Editorial

Bioterrorism†

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Historical aspects

Bioterrorism is the deliberate use of pathogenic micro-organisms for hostile purposes or in armed conflict[1]. The intent could be to inflict disease and or death on humans, animals or even plants. Therefore crops, livestock or humans are considered targets for bioterrorists. It has been recorded that ancient civilizations (Greeks, Romans and Persians) had attempted to pollute drinking water supplies of their enemies by contamination with dead animals[1]. During the 14th century siege of Kaffa, a sea port city in the now known Ukraine, Tartar forces are believed to have catapulted deceased plague victims into the city under siege to spread disease and accelerate a victory. This resulted in an outbreak of plague which is documented[2]. There are records showing that inanimate objects (eg. blankets and handkerchiefs used by small pox victims) were used as vehicles to transmit disease to enemies[1]. Between the two world wars many countries stepped up their research programmes on biological warfare. After world war II, the United States military established Fort Detrick as a testing site for investigation of agents of biological warfare[2]. Russia also established a research and manufacturing organization called Biopreparet[3]. In 1979 accidental release of aerosolized anthrax spores from a microbiology research centre in Sverdlovsk in the former Soviet Union led to 68 deaths caused by inhalation anthrax[4]. In 1984, in Dallas, Oregon, the salad bars of two restaurants were contaminated with a salmonella species by followers of Bhagwan Shree Rajneesh[5].

Types of biological weapons

The reasons for using biological weapons are varied and would be to promote separatist objectives, revengeful destruction of life, protest against governance, creating chaos or mimicking god. An "ideal" weapon should be reliable, cheap for mass production and be able to be targeted precisely at particular persons. It should also have a long shelf life and possess aerosol durability. These criteria have enabled the enumeration of tiered lists with varying potential to cause damage (Tables 1 and 2).

Table 1. Tier I agents

Bacteria	*Viruses*	*Toxins*
Bacillus anthracis	Small pox	botulinum toxin
Yersinia pestis	Viral encephalitis	staphylococcal toxin
Francisella tulerensis	Ebola, Marburg viruses	
Coxiella burnetti		
Brucella suis		

Table 2. Tier II agents

Bacteria	*Viruses*	*Parasites*	*Toxins*
Rickettsia	HIV	Ascaris suum	cholera
Salmonella	Yellow fever	Giardia lamlia	snake venom
Vibrio cholerae		Schistosoma	diphtheria
Yersinia enterocolitica		Cryptosporidium	

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Microbiological and clinical considerations of more likely agents of bioterrorism

Anthrax

Anthrax is an acute infection caused by Bacillus anthracis, which is a gram positive spore forming bacterium. This can naturally infect many species of animals like cattle and goat who ingest these spores while grazing on grass. Humans can be infected by skin contact with spores, ingestion of spores via contaminated food and inhalation. The most common method of human infection is inoculation through the skin. The method used in bioterrorism (ie. weaponizing the bacterium) is to create an aerosol and thereby cause inhalation anthrax which has a high mortality. To achieve this the spores are made to a size between 1-5 um. and the ID50 for inhalation has to be in the range of 10000-15000 spores. (ie. the infectious dose required to cause disease in 50% of those exposed to inhalation). Anthrax spores germinate into multiplying vegetative forms when they find an environment rich in amino acids, glucose and nucleosides. This type of environment is seen in tissues of a human host or an animal. The virulence of the organisms requires the presence of an anti phagocytic capsule and toxin complex composed of protective antigen, lethal factor and oedema factor. The virulence factors are encoded on two virulence plasmids px01 and px02. The expression of the virulence factors is regulated by host factors such as elevated temperature (>37°C) carbon dioxide concentration (>5 percent) and presence of serum components. The vaccine strains were made by rendering the strain free of one or both plasmids. Sterne strain is attenuated, carries px01 plasmid and can synthesize exotoxins but does not have the capsule. Pasteur strain carries px02 plasmid, is capsulated but does not express exotoxin components.

In inhalation anthrax, there is ingestion of spores by the alveolar macrophages, some of which undergo lysis and destruction. The surviving spores are transported via lymphatics to mediastinal nodes where germination of multiplying bacteria release toxins leading to haemorrhage, oedema and necrosis. Initial symptoms are non specific and resembles a minor upper respiratory infection which takes 2-8 days with an average of 5 days, although symptoms may occur even after 40-50 days as was seen in the Sverdlovsk outbreak. Typical bronchopneumonia is not seen but what does occur is haemorhagic thoracic lyphadenitis and haemorhagic mediastinitis as was seen post mortem in patients who died in Sverdlovsk. Half of these patients also had harmorhagic meningitis. 25% of those who had autopsies performed had nectrotising pneumonic lesions similar to the Ghons complex seen in tuberculosis. Early diagnosis of inhalation anthrax is difficult and requires a high index of suspicion. Usually an aerosol is fully dispersed in 24-48 hours and the first case could be seen 4-5 days later. During the early stages laboratory studies are non-specific. In some patients a brief period of apparent recovery is seen followed by progression to a fulminant stage of the illness[8]. During this stage there is fever, dyspnoea and the massive lymphadenopathy with expansion of the mediastinum leads to stridor in some cases. A chest X-ray at this stage will show a widening of the mediastinum consistent with lymphadenopathy[9].

