CHAPTER 2

ANTHRAX

2.1 Etiology

Anthrax is a widely known disease caused by *Bacillus anthracis*, a gram positive, spore-forming, rod-shaped bacterium. The organism is readily cultivable on routine nutrient medium and grows best aerobically, but will also multiply under anaerobic conditions (Turnbull 1999). The disease in livestock is usually peracute, characterized by septicaemia and sudden death in 1-3 hours with exudation of unclotted tarry coloured blood from natural orifices and incomplete rigor mortis. Post mortem examination shows splenomegaly in cattle (Parker, *et al.*, 2005). It is a potential zoonotic disease, readily transmitted to human beings via inhalation of spores, handling of infected animals, consumption of contaminated meat, contaminated carcass, contaminated fomites etc. In humans it is a subacute disease and fatal if not treated immediately. The skin lesion is painless (Shivachandra, *et al.*, 2016). *Bacillus anthracis* possess three plasmid-encoded virulence factors: a poly-$\gamma$-D-glutamic acid capsule that inhibits phagocytosis; edema toxin composed of protective antigen (PA) and edema factor (EF); and lethal toxin, a combination of PA and lethal factor (LF). Both toxins bind to a common cell receptor through PA. Production of anthrax toxin within an animal is dependent on the presence of viable *B. anthracis*, hence the detection of PA is indicative of active anthrax infection (Boyer, *et al.*, 2007).

The word ‘Anthrax’ is derived from Greek word *anthrakis*. The literal meaning of ‘Anthrax’ is ‘coal’ as it forms a lesion in skin with black eschar which is very dark in colour, like coal.

2.2 Anthrax - Synonyms

Anthrax is also called Malignant pustule, malignant oedema, Wool sorter’s disease and Rag picker’s disease. (IOWA state University, 2007). In Kannada, it is called Neradi and Gulma jwara (Shivachandra, *et al.*, 2016).
2.3 **Significance of the disease**

Anthrax is a serious zoonotic disease that affects mostly herbivore animals, although some wild mammals and several species of birds are susceptible to it. Humans and carnivores are accidental hosts to anthrax. In endemic regions, anthrax is a serious problem in unvaccinated ruminants. By initiating an early treatment, the affected animals can be cured by using antibiotics. The course of disease is usually rapid and symptomatic infections are often fatal. Epizootics in wildlife are also a matter of concern. In 2004, an outbreak in the Malilangwe Wildlife Reserve in Zimbabwe killed almost all of the approximately 500 kudu (a species of Antelope) in the reserve, as well as large numbers of other wild ruminants. Humans contract the disease usually after exposure to infected animals and their tissues. In most countries, human anthrax occurs sporadically, mainly as an occupational hazard among veterinarians, agricultural workers and workers who process hides, hair, wool and bone products. The greatest threat to mankind from anthrax is that it has a potential to be used as a bioweapon. In 2001, weaponized anthrax was delivered in letters through the United States mail, resulting in 11 cases each of inhalational and cutaneous anthrax resulting in death of five people with inhalational anthrax. Since ruminants are sensitive, anthrax outbreak in these animals might serve as an early warning of probable disease in humans ((IOWA state university 2007).

2.4 **Anthrax and environment**

2.4.1 **Spores and its life**

The anthrax bacteria are found in two different forms *viz.*, the vegetative form and the spore form. The organism is in vegetative form within the anaerobic environment of the infected host. The vegetative form is the one in which the organism grows and multiplies to cause the disease. If the organism is exposed to atmospheric free oxygen by accidental opening of the carcass of an animal that has died from anthrax, the vegetative form of the bacteria transforms into spore. When the spores invade a susceptible host, commonly by grazing contaminated vegetation or inhalation, they go back to vegetative form. When conditions are not conducive to growth and multiplication of the *bacilli*, i.e., when the host has died, they tend to form spores. Anthrax spores are highly resistant to extreme changes occurring in the environment. They are resistant to chemicals and disinfectants also. They can survive for decades and become active under favourable conditions for multiplication (Parker, *et al.*, 2002 and Anthrax OIE 4th ed.).
2.4.2 Activation of spores

The rate and extent of sporulation by vegetative cells shed from infected animals is affected in a complex method by the environmental conditions into which they drop. Temperature, pH, oxygen availability and the presence of certain cations such as Mn++ are the prime influencing factors (Turnbull, WHO. 3rd ed.).

