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## Anthrax

### Etymology

The word 'anthrax' is derived from the Greek word *anthrakis* which means 'coal'. This is linked with the characteristic dark necrotic skin-eschar in the cutaneous form of anthrax in humans.

### Synonyms

Siberian plague, black bane, charbon, splenic fever, ragpicker's disease, hide porter's disease, wool sorters' disease, Cumberland disease, malignant pustule, malignant carbuncle and Milzbrand.

### Aetiology and Pathogen Characteristics

Anthrax is an anthroozoonotic infection caused by *Bacillus anthracis*. The organism is a Gram-positive, aerobic or facultative anaerobic, non-motile, non-haemolytic, spore-forming, rod-shaped bacteria. The organism develops a capsule in the body of the host.

### Sporulation

Spores are the dormant form of bacteria that are highly resilient, with resistance to temperature extremes, drought and UV light, possibly due to the protection of DNA in a crystalline core. In the case of *B. anthracis*, sporulation may initiate due to poor nutrient availability and in the presence of oxygen. Some of the characteristics of *B. anthracis* include the following.

- Spores can survive in dry soil for 60 years [1]; the longest reported survival of spores, i.e.  $200 \pm 50$  years, is from bones retrieved during archaeological excavations at Kruger National Park in South Africa [2].
- The pathogen is categorised as a Centers for Disease Control and Prevention (CDC) 'category A' biological agent. The spores can be used as bioweapons due to their size of 2–6 microns diameter, which is an ideal size for impinging on the human lower respiratory tract. Moreover, anthrax spores lend themselves well to aerosolization.

## Historical Overview on Anthrax

- **1834:** The first case of human anthrax was detected in 1834 in the USA, and in 1938, Delafond demonstrated the causal organism microscopically in the blood of animals.
- **1877:** Robert Koch discovered the anthrax bacillus and also hypothesised Koch postulates.
- **1881:** Louis Pasteur developed the first whole-cell anthrax vaccine.
- **1930s:** Discovery of Sterne-type vaccines. The vaccine is based on an avirulent non-encapsulated strain 34F2 (pXO1<sup>+ve</sup> and pXO2<sup>-ve</sup>), which can stimulate a protective immune response. The Sterne strain is currently the predominant strain used for immunisation of domesticated animals against anthrax. It is administered to livestock in a dose containing up to 10 million viable spores.
- **1979:** Anthrax outbreak in Sverdlovsk (USSR) caused 61 deaths and 11 non-fatal cases in 6 weeks. Some researchers concluded the outbreak could have resulted from the accidental spread of anthrax spores by the wind from a microbiology facility at the local military compound [3].
- **2001:** Use of anthrax spores in a bioweapon attack in the USA by mailing of spores to seven locations, which resulted in 22 cases of anthrax (including five deaths) [4].
- **2009:** The first outbreak of injectional anthrax was reported in heroin users in Scotland. The source of contamination was proposed to be goat skins that were used to transport the heroin [5].

## Pathogenesis and Virulence Factors

The bacterium *B. anthracis* is likely to be evolved from *Bacillus cereus* that acquired two extrachromosomal plasmids, pXO1 and pXO2, from the environment through lateral genetic transfer.

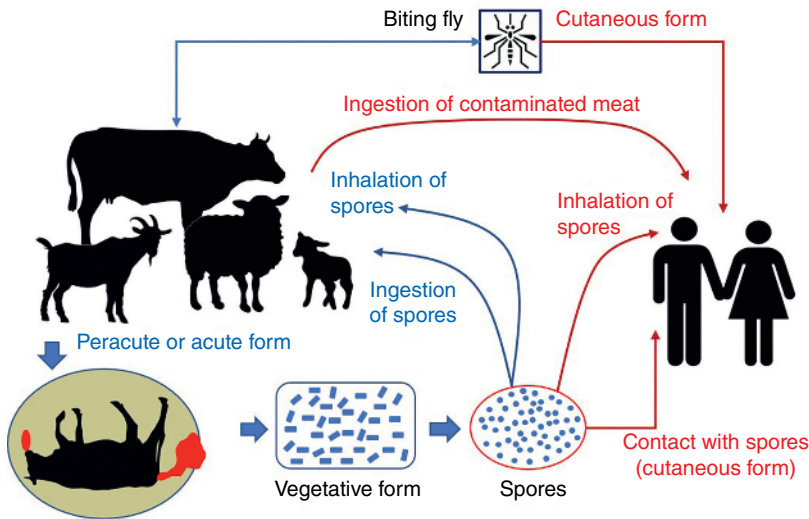
The plasmid **pXO1** encodes tripartite toxin complex as follows.

