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# *Bio-Terrorism – What Should Physicians Know?*

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## Introduction

Bio-terrorism may be defined as the unlawful use, or threatened use, of micro-organisms or toxins derived from living organisms to produce death or disease in humans, animals or plants.[1] The act is intended to create fear and/or intimidate governments or societies in the pursuit of political, religious or ideological goals. The history of bio-terrorism may be traced to the Assyrians who fouled up enemy waters with the fungus *Claviceps purpurea* (Rye ergot) in the 6th century BC, the Tartars who hurled bodies of bubonic plague victims into the city of Kaffa in 1346 and later the British who gave blankets used by smallpox patients to native American Indians "as a gesture of goodwill" .[2] More recently the use of biological agents by the Japanese in the First World War, biological warfare in Iraq and the anthrax attack on the USA in this very decade are stark reminders of the ever present spectre of bio-terrorism.[3] No bio-terrorist strike has been recorded in India to date but this may be due to very low levels of suspicion and lack of definitive investigative procedures.[4]

Recognition of a bio-terrorist attack will require first and foremost, considering its possibility when the epidemiology of an illness outbreak is unusual. This would have to be followed by rapid action specific to the affected individual, as well as initiation of action at a community level. The effects of many of the agents can be ameliorated with prompt treatment or post-exposure prophylaxis. Active participation of physicians in, hospital, local and regional disaster planning is therefore imperative.

## Bioterrorism: a real threat

The threat perception of bio-terrorism is legitimized by several factors, principal amongst which are the ease of production of microbes and toxins in laboratories with minimal facilities, access to information on the internet and access to dual use equipment. The economics of biological warfare further, makes it an attractive weapon for the poorly financed ideologue. In 1971 the cost of 50% mortality in a one square kilometer area using various weapons was calculated to be $ 2000, $ 800 and $1 using conventional weapons, nuclear weapons and anthrax respectively! On the other hand the recent anthrax attack on the US congress paralyzed their postal system and cost them $ 6 billion to clean up.

The ease of dissemination of biological agents over large areas and difficulty in detecting release, with first symptoms delayed by days or weeks, are added advantages for the terrorist who desires to terrorize but not be labelled a "dirty" terrorist. The use of bio-agents may cause panic amongst

**Table I : Bioterrorism agents/diseases**

**Category A**
- Anthrax (*Baccilus anthraxis*)
- Botulism (*C. botulinum* Toxin)
- Plague (*Yersinia pestis*)
- Smallpox (*Variola major*)
- Tularemia (*Francisella tularensis*)
- Viral Hemorrhagic Fevers (Filoviruses [e.g. *Ebola, Marburg*] and arenaviruses [e.g. *Lassa, Machupo*], Others)

**Category B**
- Brucellosis (*Brucella species*)
- Epsilon toxin of *Clostridium perfringens*
- Food safety Threats (e.g. *Salmonella species, E coli O157:H7, Shigella*)
- Glanders (*Burkholderia mallei*)
- Meliodosis (*Burkholderia pseudomallei*)
- Psittacosis (*Chlamydia psittaci*)
- Q Fever (*Coxiella burnetii*)
- Ricin Toxin from *Ricinus communis* (Castor Beans)
- Staphylococcal enterotoxin B
- Typhus fever (*Rickettsia prowazekii*)
- Viral encephalitis (alphaviruses [e.g., *Venezuelan equine encephalitis, eastern and western equine encephalitis*])
- Water safety threats (*Vibrio cholera, Cryptosporidium parvum*)

**Category C**
- Emerging infectious diseases such as *Nipah virus* and *Hantavirus*

the victim population while the perpetrator can protect himself and escape before effects occur. Bio-weapons in addition persist for long duration in the population and unless controlled by a good anti-bio-terrorism setup, can have a multiplier effect.

Against such a background at least 17 countries are known to have a biological weapons program and consequent to the collapse of the erstwhile Soviet Union microbe stocks and technology appear to have passed into terrorist hands.[5]

**The "weapons"** - While hundreds of microbes and toxins have potential as biological weapons, certain characteristics make some preferable over others. The key features of biologic agents, which may be used as bioweapons, are :

- Causes high morbidity and mortality.
- Potential for person-to-person spread.
- Low infective dose and highly infectious by aerosol.
- Lack of rapid diagnostic capability.
- Lack of universally available effective vaccine in a short time.
- Potential to cause anxiety.
- Easy availability of pathogen and feasibility of production.
- Environmental stability of the pathogen.
- Database of prior research and development.
- Potential to be "weaponized", meaning ability to be modified for greater virulence and ability to be dispersed with available weapon delivery system.[6]

Based on ease of dissemination, severity of mortality and morbidity and action required from public health agencies, CDC Atlanta has categorized bio-terror agents into categories A, B and C. Category "A" agents are those that can be easily disseminated or transmitted from person to person, result in high mortality rates, have the potential for major public health impact, might cause public panic and social disruption and require special action for public health preparedness. Category "B" diseases or agents are moderately easy to disseminate, result in moderate morbidity rates and low mortality rates and require specific enhancement of diagnostic capacity and enhanced disease surveillance. Category "C" agents are emerging pathogens that could be engineered for mass dissemination in the future because of availability, ease of production and dissemination and have a potential for high morbidity, mortality rates and major health impact. A few important bio-terrorism agents are listed by category in Table 1.[7]

The large number of potential bioterrorism agents provide the terrorist with choices to use agents to achieve any degree of impact that they desire. On the other hand by the same property the specific diagnosis of diseases caused by such agents becomes a task of gargantuan proportions

