Anthrax: A ‘Quiescent Lethal Bug’ with Zoonotic Potential

Since antiquity, anthrax has been a pestilence of livestock and subsequently of humans due to the close contact with infected animal products or proximity to anthrax-succumbed animals and thereby, the disease is generally classified as anthropozoonotic. Anthrax has been reported as one of the most important zoonoses in terms of human health impact, livestock impact, amenability to agricultural interventions, severity of disease and emergence, with approximately 10-100 thousand human incidences annually throughout the world, with significant numbers of cases in Chad, Ethiopia, Zambia, Zimbabwe and India. Many of the cases go unreported due to lack of awareness and improper monitoring and surveillance systems of the country that masks its true burden, especially in developing nations. Hence, a proper understanding of the causative agent, the nature of the disease, its transmission and maintenance strategies, clinical signs in humans and animals and proper control strategies are the need of the hour to combat the endemicity of anthrax in many regions of the country.

Introduction

Etymology: The word “anthrax” is derived from the Greek word ‘anthrakis’, meaning coal, because of the characteristic dark necrotic skin-eschar due to the cutaneous form of anthrax infection. It is also known as Siberian plague, black bane, charbon, malignant pustule, malignant oedema, malignant carbuncle, milbrand, woolsorter’s disease (inhalational anthrax), ragpicker’s disease (inhalational anthrax), hide porter’s disease (cutaneous anthrax).

Timeline of Disease

The first clinical descriptions of anthrax were given by Maret in 1752 and Fournier in 1769. In 1877, Robert Koch cultured B. anthracis and demonstrated the microbial etiology of an infectious disease in the famous “Koch postulates”. Louis Pasteur took Koch’s work a step further and in 1881 he demonstrated the life cycle of the pathogen, and also prepared a vaccine for anthrax. Anthrax has at times been used as a biological weapon and currently, the CDC categorises it as a category A biological agent. During World War I, German agents acting in the US were believed to have injected anthrax into horses, mules, and cattle. Anthrax as a bio-weapon came into the limelight in 2001 via letters containing milled anthrax spores mailed to US news organisations and Congress, resulting in 22 cases, five of whom died. Anthrax spores lend themselves well to aerosolisation and resist environmental degradation. Moreover, these spores, at 2-6 microns in diameter, are the ideal size for impinging on human lower respiratory mucosa, optimising the chances for infection to occur.

Microbiology and Biochemical Characteristics of Bacillus anthracis

Bacillus anthracis is a Gram-positive, aerobic or facultative anaerobic, non-motile, endospore-forming, rod-shaped bacterium. In blood smears, smears of tissues and in lesion fluid from diagnostic specimens, the organism appears in chains of two to a few cells in length. Smears of in vitro cultures appeared as endless strings of cells with square ends (“boxcars”). In the presence of oxygen and towards the end of the exponential phase of growth, a centrally/subterminally placed spore is formed in each cell. In the absence of oxygen and under a high partial pressure of CO₂ / in the presence of bicarbonate (HCO₃⁻), the vegetative cell secretes its polypeptide (poly D glutamic acid) capsule which prevents phagocytosis by host immune cells. MacFadyean (polychrome methylene blue stain) reaction stains blue bacilli with purple capsules.

Other characteristics of B. anthracis:

- Non-haemolytic colonies on blood agar
- Sensitive to the diagnostic ‘gamma’ phage and penicillin
- Egg yolk reaction +ve
- Oxidase +ve
- Catalase production +ve
- Gelatin hydrolysis +ve
- Nitrate reduction +ve

Phylogenetically, B. anthracis belongs to the group of spore forming soil bacilli known as Bacillus cereus sensu lato. B. anthracis appears to be one of the most monomorphic species known, i.e. isolates from whatever type of source or geographical location are almost identical phenotypically and genotypically. Recent molecular techniques enabled the determination of the phylogenetic relationships among isolates worldwide into two major clonal groups, A and B.

Characteristic colonial morphology: On blood agar the colonies have irregular borders and are non-haemolytic in nature, and on nutrient agar the characteristic colonies are described as “Medusa head” or “Comet tail”. The most commonly used selective media is polymyxin- lysozyme-EDTA-thallous acetate (PLET) agar. The inverted fir tree appearance is depicted in liquid medium.

Nature of the spore: “sporulate or die” concept: Vegetative forms shed by the dead animal die rapidly in most environmental conditions and they depend on the availability of the next animal host for their survival, and the environmental persistence of anthrax solely depends on their sporulation capacity.

