Correa, F. (2018). Natural lymphatic ("atypical") actinobacillosis in cattle caused by Actinobacillus lignieresii. *Journal of Veterinary Diagnostic Investigation*, *30*(2), 218-225.

Phillips, J. E. (1992). The genus Actinobacillus. In *The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications* (pp. 3342-3351). New York, NY: Springer New York.

Scheid, H. V., Estima-Silva, P., Monteiro, F. L., Marques, L. S., Coelho, A. C. B., Lima, M. D., ... & Schild, A. L. (2020). Actinobacillosis outbreak in cattle with clinical manifestation of hippopotamus-like face. *Pesquisa Veterinária Brasileira*, *40*, 355-359.

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