**Table 2 : Properties of Bio-Terrorism Agents**

| Agent | Transmission mode(Person to person spread -Yes/No) | Incubation and lethality |
|---|---|---|
| **Respiratory Syndromes** | | |
| Anthrax | Spores, aerosol, food(No/Rare) | 1-5 days, High unless treated |
| Plague (pneumonic/bubonic) | Aerosol droplets/flea vectors(Yes) | 1-6 days, High unless treated |
| Q Fever | Aerosol, food/water, tick bites(Rare) | 2-3 weeks, Moderate to low |
| Staphylococcus enterotoxin B | Aerosol, Contaminated food or water. (No) | 1-6 hrs, Lethality < 1% |
| Ricin | Aerosol, Contaminated food or water. (No) | Hours – days, High lethality |
| **Neurological Syndromes** | | |
| Japanese encephalitis | Mosquito bite(No) | 6-16 days, High lethality |
| Venezuelan equine encephalitis | Mosquito bite, aerosol | 1-6 days, Low lethality |
| Botulinum Toxin | Aerosol, Contaminated food or water(No) | 6 Hrs – 14 days, High lethality |
| **Hemorrhagic Fevers** | | |
| Ebola, Lassa, Marburg, | Nosocomial (possible animal reservoir)(Yes) | 2-21 days, High lethality |
| Yellow fever | Mosquito bite(Yes) | 3-6 days, High lethality |
| Dengue hemorrhagic Fever | Mosquito bite(Yes) | 4-5 days, Moderate to high lethality |
| **Fever with Rash** | | |
| Smallpox | Direct contact, body fluids(Yes) | 7-17 days, High lethality |
| Rubella | Aerosol(Yes) | 14-21 days, Moderate to high lethality |
| Epidemic Typhus | Lice (Yes) | 5-9 days, Moderate lethality |
| **Diarrheal Syndromes** | | |
| Cholera | Contaminated food or water(Yes) | Hours, 20-25% lethality, if untreated |
| Shigellosis | Contaminated food or water(Yes) | 2-3 days, High lethality with *S. Dysenteriae,* if untreated |
| Typhoid | Contaminated food or water(Yes) | 3 days-8 wks, Moderate lethality |
| E. Coli O157:H7 | Contaminated food or water(Yes) | 2-8 days, Low lethality |
| **Influenza like syndromes** | | |
| Tularemia | Aerosol, tick/insect bites, food/water(No/Rare) | 3-14 days, Moderate lethality if untreated |
| Brucellosis | Contaminated food or water, aerosol, abraded skin(Rare) | 1-3 weeks, High with Brucella endocarditis |
| **Others** | | |
| Glanders | Direct contact, aerosols, wound contamination(Yes) | 1-5 days, Low lethality with treatment |
| Melioidosis | Contaminated food and water, aerosol, wound contamination(Yes) | 2 days to years, High fatality with untreated bacteremia |
| Aflatoxin | Aerosol, Contaminated food or water(No) | Variable. Lethality depends on dose and route of exposure |

for the physician, keeping in mind especially that diseases due to most of these agents/organisms are otherwise rare. Thus it would be advantageous to adopt a syndromic approach to the diagnosis of diseases caused by such agents. The modes of transmission with specific reference to person to person transmission and incubation period of the category A, B, C agents categorized by disease syndrome are listed in Table 2.[2, 5, 8]

To counter bio-weapons, recognition of risk, accurate diagnosis and rapid treatment is necessary. For most agents specialized testing is necessary