A salient ability of anthrax spores to persist in the soil and other environments for decades has been noticed. It has been found that anthrax spores have survived in dry soil for 60 years. Unaffected by harsh environment, spores are resistant to...
high temperature, pressure, pH, chemicals, UV and deficiency of nutrients. The longest survival claim is probably from the bones retrieved during archaeological excavations at a site in the Kruger National Park, South Africa, which were estimated by carbon-dating to be 200 ± 50 years old².

**Pathogenesis:** *B. anthracis* is non-invasive and the spores must gain access to sub-epidermal tissue through a cut or abrasion before infection can occur¹.

Two plasmids are essential for the virulence of *B. anthracis*: pXO1 and pXO2. Plasmid pXO1 harbours genes for the anthrax tripartite toxin complex, which consists of three synergistically-acting proteins, protective antigen (PA, 83kDa), lethal factor (LF, 90 kDa) and oedema factor (EF, 89 kDa) which is produced during the log phase of its growth.

Plasmid pXO2 carries genes required for the biosynthesis of an anti-phagocytic poly-c-D-glutamic acid capsule (capBCA). Protective immunity against anthrax requires antibodies against the components of anthrax toxin, primarily protective antigen. Both the non-cellular human vaccines and live-spore animal vaccines confer protection by eliciting antibodies to protective antigen.

**Cycle of Infection:**
The bacilli maintain their cycle through the uptake of spores from the environment, so that anthrax is contracted to the grazing animals. Within the infected host, the spores germinate to produce the vegetative forms which multiply, eventually killing the host. A proportion of the bacilli released by the dead animal into the environment (usually soil under the carcass) sporulate, ready to be taken up by another animal, and the cycle continues.

Climatic conditions enhance the effect, either directly by influencing the way in which the animal makes contact with the spores, for example, infection usually by grazing closer to the soil in dry periods when the grass is sparse, or due to enforced grazing at restricted sites when water becomes scarce. The spiky grass and grit produce oro-gastrointestinal lesions and, since *B. anthracis* is apparently non-invasive, these lesions help for the initiation of infection and may affect indirectly the general health of the animal and its level of resistance to infection. Studies have shown that soils that are calcium-rich with neutral-to-alkaline pH can act as ‘favourable sites’ for anthrax spore development, and these in turn are called ‘anthrax belts’. Calcium is integral to the dehydration of vegetative cell genome precursors, which is necessary for its effective long-term storage in the spore. Livestock may acquire the disease continents away from the original infection source, through contaminated feedstuffs or from spores that have reached fields via industrial effluents. Biting flies, particularly Hippobosca and Tabanus species, were considered important as mechanical transmitters of disease.

**Clinical Signs in Animals:**
**Host Range:** Anthrax has been documented in a wide variety of warm-blooded animals. Some species, such as rats, chicken and dogs, are quite resistant to the disease, whereas others (notably herbivores such as cattle, sheep, and horses) are highly susceptible. Humans have intermediate susceptibility to the disease.

**Bovines, Sheep and Goats:** In bovines, anthrax often occurs as a peracute condition or as an acute febrile disease, although subacute disease with throat swellings may be encountered in tropical countries, possibly through buccal lesions from the chewing of infected bones. If no treatment is given, death usually occurs in 2-3 days, with the animal showing cramp-like symptoms and shivering. The urine may be blood-stained and blood may exude from the rectum and all other natural openings. Often advanced signs are minimal, and the apparently healthy animal may fall in an apoplectic seizure and die within a few minutes to a few hours.

**Horses:** Horses may show acute symptoms and die in 2-3 days. Intestinal lesions may result in colic and diarrhoea. Large oedematous lesions on the breast, abdomen, neck and shoulders may notice with cases transmitted by biting flies. Pigs: Pigs are regarded as more resistant to anthrax than cattle, sheep, goats and horses. Their greater resistance is reflected in the greater evidence of localised signs, such as swellings of the throat and pharyngeal and cervical lymph glands.

**Dogs and Cats:** Dogs are considered as very resistant to anthrax. Dogs that have scavenged anthrax carcasses may suffer severe inflammation and oedematous swelling of the throat, stomach, intestine, lips, jowls, tongue and gums.

**Birds:** The apoplectic type of death is usual, although less acute infection, with carbuncular lesions on the comb or extremities, also occurs. The lesions of anthrax in the ostrich, duck and eagle are similar, with haemorrhagic enteritis and oedematous swellings, particularly in the neck.

