

Genus Actinomyces

Genus name: Greek *actino* ray, *mykes* fungus (referring to the **radiating appearance of microcolonies**)
Species epithet: of the ox (*Actinomyces bovis*).

The Family Actinomycetaceae: The Genera Actinomyces, Actinobaculum, Arcanobacterium, Varibaculum, and Mobiluncus

Genus Actinomyces *Actinomyces bovis* (Ray fungus)
 Actinomyces viscosus
 Actinomyces israelii
 Actinobaculum suis (*Actinomyces suis*)

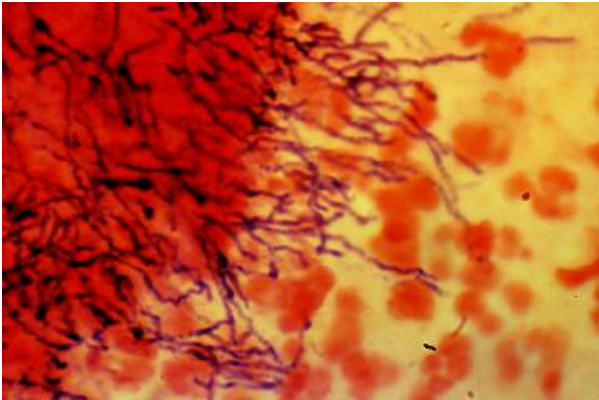
Actinomycete	Host	Disease
<i>Actinomyces bovis</i> (Syn: Ray fungus)	Cattle	Bovine actinomycosis (Lumpy jaw)
	Horses	Poll evil/Fistulous withers (occur as a mixed infection with <i>Brucella</i> species)
<i>Actinomyces viscosus</i>	Dogs	Canine actinomycosis Localised cutaneous granulomatous abscess and/or Pyothorax and granulomas in the thoracic cavity
<i>Actinomyces israelii</i>	Human	Human actinomycosis
<i>Actinobaculum suis</i> (<i>Actinomyces suis</i>)	Pigs	Pyogranulomatous mastitis, ascending pyelonephritis, cystitis.

Actinomyces bovis

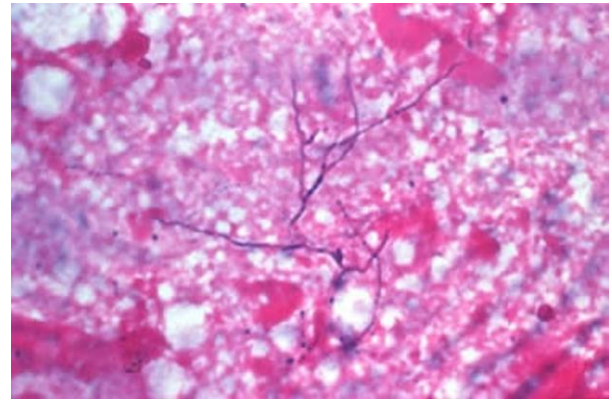
Actinomyces bovis are **anaerobic**, non-motile, nonspore forming, Gram-positive, non capsulated, nonacid fast pleomorphic rods to coccobacilli associated with 'Lumpy Jaw' in cattle. Usually long and filamentous although short V, Y, and T configuration is seen. Small bent rods (diameter: 0.2-1.0 µm), sometimes branching filaments/club shaped up to 50 µm.

Actinomyces bovis is an obligate inhabitant of the oral cavity of cattle.
Actinomyces affects skeletal muscles.

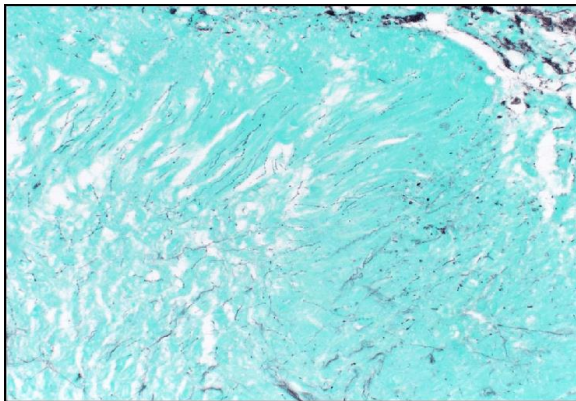
In lesions of actinomycosis, the pus contains small pale yellow granules known as '**sulfur granules**'. The sulphur granule is composed of bacterial filaments and mineralized calcium phosphate of host origin. When the granules are crushed and Gram stained, a mass of Gram-positive branching filaments 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**'.