**Table 3 : Disease description, diagnostic tests, treatment and prophylaxis for category 'A' agents**

| Bioterrorism threat disease description | Initial laboratory & other diagnostic test results | Treatment | Prophylaxis |
|---|---|---|---|
| **Inhalational Anthrax**<br>Abrupt onset of fever; chest pain; respiratory distress without radiographic findings of pneumonia; no history of trauma or chronic disease; progression to shock and death within 24-36 hours<br>Cutaneous Anthrax: papular lesion turns to fluid filled vesicle, eschar | Chest x-ray with widened mediastinum, occ. pleural effusion; gram-positive bacilli in sputum or blood; definitive testing available through public health laboratory network<br>Culture definitive 6-24 hrs. Specimen before antibiotic exposure<br>Vesicular fluid, blood for staining and culture | Ciprofloxacin, 400 mg IV q12h *or* Doxycycline, 100 mg IV q12 *plus* Clindamycin, 900 mg IV q8h and/or rifampin, 300 mg IV q12h; switch to PO when stable for 60 d total | Postexposure chemoprophylaxis:<br>Ciprofloxacin, 500 mg, PO bid x 60 days *or* Doxycycline, 100 mg PO bid x 60 days<br>Amoxicillin, 500 mg PO q8h, likely to be effective if strain penicillin sensitive<br>Anthrax vaccine adsorbed. |
| **Botulism**<br>Acute bilateral descending flaccid paralysis beginning with cranial nerve palsies | CSF protein normal; EMG with repetitive nerve stimulation shows augmentation of muscle action potential; toxin assays of serum, faeces, or gastric aspirate available through public health laboratory network. | Supportive measures including ventilation.<br>5000–9000 IU equine antitoxin. | Administration of antitoxin. |
| **Pneumonic Plague**<br>Apparent severe community-acquired pneumonia but with hemoptysis, cyanosis, gastrointestinal symptoms, shock | Gram-negative bacilli or coccobacilli in sputum, blood or lymph node aspirate; safety-pin appearance with Wright or Giemsa stain; definitive testing available through public health laboratory network. | Gentamicin, 2.0 mg/kg IV loading then 1.7 mg/kg q8h IV *or* Streptomycin, 1.0 g q12h IM or IV.<br>Alternatives include doxycycline, 100 mg bid PO or IV; chloramphenicol 500 mg bid PO or IV | Doxycycline, 100 mg PO bid (ciprofloxacin may also be active).<br>Formalin-fixed vaccine (withdrawn). |
| **Smallpox**<br>Fever with papular rash with centrifugal distribution; headache, vomiting, back pain, and delirium common | Clinical with laboratory confirmation; vaccinated, gowned and gloved person obtains specimens (scabs or swabs of vesicular or pustular fluid). Definitive testing available through public health laboratory network. | Supportive measures; consideration for cidofovir, anti-vaccinia immunoglobulin | Vaccinia immunization |
| **Tularemia (Typhoidal, Pneumonic)**<br>Fever, chills, rigors, headache, myalgias, coryza, sore throat initially; followed by weakness, anorexia, weight loss. Substernal discomfort, dry cough if pneumonic disease. | Small, faintly staining, slow-growing, gram-negative coccobacillus in smears or cultures of sputum, blood. CXR may show infiltrate, hilar adenopathy, effusion. Definitive testing available through public health laboratory network. | Streptomycin, 1 g IM bid *or* Gentamicin, 5 mg/kg per day div q8h IV for 14 days *or* Doxycycline, 100 mg IV bid *or* Chloramphenicol, 15 mg/kg IV qid *or* Ciprofloxacin, 400 mg IV bid | Doxycycline, 100 mg PO bid x 14 days *or* Ciprofloxacin, 500 mg PO bid x 14 days |
| **Viral Hemorrhagic Fever**<br>Fever with mucous membrane bleeding, petechiae, thrombocytopenia and hypotension | Definitive testing available through public health laboratory network. | Supportive measures.<br>Ribavirin 30 mg/kg up to 2 g x 1 day, followed by 16 mg/kg IV up to 1 g q6h for 4 days, followed by 8 mg/kg IV up to 0.5 g q8h x 6 days | No known chemoprophylaxis.<br>Consideration for ribavirin in high-risk situations.<br>Vaccine exists for yellow fever. |

by public health specialists or laboratories. For bacterial agents, vaccination and treatment with antibiotics and anti-toxins must be started early to prevent disease progression and death. For viral diseases vaccination is the principal form of prophylaxis: the use of antiviral drugs might be useful, but effectiveness and safety have yet to be established.[5]

Potentially hundreds of agents might be used as bioweapons, however based on threat perception the important ones have been enumerated by CDC as given in Table 1. It is not possible to discuss all bioterrorism agents here, hence only the Category A agents shall be considered. The disease description, laboratory diagnosis, treatment and prophylaxis for these agents are briefly put-forth in Table 3. [2, 9, 10]

## Bioterrorism agents/diseases – important issues

The diagnosis of these diseases which have largely non-specific features at onset, poses a diagnostic challenge for the physician. While treatment of victims of bioterrorism is of paramount importance, the most important weapon by which bioterrorism may be countered is doubtlessly effective prevention of spread of infection by early diagnosis, chemoprophylaxis where indicated, vaccination and personal protection of contacts and health staff. Thus a knowledge of the recommended disease diagnostic criteria, both clinical and laboratory, therapy and prophylaxis, both pre and post exposure with passive and active immunization and recent advances in these areas will aid us in effectively combating the threat of bio-terrorism. While most of these issues have been outlined in table 3 above, some relevant and contemporary issues are being considered in detail below.

### Anthrax

This is one of the most serious forms of biological weapons available to terrorists. The very hardy spore can survive years in the environment, although the vegetative form is incapable of surviving outside the host. Anthrax has four known forms, cutaneous, pulmonary, gastrointestinal and oro-pharyngeal. While the first is the commonest the others are rare. However in case of a bio-attack the pulmonary form would be the commonest, with frequent gastro-intestinal features. Cutaneous forms would also be seen.

#### *Diagnosis*

The definitive diagnosis of anthrax is based on isolation of *Bacillus anthracis* from body fluids and blood by culture. Current recommendations are that once bacillus species is isolated in any case, further confirmation to rule out *B anthracis* must be done. Chest CT scan may show hyperdense hilar and mediastinal nodes, mediastinal edema, infiltrates and pleural effusion. On thoracocentesis hemorrhagic pleural effusions may be found.

#### *Treatment*

Anthrax inhalation causes a fulminant systemic infection and treatment must be initiated pending laboratory confirmation. Because even one to two doses of antibiotics interfere with the culture, samples must be taken before initiating therapy.[11] Antibiotic therapy is as outlined in Table 3 and it must be emphasized that due to concerns of long surviving spores of *B anthracis,* treatment/ prophylaxis is to be given for at least 60 days.

#### *Vaccines*

Whilst an adsorbed cell-free anthrax vaccine is available, its availability is restricted and in a mass casualty setting chemoprophylaxis is the method of choice. Also the vaccine has to be given in six doses with annual boosters and is known to have side effects.

