

# ANTHRAX IN CAMELS





## DEFINITION

Anthrax is a serious infectious bacterial disease affecting all wild and domestic hoofed animals caused by *Bacillus anthracis* and takes an acute or a peracute form leading to septicaemia and sudden death, with or without clinical manifestations. The disease is well-known to nomads and camel breeders, hence the many local names and synonyms given to it by nomads and camel breeders in many countries. Infection takes place in camels mostly through the alimentary tract, by ingesting contaminated food or drinking from stagnant water. It is a zoonotic disease and is most common in wild and domestic herbivores such as cattle, sheep, goats, camels, antelopes. Humans get the infection when exposed to tissue from infected animals, to contaminated animal products, or directly to *B anthracis* spores under certain conditions.

## AETIOLOGY

*Bacillus anthracis* is a large, gram-positive, non-motile, spore-forming bacterial rod. The three virulence factors of *Bacillus anthracis* are edema toxin, lethal toxin, and a capsular antigen. *B anthracis* spores can remain viable in soil for many years. During this time, they are a potential source of infection for grazing livestock but generally do not represent a direct risk of infection for people. Grazing animals may become infected when they ingest sufficient quantities of these spores from the soil. In addition to direct transmission, biting flies may mechanically transmit *B anthracis* spores from one animal to another. It is transmitted directly by biting flies, e.g. *Tabanus* species or nasal bots (*Cephalopina titillator*). Feed contaminated with bone or other meal from infected animals can serve as a source of infection for livestock.

## EPIDEMIOLOGY

Underdiagnosis and unreliable reporting make it difficult to estimate the true incidence of anthrax worldwide. It has been reported from nearly every continent and is most common in agricultural regions with neutral or alkaline, calcareous soils. In these regions, anthrax periodically emerges as epizootics among susceptible domesticated and wild animals. These epizootics are usually associated with drought, flooding, or soil disturbance, and many years may pass between outbreaks. During interepidemic periods, sporadic cases may help maintain soil contamination.

In developing countries, each affected animal can result in up to 10 human cases because of home slaughter and sanitation issues. In cases of natural transmission, people exhibit primarily cutaneous disease in 95% cases. GI anthrax (including pharyngeal anthrax) may be seen among human populations after consumption of contaminated raw or undercooked meat. Under certain



artificial conditions (eg, laboratories, animal hair processing facilities, exposure to weaponized spore products), people may develop a highly fatal form of disease known as inhalational anthrax or woolsorter's disease. In addition to causing naturally occurring anthrax, *B anthracis* has been manufactured as a biologic warfare agent. *B anthracis* was used successfully as a weapon of terrorism in 2001, killing 5 people and causing disease in 22. Probably because of the method of delivery (via mail), no known animal disease resulted from this attack. Weaponized spores represent a threat to both human and animal populations.

## CLINICAL FINDINGS

Typically, the incubation period is 3–7 days (range 1–14 days). The clinical course ranges from peracute to chronic. The peracute form is characterized by sudden onset and a rapidly fatal course. High fever, staggering, dyspnea, trembling, collapse, a few convulsive movements, and death may occur with only a brief evidence of illness. Painful swellings sometimes develop at the throat, the base of the neck and groins. These are particularly large when the palate becomes involved due to a local puncture in the nasopharynx by bots or other means.

There may be bloody discharges from the natural body openings. Some infections are characterized by localized, subcutaneous, edematous swelling that can be quite extensive. Areas most frequently involved are the ventral neck, thorax, and shoulders. Death usually occurs within 2–3 days of onset. Depending on the route of infection, host factors, and potentially strain-specific factors, anthrax can have several different clinical presentations. In herbivores, anthrax commonly presents as an acute septicemia with a high fatality rate, often accompanied by hemorrhagic lymphadenitis.

