

Review Article

Anthrax: An Emerging Zoonotic Disease in Bangladesh

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Introduction

Anthrax has emerged as a zoonotic disease in Bangladesh during 2009-2010. The human outbreaks were preceded by animal outbreaks. The animal anthrax known as 'Torka' is believed to be enzootic for long in Bangladesh. The disease caused by the bacterium *Bacillus anthracis* is primarily a disease of herbivores and was one of the main causes of uncontrolled mortality in cattle, sheep, goats, horses and pigs worldwide until the development of an effective vaccine and advent of antibiotics. Although the organism has always been high on the list of potential agents with respect to biological warfare and bioterrorism, humans almost invariably contract the natural disease directly or indirectly from animals or animal products.¹⁻⁵

The organism

Bacillus anthracis is a Gram-positive, rod-shaped nonmotile bacterium, the only obligate aerobic pathogen in the genus *Bacillus*. It has both vegetative and spore forms. Within low oxygen environment of the host, the organisms exclusively remain in the vegetative form. When conditions are not conducive to growth and multiplication, the vegetative forms start to form spores. Sporulation requires the presence of free oxygen. Spores are formed in cultures, in the soil, and in the tissues and exudates of dead animals. Spores are ellipsoidal or oval and are centrally located and are markedly resistant to biological extremes of heat, cold, pH, desiccation, chemicals, irradiation and other such adverse conditions.^{3,6-8} The organisms grow well on most common laboratory media but for demonstration of characteristic colonial morphology, specimens should be inoculated on 5% blood agar free of antibiotics. Maximal growth is obtained at pH 7.0-7.4 under aerobic conditions at 37°C. The organisms produce large, raised, opaque, grayish white, plumose colonies 2-3 mm in

diameter, with an irregular, fringe like edge with no haemolysis. Tangled masses of long hair like curls can be seen with a colony microscope. The colony is membranous in consistency and emulsifies with difficulty. Though identification of typical virulent strains from clinical material is relatively simple but identification of strains from nonclinical material may be more difficult. *B. cereus* is easily mistaken for *B. anthracis*. The organism appears to be one of the most monomorphic species known, i.e. isolates from any source or geographical location are almost identical phenotypically and genotypically. In the past five years important progress has been achieved in determining phylogenetic relationships among isolates worldwide through multilocus variable number tandem repeat (VNTR) analysis (MLVA).^{3,9-10}

Global Situation

Through successful national programmes, there has been a progressive global reduction in animal anthrax cases over the past three decades. The disease is now absent or only sporadic in the West Europe and the North America. Anthrax is hyperendemic in many countries of Africa with epizootic form in Chad and Ethiopia. In the Middle East, the disease is sporadic. Various levels of endemicity occur in central Asian countries, in western China and some other Southeast Asian countries. Anthrax is a severe problem in southern and eastern India. Humans are moderately resistant to anthrax and the incidence of anthrax in humans is low. Reported human: animal case ratios in a country reflect the economic condition, quality of surveillance, social traditions and dietary behaviour. Whereas in northern Europe, there has been one human infection per 10 livestock cases, in Africa and Asia, there can be some 10 human cases per one livestock infection. There are approximately 10-100 thousand human incidences annually throughout the world with significant numbers of cases in Chad, Ethiopia, Zambia, Zimbabwe and India.^{4,11-15}

Cycle of infection

The spore is central to the cycle, although vegetative forms may also play a role in establishing infection. *B. anthracis* is not an invasive organism; it gains entry through breach of

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lining of surfaces. LD₅₀s ranges from <10 spores in susceptible herbivores to >10⁷ in more resistant species. The LD₅₀s by inhalational or oral routes are much higher as compared with parenteral challenges in susceptible laboratory animals. Animals acquire anthrax by ingestion of spores while grazing or browsing. Flies appear to play an important role in explosive outbreaks. Inhalation within dust may be important on occasion. Direct animal to animal transmission is insignificant.^{4,16-17}

Incubation period (IP)

Incubation period varies from few hours to even up to 60 days. For cutaneous anthrax, IP ranges from few hours to 3 weeks but most often it is 2 to 6 days; for gastrointestinal anthrax the average IP is 3-7 days. In inhalation anthrax, the median incubation period is 4 days, but may be up to 10 or 11 days.⁴

Pathogenesis

Humans become infected in one of three ways – percutaneous, inhalation and gastrointestinal. In cutaneous route, the organisms gain access through small abrasions or cuts and multiply locally with an inflammatory response. In inhalation anthrax, the organisms multiply in the lung, and are swept to the draining hilar lymph nodes leading to marked haemorrhagic necrosis. In ingestion anthrax, organisms are ingested with infected meat, with resultant invasion and ulceration of the gastrointestinal mucosa. From all three forms, invasion of blood stream and profound toxemia may occur. Metastatic infections such as meningitis may complicate the primary process. Anthrax infection in humans provides permanent immunity and second attacks are rare. Though in general, the case fatality rate (CFR) due to anthrax has been reduced due to antibiotics but all three forms are potentially fatal if untreated and CFR is substantial in situation of use anthrax as biological weapon.¹⁸⁻²²