#### *Recent advances*

Efforts to develop newer less reactogenic, easily administered vaccines are on. In this regard advantage has been taken of inducing mucosal immunity and vaccines containing recombinant protective antigen (rPA) or protective antigen (PA) have been delivered intranasally with good results in animals.[12,13] Full length PA expressed on Salmonella

Enterica serovar *Typhimurium*, administered orally to mice, has been shown to confer immunity as have fusion protein molecules of the domain 1 and 4 of PA. Thus an oral vaccine for anthrax is today a possibility.[14]

The pathological mechanisms of the anthrax bacillus have also been studied and the lethal (LeTx) and edema (ETx) toxins identified as the key virulence factors. The protective antigen, which has been found to be a component of these toxins, binds to specific cell surface receptors and allows uptake of these toxins. LeTx has the ability to cleave mitogen activated protein kinase kinases, and the evidence indicates that rather than excessive inflammatory response contributing to shock with LeTx, the immunosuppressive effects of LeTx could promote infection; however, direct endothelial dysfunction may have an important role in shock due to LeTx. Edema factor, a potent adenyl cyclase, may have a major role in shock during anthrax and it may also be immunosuppressive.[15] In light of this the reports of prophylactic and therapeautic use of recombinant and other immunoglobulins targeting the protective antigen imply that we may be able to improve on prophylaxis and treatment for anthrax soon.[16, 17]

## Botulism

Botulinum toxin is the most poisonous substance known to man and as little as 0.1 µgm IV/IM is fatal for an adult. Unfortunately botulinum toxin type-A is also a Food and Drug Administration approved agent used for a variety of disorders. Warfare use of this agent would likely take the form of an aerosolized dispersion of 0.1 to 0.3 µm particles; ideal for deposition in the distal airways. Iraq has produced over 19,000 L of botulinum toxin and equipped 13 missiles with a range of 600 kms with the toxin. The Aum Shinrikyo cult in Japan attempted to use aerosolized toxin at least three times in the early 1990's. Fortunately none of the attempts were successful.

An outbreak of multiple cases of acute flaccid paralysis with prominent bulbar palsies especially with uncommon botulinum toxin type C, D, F, G and E (not from aquatic source) should warn the clinician of a likely bioterror attack. A careful travel, activity and dietary history should be elicited and the patient must be asked if she/he is aware of anyone else with similar symptoms.

### *Diagnosis*

Botulism is frequently mis-diagnosed, most often as a polyradiculoneuropathy, myasthenia gravis, or a disease of the central nervous system. Botulism differs from other flaccid paralyses in its prominent cranial nerve palsies disproportionate to milder weakness and hypotonia below the neck, in its symmetry, and in its absence of sensory nerve damage.

### *Treatment*

Therapy for botulism consists of supportive care and passive immunization with equine antitoxin. Timely administration of passive neutralizing antibody will minimize subsequent nerve damage and severity of disease but will not reverse existent paralysis. Antitoxin should be given to patients with neurologic signs of botulism as soon as possible after clinical diagnosis. The antitoxin used is an equine antitoxin to which hypersensitivity is known and should be tested for.

### *Vaccine/anti-toxin*

Immediate immunity can be provided by passive administration of equine botulinum antitoxin or by specific human hyperimmune globulin; however use of antitoxin for postexposure prophylaxis is limited by its scarcity and its reactogenicity. Pre-exposure immunization which could be achieved by toxoid administration is currently neither recommended for, nor available to the general population. Botulinum toxoid induces immunity over several months and, so, is ineffective as post-exposure prophylaxis. The US army and government have stocks of pentavalent equine anti-toxin and recent work on recombinant human pentavalent anti-toxin has established the "proof of concept" for such therapies; but their availability is very limited.[18]

Animal studies of Fragments of heavy chains of botulinum toxin as vaccine as well as a combination of BoNT (Botulinum Neurotoxin) type A toxoid and a mutant of cholera toxin termed E112K, delivered intranasally, have shown promise but no human vaccine is available to date.[19, 20] The use of combinations of oligoclonal antibodies to the toxin has been shown to neutralize toxin 90 times greater than hyperimmune globulin and should be useful in acute severe cases.[21]

## Plague

Although it lacks the environmental stability of anthrax bacillus, the highly contagious nature and high mortality of plague make it close to an ideal agent for bio-weaponization. Intentional dissemination of plague would probably occur by an aerosol of *Y Pestis*. Symptoms would begin to occur 1-6 days following the exposure and people would die quickly following onset of symptoms. Diagnosis of pneumonic Plague following use of *Y pestis* as a biological weapon would depend on sudden appearance of many persons with fever, cough, shortness of breath, chest pain and hemoptysis, the common occurrence of gastro-intestinal features such as nausea, vomiting, abdominal pain and diarrhea and a fulminant course with high fatality.

In contrast to secondary pneumonic plague, primary pneumonic plague would be characterized by the absence of buboes (except rarely cervical buboes), and on pathological examination, pulmonary disease with areas of profound lobular exudation and bacillary aggregation.

### *Diagnosis*

Chest radiographic findings are variable but bilateral infiltrates or consolidation are common. Gram stain of sputum or blood sample may show gram negative bacilli or cocco-bacilli and Wright, Giemsa or Wayson stain may show bipolar rods. Confirmatory tests such as antigen detection, IgM enzyme immunoassay, immunostaining and polymerase chain reaction are available with specialized labs.

A rapid antigen detection test, a dipstick containing F1 antibody is sensitive and specific in field testing in Madagascar and trials are on in Congo, Tanzania, Mozambique and Malawi.[22]

### *Treatment*

Though streptomycin has traditionally been the drug of choice in community acquired plague, in the mass casualty setting of bio-terrorism doxycycline, ciprofloxacin or chloramphenicol is recommended in appropriate oral doses (Table 3) for adults, children and pregnant women. Prompt use of antibiotics can reduce case fatality rate of plague from nearly 100% to between 5 and 14% in late treated pneumonic plague. Respiratory isolation is critical when a person is suspected to have pneumonic plague because person-to-person transmission is very common. Health care workers need protection from exposure through ruptured buboes or surgical procedures that aerosolize the organism.