Gram stain of aspirate from oral abscess (x1000). *Actinomyces* frequently grows in aggregates, termed "sulfur granules." Note the branching, gram-positive filaments on the edge of this granule. *Actinomyces* is a fastidious obligate anaerobe requiring anaerobic culture. Photo by Harriet Provine



Actinomyces bovis, Gram-stained smear. *Actinomyces bovis*, gram-stained smear, high power. Note the characteristic branching nature of these slender gram-positive rods. Photo by Dr. John Prescott.



Grocott-Gomori's methenamine silver staining of the sulfur granules showing the filamentous structures of the actinomyces (dark areas) Alshati *et al.*, 2019



Actinomyces bovis: Gram Positive rods-filamentous, short forms

Cultural Characteristics:

Difficult to grow *in vitro*. Anaerobic, prefers 10-15% CO₂ for its growth.

Actinomyces bovis

Blood Agar: *Actinomyces* require enriched media for growth, grow well on sheep blood agar. Do not grow aerobically. Colonies are white, rough, and nodular, 2–3 mm in diameter; smooth and rough forms are common. The colonies adhere tenaciously to solid media and are removed with difficulty. Non-haemolytic. Gram-stained smears from growth on solid or in fluid media show gram-positive, slightly branched filaments or short hyphae. On subculture the organisms may become diphtheroidal or coccobacillary. Growth usually occurs within 2–4 days but plates should be incubated for 7 days, and thioglycollate broth for 2 weeks.

Actinomyces bovis is capnophilic (i.e. required 10-15% CO₂ for its growth).

Actinomyces viscosus will grow aerobically but 10% CO₂ will enhance their growth.

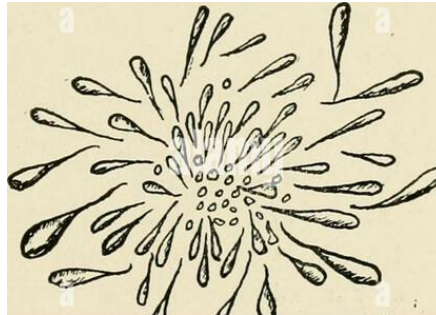
Thioglycollate medium: Grows well in thioglycollate medium, giving a characteristic diffuse growth in about 7-10 days. In broth cultures, coarse aggregates, with a granular deposit and a clear supernatant is seen.

“Sulphur granules” (Drusen)

The granules (Drusen) within the inflammatory lesions in three cases of infection by Actinomycetales. The material consisted of subcutaneous granulomas caused by *Actinomyces bovis* in a cow, epidural granulomas caused by *A. viscosus* in the spinal canal of a cat.

The granules of *A. bovis* were 2000 to 3000 micrometers in diameter. Their centers consisted of a slightly basophilic, gram- and grocott-negative material with branching, gram- and grocott-positive filaments. The periphery was slightly basophilic or eosinophilic, but gram- and grocott-positive. Its surface was made of clubs (15 x 3.5 micrometers); they were acidophilic, gram- and grocott-negative.

In the center of the granule there are numerous type a cells (cocci bacillary cells with a trilaminar cell wall of 12 nanometers) and rarely type b cells (filaments with bilaminar cell wall of 30 nanometers). The periphery was made of germinative centers of type a cells. The clubs were lytic type b cells, embedded in an amorphous material



Bestetti, G. (1978). Morphology of the “sulphur granules”(Drusen) in some actinomycotic infections.

Because many Actinomycetales are difficult to cultivate, the diagnosis may depend exclusively on the morphology of the organisms and possibly of the granules (Drusen) in tissue.

“Grains”, “granules” or “sulphur granules” (if such structures appear yellow macroscopically) are equivalent terms to the German “Drusen”. The granules, as in vivo microcolonies, are always present in *Actinomyces bovis* infections, whereas they are not regularly found in lesions caused by other Actinomycetales, such as *A. viscosus* and *Nocardia*.



