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Anthrax and Bio-terrorism

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ABSTRACT

Threat of terrorism has compelled to attract the attention to the risk posed by various microorganisms as biological weapons. Anthrax is primarily a disease of herbivorous animals, although all mammals including humans and at least some avian species can contract it. The disease has world wide distribution and is a zoonosis. Circumstantial evidence indicates that man is moderately resistant to anthrax. Cutaneous anthrax is said to account for 95% or more of human cases globally. Molecular techniques can help in identifying the diversity of the pathogen. plasmid pXO1 is of 184.5 kb pairs and encodes toxin complex, which consists of three synergistically acting proteins i.e., Protective Antigen(PA,83 KDa), Lethal Factor(LF, 87 KDa) and Oedema Factor (EF, 89 KDa) is produced during the log phase of growth of *B anthracis*. Anthrax in human is classically divided in two ways. The first type of classification, which reflects how the occupation of the individual led to exposure of anthrax occurring in farmer, butcher, knackers, veterinarians and so no is non industrial type. Other type is industrial anthrax, occurring in those employed in bones, hides, wool and other animal products. Prompt and timely antibiotic therapy usually results in dramatic recovery of the individual or animal infected with anthrax. Almost all isolates of *B anthracis* can be expected to be highly sensitive to penicillin and being cheap and readily available in most parts of the world, this remains the basis of treatment schedules. Despite improvements in treatment and prophylaxis, anthrax considered as a fatal infection. Biowarfare attacks by anthrax agents are now a serious possibility. Primary prevention depends on creating a strong global norm that stops development of such weapons. Secondary prevention depends on early detection and its prompt treatment.

Key words: Anthrax and Bio-terrorism.

INTRODUCTION

In recent times the increased threat of terrorism has compelled to attract the attention to the risk posed by various microorganisms as biological weapons Biological warfare agents are more potent than conventional and chemical weapons. During recent past, better understanding of biochemistry and immense progress in biotechnology has smoothened and simplified the development and production of biowarfare agents. Besides this, genetic engineering also potentiates its development. Wide availability of biological agents, easy process of production along with smoothly available technical know how increased its demand of possession by various developed and developing nations. Among the numerous bioterrorism agents anthrax may be an effective biological weapon since it is easy to culture, readily form spores, and can be aerosolized, which remain viable for years infecting soil and other materials long after initial attack[1]. Besides this it can cause wide spread illness and death and eventually cripple a city or region[2].

The Centre for Disease Control and Prevention (CDC), Atlanta, Georgia has categorized

bioterrorism agent into three categories. In the first category (CatagoryA) there is *Bacillus anthracis*. As per category definition this type of agents with high priority pose a risk to national security because they can be easily disseminated or transmitted from person to person, resulting in high mortality rate and have the potential for major public health impact leads to public and social disruption which compelled for requirement of special action for public health preparedness. Transmission of *Bacillus anthracis* is some how well known, which reveals a truth that "Inhalation form" most likely occurs following an intentional release, results from inhaling anthrax spore[3]. Mortality rates in inhalation anthrax patients are very high despite appropriate antibiotic treatment. On 1972, a convention on the "Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction" also known as BWC, was held and it prohibited offensive bio- weapons research and development and was signed by most countries[2]. However former Soviet Union and Iraq, both signatories of the convention, have subsequently acknowledged offensive bio-weapons research and production[4]. Demonstration by the "anthrax letter" in the following time of World Trade Center attack in September 11, 2001, the occurrence of only a small number of infections can create an enormous psychological breakdown and uncertainty feeling, leads to the belief that anthrax as a bio-terrorist weapon possessed a high credibility in coming era. This threat is a high challenge for medical community to prepare and educate itself for response as perfectly as possible to this terrorism.