### *Post-exposure prophylaxis*

It is suggested in all people during an epidemic/ attack developing a fever of 38.5° C or higher and/or cough. In children dyspnea would be an additional indication for post-exposure prophylaxis. Asymptomatic close contacts of untreated patients should also receive antibiotics for 7 days and watch for fever or cough. Tetracyclines, Doxycycline, Chloramphenicol, Sulfonamides and Fluoroquinolones can all be used.[23]

### *Vaccines*

Vaccines for plague which were available for high risk personnel have been withdrawn and currently no vaccine is available. The killed vaccine was found to be ineffective against primary pneumonic plague while the live vaccines have enough virulence to be unsuitable for human use. Many prospective vaccines have been developed based on combinations of protective plasmid-specified protein antigens F1 and LcrV. A subunit vaccine which combines these antigens and is protective in mice is in phase II trials. Passive aerosolized

antibodies are also protective against respiratory infection in mice and could be useful in emergency treatment.[22]

## Smallpox

It is caused by the Orthopoxvirus Variola and is highly contagious, Because of its high person-to-person infectivity, its viability outside the human host and high case fatality rate, the intentional release of the smallpox virus could cause colossal damage.

The majority of smallpox cases present with a characteristic rash that is centrifugal in distribution, i.e., most dense on the face and extremities. The lesions appear during a 1- to 2-day period and evolve at the same rate. On any given part of the body, they are generally at the same stage of development i.e., from papule to vesicle to pustule. The distribution of varicella (chickenpox) lesions is centripetal, with a greater concentration of lesions on the trunk than on the face and extremities. Chickenpox is most commonly confused with smallpox. The signs and symptoms of both hemorrhagic and malignant smallpox were such that smallpox was seldom suspected until more typical cases were seen and it was recognized that a smallpox outbreak was in progress. Hemorrhagic cases were most often initially identified as meningococcemia or severe acute leukemia. Malignant cases likewise posed diagnostic problems, most often being mistaken for hemorrhagic chickenpox or prompting surgery because of severe abdominal pain.

### *Diagnosis*

Laboratory confirmation of the diagnosis in a smallpox outbreak is important in view of the fact that the disease has been globally eradicated and vaccination stopped worldwide 1980 onwards. Once it is established that the epidemic is caused by smallpox virus, clinically typical cases would not require further laboratory confirmation. Definitive laboratory identification and characterization of the virus involves growth of the virus in cell culture or on chorioallantoic egg membrane and characterization of strains by use of various biologic assays, including polymerase chain reaction techniques and restriction fragment-length polymorphisms. The latter studies can be completed within a few hours.

### *Treatment*

There is no specific therapy for smallpox as yet and the same remains supportive primarily. No antiviral substances have yet proved effective for the treatment of smallpox. Recent studies in tissue culture, mice, and a small number of monkeys have suggested the possibility that cidofovir, a nucleoside analog DNA polymerase inhibitor, might prove useful in preventing smallpox infection if administered within 1 or 2 days after exposure. At this time, there is no evidence to suggest that cidofovir would be effective in the treatment of smallpox once symptoms have appeared. Moreover, the potential utility of this drug would be of limited value in an epidemic, given the fact that it must be administered intravenously and its use is sometimes accompanied by serious renal toxicity.

Any confirmed case of smallpox would be considered an international health emergency. Strict quarantine with respiratory isolation should be imposed for at least 17 days for all suspected contacts of a case of smallpox.

### *Vaccination*

Vaccination is the only effective pre and post exposure prophylaxis available. Currently two vaccines the calf-lymph derived and cell-culture derived vaccines are available and in a survey have been found to be of equal efficacy and equal incidence of adverse effects. A 1:10 dilution was found not to reduce vaccine potential.[24] However individuals who have received a single dose immunization as children have been found not to have lifelong immunity except in endemic areas. Accordingly the United States military has begun to revaccinate personnel who are being deployed outside the USA.

As soon as smallpox is suspected all cases should be isolated and contacts vaccinated and put under surveillance. Vaccination administered within

4 days of first exposure has been shown to offer some protection against acquiring infection and significant protection against a fatal outcome. An emergency vaccination program for all health and disaster response personnel is indicated. However vaccination is not without risk.

Five groups of persons are ordinarily considered at special risk of smallpox vaccine complications: (1) persons with eczema or other significant exfoliative skin conditions; (2) patients with leukemia, lymphoma, or generalized malignancy who are receiving therapy with alkylating agents, antimetabolites, radiation, or large doses of corticosteroids; (3) patients with human immunodeficiency virus (HIV) infection; (4) persons with hereditary immune deficiency disorders; and (5) pregnant women. If persons with contraindications have been in close contact with a smallpox patient or the individual is at risk for occupational reasons, vaccinia immune globulin (VIG), if available, may be given simultaneously with vaccination in a dose of 0.3 mL/kg of body weight to prevent complications. This does not alter vaccine efficacy. If VIG is not available vaccination may still be considered.[25]

## Tularemia

Tularemia, a bacterial zoonosis, is caused by *Francisella tularensis*, one of the most infectious pathogenic bacterias known. Inoculation or inhalation of as few as 10 organisms can cause disease. Its most likely use as a biological weapon would be an aerosol release. Airborne *F. Tularensis* would be expected to principally cause pleuropneumonitis, but ocular, ulceroglandular and oropharyngeal disease with cervical lymphadenitis may also occur. Release in a densely populated area would be expected to result in an abrupt onset of large number of acute, non-specific febrile illness beginning 3–5 days later (incubation range 1–14 days), with pleuropneumonitis/bronchopneumonia developing in a significant proportion of cases during the ensuing days and weeks.

