BIOTERRORISM : A GLOBAL PROBLEM

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ABSTRACT
Bioterrorism is defined as the use of biological agents to inflict disease and/or death in humans, animals and plants. The intentional introduction of a poisonous additive to the food supply of livestock animals is potential means of executing a terrorist attack. Various microbes can be used for bioterrorism viz. Bacillus anthracis, Francisella tularensis, Yersinia pestis, Brucella sp., Coxiella burnetti, Staphylococcal, Clostridium, Variola virus. A diverse view exists on preparedness to be maintained against bioterrorism. In the 21st century we are likely to see the emergence of new pathogens and the spread of existing organism to new demographic areas and hosts. Living in the current world India is not immune to acts of bioterrorism. In order to counteract that there should exit a proper link between physician, agriculturist, veterinarians and the public.

KEYWORDS: Bioterrorism, veterinarians, public health, microorganism.

INTRODUCTION
Bioterrorism is defined as the use of biological agents to inflict disease and/or death in humans, animals and plants (Kleitmann and Rouff, 2001). Bioterrorist acts could have political, religious, ideological or criminal motivation and could be planned by groups or individuals.

The term biological agent applies to a diverse group of microorganisms as well as toxins of microorganisms, plants and animals. Although bioterrorist acts are expected to be infrequent, yet they are regarded as low probability, high consequence events (Tucker, 1997).

Cole (1996) stated that the fear of nuclear, chemical and biological weapons of mass destruction is well founded and perhaps reflects an annate human revulsion against the use of weapons producing catastrophic suffering in fellow humans even those constructed as enemies. The development if biological weapons became much more focused in the 20th century, while the media encouraged and highlighted public concern about bioterrorism.

According to the Centre For disease Control (1999) the biological agents considered to be the most likely weapons of bioterrorism include Bacillus anthracis (anthrax), Francisella tularensis (tularemia), Yersinia pestis (plague), Clostridium botulism toxin, Staphyloccocus...
enterotoxin, *Coxiella burnetti* (Q fever), *Variola virus* (small pox), and agents of viral haemorrhagic fever.

Brucella species (brucellosis), *Vibrio cholerae* (cholera), *Burkholderia pseudomallei* (glanders) and mycotoxins were removed from the list of most likely agents, but continue to remain possible threats.

While some biological agents could harm the exposed population (botulinum toxin), infectious agents producing contagious diseases like small pox could disseminate through susceptible populations unaffected directly by the initial bioterrorist event.

**Mode of Use:**
The concept of biological agents as weapons is not a new idea. History offers many instances of their use in order to inflict harm upon enemies. In order to deliver a biological agent to its target it first has to be weaponised or produced in a sufficient quantity, and in a form that would be relatively stable and easily disseminated.

The most efficient method of delivering biological agents is thought to be air borne route, with agents dispersed in aerosols. Wide dissemination of infectious agents, even toxin can be achieved by this method. Suspended in air for hours aerosols are sufficiently small and make their way into distant bronchioles and terminal alveoli after inhalation. They can be delivered from stationary point sources, aircrafts, boats and missiles. In 1937, the Japanese attacked 11 Chinese cities with plague–infected fleas sprayed from aircrafts. Heavy air contamination occurred when allied forces produced and tested anthrax bombs on Grinurd Island located off the coast of Scotland. This resulted in quarantine of the island for forty years, and only recently has the Island been declared safe (Christopher *et al.*, 1997).

The obvious and age-old method of contaminating food or water supplies is considered a plausible modern route for dissemination of some biological agents. Contaminating and poisoning enemy drinking water supplies with dead animals was employed in ancient civilization and in the 19th century during the American civil war (Poupard *et al.*, 1992).