History

Anthrax is primarily a disease of herbivorous animals, although all mammals including humans and at least some avian species can contract it. The disease has world wide distribution and is a zoonosis. From earliest historical records until the development of effective veterinary vaccine mid-way through the present century, together with the subsequent advent of antibiotics the disease was one of the foremost causes of uncontrolled mortality in herbivores i.e. cattle, sheep, goats, horses and pigs. The major sources of human anthrax infection in its various forms (inhalation, cutaneous and gastro- intestinal) are direct or indirect contact with infected animals, or occupational exposure to infected or contaminated animal products. Other possible sources are rare and epidemiologically trivial. The bacillus responsible for causing anthrax has a long history of interaction with humans. Described as far back as 1500 BC, it is thought to be the etiologic agent of the fifth Egyptian plague[5]. It has been suggested in texts of antiquity that the famous plague of Athens (430-427 BC) was an epidemic of inhalation anthrax[6]. The species name is derived from Greek word "anthracites", means coal like, indicative to the typical black eschar seen in the cutaneous form of the disease. There are accurate records of infections in ancient Rome depicted by the Roman Poet Virgil (70-19 BC)[7] in his third "Georgics" which is devoted to animal husbandry and contains a section of veterinary medicine. He detailed an epizootic that occurred in the Noricum district of Rome (ancient name of the Danube River delta and the eastern Alps), where disease affected cattle, horses, sheep as well as dogs and other domestic and wild animals. The description of the symptoms of anthrax are narrative with poetic touch, which non-considering some factual materials expresses the understanding of Virgil about the infectious source, as well as the potential for transmission among animals and humans. Over the centuries, it was known as the "Black Bane" and the "malignant pustule" due to its cutaneous manifestations, although it is neither pustulant nor necessarily deadly. Other names include "Rag pickers' disease" Charbon, Milzbrand, "Tanners' disease", Siberian (splenic) fever etc. It was also known as " Wool sorters' disease" because of occupational hazards frequently observed among mill workers exposed to animal fibres contaminated with *B. arthracis* spores[4,5].

In 19th century, anthrax was a major point of interest in biological research. Pierre Rayer and his assistant Casimir – Joseph Davaine in 1850 were the first to observe non motile, filiform bodies in the blood of sheep having died of anthrax. In 1855 Pollender, 1857-58 Brauell, 1859 Fuchs and 1860 Delafond also identified that same agents in anthrax affected animals. Among them Brauell, in 1857-58 first reported the transmission of anthrax from man to sheep. In a series of studies between the years 1863 and 1868, Davaine definitely established the presence of filiform bodies in the blood of animals,

which had died of anthrax. He gave the name "bacterides" to these bodies[8].

In 1871, Tiegel and in 1877 Louis Pasteur and Joubert, showed that anthrax was caused by the small "bacterides" of Davaine because filtrate from which the organisms had been removed did not produce disease[8]. In 1877, Koch postulated that the anthrax bacilli could be transmitted from one host to another and he grew the organism in vitro and induced the disease in healthy animals by inoculating them with materials from these bacterial cultures[4]. He was able to trace the complete life cycle of this bacilli for first time (1876) and also explored that it remain viable for long periods in unfavourable environment[9]. Koch proved his postulates by isolating the organism from the infected animal[8].

Contributions to the immunity of anthrax were made in 1880 by Chauveau and in the same year by Toussaint. Final and undoubted proof of the value of vaccination, however, resulted from the famous experiments of Pasteur at Pouilly-le-Fort, a small village out-side of Paris, in 1881. He inoculated 25 cattle with his anthrax vaccine, which contained live attenuated organisms. Subsequently, he inoculated the vaccinated as well as other cattle with a virulent strain of anthrax bacilli. All the vaccinated animal survived, but others died[8,9]. However it is time that both Koch and Pasteur pave the way for further work in medical microbiology.