Cutaneous anthrax occurs following inoculation of the bacterium through a break in continuity of intact skin.The areas are mainly in the hands, face and neck. The lesion starts as a painless pruritic papule. This may enlarge in size and progress to vesicular lesions which could discharge serous fluid. This vesicular fluid contains the organisms which could be seen under a Gram stain. In 4-7 days time black necrotic eschar would develop with surrounding oedema. The eschar dries up, loosens and then falls off in 1-2 weeks. Lymphangitis and painful lymphadenitis can occur with associated systemic symptoms.

Gastrointestinal anthrax is seen after ingestion of spores through contaminated food. The spores germinate in the upper or lower gastrointestinal tract resulting in an oropharyngeal ulcer or an intestinal ulcer respectively. This is followed by regional adenopathy, oedema and necrosis leading to intestinal lesions as well. Initial symptoms of nausea, vomiting and malaise are followed by bloody diarrhoea and an acute abdomen. Advanced gastro-intestinal anthrax could resemble the sepsis syndrome following inhalation anthrax.

Diagnosis carried out in laboratories with at least category II safety cabinets is based on direct Gram stained examination of skin lesion (vesicular fluid or eschar), cerebrospinal fluid or blood showing encapsulated broad gram positive bacilli. Cultures on sheep blood agar would show non haemolytic colonies. These organisms are non motile and do not grow on McConkey's agar. Confirmation is by looking for the capsule with India ink stain. Other confirmatory tests include visualising blue coloured bacilli with red capsular material on Mc Fadyean stain, lysis by a gamma phage or direct fluorescent antibody staining of cell wall polysaccharide antigen. Rapid tests for antigen detection via polymerase chain reaction and enzyme linked immunosorbent assay (ELISA) also are available in specialized centres.

Penicillin has been the drug of choice for anthrax for many decades and penicillin resistance is not known among naturally occuring strains. No evidence based clinical studies on antmicrobial therapy of

human anthrax are available. Animal studies have shown doxycycline and ciprofloxacin to have excellent efficacy. Other respiratory fluoroquinolones like levofloxacin, gatifloxacin and grepafloxacin are believed to be equally efficacious. In vitro Bacillus anthracis is additionally susceptible to macrolides, aminoglycosides and carbapenems. They also show response to first generation cephalosporins but are resistant to cefotaxime, ceftazidime, and also to monobactams. Ciprofloxacin and doxycycline are also recommended for chemoprophylaxis.

Since there is no risk of person to person transmission even in the case of patients with inhalation anthrax, patients could be hospitalised and nursed in wards using standard precautions. Dressings containing drainage from cutaneous lesions need to be incinerated or autoclaved. The epidemiologist should be notified of a possible bioterrorist attack if a single case of inhalation anthrax is seen. Notification should be considered if a diagnosis of cutaneous anthrax is confirmed in an individual who has no occupational hazard of contracting the disease or if two or more cases of anthrax are linked ie. from the same place at the same time. For decontamination of a contaminated area (possibly after spillage of spores from an envelope sent to an individual with a terrorist intention) a sporicidal solution need to be used. Commercially available bleach or a 0.5% percent hypochlorite solution (ie. 1 in 10 dilution of household bleach) are alternatives.

Smallpox

There is concern that Variola major (small pox) virus may be used as a biological weapon. If used this would be a serious threat to humans as the case fatality rate is 30% or more among unvaccinated people. The last case of small pox in the world was seen in 1977 and since the declaration of global eradication in 1980 there is a large population unvaccinated. Only 15-16 million doses of smallpox vaccine produced over 20 years ago are available in the United States[11]. In September 2000 the Centre for Disease Control (CDC) in the USA awarded a contract to OraVax (Cambridge, MA) for large scale production of smallpox vaccine. This highlights the growing concern in the USA about the threat of smallpox virus as a biological weapon. This new vaccine is expected to be produced using modern technology including virus culture on MRC-5, a human diploid lung cell line, approved for the manufacture of other live attenuated vaccines. Work with variola virus need to be carried out under biosafety level 4 containment conditions. Natural infection follows implantation of the virus in the oro-pharyngeal region or the respiratory mucosa. After multiplication in the regional nodes a viraemia results at which time the patient may be asymptomatic. Localizing in the spleen and bone marrow enhances further multiplication for a secondary viraemia to occur followed by fever and toxaemia. Then the virus contained in the white cells localize in small vessels of the dermis and under the oral mucosa. One to three days after acquisition of the secondary viraemia there is high fever, prostration with severe headache and backache. A macular papular rash appears in the mucosa of the mouth face and hands which may spread to the trunk and legs. This rash becomes vesicular and then progresses to the pustular stage. Except for skin and mucous membrane involvement other organs are rarely involved. In the fatal malignant type, the onset of constitutional symptoms are abrupt, and progresses rapidly followed by haemorrhages into the skin and mucous membranes. Death occurs 5-6 days after the onset of rash. The discovery of a single suspected case of small pox is considered a health emergency and the epidemiologist has to be informed.