2.4.3 Pathogenesis of the disease

Usually, the spores of *Bacillus anthracis* enter the host body through inhalation or ingestion. These spores are phagocytosed by macrophages followed by intracellular germination. These macrophages get lodged in the regional lymph nodes and release the vegetative form of the organism into the circulation leading to massive septicaemia. These vegetative forms also produce the toxin that gets into circulation causing severe toxaemia. The released toxin spreads throughout the body and causes pathological changes leading to death of the cells (Savransky, *et al.*, 2013).

2.4.4 Anthrax and season

Anthrax is a seasonal disease. Anthrax enzootic areas are usually found in warm climates. The anthrax outbreak association with warmer climates is probably attributable to the relationship between temperature, water sources and rates of sporulation of *bacilli* shed from victims of the disease. The vegetative form appears to survive poorly outside the animal host and sporulation depends on the temperature. An outbreak of anthrax in an enzootic area is usually after a prolonged hot dry spell, which in turn was preceded by heavy rains or flooding or with rain ending a period of drought (Anthrax OIE, 4th ed.).

The outbreak of anthrax has a seasonal occurrence. Climate is the major predisposing factor in disease precipitation. It has been well reported in the literature that the incidence of the disease is related to temperature, rains or drought. During drought, the animals graze closer to the soil thereby picking up the anthrax spores or during heavy rains the spores get washed up and come in contact with the host (Turnbull, WHO. 3rd ed.).
2.5 Anthrax in animals

2.5.1 Herbivores disease

The outbreaks of anthrax are mainly recorded in herbivore animals, although some wild mammals are also susceptible to it. Cattle, sheep, goats, horses and pigs are highly susceptible to anthrax. Humans and carnivores are accidental hosts to anthrax.

2.5.2 Incubation period

The incubation period is considered to be from 1 to 20 days. In herbivores, infections appear after 3 to 7 days. The incubation period in pigs is usually 1 to 2 weeks. In highly susceptible species with peracute disease, the period between onset of visible symptoms and death may be just a few hours. The course of these events is more protracted in more resistant species (Turnbull, WHO. 3rd ed.).

Anthrax is always suspected in the event of sudden death in the susceptible hosts in those areas where there is a history of the disease in the recent past. Further, it is more suspected if the land operations such as digging, ploughing, dredging of watercourses etc., have taken place. Anthrax should always be considered as a possibility in the event of sudden deaths in zoos or other captive facilities where meat from knackeries or the equivalent are regularly fed to animals (Anthrax OIE, 4th ed.).

2.5.3 Clinical signs

Anthrax occurs as a peracute, acute, subacute or chronic disease in animals.