Fig. Thoracic exudate in Petri dish containing numerous macroscopic sulfur granules. (Photo by David F. Edwards, University of Tennessee, Knoxville, TN.)

Biochemical Properties, Resistance, Antigens and Toxins

Biochemical tests

- ☐ *Actinomyces bovis* is **catalase negative**, ferments several sugars, 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 minutes and are susceptible to various disinfectants.

Antigens and toxins

- ☐ *Actinomyces* species have not been shown to produce any toxin.

Pathogenesis

The organism gains access to bony structures via lymphatic system from the oral cavity through wounds of the buccal mucosae, ulcerative lesion of the buccal mucosae, tooth root abscess or tooth eruption. The bacteria enter through cuts and abrasions (i.e. due to teeth eruptions or coarse feed) and migrate to the bone, leading to osteomyelitis (inflammation and infection of the bony tissues). The mandible is affected more commonly than the maxilla. The defining feature of actinomycosis is the presence of a **non-painful swelling under the jaw**. This swelling can rupture and drain pus-type, smelly fluid which contaminates the environment. It is common to have a concurrent infection with *Staphylococcus aureus* *Staphylococcus* spp and *Trueperella (Arcanobacterium) pyogenes*.

Pyogranulomatous inflammation results in suppurative tracts that permeate the medullary spaces leading to osteomyelitis and multiple foci of bone reabsorption and proliferation.

- Exostosis occurs resulting in gross deformities of the affected bone. The exostosis can result in the mal-alignment of the molars and premolars and impair feeding and rumination.

Discharging sinuses may form, most commonly on the underside of the affected jaw.

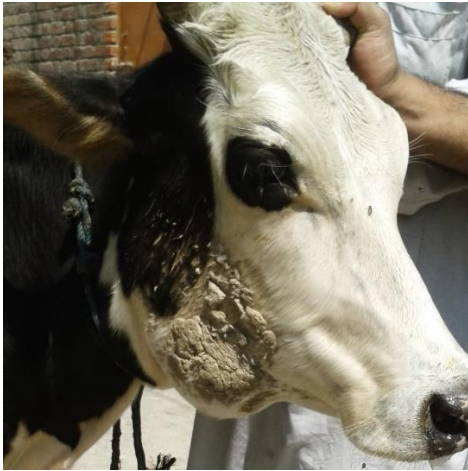
The thick pus (resembles honeycomb) discharging from the sinus contains numerous yellow granules, approximately 1-2mm in diameter ('sulphur granules').

Time course

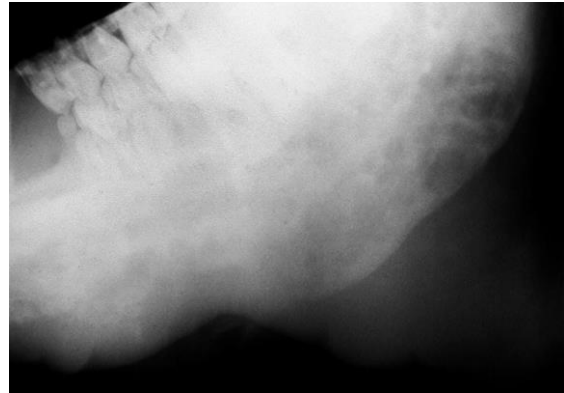
- Enlargement and progression the lesion may occur rapidly (over several weeks) or slowly (over several months).

Clinical Signs

- Hard, immobile, bony mass on the mandible
- Late in the disease, draining tracts may erupt
- Inflammation of the lymph nodes (lymphadenitis)
- Swelling of the pharynx (back of the throat)
- Excessive salivation due to pain
- Abnormal eating, dropping of feed from the mouth, inability to grasp food properly
- Fever in the early stages of disease.
- Honey Comb structure of affected bone-Hole-riddled bone (**Due to resorption of minerals from bone**). The weakened jaw bone is prone to fracturing. Hard lumps along the jaw, or rarely, other facial bones.