Torok *et al.*, (1997) reported in 1984 that salad contaminated with Salmonella typhimurium in Oregon resulted in numerous cases of food borne infection. The bioterrorist act was done to sicken citizens and prevent them from voting. Directly throwing diseased carcasses into enemy territory in order to spread the disease and hasten victory has been practiced since long. Inanimate fomites as vehicle for spreading disease among enemies have been practiced since a long time, (Bardi, 1999). Blankets and handkerchiefs used by British troops suffering from...
suffering from pox were deliberately distributed among Native Americans resulting in an epidemic (Bremann and Henderson, 1998).

The intentional introduction of a poisonous additive to the food supply of livestock animals is potential means of executing a terrorist attack. The primary motive being to cause economic and social disruption. During World War II the British prepared over five million anthrax-infected cattle cakes and dropped them into German fields.

As of March 2004, thirty-one cases of terrorism involving animals were found in the weapons of mass destruction (WMD) database (Satheesha, 2005).

According to Cohen et al (1999) the preference for use of bioweapons buy terrorist organizations is attributed to the comparatively easy access to a wide range of disease producing biological agents, their low production costs, their non-detection by routine security systems and their transportation from one place to another. Because of their low production costs they have been apply termed as the “poor man’s atomic bomb” and “poor man’s weapons of mass destruction”.

Arora and Gautam (2001) reported that for atomic bombs, conventional weapons and nerve gas weapons the cost per causality is estimated to be approximately $2000, $800 and $600 respectively, while for biological weapons the cost would be about $1 per causality. The study showed that the economic impact of a bioterrorist attack can range from an estimated $477.7 million per 100,000 persons exposed (brucellosis scenario) to $ 26.2 million per 100,000 persons exposed (anthrax scenario).

MICROBES USED FOR BIOTERRORISM

*Bacillus anthracis:*

As a result of recent terrorist activities the genes of this bacterium have become an area of heated investigation. Isolated and characterized by Robert Koch the bacterium was used by him to proof the Koch’s postulates. The cells are rod shaped (1 to 1.5 mm by 4 to 10mm) facultative anaerobes, non-motile, encapsulated and arranged in chains. Subterminal spores are formed, only in the presence of oxygen, and are reported to survive in soil for upto 40 years. On sheep blood agar it forms non-haemolytic colonies referred to as Medusa head. The characteristics cotton wool like appearance is seen in liquid media (Inglesly, 1999).

Depending on the site of infection anthrax has different has clinical manifestations. In cutaneous anthrax spores are introduced into the skin, germination occurs within hours and the vegetative cells produce toxin. A red maculae is formed at the site, which later ulcerates and develops into a blackened necrotic eschar (anthrax meaning coal got its name due to this appearance).
Spontaneous healing occurs in 80 to 90% of untreated cases, but when bacterimia develops it leads to high fever and death (Swartz, 1999).

On inhalation, anthrax (Wool sorter’s disease) spores enter alveoli of lungs and are phagocytosed by alveolar macrophages and begin to germinate. Some germinate in lymph nodes. This results in hemorrhagic mediastinitis and bacterimia accompanied by secondary pneumonia. Meningitis may also occur as a complication of bacterimia (Moran, 1999).

Gastrointestinal anthrax is contracted by the ingestion of contaminated meat that is not thoroughly cooked, resulting in oropharyngeal infection or intestinal infection. The symptoms vary and include fever, vomiting, abdominal pain, bloody diarrhea and hemoconcentration.

Diagnosis of anthrax can be done by direct examination of blood films, immunological tests and animal inoculation.

Antibiotics like Penicillin, Erythromycin, Ciprofloxacin and Vancomycin have been successfully used in the treatment of anthrax. Control and preventive measures include vaccination, rapid identification of infected carcasses and their disposal by burning or burying. Anthrax spores exposed to the atmosphere on hides can be killed by exposure to liquid nitrogen oxide gas.

***Francisella tularensis:***

The bacterium was first isolated by McCoy and Chapin from Tulare country, California. It is gram negative coccobacilli, non-motile, non-spore forming and a strict aerobe. On cysteine heart agar it forms small greenish opalescent colonies. It requires inoculation or inhalation of as few as 10 organisms to cause disease. The organism survives for weeks at low temperature in water, moist soil, hay, straw and decaying animal carcasses.