Anthrax is world wide in its distribution. It is particularly prevalent in countries where no organized control of animal disease exists. India, China, Siberia, Russia, northern Africa, some parts of South America and Mexico has anthrax as a major livestock problem. Germany, France, Italy, Great Britain and the United States keep the disease well under control. Anthrax is enzootic in southern India but is less frequent to absent in the Northern Indian states where the soil is more acid, while in Nepal it is endemic. Hansen believes that anthrax was introduced into the Ohio River Valley during the early days of westward migration, and he cites that Kercheval was the first person to describe an outbreak of the disease in cattle and infection in four farmers in the United States in 1824. An extensive epizootic of anthrax occurred in northeastern Oklahoma and southeastern Kansas in 1957, with a loss of 1627 animals on 741 premises as reported by Van Ness and associates[8]. As countries become free of anthrax or the annual incidence of outbreaks approaches unity, the numbers of animals, affected in an outbreak increase. This seems to be due to the decreasing veterinary experience in recognizing cases and in dealing appropriately with outbreaks. The mere absence of reported livestock anthrax does not mean that a country is free of the disease. Reporting deficiencies and insufficient examination of unexpected livestock deaths are common throughout the world[10,11].

Circumstantial evidence indicates that man is moderately resistant to anthrax. Before vaccines and antibiotics became available, and at a time when understanding of industrial hygiene was relatively basic, workers in at risk industrial occupations processing animal products were exposed to significant numbers of anthrax spores on a daily basis. In Britain, 354 cases of Anthrax in such industries were notified during 13-year period 1899-1912[12]. Although the numbers of persons exposed is not known, it must have been many thousands, and the number of cases represented only a very small proportion of the number exposed. With improvements in industrial hygiene practices and restrictions on imported animal products, the number of cases fell in considerable level in latter parts of the 20th Century. However, death rates remained high (>85%) when inhalation anthrax occurred[4].

Major sources of human anthrax infection are direct or indirect contact with infected animals or occupational exposure to infected or contaminated animal products. Other possible sources are rare and epidemiologically trivial. Historical analysis of epidemiological data globally reveals the following approximate ratios: a) One human cutaneous anthrax case to ten anthrax livestock carcasses; b) One incident of enteric human anthrax to 30-60 anthrax-infected animals eaten; c) in humans, 100-200 cutaneous cases for each enteric case that occurs.

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. These relationships are essentially all that can be used for many countries where human anthrax is infrequently, erratically or incompletely reported. In addition, certain countries suppress anthrax reporting at the local or national levels.

Human case rates for anthrax are highest in Africa, the Middle East and central and southern Asia. Where the disease is infrequent or rare in livestock it is rarely seen in humans[13].

In the 1950s, US Army Chemical Corps was developed human anthrax vaccine which was replaced by new and improved vaccine in 1970 with its license. In 1997, the US armed forces mandated vaccination for all reserve and active troops[4,14].

Cutaneous anthrax is said to account for 95% or more of human cases globally. However, serological and epidemiological evidence suggest that undiagnosed low-grade gastrointestinal tract or pulmonary anthrax with recovery can also occur, and may not be infrequent, among exposed groups[15,16].

The outbreak in a mill in New Hampshire, USA, in 1957 was not associated with any unusual change in occupational exposure but seems to have been an isolated event within a prolonged period of exposure. The US Department of Defense bases its strategies on an estimate that the LD50 for humans is 8000 to 10,000spores. However, in this relation it was found that, in one mill in USA, workers were found to be inhaling 600to 1300 anthrax spores over an 8-hour shift without ill effect and in two goat –hair mills,

B anthracis was recovered from the nose and pharynx of 14 of 101 healthy persons. In this context, it is, furthermore, well established that, at sizes above 5 mm profiles face increasing difficulty in reaching the alveoli of the lung. The likelihood of inhaled spores penetrating far enough to induce inhalation anthrax therefore depends greatly on the size of the particles to which they are attached[13,17-18]. Outbreaks and epidemics do occur in humans, sometimes these are sizeable, such as the epidemic in Zimbabwe which began in 1979, was still smouldering in 1984-85 and had by that time affected many thousands of persons, albeit with a low case fatality rate[19,20].