### Lesions:

Rigor mortis is frequently absent or incomplete. Dark blood may ooze from the mouth, nostrils, and anus with marked bloating and rapid body decomposition. If the carcass is inadvertently opened, septicemic lesions are seen. The blood is dark and thickened and fails to clot readily. Hemorrhages of various sizes are common on the serosal surfaces of the abdomen and thorax as well as on the epicardium and endocardium. Edematous, red-tinged effusions commonly are present under the serosa of various organs, between skeletal muscle groups, and in the subcutis. Hemorrhages frequently occur along the GI tract mucosa, and ulcers, particularly over Peyer's patches, may be present. An enlarged, dark red or black, soft, semifluid spleen is common. The liver, kidneys, and lymph nodes usually are congested and enlarged. Meningitis may be found if the skull is opened.



## DIAGNOSIS

### 1. Clinical diagnosis

Diagnosis based on clinical signs alone is difficult. Typical signs like sudden death without premonitory signs, dark foamy blood oozing from the natural orifices, dyspnea, staggering etc. can be suggestive of Anthrax

### 2. Identification of the agent

Demonstration of encapsulated *B. anthracis* in smears of blood or tissues from fresh anthrax-infected carcasses and growth of the organism on blood agar plates is relatively uncomplicated. Recovery of *B. anthracis* from old decomposed carcasses, processed specimens (bone meal, hides), or environmental samples (contaminated soil) is often difficult, requiring demanding and labour-intensive procedures. However live spores may be recovered from the turbinates of dead livestock and wildlife for an extended period after death

#### a) Culture and identification of *Bacillus anthracis*

##### i) Fresh specimens

*Bacillus anthracis* grows readily on most types of nutrient agar, however, 5–7% horse or sheep blood agar is the diagnostic medium of choice. Blood is the primary clinical material to examine. Swabs of blood, other body fluids or swabs taken from incisions in tissues or organs can be spread over blood agar plates. After overnight incubation at 37°C, *B. anthracis* colonies are grey-white to white, 0.3–0.5 cm in diameter, nonhaemolytic, with a ground-glass surface, and very tacky when teased with an inoculating loop. Tailing and prominent wisps of growth trailing back toward the parent colony, all in the same direction, are sometimes seen. This characteristic has been described as a 'medusa head' or 'curled hair' appearance. Confirmation of *B. anthracis* should be accomplished by the demonstration of a capsulated, spore-forming, Gram-positive rod in blood culture.

##### ii) Capsule visualisation

Virulent encapsulated *B. anthracis* is present in tissues and blood and other body fluids from animals that have died from anthrax. Thin smears may be prepared from blood from ear veins or other peripheral veins and exudate from orifices. However if the animal has been dead more than 24 hours, the capsule may be difficult to detect. The bacteria should be looked for in smears of these specimens that have been dried, fixed either using heat or by dipping the smear in 95–100% alcohol for about 1 minute and air dried and then stained with polychrome methylene blue (MacFadyean's reaction). The capsule stains pink, whereas the bacillus cells stain dark blue.

The cells are found in pairs or short chains and are often square-ended (the chains are sometimes likened to a set of railway carriages – so-called 'box-car' or 'jointed bamboo-rod' appearance).



Identification of *B. anthracis* from old, decomposed specimens, processed materials, and environmental samples, including soil, is possible but these samples often have saprophytic contaminants that outgrow and obscure *B. anthracis* on non-selective agars.

**i) Ascoli test**

Ascoli (1911) published a procedure for the detection of thermostable anthrax antigen in animal tissue being used for by-products. This uses antiserum raised in rabbits to produce a precipitin reaction. The test lacks high specificity, in that the thermostable antigens of *B. anthracis* are shared by other *Bacillus* spp., and is dependent on the probability that only *B. anthracis* would proliferate throughout the animal and deposit sufficient antigen to give a positive reaction. To perform the Ascoli test, put approximately 2 g of sample in 5 ml of saline containing 1/100 final concentration of acetic acid and boil for 5 minutes. The resultant solution is cooled and filtered through filter paper. A few drops of rabbit antiserum (see preparation below) are placed in a small test tube. The filtrate from the previous step is gently layered over the top of the antiserum. A positive test is the formation of a visible precipitin band in under 15 minutes. Positive and negative control specimen suspensions should be included.