Tularaemia is principally a disease of wild animals; however, humans as well as some domestic animals are susceptible. The disease is transmitted through arthropod bites, direct contact with infected animal tissues, inhalation and ingestion of contaminated food and water.

The clinical manifestation of tularaemia is related to the route of exposure. Thus, infection may be classified as ulceroglandular, occuloglandular, pharyngeal of pneumonic. The disease is greatly under recognized and under reported. Its release in a densely populated area would be expected to result in an abrupt onset of large number of cases of acute, non-specific febrile illness (Cross and Penn, 2000).

Francisella tularensis can be isolated from blood, pleural fluid, sputum, lymph nodes, wounds or gastric aspirates. The serological tests commonly used for diagnosis are Enzyme linked Immuno Sorbent Assay (ELISA) and Flouruscent Antibody test (FAT).
Streptomycin and Gentamicin are the drugs of choice for the treatment. An effective vaccine against the disease is still under development.

Although live attenuated vaccine derived from avirulent F. tularensis is being used in laboratorians working with the bacterium.

**Yersinia pestis:**
The causative agent of plague the bacterium on staining shows the typical bipolar appearance. It is rod shaped non motile and can be grown on both blood and Mac Conkeys agar (Aleksie et al., 1999).

It causes disease of rodents and other animals, and is transmitted to humans via flea bites, resulting in Bubonic plague, characterized by the sudden onset of fever, malaise and lymphadenitis.

Inhalation of the bacterium leads to pneumonic form of disease. The form can also occur due to hematogenous spread of bacilli from bubos to the lungs. This form spreads very fast via air borne droplets, which makes it easy for a bioterrorist attack (Butler, 2000).

World Health organization (WHO) reported, if 50 kg of *Y. pestis* is released as an aerosol over a city of 5 million, pneumonic plague could occur in as many as 1,50,000 person, 36,000 of whom would be expected to die. The plague bacilli can remain viable as an aerosol for 1 hr for a distance of up to 10 km.

**Brucella sp.** – Members of this genus are all pathogenic, intracellular parasites with predilection for organs rich in sugar erythritol such as the reticulo-endothelial system and reproductive tract. The bacteria are gram negative, non motile, non-sporing coccobacilli producing non hemolytic colonies on blood agar.

Brucellosis is a zoonotic disease contracted by humans as a result of direct or indirect contact with animals that are infected with the microbe. Infections can be established via cutaneous, respiratory or gastrointestinal routes (Barham et. al., 1993). Symptoms of brucellosis are fairly non-specific and the onset of illness may be acute. As result of systemic nature of brucellosis almost any organ in the body may become infected. in the event of deliberate exposure to brucellosis., the respiratory route of exposure will be almost likely, although food-borne exposure is possible. The most common clinical manifestation being – “undulant fever”, headache, chills, myalgia, weakness and malaise. in contrast to animals, abortion is not seen in humans (Sharpie and Wong, 1999).
Diagnosis of brucella can be made by culture of organism from clinical specimens on special or enriched medium and incubating in an environment having high CO2 concentration. The serological tests used include agglutination complement fixation.

Preventive and control measures include vaccination and elimination of infected animals from the herd as is practiced in some developed countries. In humans treatment with antibiotic like doxycycline and rifampin has been found effective.

**Coxilla burnetti**

It is an obligate intracellular, gram negative, non-sporing short, rod like, non- motile. Aerobic rickettsia that is the casual agent of Q fever, a zoonotic disease. It is a potential bioweapon. it is highly effective to both humans and livestock and is capable of inducing acute infections in human resulting in isolated bouts of fever, pneumonia, granulomatous hepatitis, abortion or meningoencephalitis. The organism can induce abortion in domestic mammals and ruminants, and these animals represent their main reservoir. The danger posed by Coxiella is that they can be excreted by animals exhibiting no apparent clinical signs of the disease a be transported to human via inhalation or tick bite. The bacterium is difficult to study because it cannot be culture on artificial media (Downie, 1965).