In ruminants, peracute form of the disease is very common and only sign that may be observed is sudden death. Some animals show staggering, trembling and dyspnea, followed by rapid collapse, terminal convulsions and death. The body temperature may reach up to 42°C (Parker, et al., 2005). In the acute form of the disease, the animal will show clinical signs such as fever, depression, stupor, disorientation, muscle tremors, dyspnea and congested mucous membranes. Abortion may occur in pregnant cows and marked drop in milk production. Bloody discharges from the nose, mouth and anus are also sometimes seen. Occasionally, subcutaneous edematous swellings, most often in the ventral neck, thorax and shoulders are observed in ruminants. Anthrax in wild herbivores varies with the species, but
usually resembles the disease in cattle. Horses typically develop acute disease. Common symptoms include fever, chills, anorexia, depression, severe colic and bloody diarrhoea. Swellings are usually seen in the neck, sternum, lower abdomen and external genitalia. Dyspnea can occur due to the swelling of the neck. This follows death within 1 to 3 days and rarely a few survive up to a week. Occasionally, septicaemia and sudden death occurs in pigs. Some animals develop rapidly progressive swelling of the throat, with dyspnea and difficulty in swallowing. These animals may suffocate. Intestinal involvement, anorexia, vomiting, diarrhoea or constipation is rarely reported in pigs. Some pigs with anthrax do recover. On necropsy examination, localized infection of tonsils and cervical lymph nodes has been recorded. Dogs, cats and wild carnivores show clinical signs similar to pigs. A study conducted on dogs observed massive swelling of the head, neck and mediastinum as the most common lesion. Death was usually due to toxemia and shock, but swelling of the throat and suffocation could also have been a factor for the death. In addition to the common lesions, Hemorrhagic gastroenteritis, swollen foreleg and ptyalism have been reported in a dog (IOWA state University 2007).

2.5.4 Infection and its severity

Ingestion of contaminated feed and water is the most common mode of transmission of the disease. The disease may be transmitted mechanically by insect bites by those insects which have fed on an infected carcass. The disease is generally severe with the affected animal dying within a few hours of infection. Except for sudden death, the animals may not show any clinical signs. Tarry coloured, unclotted blood may ooze out of natural orifices. This blood contains the organisms which form spores on exposure to the atmosphere.

2.5.5 Factors influencing anthrax

Much has been written about the effects of season, temperature, rainfall, vegetation soil, host condition and population density on the epidemiology of anthrax, but little consensus exists on the roles played by these factors in the incidence of the disease. Majority of the studies are built on conception of conditions under which B. anthracis may germinate and multiply in the environment but strong scientific encouraging data are not available. No single model adequately explains the differing observations on the relationship between the factors listed above and the incidence and persistence of anthrax in a locality. Further detailed scientific
analysis is necessary to prove the effect of the risk factors listed above on the disease (Turnbull, WHO. 3rd ed.).

Anthrax mainly occurs in alkaline soils with high nitrogen levels caused by decaying vegetation, alternating periods of rain and drought and temperatures in excess of 16° C (Parker, et al., 2005).

2.5.6 Germination of spores

2.5.6.1 Germination in host

The bacillus, on entry into the host, transforms from spore to vegetative form by germination. It is in vegetative state in the infected host. They grow and multiply, eventually killing the host.

2.5.6.2 Germination in the environment

When conditions are not conducive to growth and multiplication of the vegetative forms of Bacillus anthracis, that is, when they are not inside the host, the fragile vegetative form dies immediately. Outside the host body, i.e., in the environment, they start to form spores.

Anthrax spores germinate in the environment if it is favourable to them, like temperature between 8°C and 45°C, pH between 5 and 9, relative humidity greater than 96% and presence of adequate soil nutrients in the soil (Sussman & Halvorson, 1966 cited in Anthrax OIE, 4th ed.).

2.5.6.3 Findings on Temperature, pH and Relative humidity.

According to World Organisation for Animal Health (OIE), there is a shortage of reliable data on germination, multiplication and sporulation of Bacillus anthracis under different conditions of temperature, water activity and pH. Perusal of literature revealed a few of the findings on these conditions worked out by the earlier researchers as listed below.

The laboratory study of Davies (1960) illustrates the extent to which temperature and relative humidity affect sporulation and how temperature affects germination. His study indicated that the quickest sporulation was at 37°C and 100% Relative Humidity (6 Hours.) and as the Relative Humidity decreased, the time required for sporulation increased. Further, with
decreased temperature (26°C), longer time was required for the sporulation. Similar results were obtained by Sussman and Halvorson in 1966 (cited in Anthrax OIE, 4th ed.)