Clinical manifestation

Cutaneous anthrax accounts for more than 95% of human cases. It begins 2 -5 days after infection as a small papule resembling an insect bite developing within a few days into a vesicle filled with dark bluish black fluid. Rupture of the vesicle reveals a black eschar at the base with a very prominent inflammatory ring around the eschar. This is sometimes referred to as malignant pustule. The lesion is classically found on the hands, forearms or head and is painless. It is rarely found on the trunk or lower extremity. Generally, cutaneous lesions are single, but sometimes two or more lesions are present. The cutaneous form is more often self-limiting and with treatment, CFR is < 1% cases are fatal (Gold, 1967).²²⁻²⁵

Ingestion anthrax

There are two clinical manifestations of ingestion anthrax –

oropharyngeal anthrax and gastrointestinal anthrax. Oropharyngeal lesion is generally localized in the oral cavity covered with a grey pseudomembrane surrounded by extensive oedema. The main clinical features are sore throat, dysphagia and painful regional lymphadenopathy in the involved side of the neck. The gastrointestinal anthrax lesions are ulcerative and occur mostly in the ileum and caecum. The lesions may lead to haemorrhage, obstruction, perforation or any combination of these. Some cases are complicated with massive ascitis and this leads to shock and death.^{22, 26,27}

Inhalational anthrax

Inhalation anthrax occurs in persons who handle raw wool, hides or horsehair through inhalation of spores. The symptoms are typically those of respiratory tract infection with fever, malaise, myalgia and an unproductive cough. Within several days, it rapidly progresses to a severe infection with marked respiratory distress and cyanosis, with the sudden worsening and death may occur within 24 hours.⁴

Anthrax meningitis and sepsis

Meningitis due to anthrax is a serious clinical development with symptoms of neck pain with or without flexion, headache, changes in mental state, vomiting and high-grade fever. A markedly elevated cerebrospinal fluid (CSF) pressure and the appearance of blood in the CSF are followed rapidly by disorientation, loss of consciousness and death. Anthrax sepsis develops after the lymphohaematogenous spread of *B. anthracis* from a primary lesion. Clinical features are high fever, toxemia and shock followed by death.^{21,29-34}

Laboratory Diagnosis

Microscopy with Gram stain and Polychrome methylene blue stain reveals respectively Gram positive rods and the capsule. On site anthrax specific antigen test by ICT may be done. Culture of specimen obtained from skin lesion, sputum or blood will confirm the diagnosis.

Direct Polymerase Chain Reaction (PCR) on clinical specimens is regarded as an acceptable diagnostic procedure. Immunohistochemistry of pulmonary effusions or of bronchial biopsies is often proved to be valuable. In the case of patients who survive, antitoxin antibodies may be demonstrable in convalescent sera. Post mortem examination may reveal the capsulated *B. anthracis* usually visible in capsule-stained smears of these fluids, and should be easily isolated from them by bacteriological culture. Again, tests for toxin or PCR on these fluids would still be positive when treatment has rendered them smear- or culture-negative.^{4, 26, 27,36-42}

Treatment of anthrax

B. anthracis is quite responsive to antibiotic if administered early in the course of infection. Ciprofloxacin and doxycycline

have received high profiles as primary treatment alternative in recent years. Doxycycline has the disadvantage of poor penetration to the Central Nervous System (CNS). In severe life threatening cases, combination therapy with penicillin/ciprofloxacin and another antibiotic, preferably one which has good penetration into CNS are advisable. Clarithromycin, clindamycin, vancomycin or rifampicin are suggested for pulmonary anthrax and vancomycin or rifampicin is preferable for anthrax meningitis. *B. anthracis* is not susceptible to cephalosporin or trimethoprim-sulfamethoxazole. Although susceptible to penicillin and ampicillin, due to presence of inducible beta-lactamase, these drugs are not recommended.^{4,43}

Prevention of Anthrax

Control of human infection ultimately depends on control of the disease in animals. To cremate the carcass or to bury it deeply in lime to prevent soil contamination. Burning of the field may also be required. Active immunization is the only known method of preventing anthrax in herbivorous animals in areas where the pasture land is already contaminated with spores. Pasture's famous attenuated living anthrax vaccine was effective but difficult to maintain at a desired level of virulence. It has been superseded by a living spore vaccine derived from a nonencapsulated strain of *B. anthracis* (Sterne strain). The widespread use of a living spore vaccine in South Africa has reduced the incidence of anthrax in cattle of this area by more than 99%.⁴