Most acute of Q fever begin with sudden onset of high fever (104-106°F) severe headache, general malaise, myelagia. Sore throat, nausea, vomiting, diarrhoea, abdominal and chest pain. *Coxiella burnetti* is considered by the U.S. government as possible agent for bioterrorism as it is highly infectious and quite resistant to heat and drying. It can become air borne and be inhaled by humans.

The serological tests employed for the diagnosis of Q-fever include immunofluoroscence , complement fixation test (CFT) and ELISA.

Antibiotics like Tetracycline and erythromycin have been found most effective when administered within the first three days of illness. Recently a combination of doxycycline with erythromycin of Rifampin has been suggested.

The preventive and control measures include vaccination, proper disposal of infected material. Tick control and pasteurization of milk by flash method.

**Staphylococcal enterotoxins**

These are protein toxins produced by coagulase positive staphylococci capable of irritating the gastro-intestinal tract. Six serologically distinct enterotoxins (A to F) have so far been recognized, of which enterotoxins A is by far most frequently encountered in food poisoning out breaks.
Enterotoxin F has recently been identified as a result of its association with toxic shock syndrome. The enterotoxins are fairly resistant to most proteolytic enzymes and heat. Although heating at 100°C lessens the toxicity of foods to is inefficient to inactivate them completely (Carter and Wise, 2004).

Some individual may not always demonstrate all he symptoms associated with the illness. The main clinical signs associated with enterotoxin food poisoning are headache, muscle cramping, nausea, vomiting, abdominal pain, diarrhoea, transient changes in blood pressure and pulse rate may occur. Developing 2 to 6 hours after ingestion of contaminated food, the illness is self-limiting and recovery usually occurs within a day or two.

Staphylococcal food poisoning results from ingestion of contaminated food containing performed toxin. A toxin dose of less than 1.0 mcg in contaminate food will produce symptoms of intoxication.

The detection of staphylococcal enterotoxin is done by serological methods like ELISA, Reverse passive latex agglutination using monoclonal antibodies. The disease is usually self-limiting. Intravenous fluids are administered for severally dehydrated cases. Antibiotics have not been found to have any efficacy.

**Botulism toxin:**

Clostridium botulism strings and some isolated of Clostridia such as *Cl. butyricum, Cl. barati* and *Cl. argenteuse* produce a family of seven immunologically distinct potent heat labile neurotoxins designated from A through G. type A, B, E and F are the usual agents of botulism in humans and cause the clinical syndromes identified as food botulism, wound botulism caused by toxin production, after clostridial colonization of the intestine. The toxin is released when the organ undergo autolysis. The toxins spread through the blood stream and appear to be especially directed towards peripheral nerve and exert their effect at neuromuscular junctions where they inhibit the release of acetylcholine. He classical presentation of botulism is acute flaccid paralysis that begins the head and descends symmetrically. Breathing becomes impaired. There is double vision, blurred vision, drooping eyelids, slurred speech and difficulty in swallowing. if untreated, these symptoms may progress to cause paralysis of the arms, legs, trunk and respiratory muscles. In food borne botulism symptoms generally begin 18 to 36 hours after eating of contaminated food, but they can even occur as early as 6 hours or as late as 10 days. a toxin dose of 0.001 mcg is sufficient to produce symptoms of intoxication.

The average clinical laboratory is not involved in diagnosis of this highly potent toxin. 1 mg of the neurotoxin contains more than 120 million mouse lethal doses.
Serum specimens, vomitus, stool or tissue debraded form infected wounds are tested for toxin. If diagnosed early, the action of toxin is blocked by the administration of antitoxin.

**Variola virus**- the brick shaped virus is among the largest animal viruses and exists in two forms; variola major causing severe small pox and variola minor causing mild small pox. Humans are the only natural host for small pox (Douglas, 2003).