Diagnosis rests on microscopic examination of the vesicular or pustular fluid by electron microscopy and growth of the virus on cell culture. Enzyme immunoassay technology was in its infancy at the time small pox was eradicated. The polyclonal and monoclonal antibodies are been evaluated with inactivated antigens. Nucleic acid based diagnostics are also being examined. A large number of antiviral agents have been looked at for activity as well as their therapeutic indices against variola and other pox viruses. A nucleoside phosphate DNA polymerase inhibitor cidofovir has shown promise. Another group of drugs undergoing evaluation is S-adenosylhomocystein hydrolase inhibitors.

Before the 1970s smallpox patients were looked after in separate hospitals. In the event of a limited outbreak (as would be the case if there is a bioterrorist attack) single room isolation with respiratory precautions (the use of masks gloves gowns etc.) need to be instituted .The waste would need segregation and disposal through incineration or autoclaving.

Plague

Human plague caused by a bacterium Yersinia pestis occurs when plague infested fleas bite humans. These patients develop bubonic plague. Before humans develop plague, epidemics occur among rats precipitating movement of fleas from rats (which is the reservoir) to humans. A small number develop sepsis with no bubo and this is termed septicaemic plague. Pneumonic plague occurs as a result of haematogenous spread of the bacillus from a bubo to lungs or after inhalation of the organism. This inhalation

form will be the likely route of infection as a result of a bioterrorist attack. Neither the bubonic form, nor the septicaemic form, spreads directly from person to person but the pneumonic form can spread via air borne droplets. Indication that plague has been spread artificially would be the occurrence of cases of pneumonic form in areas where death of rats has not been reported. The humans develop fever with cough and dyspnoea 1-2 days after aerosol dissemination progressing rapidly to pneumonia.

Microbiological diagnosis off sputum and blood rests on growth of the gram negative lactose fermenting Yersinia pestis on Mc Conkey agar and its identification. Historically the treatment of choice has been with streptomycin. The newer aminoglycosides like gentamicin netilmicin and amikacin has not been approved with evidence based studies. Tetracycline and doxycycline have been used successfully although no data from controlled clinical studies are available. The fluoroquinolones particularly ciprofloxacin has demonstrated efficacy in animal studies and shown to be as efficacious as aminoglycosides and tetracyclines[10].

Botulism

Clostridium botulinum which is a gram positive spore forming obligate anaerobe produces potent neurotoxins designated A,B,E and F. These are responsible for botulism in humans and cause clinical syndromes identified as food borne botulism and wound botulism. Botulism caused by toxin production after clostridial colonisation of intestines of infants is known as infant botulism. Botulinum toxin poses a bioterrorist threat because of its extreme potency and lethality. The toxin can spread through the blood stream and exert its effects at the neuromuscular junction where it inhibits the release of acetylcholine. The toxin is a zinc proteinase that cleaves one or more of fusion proteins by which neuronal vesicles release acetylcholine into the neuromuscular junction. It is regrettable that toxin needs to be considered as a biological agent when it has just been licenced for the treatment of cervical torticollis, strabismus, and blepharospasm associated dystonia.

Terrorists have attempted to use the botulinum toxin as an aerosol. The Japanese cult Aum Shinrikyo attempted its use in Tokyo and at US military installations in Japan. The man made form of aerosolised botulinum toxin leads to the inhalation type of botulism.

Definitive diagnosis of botulism rests on a bioassay of the toxin and is available in highly specialised centres only. The main line of treatment is the use of antitoxin in addition to other supportive measures.

Preparedness

The possible use of biological agents as weapons has generated considerable interest round the world. With the increasing awareness an attempt has been made to defend against the possibility of biological warfare. Healthcare providers as well as military establishments have begun to train teams to prepare attacks of bioterrorism. The Ministry of Health in Sri Lanka has formed a task force for this purpose. Bioterrorism preparedness programme would need to address this problem by way of guidelines. For disease detection through proper laboratory diagnosis, special centres need to be set up. This would have to be followed by tight surveillance and epidemiological investigation through an effective communication between curative and public health sectors.

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