**c) Confirmation of virulence with the polymerase chain reaction**

Confirmation of virulence can be carried out using the PCR. Template DNA for PCR can be prepared from a fresh colony of *B. anthracis* on nutrient agar by suspension of a loop of growth in 25 µl sterile deionised (or distilled) water and heating to 95°C for 20 minutes. Following cooling to approximately 4°C, and brief centrifugation, the supernatant can be used for the PCR reaction.

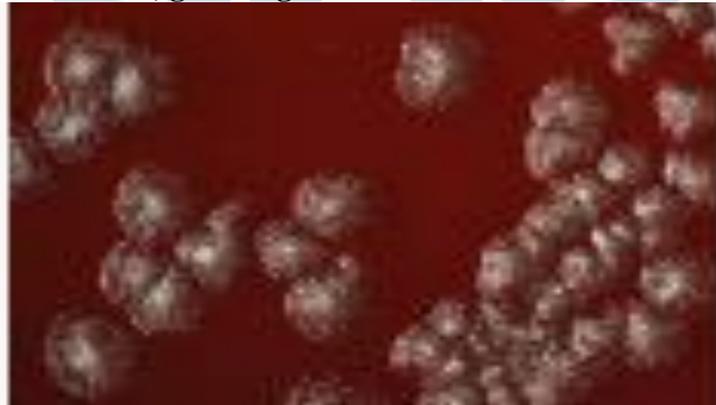
- 1. Confirmatory laboratory examination** should be attempted if anthrax is suspected. Because the vegetative cell is not robust and will not survive 3 days in transit, the optimal sample is a cotton swab dipped in the blood and allowed to dry. This results in sporulation and the death of other bacteria and contaminants. For carcasses dead >3 days, either the nasal turbinates should be swabbed or turbinate samples removed.. Before submission, the receiving reference laboratory should be contacted regarding appropriate specimen labelling, handling, and shipping procedures.

Specific diagnostic tests include bacterial culture, PCR tests, and fluorescent antibody stains to demonstrate the agent in blood films or tissues. Western blot and ELISA tests for antibody detection are available in some reference laboratories.

**Bacillus anthracis, methylene blue stain**



Source-Merck veterinary Manual  
**Bacillus anthracis, ground glass colonies**



Source-Merck veterinary Manual

In Camels, anthrax must be differentiated from other conditions that cause sudden death. Clostridial infections, bloat, and lightning strike (or any cause of sudden death) may be confused with anthrax. Also, acute leptospirosis, bacillary hemoglobinuria, anaplasmosis, and acute poisonings by bracken fern, sweet clover, and lead must be considered.



## TREATMENT AND CONTROL

Early treatment and vigorous implementation of a preventive program are essential to reduce losses. Camels at risk should be immediately treated with a long-acting antibiotic to stop all potential incubating infections. This is followed by vaccination 7–10 days after antibiotic treatment. Simultaneous use of antibiotics and vaccine is inappropriate, because available commercial vaccines are live vaccines. Suspected contaminated feed should be immediately removed. Parenteral administration of penicillin (10000 IU per Kg ) may be tried in the early stages of the disease. Oxytetracycline given daily in divided doses also is effective. Other antibacterials, including amoxicillin, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, gentamicin, streptomycin, and sulfonamides also can be used, but their effectiveness in comparison with penicillin and the tetracyclines has not been evaluated under field conditions.