The usual route of infection is through inhalation of droplets containing infectious virus particles. The virus multiplies in mucosal cells and regional lymph nodes and a transient viraemia develops spreading the virus to internal organs and the skin. Subsequent multiplication leads to the toxemic phase, which is characterized by prodromal rashes, high fever, headache and prostration. The rash starting on the tongue and roof of the mouth spreads to the face and through out the body leading to the development of skin lesions (Henderson, 1999).

In laboratories equipped to handle small pox specimens, a presumptive diagnosis can be made with Giemsa stained smears of material from skin lesions in which Guarneri inclusions bodies may be seen. Isolation of virus from the clinical material can be done on the CAM of embryonated eggs. Serological tests used for the antigen detection include the CFT, precipitation and immune flouroscence tests (Douglas, 2003).

Other than vaccines, which may be effective in the first few days post infection there is no proven antiviral drug treatment available for clinical small pox. there is no licensed antiviral for small pox although “cidofovir” is in the experimental stage (Donald, 2006).

Preventive measures include primary vaccination of infants and revaccination of children upon entry into school with exposure to high risk of infection. All cases have to be reported to public health authorities and kept isolated till the disappearance of crusts. persons having contact with infected patients should be vaccinated and kept under surveillance for 16 days from time of last contact.

**Agents of viral hemorrhagic fevers:**

A diverse group of viruses like the Ebola and Marburg virus, Rift valley fever virus, Yellow fever and Dengue viruses are capable of causing viral hemorrhagic fever syndrome. Humans are exposed to these agents by contact with infected animals and via arthropod vectors. The symptoms include fever, mylagia, hemorrhage in mucous membrane and shock. There can be high morbidity or mortality in some cases (Peters, 1997).

Diagnosis of viral hemorrhage fever includes antigen detection by ELISA. RT-PCR and viral isolation.
Treatment is supportive with maintenance of fluid and electrolyte balance. There is no antiviral drug approved for treatment. Ribavirin has some activity against Ebola and Marburg virus and Rift valley fever but no utility against Dengue virus and yellow fiver virus. Due to lack of effective therapies and vaccines, effort to prevent transmission of infection, meticulous implementation of infection control measures are done.

Research on microbes intended for biological warfare is reported to involve methods of genetic engineering aimed at increasing their ability to express toxins, increase virulence, develop resistance to antibiotics or improve their survival during storage and application (Davis, 1999).

**Efforts to establish preparedness:**

A diverse view exists on preparedness to be maintained against bioterrorism. On the one hand developed nations are reported to be strongly committed to developing extensive biological weapon defense plans for both their military and civilian population, while on the other there were views like that of Leonard Cole who stated that emphasis on the ethical and moral reasons against the use of biological weapons, along with strong treaties, verification regimens and surveillance are the best methods for preventing the use of such weapons (Cole, 1996).

Some advanced countries are developing plans to deal with possible bioterrorist events. According to the Centre for Disease Control (CDC, 1999) comprehensive public health response to bioterrorism includes detection, rapid laboratory diagnosis, epidemiological investigation, communication and preparedness.

Clinical microbiology laboratories play a key role in the detection and identification of biological agents likely to be used in bioterrorist events. The CDC working with public health authorities is developing a nationwide plan for bioterrorist preparedness.

In the LRN (Laboratory Response Network) set up by the WHO for bioterrorism, laboratories are classified into four levels depending on their testing facilities and abilities.

**Level A:** the laboratories culture and identify routinely isolated pathogens. Depending on the results after performing a small number of simple rule out the tests the organisms are referred to a higher-level laboratory for further testing performed in a biological safety cabinet.

**Level-B:** the laboratories contain Biosafety Level (BSL) 3 facilities. Their activity includes test for rapid presumptive identification and antimicrobial susceptibility testing.