Typically, gastrointestinal anthrax follows the consumption of insufficiently cooked contaminated meat. In 1987, 14 cases of gastrointestinal and oropharyngeal anthrax were reported from northern Thailand[21].

Anthrax related biological warfare

If we go through the history of attempts of using diseases in biological warfare, it illustrates the difficulty of differentiating between a naturally occurring epidemic and an alleged or attempted biological warfare attack. This problem has continued into present times. The conception of Koch's postulates and the development of modern microbiology during the 19th century made the isolation and production of –stocks of specific pathogen possible and many countries worked to develop these agents for biological warfare purpose[2,4]. So, the use of biological warfare became sophisticated during the nineteenth century.

World war I

Substantial evidence suggests the existence of an ambitious biological warfare program in Germany, England and France during World War-I. This program allegedly featured covert operations. During World War- I reports circulated of attempts by German to ship horses and cattle inoculated with disease producing bacteria, such as *Bacillus anthracis* (anthrax) and *Pseudomonas pseudomalli* (glanders), to the USA and other countries[4,22]. Though latter no hard evidence of using such arms was found against Germany, but as a result of diplomatic response in International level towards baring of use of such weapons, on 17th June, 1925 "Protocol for the Use in War of Asphyxiating, Poisonous or Other Gases and of Bacteriological Methods of Warfare" i.e. Geneva Protocol was signed by 108 nations. Later it was proved to be less meaningful Protocol and that's why several countries like Canada, Belgium, France, Great Britain, Italy, the Netherlands, Japan, Poland and the Soviet Union began to develop biological weapons soon after its official implications. The USA remained apart from its implication until 1975[2].

World war II

During World War II Germany, Canada, United Kingdom, Japan, the Soviet Union, the USA began ambitious biological warfare research program. Various allegations and counter charges clouded the events during and after World War-II. Japanese biowarfare program was known as "Unit 731" and was conducted in occupied Manchuria near the town of Pingfan from 1932 until the end of World War-II. The program was under the direction of Shiro Ishii (1932-1942) and Kitano Misagi (1942-1945) and consisted of more than 150 buildings in Pingfan, 5 satellite camps, and a staff of more than 3000 scientist[23,24]. *Bacillus anthracis* was one of the organisms of interest which had extensively researched and used. The Japanese government accused the Soviet Union of experimentation of biowarfare agents *like B. anthracis*, *Shigella, V. cholerae* on the basis of recovering such agents from Russian spies[2]. Though German medical Researchers infected prisoners with disease producing organisms like *Rickettsia prowazeki*, hepatitis A virus, malaria, but despite this effort German offensive biological warfare program was not completely materialized[22]. On the other hand, German official accused the alies for using biowarfare agents as weapons. In fact it was believable by many that, the British were actually experimenting with atleast one organism as biological warfare agents i.e. *Bacillus anthracis* under the control of Dr. Paul Fildes in 1940s. Bomb experiments of weaponised spores of *B anthracis*were conducted on Gruinard Island near the northwest coast of Scotland, which lead to a heavy contamination of the island with persistence of viable spores. In 1986, the island was finally decontaminated by using formaldehyde and seawater[24,25]. Offensive Biological warfare program was begun in USA on 1942 at Camp Detrick (recent name Fort Detrick), Maryland where are of the organisms of interest was *B. anthracis*. Canada also started biowarfare program with small number of workers where *B anthracis*was also under consideration during world war –II.

After world war –II

In post World War-II Scenario during the Korean War, the Soviet Union, China, and North Korea accused the USA of using anthrax spores against the Chinese and North Koreans, although this has not been proved. But credibility of the USA in this respect was remained questionable due to its failure to ratify Geneva Protocol of 1925 as well as by suspicions of collaboration with former Unit 731 scientist for its own offensive biological warfare program[2,5].