Clinical presentation of a calf at ICD Centre Gadoora Ganderbal with unilateral actinomycotic lesions in the mandible. Actinomycotic lesion appeared as hard swelling in the mandible. Ahad Wani, 2020



Actinomycosis in cattle, radiograph
Courtesy of Dr. Geoffrey Smith



Honey Comb structure

Hole-riddled bone shows the animal suffered from lumpy jaw

<https://www.producer.com/livestock/painful-lumpy-jaw-disease-requires-intervention/>

As the name implies, cattle infected with the condition known as lumpy jaw develop hard lumps along the jaw, or rarely, other facial bones. The affected area takes on a honey-comb appearance and this weakened jaw bone is prone to fracturing.

Animals develop lumpy jaw after a break in the inner surface of the mouth. This allows the causative bacteria, *Actinomyces bovis*, to enter and begin the infection. These bacteria are particularly aggressive and invasive. In some ways, they behave more like a fungal infection than a bacterium.

Diagnosis: Presumptive diagnosis is often based on clinical signs. The diagnosis can be confirmed by culture of the organism from the lesion; however, this requires anaerobic conditions and is frequently negative.

1. **Isolation of bacteria and identification** (Material Collection, Morphology and Cultural characteristics)

Material to be collected

Pus, exudates, aspirates, tissue and scrapings from the wall of abscesses.

Good volume of fluid or pus should be collected and submitted, if possible, rather than just a small amount .

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. On staining with Gram's, the ray fungus can be demonstrated.

2. **Polymerase chain reaction**

3. X Ray

4. Histopathology

Prevention & Control:

1. No vaccine available

2. Avoiding and Treating oral injuries in time-eliminating foreign/sharp material from feed bunks is important.

3. **There is no curative treatment.** Multiple intravenous injections of **sodium iodine**, sometimes **in combination with antibiotics**, are used to stop the progression of the infection. The boney mass will remain but may spread slower. In animals with advanced disease, **humane culling is the best option**. The goal of treatment for actinomycosis is to kill the bacteria and stop the spread of the lesion.

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

5. Prevention is a challenge since the bacteria are normally present in the mouth of cattle without causing disease. There is no protective vaccine available.

6. Affected animals premises should be disinfected. A strong disinfectant such as hypochlorite (1:10 household bleach) is a good choice.

References

Ahad, Wani. (2020). Clinical Management of Bovine Actinomycosis (Lumpy Jaw) in Cattle: A Case Report. 10. 24868-24870.

Alshati, Ali & Appleton, Leslie & Maddux, Jacob & Almohammedawi, Mays & Dangerfield, Benjamin. (2019). Esophageal Actinomycosis Presenting as an Obstructive Esophageal Mass. ACG Case Reports Journal. 6. 1. 10.14309/crj.000000000000140.

Bestetti, G. (1978). Morphology of the “sulphur granules”(Drusen) in some actinomycotic infections. A light and electron microscopic study. *Veterinary pathology*, 15(4), 506-518.

Schaal, K.P., Yassin, A.F., Stackebrandt, E. (2006). The Family Actinomycetaceae: The Genera *Actinomyces*, *Actinobaculum*, *Arcanobacterium*, *Varibaculum*, and *Mobiluncus*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, KH., Stackebrandt, E. (eds) *The Prokaryotes*. Springer, New York, NY. https://doi.org/10.1007/0-387-30743-5_21

Rzewuska, Magdalena, Ewelina Kwiecień, Dorota Chrobak-Chmiel, Magdalena Kizerwetter-Świda, Ilona Stefańska, and Małgorzata Gieryńska. 2019. "Pathogenicity and Virulence of *Trueperella pyogenes*: A Review" *International Journal of Molecular Sciences* 20, no. 11: 2737. <https://doi.org/10.3390/ijms20112737>.

Learn More:

Sulphur Granules

The “clubs”, found at the periphery of the “sulfur granules” in *Actinomyces* infection, are composed chiefly of a highly polymerized basic protein rich in guanidyl, indole, and phenolic groups; sulfhydryl groups are absent. The clubs appear to be made of a material actively secreted by the filaments. It is hypothesized that some product or products of animal protein decomposition which are metabolized by the actinomycete trigger and maintain club formation in vivo, and that the club prevents phagocytosis or enzymatic digestion of the filaments on the one hand but proscribes further growth of the filaments on the other. New growth of the parasite takes place by extension of non-clubbed filaments at the periphery of the granule or by deposition of coccoid elements phagocytized by leucocytes.