**Level C:** the laboratories perform strain typing procedure.

**Level D:** Critical biological agents are finally refereed to these laboratories.
EFFORTS MADE BY THE INDIAN GOVERNMENT:

Indian has always been gripped by a host of diseases. Poverty, a lack of hygiene and sanitation, poor water supply and frequent disasters, provide green pastures for disease outbreaks. Experts recommended that India expand its disease surveillance network and its ability to monitor bioterrorism so that bioterrorist attacks are not passed over as natural disease outbreaks or outbreaks of unknown origin, or be classified as an emerging infectious disease. The need for international partnership was announced in U.N. General Assembly in 2005 by President Bush, whose country had been the target of anthrax attacks shortly after the terrostriles on the World Trade Tower in 2001.

In “The Hitavada” of January 2007, the suggestions made by Rajiv Venkaya, Special Assistant to U.S. President for bio-defence, regarding the development of a agile and large network of laboratories in India to identify and counter pandemic strains was published. He stated that “advance preparedness would help in tackling ant threats of pandemic whether they are caused by nature or a bioterrorist attack.”

The threat of biological warfare has been engaging the attention on Indian defence and medical experts for a long time. During the Indo-pakistan war of 1965, a Scrub typhus pot break in northeastern India came under suspicion. In September, 2001 Colonel Nagendra, Head Of Microbiology at the Armed Forces Medical College, Pune, speaking at a conference in the National Institute of Communicable Disease, New Delhi, stated that “terrorist incidents have occurred of Pakistan and Osama Bin Laden’s terror network, in which the use of biological agents cannot be ruled out.”

India’s defense and intelligence outfits were alert to the outbreak in Surat of Pneumonic plague and of Bubonic plague in Beed in 1994, which caused several deaths and sizable economic loss. Dr. Batra, Joint Director of defense research and Development stated “The Yersinia pestis strains that are percolating in established plague foci in India are very much les virulent and different from those from the plague outbreak region.

An infrastructure consisting of trained infectious disease physician, microbiologist, and epidemiologist and policy makers is required. Incorporating bioterrorism response measures into disaster management plans in emergency medicine is the best solution.

In India, a formidable portion of the annual budget goes into weaponry defense and a miniscule portion is allocated to health. It will be a challenge to the Indian administration, now, to build a biological defense infrastructure, which falls in between zones of both defense and health.
The Indian Defence Research and Development committee has made eight recommendations involving potentially simpler, faster and less expensive mechanisms for the use of developing countries (Rao, C.K., 2003). The recommendations include-

1. financial support for improvement of surveillance
2. Survey of metropolitan hospitals for drugs and equipments availability
3. Sharing information on disease and drugs between Government and private agencies
4. Develop incentives for hospitals to posses’ appropriate personal protective equipments and expandable decontamination facilities and train emergency departments in their use.
5. An urban medical strike team should b organized and equipped in high risk areas throughout the country.

CONCLUSION

In wake of the September 11, 2001 tragedy and the subsequent discovery of U.S. Mail contaminated with anthrax spores in the states of New York, Washington D.C. and Florida, the specter of bioterrorism has been raised. Many nations are reported to posse offensive biological weapons programme.

A ounce of prevention is worth a pound of care. In the 21st century we are likely to see the emergence of new pathogens and the spread of existing organism to new demographic areas and hosts. Under the circumstances a coordination of the medical infrastructures together with the anti-bioterrorism initiative will strengthen antions ability to respond with vigor and efficiency against an attack or an introduced epidemic (Bryan et al., 1999).

It is indeed a fact; living in the current world India is not immune to acts of bioterrorism. In order to counteract that there should exist a proper link between physician, agriculturist, veterinarians and the public. The role of veterinarian in detecting and combating bioterrorism should never be over looked or underestimated as this is the only profession which is familiar with both animal and human diseases. Keys enabling the introduction of biological agents should be developed (Lillibridge et. al., 1999).

Preparedness is always beneficial. Dr. Sagar Galankar, Founder emergency Medicine, India, has rightly stated “the defender has to be lucky all the time, but the destroyer has to be lucky just once.”

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