## VACCINATION

Camels can be immunised passively or actively by using hyperimmune serum, vaccine or both. A suspension of viable *Bacillus anthracis* Sterne Strain 34F2 spores in saponin is currently available. In areas where anthrax is known to be a problem, it is advisable to revaccinate annually approximately 4 weeks prior to the time the disease usually appears. For control of outbreaks, vaccination of all animals not showing symptoms is recommended. Not all animals will be protected by this procedure but taking action as suggested may stop further spread of the disease. It is also recommended that animals showing symptoms be isolated and treated with antibiotics as permitted. Animals being vaccinated or recently vaccinated should not receive antibiotics, as antibiotics will interfere with effective vaccination. Inject 1 ml subcutaneously into each animal. Revaccinate in 2-3 weeks in heavily contaminated areas

In addition to therapy and immunization, specific control procedures are necessary to contain the disease and prevent its spread. Human infection is controlled through reducing infection in livestock, veterinary supervision of animal production and slaughter to reduce human contact with potentially infected livestock or animal products, and in some settings either pre- or postexposure prophylaxis. In countries where anthrax is common and vaccination coverage in livestock is low, people should avoid contact with livestock and animal products that were not inspected before and after slaughter. In general, consumption of meat from animals that have exhibited sudden death, meat obtained via emergency slaughter, and meat of uncertain origin should be avoided. Routine vaccination against anthrax is indicated for individuals engaged in work involving large quantities or concentrations of *B anthracis* cultures or activities with a high potential for aerosol production.



Control measures are aimed at breaking the cycle of infection. Each of the following actions must be rigorously implemented:

- Cut off infection source and dispose of anthrax carcasses correctly
- Correctly disinfect, decontaminate and dispose of contaminated materials
- Vaccinate exposed susceptible animals and, where possible, humans in at-risk occupations
- Observation of general sanitary procedures by people who handle diseased animals, both for their own safety and to prevent spread of the disease. Contaminated soils are very difficult to completely decontaminate, but formaldehyde will be successful if the level is not excessive. The process generally requires removal of soil

#### **Discontinuation of infection source**

In outbreaks having a defined infection source, clearly discontinuing this source is an essential first step to breaking the cycle of infection. The feed source should be immediately withdrawn and destroyed. Moving other animals away from the affected area is an important early action. If flies are suspected of being important vectors, fly control should be considered.

#### **Disposal of anthrax (animal) carcasses**

The preferred method of disposal of an anthrax carcass is incineration. Burial is the remaining less satisfactory alternative. New outbreaks following disturbance of old burial sites are reported. Consideration might be given to treating anthrax carcasses with 10% formalin, leaving them in situ for some days before disposal while natural putrefaction processes within the carcass kill the vegetative anthrax organisms. The formalin would have the action of killing anthrax organisms shed by the dead animal, preserving the skin so that it retains the anaerobic environment within the putrefying carcass. It may also deter scavengers that would otherwise open up the carcass and thereby increase the contamination, and flies that might spread the disease. Ideally, the soil surrounding and under the carcass, particularly around the nasal and anal regions, should be decontaminated and then incinerated with the carcass.

#### **Disinfection**

Anthrax spores are resistant to heat, sunlight, drying and many disinfectants. They can be killed with formaldehyde or 2% glutaraldehyde; overnight soaking is recommended. A 10% NaOH or 5% formaldehyde solution can be used for stockyards, pens and other equipment. Sodium hypochlorite has also been recommended for some purposes. Household bleach must be diluted with water to increase the free available chlorine, and adjusted to pH 7. Prolonged contact is recommended. Gaseous sterilization can be accomplished with chlorine dioxide, formaldehyde gas and other methods, under specific conditions of humidity and temperature. Sterilization is also possible by heating to 121°C (250°F) for at least 30 min. Gamma radiation has been used to decontaminate animal products, as well as mail from contaminated postal facilities. Exposed arms and hands can be washed with soap and hot water then immersed for one minute in a



## REFERENCES

1. OIE Terrestrial Animal Health Code. [www.oie.int/en/international-standard-setting/terrestrial-code/access-online/](http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/)
2. Anthrax (4<sup>th</sup> Edition) WHO 2008.
3. The Merck Veterinary Manual. 2015 Veterinary Edition.