Human Immunization

For the protection of humans in high-risk work situation, a nonliving vaccine consisting of aluminum hydroxide-adsorbed supernatant material from fermentor cultures of a toxigenic but nonencapsulated strain of *B. anthracis* is used. The antigen appears to be effective but requires multiple doses over long periods of time. The cell free filtrate vaccine is available for veterinarians and others who work with livestock. Six inoculations over an 18-months period are 93% effective. Only four countries can produce the vaccine. In the USA, there is no vaccine for civilian use, but since 1998, all military personnel receive the cell-free filtrate vaccine. Prolonged antibiotic prophylaxis is only recommended to those exposed to deliberate use of anthrax spore. Antibiotic prophylaxis should not be used for naturally occurring anthrax. Use of personal protective equipment has to be encouraged.⁴

Bangladesh situation

Though there are not many reports, but anthrax in animal locally known as 'Torka' is believed to be enzootic in Bangladesh. Sixty two animal infections were recorded with 69% deaths from Pabna milk shed areas during 1980-84. In another report, there were found to be 333 animal cases during 1989-96 from Bangladesh. A number of outbreaks of

animal anthrax were reported during 2009-10 infecting 140 cattle and goat in different districts. The country has an estimated 23 million cattle, 1 million buffalo, 21 million goat and 3 million sheep. To prevent anthrax vaccination is practiced in the country since long. In 2009-2010, the total vaccine production was 38.29 million doses. Various reports indicate that the disease is more prevalent in areas-Pabna, Sirajganj and Tangail having greater cattle population.^{2,4,44}

Like that of animal anthrax, reports for human anthrax are also lacking. So the true picture of anthrax is not available. As the disease is thought to be enzootic and in agricultural setting, humans always get infection from infected animal, so anthrax is also likely to be prevailing in the country in endemic form. Samad and Haque (1986) reported 27 human cases of anthrax during 1980-84 and a research team of Institute of Epidemiology, Disease Control and Research (IEDCR) detected 19 cases out of 624 tannery workers of Dhaka city in 1997. In 2009 and 2010, there were twenty nine human anthrax outbreaks with 706 outbreaks in 12 districts.^{2,4,44}

Table I: Reported findings of anthrax in animal and humans in Bangladesh

□	Year	Animal case	Human case
□	1980	6	00
□	1981	7	00
□	1982	16	17
□	1983	21	10
□	1984	12	-
□	1989-96	333	-
□	1997	00	19
□	October 2009 – June 2010	55	99
□	August 2010 – October 2010	140	607
□	Total □	450 □	725

All the human cases were cutaneous anthrax and no inhalation or ingestion anthrax was ever reported from Bangladesh. The skin lesion was characterized by the presence of papule and/or vesicle, ulcer, erythema, central black eschar, surrounding oedema and tenderness. The lesions were mostly distributed on the upper limbs (75%), but were also present on lower limbs, face, chest, back, neck and scalp. Almost all of the cases with cutaneous lesion (97%) had slaughtered sick cattle or goats, or handled raw meat or were present at the slaughtering site.²

Microbiologists of the IEDCR identified *Bacillus anthracis* from three vesicular swab samples tested from the first outbreak and from eight out of 12 vesicular samples tested from the fourth outbreak and did the sensitivity test. All the isolates were sensitive to ciprofloxacin and doxycycline, which were identified as the drug of choice for the cases.² Centre for Disease Control (CDC), USA isolated *B. anthracis* from swabs of two patients. Multiple-locus variable-number

tandem repeat analysis (MLVA) was performed at the CDC on the animal and human isolates and confirmed that both were infected by isolates of the same genotype. *B. anthracis* was also detected in three tissue biopsies by Immunohistochemistry. It was also detected from four vesicular swab smears by M'Fadyean staining. Fifteen of the 26 case-patients, who had acute and convalescent sera tested at CDC, exhibited a four-fold increase in anti-PA (protective antigen) titre.²

Conclusion

Anthrax is rare in humans but occur among cattle in many parts of the world. *B. anthracis* remains in two forms: vegetative and spore. Cattle get infection exclusively with spore form. Human gets infection both in spore and vegetative forms. Human gets infection from three sources - agricultural setting, industrial setting and deliberate use (bioterrorism). Human infection occurs in three forms cutaneous, intestinal and pulmonary. Cutaneous infection is the most common (>95%). It can be caused by vegetative form or spore form and the organism enters through breached skin. The clinical diagnosis is made with epilink, characteristic ulcer, microscopy and culture. For uncomplicated anthrax, ciprofloxacin is the drug of choice. Prevention of human infection is based on prevention of anthrax in cattle through vaccination, avoidance of slaughtering sick cattle and use of PPE during handling of carcass.

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