Biological weapon convention 1972

As it was already obvious that Geneva Protocol 1925 established itself a toothless measure for control & proliferation of biological weapons, so risk of unpredictable nature as well as lack of its epidemiological control measure for biological weapons became a serious concern internationally during late sixties. In this sequence, in July 1969, Great Britain and in September 1969, the Warsaw Pact Nations leaded by the Soviet Union submit a proposal for need of prohibition of biological weapons. This proposal was strengthen by a report issued by World Health Organization on November, 1969 regarding the possible consequences of the use of biological weapons, which revealed that anthrax is more dangerous threat to the people because it showed that release of 50Kg aerosolized anthrax agents by aircraft over an urban area along a 2-Km line upwind of a population center of 500,000, causes 95,000 death and 1, 25,000 severe incapacitation[2,4,26,27]. In the way of on going process, on 1972 "Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction" known as BWC, was developed. This BWC were signed by 103 nations and implicated officially in April 1972, which helped in prohibition of development, production, stockpiling as well as technology transfer of biological warfare agents.

Post BWC scenario

Despite BWC agreement on 1972, some signatories continued that offensive bio-warfare program. On April, 1972 an large epidemic of (inhalation) anthrax accured among citizens of Sverdlovsk (now Ekaterinburg), Russia, which was populated by 1.2 million people and it was situated 1400 Km east of Moscow. The epidemic suffered by those who lived and worked near a soviet military microbiology facility, Known as Compound 19 and many livestock also died of anthrax in same area within 50 Km from that Compound in Sverdlovsk.

European and US intelligence suspected that this epidemic might be attributed to an accidental release of anthrax spore from Compound 19 where biological warfare research was conducted. In this context on 1980, February, the well circulated German daily "Bild Zeitung" revealed a story about an accident in Soviet military settlement in Sverdlovsk in which an anthrax cloud had resulted. Soviet officials attributed the human cases of anthrax from the ingestion of contaminated meat. Epidemic of Sverdlovsk which claimed thousands of life requires independent scientific investigation, this patterns of request was renewed by Matthew Meselson (Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts) in 1986 after several unsuccessful trial, which ultimately turned into invitation to come to Moscow for discussion with 4 Soviet physicians engaged in Sverdlovsk outbreak. But outcome of this meeting was that epidemiological and pathoanatomical data was needed for further investigation. The Soviet Union maintained that this outbreak was due to consumption of anthrax contaminated meat from black market. But soon after collapse of the Soviet Union, in 1992, the then Russian President Boris Yeltsin admitted that the compound 19 was a part of offensive Biological warfare program and the epidemic was caused by an accidental release of anthrax spore. Then, returning to Russia Meselson and his team joined in investigation and reviewed demographic, ecologic, atmospheric as well as private pathologist's data, which leaded them to the conclusion that 42 cases of fatal anthrax bacteremia and toxemia were typically of inhalation anthrax as seen in experimentally infected nonhuman primates. This focus the fact that, narrow zone of human and animal anthrax cases extending downwind from compound 19 indicated that the outbreak resulted from an aerosal that originated there. After this incident, the research was continued at a remote military facility in the isolated city of Stepnogorsk in Kazakhstan, for producing more virulent anthrax strain[2,4,17,28].

In August 1991, at the end of Persian Gulf War, during the process of UN inspection, representative from Iraqi government announced to the UN special commission Team that Iraq had conducted research into the offensive Biological weapon program in which one of the agents of interest is *B anthracis*. Besides this, bioterrorism attack in Japan using anthrax and botulinum toxin was also conducted unsuccessfully by Aum Shinrikyo before March 1995[2,17].