The onset of Tularemia is usually abrupt, with fever (38°C–40°C), headache, chills and rigors, generalized body aches (often prominent in the low back), coryza, and sore throat. Pulse-temperature dissociation, with relative bradycardia, has been noted in as many as 42% of patients. A dry or slightly productive cough and substernal pain or tightness frequently occur with or without objective signs of pneumonia, such as purulent sputum, dyspnea, tachypnea, pleuritic pain, or hemoptysis. Nausea, vomiting, and diarrhea may occur. Sweats, fever, chills, progressive weakness, malaise, anorexia, and weight loss characterize the continuing illness. In general tularemia would be expected to have a slower progression and lesser fatality than anthrax or plague. Its milder forms may resemble Q fever, another agent of bioterrorism.

### *Diagnosis*

Rapid diagnostic testing for Tularemia is not widely available. Inhalation tularemia should be suspected in patients presenting with atypical pneumonia, pleuritis, and hilar lymphadenopathy and specimens of respiratory secretions and blood should be collected for special diagnostic procedures. The diagnosis is established by direct examination of secretions, exudates, or biopsy specimens using Gram stain, direct fluorescent antibody, or immunohistochemical stains and microscopic demonstration of *F. Tularensis* using fluorescent-labeled antibodies. The definitive diagnosis is by growth in culture. It can be grown from pharyngeal washings, sputum specimens, and even fasting gastric aspirates in a high proportion of patients with inhalation tularemia. It is only occasionally isolated from blood.

### *Treatment*

In a mass casualty setting, Doxycycline (100 mg PO BD for 14-21 days) or Ciprofloxacin (500 mg PO BD for 10 days), administered orally, are the preferred choices. The same drugs in pediatric doses are advised for children while Ciprofloxacin is preferred to Doxycycline in pregnant women.[26]

*Vaccine*

In the United States, a live attenuated vaccine derived from avirulent *F. Tularensis* biovar palaearctica (type B) has been used to protect laboratory staff routinely working with the bacterium, in the past. Recently work in animal models has shown promising results with a live mutant (attenuated) vaccine strain and live attenuated strain with concomitant administration of interleukin -12. These vaccines are being developed for oral/intranasal use.[27, 28]

*Chemoprophylaxis*

Chemoprophylaxis with 14 days oral administration of Doxycycline/Ciprofloxacin is suggested in the unlikely scenario of an attack being discovered before individuals become ill. If an attack is discovered only after individuals become ill, persons potentially exposed but not ill, should begin a fever watch. Those who develop an otherwise unexplained fever or flu-like illness within 14 days of presumed exposure should begin treatment as outlined above.

**Viral hemorrhagic fevers**

The hemorrhagic fevers viruses are transmitted naturally to humans by animal contact or arthropod vectors. The mode of transmission, clinical illness and mortality of these diseases vary but all are characterized by their capability to cause a hemorrhagic fever syndrome. The virus of this group likely to be used as biowarfare weapons are

- Family Filoviridae – Ebola and Marburg hemorrhagic fever
- Family Arenaviridae – Lassa fever, New World hemorrhagic fever
- Family Bunyaviridae – Crimean-Congo hemorrhagic fever, Rift Valley fever, Agents of hemorrhagic fever with renal syndrome (Hantavirus)
- Family Flaviviridae – Dengue, Yellow fever, Omsk Hemorrhagic fever, Kyasanur forest disease.

Hemorrhagic fever viruses have been weaponized by the former Soviet Union, Russia and the US. North Korea may have weaponized the Yellow fever virus.

*Clinical Profile*

Infected individuals display a non-specific prodrome usually lasting a week with high fever, headache, malaise, myalgia, arthralgia, nausea, abdominal pain and non-bloody diarrhea. Signs at this stage include fever, hypotension, relative bradycardia, tachypnea, conjunctivitis, pharyngitis, cutaneous flushing or rash. Suspected index case may be identified by the following criteria

- temperature $>$ 101° F (38.3° C) of $<$ 3 weeks' duration;
- severe illness
- at least 2 of the following hemorrhagic symptoms: hemorrhagic or purple rash, epistaxis, hematemesis, hemoptysis, blood in stools, other with no predisposing factors for hemorrhagic manifestations; and
- no established alternative diagnosis.

*Diagnosis*

Methods of diagnosis at specialized laboratories include antigen detection by antigen-capture ELISA, IgM antibody detection by antibody-capture ELISA, reverse transcriptase polymerase chain reaction (RT-PCR), and viral isolation. Antigen detection (by ELISA) and reverse transcriptase polymerase chain reaction are the most useful diagnostic techniques in the acute clinical setting.

*Treatment*

On clinical diagnosis of viral hemorrhagic fever (VHF), VHF specific barrier nursing should be instituted. The mainstay of treatment of viral hemorrhagic fever is supportive, with careful maintenance of fluid and electrolyte balance, circulatory volume, and blood pressure. Because in some cases intravenous fluids have not reversed hypotension and may have contributed to pulmonary edema, consideration should be given

to early vasopressor support with hemodynamic monitoring. Mechanical ventilation, renal dialysis, and antiseizure therapy may be required. Intramuscular injections, aspirin, nonsteroidal anti-inflammatory drugs, and anticoagulant therapies are contraindicated. Steroids are not indicated.