- 1) **Protective antigen** (PA, 83 kDa): This permits the entry of toxins into the host cell.
- 2) **Oedema factor** (EF, 90 kDa): This is responsible for oedema toxin (PA + EF). Due to this toxin, the calmodulin-dependent adenylate cyclase increases intracytoplasmic levels of cAMP that lead to alteration of water homeostasis which results in oedema. The oedema toxin can induce lethality in the host mainly by targeting hepatocytes.
- 3) **Lethal factor** (LF, 89 kDa): This is responsible for lethal toxin (PA + LF). It is a zinc metalloprotease toxin that can cause the hyperinflammatory condition in macrophages by activating the oxidative burst pathway and release of reactive O<sub>2</sub> intermediates. It cleaves and inactivates mitogen-activated protein kinase kinases (MAPKKs) 1–4, 6 and 7, which play a crucial role in responses to diverse stimuli, such as mitogens, heat shock, proinflammatory cytokines and cellular stresses. It is responsible for the production of proinflammatory cytokines (TNF- $\alpha$  and IL-1 $\beta$ ). The lethal toxin causes lethality by targeting the cardiovascular system, in particular cardiomyocytes and vascular smooth muscle cells.

The plasmid **pXO2** encodes proteins that synthesise a poly- $\gamma$ -D-glutamic acid capsule which confers resistance to phagocytosis.

## Transmission Cycle

Most mammals are susceptible to anthrax. The disease is most commonly seen in herbivores (e.g. cattle, sheep, goats) whereas pigs, equines, dogs and camels are reported to be moderately susceptible. The disease has also been commonly reported in wild animals (e.g. lion, hyena, elephant, jackal, giraffe, zebra, etc.).



**Figure 1.1** The transmission cycle of anthrax between animals and humans.

Herbivores are considered the primary host for anthrax. Upon the death of the host, bacteria in the carcass are exposed to air through haemorrhages, opening of the carcass by scavengers, etc. On exposure, the bacteria sporulate and persist in the soil for prolonged periods which can be the source of infection to other animals or humans. The soil can act as a long-term reservoir for spores of anthrax bacilli. In addition, regions with high humidity, alkaline soils and a high amount of organic matter are categorised as ‘incubator areas’ for the survival or persistence of anthrax spores. An overview of the transmission cycle of anthrax in animals and humans is provided in Figure 1.1.

## Factors Affecting the Transmission of Anthrax

### Transmission in Animals

- Ingestion of contaminated fodder, water and processed feed (meat/bone meal, meat scraps).
  - Inhalation of spores during wallowing in contaminated water sources.
  - Climatic conditions may influence the animal’s contact with spores.
    - Grazing closer to contaminated soil in dry periods when grass is sparse increases the chances of animal contact with spores.
    - Enforced grazing at restricted sites (contaminated areas/burial sites) when water sources become scarce is also considered an important risk factor.
  - Spiky grass and grits can cause orogastrointestinal lesions in animals which can be infected by germination of spores.
  - Calcium-rich soils with neutral-to-alkaline pH can act as favourable sites for spore development. Such regions are also known as ‘anthrax belts’.
- Note: *Role of calcium in spore formation*: calcium is integral to the dehydration of vegetative cell genome precursors, which is necessary for its effective long-term storage in spore form.
- Mechanical transmission of the pathogen can occur by biting flies (e.g. *Hippobosca* spp., *Tabanus* spp.).
  - The use of contaminated surgical instruments for dehorning and docking may cause disease transmission.