As per reports (Anthrax OIE, 4th ed.) moist alkaline (pH 9) and calcium-rich environment favours spore survival for long periods.

2.5.7 Anthrax transmission

2.5.7.1 Disease acquisition

The vegetative form of *Bacillus anthracis* are shed from the infected animal to the environment. Once outside the infected host, the *bacilli* form spores. These spores become the source of infection to other animals when they are ingested by animals while grazing.

2.5.7.2 Disease Transmission

The anthrax infected animal dies with thick, tarry coloured bloody discharge from the natural orifices such as mouth, nostrils and anus. This fluid contains the *bacillus* which on contact with the air form spores. These spores contaminate the environment. The spores remain viable for long period. When the animals graze on such contaminated field, they get infected. Secondly, these spores get mixed with dry soil and get blown over distance as dust particles. The animals inhaling such contaminated air get infected. Similar mechanism is also put forth in humans while handling the infected carcasses for hide, hair or bone purpose (Henry 1936, cited in Anthrax OIE, 4th ed.).

2.5.7.3 Transmission due to deliberate release – bio aggression

Taking the advantage of the knowledge of the ability of the anthrax spores to survive in the environment and the devastating mayhem it brings in the human and animal population, the spores of anthrax were deliberately used during World War I and II by Germany, Japan and United Kingdom (Christopher, *et al.*, 1997). Similar tactics were used by Iraq during the Gulf war. It is on record that the accidental release of the spores from the research facility involved in experimenting on anthrax spores as bioweapons killed 66 people in Sverdlovsk in Russia during 1979 (Abramova, *et al.*, 1993; Meselson, *et al.*, 1994, cited in Anthrax OIE 4th ed.). Powder containing anthrax spores were mailed to government offices in United States of
America. The inhalation of these spores had caused disease in 22 people, killing five of them (Anthrax OIE, 4th ed.).

2.5.8 Treatment

Anthrax is generally a peracute disease in animals with hardly any time available for treating the animals. However, in the event of the disease being early diagnosed and available for treatment, Penicillin is the drug of choice. In humans where the disease is subacute to chronic, many antibiotics have been used along with the palliative supportive treatment.

2.5.9 Prevention

2.5.9.1 Vaccination

The disease in livestock of the endemic area is generally prevented by annual vaccination using live attenuated anthrax vaccine. Similar vaccine is available for human use.

2.5.9.2 Management methods

Since it is very difficult to eradicate the spores in an endemic area, a better management of the diseased and dead anthrax animals will prevent or reduce the re-occurrence of the disease. The major management practices are listed below.

- Isolation of the affected and in-contact animals
- The anthrax suspected carcass should not be opened.
- The carcass should be buried deep with sufficient quantity of lime so that the stray dogs do not dig it up. Wherever possible, the dead animal should be incinerated.
- Proper hygienic precaution should be taken by those handling carcasses or products from dead animals.

2.6 Anthrax in human beings

Natural case of human anthrax will be as a result of close contact with the diseased animal. It is generally seen in Veterinarians, para-vets and animal handlers who come in contact with the diseased animals. Based on the clinical signs, anthrax in humans is classified into cutaneous infection, inhalation infection, gastrointestinal infection and meningitis. Of these, the cutaneous infection is the most common. The infection sets in through the breaks in the
skin. The spores gain entry into the body through these breaks. This form is rarely fatal except in cases where the lesion obstructs the air passage or leads to meningitis. Inhalation infections are generally fatal due to the fact that they go undiagnosed till the late stages of the disease. The spores in the form of aerosol get inhaled while handling the diseased animal or as described earlier by deliberate release as in case of bio-warfare and get entry into the body.

The gastrointestinal infection occurs when the infected carcass is consumed. Since the spores can withstand high temperature, they probably survive the cooking process. Once they gain entry into the body, they multiply and cause the disease. This form of anthrax is more common in endemic countries where the poor and socio-economically backward people consume the infected meat. The infected people die suddenly and in majority of instances these cases go unreported. Meningeal infection can occur due to any form of anthrax. This is highly fatal. Such cases have been reported from India (George, et al., 1994).