Ribavirin, a nucleoside analog, has some *in vitro* and *in vivo* activity against Arenaviridae and Bunyaviridae but no utility against Filoviridae or Flaviviridae. Intravenous ribavirin is suggested in a controlled casualty situation, while in a mass casualty situation like a bio-attack oral treatment is preferable. For pregnant women and children, in consideration of the benefit to risk, ribavirin is recommended in suspected VHF.

### *Vaccine*

A vaccine exists only for yellow fever. In the absence of drugs and vaccines and very limited data with ribavirin, post-exposure prophylaxis is hoped to be achieved by medical surveillance of high-risk individuals and contacts of patients, after a suspected bio-attack. If a temperature of 101° F (38.3° C) or higher develops, ribavirin therapy should be initiated promptly as presumptive treatment of viral hemorrhagic fever.[6, 29]

### *Recent advances*

Ongoing research to develop effective vaccines has shown encouraging results. A common pediatric respiratory pathogen, human para-influenza virus type 3 (HPIV3), has been used as a vaccine vector against Ebola virus. HPIV3 recombinants expressing the Ebola virus (Zaire species) surface glycoprotein (GP) alone or in combination with the nucleocapsid protein (NP) or with the cytokine adjuvant granulocyte-macrophage colony-stimulating factor have been tried with good results.[30] The vesicular stomatitis live attenuated virus has also been used as a vector for the Ebola glycoprotein and has been shown to confer good post-exposure protection too.[31] Similar strategies have been employed for the Marburg virus and Lassa fever.[31, 32] Monoclonal antibodies have been used in combination (more than one Ab) to show a protective effect against Ebola when used 2 days after a challenge and partial protection when used 3-4 days after an Ebola challenge.[33] The glycoprotein (GP) and nucleocapsid (NC) genes of Rift Valley fever virus (RVFV) have been expressed in different expression systems and evaluated for their ability to protect mice from virulent challenge using a prime-boost regime, with encouraging results.[34]

## Combating bioterrorist attacks

The key element in combating a bio-terrorism strike is rapid identification of a strike and the agent used; so that effective countermeasures may be instituted before the agent disseminates widely. The anthrax attack on the US claimed few victims - thanks to rapid intervention by bio-weapons specialists on the suspicion of an alert physician.[5] Identification of bio-attacks however may be expected to pose problems because of:

- occurrence of rare hence ill-recognized diseases
- many agents of biological warfare also cause naturally occurring disease,
- resemblance of biological toxins to chemical agents rather than infectious organisms, and
- the concomitant use of more than one agent.[5, 9]

The Iraqi government used mustard gas, a nerve agent and anthrax or aflatoxin together in their northern provinces. Thus detection of the agent and subsequent decontamination is difficult, symptoms are complicated and mortality much greater.

*Factors which should arouse suspicion of bio-terrorism are*:

## Clinical Settings

- Suddenness of onset of disease in many people and close clustering of cases
- Unusually large numbers of cases of a particular disease
- Unusual geographic or demographic distribution. An unusual geographical distribution of persons or animals at the time of their probable exposure could point to deliberate use. Aerosol release

resulting in an airborne cloud, for example, would give a distribution consistent with meteorological conditions at the time. Other unusual distributions or association with suspicious objects or activities may also be indicative of deliberate use.

- Rareness. The unexplained appearance of an infectious disease of humans or animals that is ordinarily very rare or absent in a region may indicate deliberate use.
- Severity of disease following inhalatory infection. Disease initiated by inhalatory infection may follow a course and exhibit symptoms differing from and more severe than those characteristic of other natural, routes of entry.[35]

## Clinical syndromes

- Acute severe pneumonia or respiratory distress.
- Encephalopathy.
- Acute onset neuromuscular symptoms.
- Otherwise unexplained rash with fever.
- Fever with mucous membrane bleeding.
- Unexplained acute icteric syndromes.
- Massive diarrhea with dehydration and collapse.

## Protection and Precautions

Individual protection focuses on the use of suits, masks, self contained breathing apparatus, respirators etc. however none of the methods is foolproof and the effectiveness of individual protection is a matter of duration of exposure, and the type and dose of the agent to which one is exposed.[36] Physicians and healthcare personnel are amongst the prime respondents in case of a bio-terrorism strike and because of the very nature of the diseases in such settings they may be exposed to the agents, especially those that spread by contact or from person to person, before it is realized that a bio-terror attack has occurred.

During the spread of a biological aerosol, the primary route of exposure will be via the airways and respiratory tract. Respiratory protection will then be the most important component of physical protection. Particulate filters are generally adequate. Most of the agents of special concern do not cause contagious disease, but some do, and if these become established in the population, the spread of aerosol droplets, contact between infected body fluids and mucous membranes or broken skin, and even ingestion may all be involved in the secondary spread of the agent. Universal precautions for dealing with potentially infective materials should therefore always be strictly and assiduously followed.

The protection of responders should be based on the standard principles of barrier nursing and infection control.[37] VHF-specific precautions involving strict hand hygiene, double gloves, impermeable gowns, N-95 masks or air purifying respirators, leg and shoe coverings, face shields, goggles, restricted access, dedicated medical equipment, environmental disinfection (e.g., 1:100 household bleach) and caring for all affected cases in the same part of the hospital become of paramount importance when treating cases of suspected viral hemorrhagic fevers.[29]

Vaccination or prophylactic antibiotic treatment of those involved in response may have to be considered. This is more likely to be useful in the management of any secondary spread of the infection than for the primary manifestations of the attack. Pre-attack vaccination of healthcare providers may be considered if appropriate vaccines are widely available (e.g. for smallpox and possibly anthrax).