## Transmission in Humans

Animal products including meat, hide, hair or bone from infected animals can be heavily contaminated with anthrax spores, which can act as important sources for human infection. Anthrax is considered an occupational hazard among butchers, textile workers, wool industry workers, farmers, knackers, veterinarians, workers concerned with the processing of animal products (e.g. tannery) and laboratory workers.

## Anthrax in Animals

The susceptibility and clinical signs of anthrax in different species of animals are described below.

- **Herbivores** (bovines, sheep, and goats): Herbivores generally exhibit per-acute infection which may lead to sudden death. At death, blood exudes from the rectum and other natural openings of the animal. The blood of the infected dead animal does not clot and there is absence of rigor mortis in the carcass. It has been found that the blood of the infected animal may contain  $>10^8$  bacilli/mL [6].
- **Horses**: Equines mainly exhibit acute symptoms and die within 2–3 days of infection. In some animals, biting flies may transmit the pathogen and cause large oedematous lesions on breast, abdomen, neck and shoulders.
- **Pigs**: Pigs are more resistant to anthrax than bovines and mainly exhibit localised signs which include oedema of the throat, pharyngeal and cervical lymph nodes.
- **Dogs and cats**: Dogs and cats are considered to be resistant to anthrax. Dogs that have scavenged anthrax carcasses may suffer from severe inflammation and oedematous swelling of the throat, stomach, intestine, lips, jowl, tongue and gums.
- **Birds**: In birds, apoplectic type of death is observed due to anthrax whereas less acute cases may exhibit carbuncular lesions on comb or extremities.

## Anthrax in Humans

The clinical forms of anthrax in humans are described below.

- 1) **Cutaneous anthrax**: The cutaneous form of anthrax is responsible for 95% of global human cases and is mainly reported in developing countries following contact with infected animals and their products. Cutaneous anthrax usually develops 1–7 days after exposure, but incubation periods as long as 17 days have been reported [7]. The characteristic clinical signs are anthrax eschars on exposed regions of the body, i.e. face, neck, hands and wrists. Malignant oedema is a rare complication of the cutaneous form which is characterised by severe oedema, induration, multiple bullae and symptoms of shock (Note: The common description of this form as ‘malignant pustule’ is a misnomer because the lesion is not purulent and painless.)
- 2) **Inhalation anthrax**: This occurs mainly due to inhalation of spores (size  $<5\ \mu\text{m}$ ) which reach the lower respiratory tract. The incubation period ranges from 1 to 60 days.

The alveolar macrophages then phagocytise these spores and transport them to hilar and mediastinal lymph nodes, where they germinate, proliferate and spread systemically. There is also the possibility that spores gain entry to subepithelial and lymphatic tissues in the upper airways where germination occurs and vegetative forms can spread. The initial symptoms are fever, cough, myalgia, malaise, chest pain and acute respiratory distress. However, in the septicemic form, severe cases involve high fever, dyspnoea, cyanosis, haemorrhagic mediastinitis and effusion followed by rapid progression of shock. In untreated cases, the mortality rate is nearly 100%.

- 3) **Gastrointestinal anthrax:** Gastrointestinal anthrax mainly occurs after consumption of contaminated meat from infected animals. The two forms of gastrointestinal anthrax are:
  - **oropharyngeal form:** characterized by the development of oral or oesophageal ulcers followed by regional lymphadenopathy, oedema and sepsis
  - **lower gastrointestinal form:** exhibits intestinal lesions mainly in the terminal ileum or caecum.
- 4) **Injectional anthrax:** The heroin (drug)-associated anthrax resulting from direct injection or injection under the skin, or 'skin popping', among persons who inject drugs (PWIDs) is a distinct form of anthrax which was reported during the 2009–2010 outbreak in Scotland and England and again during 2012–2013 in northern Europe and Germany [5].

## Laboratory Diagnosis

### Growth Media and Biochemical Characteristics of *B. anthracis*

- **Selective media:** Polymyxin-lysozyme-EDTA-thallos acetate (PLET) agar.
- **On blood agar:** Non-haemolytic colonies with irregular borders.
- **In liquid medium:** Inverted fir tree appearance.
- **On nutrient agar:** Medusa head or comet tail appearance.
- **McFadyean reaction:** Polychrome methylene blue stain (blue bacilli with purple capsule).