**Vulture and Anthrax:** There are contradictory views about the role of vultures in anthrax transmission. The most supported view is that water gets polluted by the anthrax organism when vultures “wash” their beaks and feathers and this is referred to as “the water cycle” of anthrax⁴. On the other hand, it has also been assessed that by rapidly consuming the bacilli-laden soft tissues of the anthrax victim before the anthrax bacilli have a chance to sporulate (the vegetative bacilli are destroyed in the vultures’ digestive tracts), vultures minimise residual contamination and therefore have a role in curtailting spread of the disease.

**Disease in Humans:** Anthrax in humans is classically divided in two ways. The first type of classification, which reflects how the occupation of the individual led to exposure, differentiates between non-industrial anthrax, occurring in farmers, butchers, knackers/renderers, veterinarians and so on, and industrial anthrax, occurring in those employed in the processing of bones, hides, wool and other animal products. The second type of classification reflects the route by which the disease was acquired, i.e., cutaneous anthrax, inhalation anthrax and alimentary form.
Cutaneous Anthrax: Accounts for > 95% of human cases globally. The cutaneous form is less fatal and more often self-limiting. The incubation period ranges from as little as nine hours to three weeks, mostly two to seven days.

Anthrax eschars are generally seen on the exposed, unprotected regions of the body, mostly on the face, neck, hands and wrists. The common description of “malignant pustule” is actually a misnomer, because the cutaneous lesion is not purulent and is characteristically painless. A painful, pustular eschar in a febrile patient indicates a secondary infection, most often with staphylococcus or streptococcus.

Malignant oedema is a rare complication characterised by severe oedema, induration, multiple bullae, and symptoms of shock. Malignant oedema involving the neck and thoracic region often leads to breathing difficulties that require corticosteroid therapy or intubation.

Inhalation Anthrax: The inhaled spores are carried on by the macrophages from the lungs, where there is no overt infection, to the lymphatic system, where the infection progresses. The typical clinical course of this form of the disease is consistent with the lesion development within the mediastinal lymph nodes before the development of bacteraemia. As initial symptoms resemble those of flu, its early diagnosis is difficult; by the time disease is correctly recognised it is too late.

Ingestion (Oral Route/Enteric) Anthrax: The incubation period is commonly three to seven days. There are two clinical manifestations of anthrax that may result from ingestion of B. anthracis in contaminated food or drink – oropharyngeal anthrax and gastrointestinal anthrax. The oropharyngeal form is the less commonly seen.

Oropharyngeal anthrax is mainly reported in persons eating undercooked meat. Intestinal manifestations presumably result from the presence of spores which survive in the gastric juices. The main clinical features are sore throat, dysphagia and painful regional lymphadenopathy in the involved side of the neck.

The gastrointestinal anthrax lesion may occur anywhere within the gastrointestinal tract, but mostly in the ileum and caecum. The lesion is generally ulcerative, usually multiple and superficial, surrounded by oedema. These lesions may bleed, resulting in fatal haemorrhages and in some cases leading to stomach infection.

Anthrax Meningitis: Meningitis due to anthrax is a serious clinical development which may follow any of the other three forms of anthrax. Anthrax meningitis is a haemorrhagic leptomenigitis with the symptoms of neck pain with or without flexion, headache, alteration in mental state, vomiting and high-grade fever.

In countries such as Thailand, ingestion anthrax is often associated with the consumption of undercooked meats and in sub-Saharan Africa, the value of the meat from an animal that has died unexpectedly outweighs the perceived risks of illness that might result from eating it.

Industrial anthrax incidence data can be inferred from the volume and weight of potentially affected materials handled or imported, taking into account the quality of prevention, such as vaccination of personnel and forced ventilation of the workplace.

Diagnosis:

Blood Smear Examination: In most species which die of anthrax (the pig being a notable exception) the blood is usually tarry and unclotted, teeming with the capsulated anthrax bacilli from all its natural orifices. In a carcass that remains intact and unsacavenged, care should be taken to obtain the minimum drops of blood for performing smears and/or cultures by means of a syringe from an appropriately accessible vein (generally an ear vein) within about 24 hours after death.

B. anthracis does not compete well with the putrefactive bacteria and, with increasing age of the carcass, the capsulated bacilli become more difficult to visualise. When a carcass is old or putrefied, B. anthracis can often be cultured from residual skin or bloodstained materials.