The incubation period in humans varies with the form of the disease. The incubation period is usually 1 to 7 days. For cutaneous anthrax, symptoms appear after 2 to 3 days. For gastrointestinal form, the incubation period is usually 2 to 5 days, but may be as short as 15 hours also. The incubation period for inhalational anthrax varies a lot. This disease can appear after two days, but spores may remain viable in the lungs for several weeks (IOWA state University 2007).

2.6.1 Clinical signs

In Cutaneous anthrax, a skin lesion appears at the site of infection which is mostly painless and surrounded by edema. The lesions on the face or neck may sometimes lead to meningitis. The disease is curable if treated with appropriate medicine. However, in the untreated cases, secondary infection sets in with fever, pus and pain. Cutaneous anthrax has a low percentage of fatality (IOWA state University 2007).

The clinical signs of gastrointestinal anthrax are ulcers anywhere in the GI tract which may be haemorrhagic leading to obstruction and intestinal perforation. Gastrointestinal anthrax has been further divided into abdominal anthrax and oropharyngeal anthrax. The initial symptoms of the abdominal anthrax are low grade fever with mild gastrointestinal symptoms like nausea, vomiting, diarrhoea and anorexia. This is followed by the acute onset of severe gastrointestinal symptoms like severe abdominal pain, haematemesis and bloody diarrhoea.
In addition, there may be other signs of septicaemia. In severe gastrointestinal anthrax, people quickly go into shock, coma and then death.

The initial symptoms of oropharyngeal are sore throat, dysphagia, fever, hoarseness and swelling of the neck. The neck swelling is caused by edema and cervical lymphadenopathy, and can result in airway compression leading to death due to asphyxiation.

The clinical signs of inhalation anthrax appear slowly and are nonspecific. Inhalation anthrax starts initially with fever, chills, tiredness and malaise; a non-productive cough and mild chest pain may be present. The symptoms sometimes improve for several hours to three days. Then there is acute onset of severe respiratory distress, tachycardia, diaphoresis, stridor and cyanosis, followed by fatal septicemia and shock within one to two days (IOWA state University 2007).

2.6.2 Transmission

Anthrax is an infectious disease, but transmission from one person to another is generally not observed. However, transmission due to contact may occur in the cutaneous form of the disease.

2.6.3 Treatment

Effective treatment depends on early detection of the disease. Detecting cutaneous form is easier compared to inhalational and gastrointestinal form. So, treatment for cutaneous anthrax is usually effective. Mortality rate is more in inhalational and gastrointestinal forms as they are difficult to diagnose early (IOWA state University 2007). U.S Centers for Disease Control and Prevention (CDC) recommends antibiotics as the initial treatment. Antibiotics are effective only against the vegetative stage of *Bacillus anthracis* and not against spores. Supportive care such as oxygen inhalation, respiratory support and treatment of shock may be undertaken (Guidelines for Prevention and Control of Anthrax, WHO. 2006).

2.6.4 Prevention

Prevention of anthrax largely depends on preventing the disease in animals. Thus the disease management in livestock sector is more important. The foremost precondition for prevention is that the disease should be diagnosed early and the body of the confirmed and the suspected
cases should not be opened for necropsy examination. Such animals must be buried deep (approximately 6 feet) with sufficient quantity of lime. Care must be taken while importing the animal products from countries where anthrax has been reported. Proper hygienic measures should be practiced in industries dealing with livestock products such as hides, bones, hair etc. The workers should be educated about the disease and any sign of the disease should be reported to the medical authorities immediately. Animals should be vaccinated regularly to build up the herd immunity.

2.6.5 Vaccination

The herbivore animals are the natural host of anthrax. Thus control of the disease in these animals will indirectly help in prevention and control of human anthrax. Good management practices, proper disposal of the animals that has died due to anthrax and treatment of the disease wherever possible will go a great length in control of the disease.