**Table 1.1** Differences between *B. anthracis* and other bacilli (anthracoid).

Characteristics	Anthrax	Other bacilli
Capsule	Present	Absent
Motility	Non-motile	Motile
On blood agar	Non-haemolytic	Haemolytic
Gelatin liquefaction	Slow	Rapid
Susceptibility to penicillin	Susceptible	Not susceptible
Susceptible to $\gamma$ phage	Susceptible	Not susceptible
Animal pathogenicity	Pathogenic	Non-pathogenic

## Diagnostic Tests

- **Ascoli test:** This is a thermostable antigen precipitin test developed in 1911. It is an old method but is still used in several countries to detect residual antigens in animal tissue(s). It is not a highly specific test as the antigens being detected are shared by other *Bacillus* spp.
- **McFadyean reaction:** The McFadyean stain remains important for the rapid diagnosis of anthrax. It is a staining procedure for blood or tissue smears from dead animals. The capsular material of the organism can be detected by the McFadyean reaction which involves staining with polychrome methylene blue. The positive observation includes blue rods in a background of purple/pink-stained capsular material.
- **Molecular diagnosis:** Polymerase chain reaction (PCR) is commonly used to target the specific genes of the organism. In addition, molecular typing of the isolates can be carried out by appropriate tools including DNA microarrays, pulsed-field gel electrophoresis (PFGE), multilocus variable number tandem repeat analysis (MLVA), etc.
- **Serology:** The commonly used serological procedure is enzyme-linked immunosorbent assay (ELISA) in microwell plates coated with protective antigen (PA) and lethal factor (LF). Other tests including direct fluorescent assay (DFA) and fluorescence resonance energy transfer (FRET) assay can be used as per availability.

## Vaccination

### Animals

Globally, the Sterne strain 34F2 anthrax vaccine (non-capsulating [ $pXO1^+/pXO2^-$ ]) is used in animals. This vaccine affords immunological protection primarily due to antibodies specific for the protective antigen (PA). In vaccinated animals, observation of the withholding period for meat (i.e. 3–6 weeks before slaughter) is highly important for human consumption. There is no withholding period for milk in vaccinated animals.

### Humans

In humans, immunisation with live spores has been limited to the former USSR and China. Other cell-free human vaccines like Biothrax™ are available in the UK and USA.

## Treatment in Humans

The drugs commonly used for anthrax treatment are ciprofloxacin and doxycycline (usually administered together) [8]. It is important to start oral antibiotics within 24 hours of exposure. Advocated antibiotics course durations are:

- 60 days – without vaccine
- 30 days – with three doses of vaccine.

## Prevention and Control Measures

The epidemiology of anthrax involves livestock, wildlife, human and environmental components. This complex cycling of the pathogen makes anthrax an ideal example for discussion in the One

Health context. Therefore, prevention and control measures should target the relevant chain of transmission. Some of the measures are listed below.

### In Animals

- Vaccination of livestock to generate herd immunity in endemic areas.
- Restrict grazing on contaminated pastures.
- Proper quarantine of imported animals.
- Respect import bans from endemic areas.
- Implementation of laws on prohibition of slaughter and consumption of meat and animal products from infected animals.
- Adequate tracing and destruction of contaminated meat and animal products.
- During an outbreak:
  - avoid opening or postmortem of ‘suspected’ dead animals
  - plug orifices of dead animals with cotton soaked in carbolic acid/lysol
  - safe disposal of the carcass as per the guidelines
  - disinfect the site of the dead animal with lysol or 3–5% formaldehyde
  - disinfect slaughter sites, processing factories and retail outlets as per the guidelines.

### In Humans

- Rapid detection and confirmation of cases by laboratory diagnostics is essential.
- Robust surveillance and tracing of cases should be the priority in endemic areas.
- Appropriate medical interventions during outbreaks.
- Community education and awareness programmes for occupational risk groups.

### Environment

Environmental contamination from stray or wild animal carcasses or even from soil disturbance over historic animal graves is possible. Therefore, extra care is required in endemic regions, especially in context with an extensive livestock production system.

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