2001 Scenario

Demonstration of "anthrax letters" associated with intentional release of the anthrax organism following World Trade Center attack in September 2001, was first confirmed in a journalist in Florida on October 2001. Latter some cases of cutaneous and inhalation anthrax was seen in postal workers who handled those types of mails. Thorough analysis of these cases suggested that this bioterrorism was conducted by using Ames strain of *B. anthracis*[2,4].

In November 2, 2001 a sample from an envelope containing suspicious white powder received by the office of Deputy Chief Minister Chhagan Bhujbal has tested positive for the presence of anthrax spores, making this the first confirmed case of "anthrax mail" in India. This sample was confirmed by Molecular Diagnostics Pvt. Ltd. in Thane[29].

Microbiology

B anthracis, the pathogen responcible for anthrax, is a gram-positive, endospore forming rods up to 4 to 10 µm by 1-1.5 µm in size. It is an facultative anaerobic and motile. In blood smears of tissue or lesion fluid from diagnostic specimens, these chains are two to a few cells in length; in-suspension made from agar plate cultures, they can appear as endless strings of cells – responsible for the tackiness of the colonies. Also characteristic is the square-ended i.e. "box- car" shaped appearance traditionally associated with *B anthracis* vegetative cells, although this is not always very clear. Ellipsoidal central spores, which do not swell the sporangium, are formed at the end of the exponential growth phase in presence of oxygen. Under anaerobic condition the bacilli in infected tissue secretes a polypeptide [poly- (D-glutamic acid)] capsule, but this character is lost when the bacterium is grown aerobically in vitro. The capsule can be induced by incubating in defibrinated horse blood for at least 5 hrs, or by culturing the isolate on nutrient agar containing 0.7% sodium bicarbonate

with incubation at 37ºC in presence of 5% to 10% carbon dioxide. This capsule can be identified under microscope by the staining with polychrome methylene blue (McFadyean's reaction), where capsule stains pink and bacillus cells stains dark blue or may be highlighted with negative staining[4].

Bacillus anthracis grows readily on different types of media at 37ºC. A slightly alkaline medium, pH 7.5 to 7.8 is most conclusive to good growth. *B anthracis* colonies are up to 2 mm to 5 mm in diameter, flat, dry, grayish and with a "ground glass" appearance after incubation for 48 hours. At low magnification, curled outgrowths from the edge of the colony impart a characteristic, "medusa head" appearance. In gelatin stab culture, filaments of growth radiate from the line of puncture and give the appearance of an inverted fir tree. It is generally non-haemolytic on sheep blood agar media. It also shows weak and slow lecithinase activity on egg yolk agar. Biochemically it is non-lactose fermenting; Indole and H_2S are not produced; Nitrates are reduced to Nitrites; Ammonia is produced. The organism is Methyl red positive and Voges-Proskauer variable. But its most diagnostic feature is that, it shows Mc Fadyean reactions, susceptibility to diagnostic gamma phage and penicillin and its colony characteristics[8, 31].

Bacilli will form spores in the environment outer from host body. Under favorable conditions, anthrax spores germinate and rapidly multiply into vegetative form. Little information exists regarding lifestyle of this pathogen outside of the host. Recently it was found that spores of *B.anthracis* have the capacity to germinate in the rhizosphere of grass plants and to establish populations of vegetative cells that could support horizontal gene transfer in the soil and helps in the evolution of this species under *Bacillus cereus* group as a saprophytic organism outside the host[32].

Multiple locus enzyme electrophoresis (MEE) and Multiple locus sequence typing (MLST) have shown the lack of genetic diversity of *B anthracis* . It is one of the most molecularly monomorphic bacteria known. Amplified fragment length polymorphism (AFLP) analysis helps to detect difference between *B anthracis* isolates and to examine phylogenetic relationship between *B anthracis* and its close relatives. Multiple locus variable number tandem repeat analysis (MLVA) which, unlike AFLP designed to subtype *B anthracis* specifically and can not be used to address phylogenetic relationship between *Bacillus* species. MLVA determines the copy numbers of variable number tandem repeat (VNTR) in the region of VrrA genes as well as on two plasmids to differentiate into 89 distinct genotypes of these bacilli. Besides this, Single nucleotide polymorphism (SNP) also helps in genome-based analysis of bacilli. Besides this, full confirmation of virulence can be carried out using the polymerase chain reaction (PCR).