Live attenuated anthrax vaccine is available in almost all countries that report the disease (Anthrax OIE, 4th ed.). Generally, annual vaccination is practiced in most of the countries. Some of the countries vaccinate only in the known endemic areas.

2.6.6 Disinfection

Anthrax spores are highly resistant to heat, sunlight, drying and many disinfectants, however they can be killed with formaldehyde (CH₂O) or 2% glutaraldehyde (C₅H₄O₂). A 10% Sodium hydroxide (NaOH) or 5% formaldehyde solution can be used for stockyards, pens and other equipment. Sodium hypochlorite (NaClO) has also been recommended. The sporicidal effectiveness of hypochlorite solutions varies with the pH and the concentration of free available chlorine. To become an effective sporicidal agent, household bleach can be diluted with water to increase the free available chlorine, and adjusted to pH 7. 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 minutes. Gamma radiation can be used to disinfect animal products. Exposed arms and hands can be washed with soap and hot water and then immersed for one minute in a disinfectant such as an organic iodine solution or 1 p.p.m. solution of mercuric perchloride. Clothing should be cleaned properly and boiled (IOWA state University 2007).
2.7 Geographic Distribution

The reports of Office international des épizooties, Paris, France (OIE 1997a) show that the disease is still enzootic in most countries of Africa and Asia, a number of European countries and countries/areas of the American continent and certain areas of Australia. It occurs sporadically in many other countries (Turnbull WHO/EMC/ZDI/98.6).

Anthrax is common in parts of Africa, Asia and the Middle East. In the United States, this disease has been reported from most states, but it occurs most often in the Midwest and West. As a result of continuous preventive measures taken, the disease is absent or sporadic in the middle and the higher latitudes of Europe and the Russian federation. In China, the disease is problematic in western China, but sporadic in the eastern provinces (Anthrax WHO 4th ed.). An estimated 20,000 to 100,000 cases occur in humans worldwide per year, mostly in developing countries (Turnbull 2008).

2.8 Anthrax in India

Anthrax in livestock is highly endemic in states of Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Kerala, Odisha, Jharkhand, Chattishgarh and West Bengal. The disease has also been reported from Madhya Pradesh, Gujarat, Rajasthan, Punjab and Bihar (Shivachandra, et al., 2016).

In India, anthrax is being reported since many years both from large and small ruminants. It is one of the top ten livestock diseases reported from the country. National institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) has compiled the reported anthrax outbreak data since 1987. It is observed from the data that the disease is endemic in India with southern India reporting more number of cases than the rest. Out of a total of 36 states (including union territories), 19 have reported the disease consistently during the period 1987 – 2016. The south Indian states top the list. There has been a constant variation in the percentage of states reporting anthrax (calculated as percentage of total states reporting disease data), but an overall increase was seen since 1999 onwards which has been attributed to the gradual spread of the disease. The occurrence of anthrax showed a progressive trend with a gradual increase in the number of outbreaks since 1992 with a peak seen between 2000 and 2002. From 2002 onwards, there is a gradual decline in the occurrence of anthrax. Figure
2.1 shows cumulative anthrax outbreaks with number of states reporting the disease (Suresh, et al., 2014).

![Anthrax Reporting States](image)

**Fig 2.1:** Cumulative anthrax outbreak reports with number of states reporting the disease in India (1987 – 2014)

Reproduced from Suresh et al. (2014).

In India, the occurrence of anthrax varies greatly in different districts of the country due to climatic and ecological factors. Anthrax being reported consistently by the states of southern India indicates not only the endemicity of the disease but also, good reporting system. Figure 2.2 shows national cumulative anthrax outbreaks.
Figure 2.2: District-wise cumulative Anthrax outbreak reports (1987-2014)

Reproduced from Suresh et al. (2014).