## Laboratory response

The Laboratory Response Network for Bioterrorism, formulated by the Centre for Disease Control and Prevention is an internationally approved consortium of academic, private and public health laboratories that follow consensus protocols to rule out and identify micro-organisms that may be used

in bioterrorism. The laboratories are classified as "Sentinel" (screening) laboratories which carry out simple tests on clinical specimens only and can help in early detection, presumptive identification and "ruling-out" of organisms, but are not equipped to make a definitive diagnosis. They may send samples to "Confirmatory" laboratories which are major public health laboratories and can perform definitive testing and further characterization. Confirmatory labs in India are Microbial containment center NIV Pune, NICD Delhi, NICED Kolkata, TRC Chennai, EVRC Mumbai, PGI Chandigarh and JALMA Agra. The CDC and USMARIID in the United States of America are "Reference" laboratories capable of high level tests, probing for the universe of organisms and archiving of samples.

### Biosafety levels (BSL)

The laboratories which are a part of the laboratory response network for terrorism conform to biosafety levels. Four biosafety levels are described which consist of combinations of laboratory practices and techniques, safety equipment and laboratory facilities. BSL 1 represents a basic level of containment that relies on standard microbiological practises with no special primary or secondary barriers. Labs with this level of safety e.g. in teaching institutions, are competent to carry out work with well characterized strains of viable organisms not known to cause disease in healthy adult humans. Laboratories with BSL 2 practices handle a broad spectrum of indigenous moderate-risk agents known to cause disease of varying severity. This level of safety involves good microbiological techniques, use of protective clothing and a combination of open bench-work and use of biosafety cabinets (BSC) for potential aerosols. BSL 3 practices are designed for special diagnostic services and research and involve use of special clothing, directional air-flow and controlled access. All work is carried out in biosafety cabinets. No bench-work is permitted. BSL 4 (Maximum containment) practices are meant for work with dangerous and exotic agents which cause disease for which no known vaccine or therapy exists. The lab practices in addition to BSL 3 include air-lock entry, shower exit, special waste disposal systems, use of Class II BSC (or Class I BSC with pressure suits), double ended (through the wall) autoclaves and filtered air through special air handling units. The biosafety levels recommended for reference, confirmatory and screening laboratories are level 4, 2 or 3 and 2 respectively.

### Biosafety cabinets (BSC)

These are classified as Class I to Class III. They work on the principle of removal of all aerosolized material within them by means of a draught of air. Thus they ensure that the hazardous organism does not contaminate the technician or the lab. The BSC Class I provides personnel and environment protection but because of non-sterile room air passing over the sample does not allow its protection from contaminants. BSC Class II provides personnel and sample protection and can be used for processing dangerous specimens if the technician uses a pressure suit. The exhaust from BSC Class I and II is passed through high-efficiency particle air (HEPA) filters to prevent environmental contamination. BSC Class III has HEPA filtered air inlets and outlets, all penetrations are sealed, the interior is at negative pressure, is accessed through heavy duty rubber gloves attached to ports in the cabin and can be connected to an autoclave to decontaminate all material entering or exiting it. It provides the highest level of personnel safety and is suitable for BSL 3 or 4 laboratories. Thus a high level of preparedness is required in our laboratories to be able to tackle the specific problems of bioterrorist attacks.

While the prospect of a deliberate attack on civilian populations with disease producing agents may seem to be an act of incomprehensible evil, history shows us that it is something that has been done in the past and will likely be done again in the future. Physicians and healthcare workers shoulder a huge responsibility where bio-terrorism is concerned. They are amongst the prime respondents in an overt attack, and

are responsible for discovery of covert attacks. Terrorist attacks in our cities, using conventional weapons are a reality we live with. With the low cost of producing bio-weapons the spectre of bio-terrorism is a possibility that can no longer be ignored. For a fast and effective response to any bio-terrorist attack in the future it is imperative that physicians have a thorough knowledge of the diseases caused by bio-terrorist agents, have a high index of suspicion and always be alert to the possibility of bio-terrorism.

## Summary

Bio-terrorism entails the unlawful use, or threatened use, of micro-organisms or toxins derived from living organisms to produce death or disease in humans, animals or plants. The recent uses of weapons of biological warfare by the government of Iraq in its northern provinces, and the anthrax attacks in the USA have validated the perception of the threat of bio-terrorism as a real possibility. The economics of bio-terrorism – cheap for the terrorist and costly for the institution attacked – further makes it an attractive option to the terrorist.

The list of potential bio-terrorist agents is large. However some of these agents have been identified as more likely to be used by terrorists because of their ease of production, low infective dose, high virulence, capacity for person-to-person spread, lack of rapid diagnostic capability, lack of universally available effective vaccine in a short time, and potential to be "weaponized". Anthrax, tularemia, plague, smallpox, botulism and viral hemorrhagic fever are some of the diseases which are perceived to pose the highest threat.

To tackle the threat of bio-terrorism effectively, it is imperative that physicians, who shall be amongst the primary responders in case of a bio-terrorism attack, maintain a very high degree of suspicion, have a thorough knowledge of the disease profile, therapy, and pre and post-exposure prophylaxis of bio-terrorist agents.

This article summarizes information on diseases caused by high threat bio-terrorism agents, their clinical picture, laboratory diagnosis, therapy and prophylaxis, for the benefit of the clinician. An attempt has also been made to touch on the entire range of precautionary measures, in clinical practice as well as laboratory processing of samples obtained from patients affected by weapons of bio-terrorism. Factors that should arouse a physician's suspicion of a bio-terrorist attack have been brought out to better sensitize us to the ever present threat of bio-terrorism.

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