Pathogenesis

The virulence of *B anthracis* derives from the presence of a capsule and the ability to produce a complex toxin. Both virulence factors are encoded by plasmids and are required for disease production. The expression of virulence factors is regulated by host temperature and carbondioxide concentration. The capsule is composed of high molecular weight poly peptide (poly-D-glutamic acid) and is encoded by the pXO2 plasmid. This small plasmid is of 95.3 kilobase pairs and encodes the three genes cap B, cap C, cap A. The capsule inhibits phagocytosis of the vegetative form of *B anthracis*.

Another plasmid pXO1 is of 184.5 kb pairs and encodes toxin complex, which consists of three synergistically acting proteins i.e., Protective Antigen(PA,83 KDa), Lethal Factor(LF, 87 KDa) and Oedema Factor (EF, 89 KDa) is produced during the log phase of growth of *B.anthracis*[4,13]. Recent study provides evidence that pXO2 is necessary for the maximal expression of pXO1[35]. Individually each factor lacks toxic activity in experimental animals, although protective antigen induces antibodies which confer partial immunity. LF in combination with PA forms lethal toxin and EF in combination with PA for oedematoxin. Both these toxins are now regarded as responsible for characteristic signs and symptoms of anthrax.

Oedema Factor or EF is a Calmodulin-dependent adenylate cyclase pwhich by catalyzing the abnormal production of cyclic-AMP (cAMP) produces them altered water and ion movements that lead to the characteristic oedema of anthrax. Lethal Factor or LF appears to a Calcium and Zinc-

dependent metalloenzyme endopeptidase. It has recently been shown that it cleaves the amino terminus of two mitogen-activated protein kinase kinases and thereby disrupts a pathway in the eukaryotic cell concerned with regulating the activity of other molecules by attaching phosphate groups to them. This signaling pathway is known to be involved in cell growth and maturation; the manner in which its disruption leads to the known effects of LF has yet to be elucidated. On the basis of mouse and tissue culture models, macrophages are major target of lethal toxin which is cytolytic in these. The initial response of sensitive macrophages to lethal toxin which is the synthesis of high level of Tumour Necrosis Factor i.e., TNF alpha and Interleukin-1 beta cytokines and it seems probable that death in anthrax results from a septic shock type mechanism resulting from the release of these cytokines.

In inhalation anthrax, spores (1-2 µm in diameter) are inhaled and deposited in the alveolar spaces. From there, they are transported to local lymphatics and the mediastinal lymphnodes, where they germinate and cause haemorrhagic lymphadenitis. Vegetative bacilli then further spread via the blood stream and lymphatics, causing septicaemia. The large amount of toxin produced by the bacilli, together with the host response i.e., release of TNF-alpha and Interleukin-1 are responsible for the rapid decline and the overt symptoms of the host organism. Recently, several newly identified putative virulence factors were observed, these include enolase, high affinity zinc uptake transporter, the peroxide stress related alkyl hydoperoxide reductase, isocitrate lyase, and the cell surface protein A[4,35].