In pigs, body-fluid smears made from fine needle aspirates of the affected area, such as submandibular swellings, regional lymph nodes or mesenteric fluid would be appropriate, in which terminal bacteraemia is invariably low compared with most other species.

Milk is not generally regarded as being useful for diagnostic purposes. There appear to be very few reports of isolation of B. anthracis from the milk of affected animals. If the carcass has been opened and residual lymph node or spleen samples are available, these should be taken for culture. Diagnosis becomes increasingly dependent on the time of isolation of spores from surface swabs of the remaining skeleton, particularly in the nostrils and eye sockets, or from soil or other environmental samples around or under the carcass that had been contaminated by the oronasal or anal exudates and spillages of body fluids.

It may not be possible to find the bacilli in smears or to isolate B. anthracis from animals that were treated before death; treatment can sterilise the blood and tissues but, if sufficient toxin has been formed, the animal may still die. Tests based on antigens, if available, may be the only practical approach.

Diagnosis Based on Tests for Antigens

The thermostable antigen precipitin test devised by Ascoli in 1911 is still used in several countries for detecting residual antigens in tissue in which it is no longer possible to demonstrate B. anthracis microscopically or by culture. However, it is not a highly specific test; the antigens being
detected are shared by other Bacillus species. Care has to be taken if the tissue being examined has been grossly contaminated with environmental materials (soil, sand, etc.) which frequently harbour large numbers of other Bacillus species.

**Molecular and Serological Diagnosis:**
PCR is becoming more widely available as a means of confirming the presence of the virulence factor (capsule and toxin) genes, and hence to confirm an isolate is, or is not, virulent *B. anthracis*.

Currently accepted as the best serological procedure is the ELISA in microtitre plates coated with the protective antigen (PA) and lethal factor (LF) components of the anthrax toxin. The toxin antigens appear to be truly specific for *B. anthracis*.

**Differential Diagnosis:**
For differential diagnosis, other causes of sudden death should be considered. Among these are African horse sickness, botulism, blackleg (*Clostridium chauvoei*), peracute babesiosis, chemical poisoning (heavy metals, other poisons), ingestion of toxic plants, snake bite, lightning strike or metabolic disorders such as lactic acidosis, magnesium deficiency, bloat.

**Vaccination and Treatment**
The attenuated live vaccine strain developed by Sterne in 1937, which is still the basis of most anthrax vaccines for livestock, lacks pXO2 and is therefore Cap– Tox+. The protection afforded by such vaccines apparently is related primarily to antibodies specific for the protective antigen component of the toxin.

Most anthrax vaccines for animals in use around the world today utilise the toxigenic, non-capsulating (pXO1+/pXO2–) *B. anthracis* strain 34F2 derived from a virulent bovine isolate in the 1930s.

All animal vaccines are live vaccines, and their use requires a withholding period prior to slaughter for human consumption. This period varies from three to six weeks. There is no withholding period for milk destined for human consumption following vaccination of milking animals with Sterne 34F2 strain vaccine.

**Treatment**
Pencilllin was considered as the ideal drug for anthrax. But inducible β-lactamase production in a number of strains is a major factor of concern. A second reason given for concerns about the use of penicillin was the poor penetration of β-lactams into macrophages, the site where *B. anthracis* spores germinate.

Many experts consider ciprofloxacin (400 mg intravenously (i.v.) q 12 h) as the drug of choice for treating victims of terrorism or warfare. Doxycycline (100 mg i.v. q 12 h) is an acceptable alternative, although rare doxycycline-resistant strains of *B. anthracis* are known.

**Control Measures**
Control measures should be aimed at breaking the cycle of infection. The following actions must be rigorously implemented:

- Cut off infection source
- Dispose of anthrax carcasses correctly
- Correctly disinfect, decontaminate and dispose of the contaminated materials
- Vaccinate exposed susceptible animals and, where possible, humans in at-risk occupations

**References:**
5. Ascoli, A. [Precipitin diagnosis of anthrax]. Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. 58: 63 (1911)

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**Dr Pankaj Dhaka**, Ph.D. Scholar, Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, India. Has international papers and many popular articles on animal health and zoonoses published in various journals and magazines. Awarded with various scholarships including, UGC-JRF: Social Medicine and Community Health and ICAR-JRF Scholarship. Email: pankaj.dhaka2@gmail.com

**Dr Deepthi Vijay**, Assistant Professor, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India. Has experience of writing international research papers and many popular articles on zoonoses, food safety and public health in various journals and magazines. Email: deepschinnus@gmail.com