Clinical symptoms and pathology of anthrax

In cutaneons cases incubation period ranges as little as 9 hours to 2 weeks, mostly 2 to 6 or 7 days. *B anthracis*(usually as spores) entered through skin lesion like cut, abrasion, insect bite etc. A small pimple or papule appears; gradually a ring of vesicles develops around it. Marked oedema starts to develop. Painful lymphadenitis may occur in the regional lymphnodes. The original papule ulcerates to form the characteristic eschar. Oedema extends some distance from the lesion. Clinical symptoms may be more serve if the lesion is located in the face, neck or chest. In these more serve forms, clinical findings are high fever, toxaemia, regional painful adenomegaly and extensive oedema, shock and death may ensue. Generally, eschar resolves within six weeks[13]. In untreated cutaneous anthrax, about 20% of patients develop septicemia and die. However, with the use of appropriate antibiotics, the mortality rate is < 1% [36]. In this context, seven confirmed and four suspected cases of cutaneous anthrax were identified during the 2001 out break. Skin trauma was not associated with these cases of cutaneous anthrax. Exposure to contaminated mail was the apparent source of infection in all patients.

There are two clinical forms of gastrointestinal anthrax which may present following ingestion of *B anthracis*in contaminated food or drink. Intestinal forms include symptoms like nausea, vomiting, fever, abdominal pain, haematemesis, bloody diarrhea and massive ascites. In this form ulcers and necrosis usually form in the wall of the terminal ileum; sometimes caecum, colon, stomach and duodenum can also be involved[4]. In Oropharyngeal form, the main clinical features are sore throat, dysphagia, fever, regional lymphadenopathy in the neck and toxaemia. Even with treatment, the mortality is about 50%[37].

In case of pulmonary form of anthrax symptoms prior to the onset of the final hyperacute phase are non-specific and suspicion of anthrax depends on the knowledge of the patient's history. Early symptoms are non-specific and "Flu-like" begin insidiously with mild fever, fatigue lasting ane to several days[38]. Headache, muscle aches, chills, fever, drenching sweats, minimally productive cough, nausea or vomiting, mild chest pain were symptoms recorded in diseased patient's chest radiography at initial examination showed mediastinal widening, paratracheal fullness, hilar fullness, pleural effusions or infiltrates or both; chest computed tomography scan is helpful in detecting haemorrhagic mediastinal lymphnodes and oedema, peribronchial thickening, hyperdense mediastinal and hilar adenopathy findings and inflamation of meninges seen in inhalation anthrax. This mild initial phase was followed by the sudden development of dyspnoea, cyanosis, disorientation with coma and death. Death occurred within 24 hours onset of the hyperacute phase. In 2001

outbreak of bioterrorism related anthrax 11 patients were identified for inhalation anthrax are believed to have been exposed to mail containing or contaminated with *B anthracis*spores.

Prompt and timely antibiotic therapy usually results in dramatic recovery of the individual or animal infected with anthrax. Almost all isolates of *B anthracis*can be expected to be highly sensitive to penicillin and being cheap and readily available in most parts of the world, this remains the basis of treatment schedules, particularly in animals and in humans in developing countries.

CONCLUSION

Though anthrax is a common and naturally occurring disease in domestic animals, but it is gradually coming up on the surface as a devastating biological weapon. Research and development of anthrax as a biological weapon during the first half of the 20th century focused on easy dissemination of the bioweapon and the development of multidrug resistant strains[4]. But this bioweapon would not only cause sickness and death but also aim to create fear, panic and paralyzing uncertainty. Its goal is disruption of social and economic activity, the breakdown of government authority and the impairment of military responses. Besides this, anthrax causes residual contamination of the ground for a long period along with great devastation in the civilized population as estimated by WHO in 1970[2]. Recent demonstration by the "anthrax letter" in the aftermath of the World Trade Centre attack in September'2001, the occurrence of only a small number of infections can cause an enormous psychological breakdown. More importantly, these attacks fueled fears that, rather than end of terrorism, future attacks might be more extensive. Despite improvements in treatment and prophylaxis, anthrax considered as a fatal infection. Biowarfare attacks by anthrax agents are now a serious possibility. Primary prevention depends on creating a strong global norm that stops development of such weapons. Secondary prevention depends on early detection and its prompt treatment. As BWC is prepared to assist nations those have been targets of biological weapons, the medical community must be in a process to face challenge of biowarfare threat and nobody knows what will happen next.

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