

# Biological Warfare — An Emerging Threat

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## Abstract

As we approach the 21st century, there is an increasing worldwide awareness and threat regarding the use of biological warfare agents both for war and terrorist attack. Biological agents include microorganisms or biological toxins that are used to produce death in humans, animals and plants. They are characterized by low visibility, high potency, substantial accessibility and relatively easy delivery. Biological warfare agents are unconventional weapons that can be delivered by unconventional means like aerosol sprays, food and water contamination, conventional explosive munitions or by covert injections. Because of their concealed delivery, easy transportation and difficult identification they are readily adaptable for terrorist operations or to gain political advantages. The detection of such attack requires recognition of the clinical syndromes associated with various biological warfare agents. Diagnosis can be made on clinical grounds and on investigations. Protective measures can be taken against biological warfare agents. These should be implemented early (if warning is received) or later (once suspicion of agent use is made). After the confirmation of diagnosis emergency medical treatment and decontamination are performed in rapid sequence. Patients are then evacuated and specific therapy is given according to the agent involved. Appropriate emergency department and hospital response could significantly limit the morbidity and mortality of biological warfare agents. ©

## INTRODUCTION

Biological warfare has been waged intermittently for nearly 2,500 years<sup>1</sup> and the deliberate use of microorganisms and toxins as weapons has been attempted throughout the history. Biological warfare has evolved from the crude use of cadavers to contaminate water supplies to the development of specialized munitions for battlefield and covert use. The modern development of biological agents as weapons has paralleled advances in basic and applied microbiology. These include the identification of virulent pathogens suitable for aerosol delivery and industrial scale fermentation process to produce large quantities of pathogens and toxins.<sup>2</sup> Biological weapons are cheap, can cause mass casualties and are relatively easy to produce, even by developing nations. Long standing sources assign the following costs to prosecute a war : conventional arms \$ 2,000/Km<sup>2</sup> vs chemical weapons \$ 600 /Km<sup>2</sup> vs biological weapons \$1Km<sup>2</sup>.<sup>3</sup> These factors have led to the attraction of terrorist groups as well to chemical and biological weapons. The deployment of these agents is no longer a hypothetical scenario but a life-threatening contingency. It presents serious challenges for patient

treatment and for prophylaxis of exposed persons. Environmental pollution could pose continuing threats. We have tried to present in this article a comprehensive review on biological warfare and its consequences.

## HISTORIC BACKGROUND

The history of biological warfare is difficult to assess because of a number of confounding factors. These include difficulties in verification of alleged or attempted biological attacks, the use of allegations of biological attacks for propaganda purposes, the paucity of pertinent microbiological or epidemiological data and the incidence of naturally occurring endemic or epidemic diseases during hostilities.<sup>2</sup>

The use of biological agent is not a new concept<sup>2</sup> and history is replete with examples of biological weapon use. Attempts to use biological weapons date back to antiquity. Scythian archers infected their arrows by dipping them in decomposing bodies or in blood mixed with manure as far back as 400 BC.<sup>1</sup> Persian, Greek and Roman literature from 300 BC quote examples of the use of animal cadavers to contaminate wells and other sources of water.

In 190 BC, at the battle of Eurymedan, Hannibal won a naval victory over King Eumenes II of Pergamon by firing earthen vessels full of venomous snakes into the enemy ships.

In 18th century AD, British forces distributed small pox infected blankets to native Americans to create transmission

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of disease. During First World War, Germans developed anthrax, glanders, cholera and a wheat fungus for use as biological weapons.<sup>4</sup> Likewise, during Second World War, Japanese operated a secret biological warfare research and carried out human experiments with plague, anthrax, syphilis on Chinese prisoners.<sup>4</sup> In 1940s and 50s, United States and Britain continued research on various offensive biological weapons like anthrax and botulinum toxin and also continued to the 60s. In 1970s, USSR and its allies were suspected of having used yellow rain (trichothecene mycotoxins) during campaigns in Cambodia and Afghanistan, which caused alimentary toxic aleukia (ATA) in civilians.<sup>5</sup> In 1979, 66 people were killed due to accidental release of anthrax from a weapons facility in Sverdlovsk, USSR.<sup>6</sup> Since the 1980s, terrorist organizations have become users of biological agents. The most frequent bioterrorism episodes have involved contamination of food and water. In September, 1984, international contamination of restaurant salad bars in Oregon by followers of Bhagwan Rajneesh infected 751 persons with *Salmonella typhimurium*.<sup>7</sup> Recently, in a short span of time, i.e. from Sept. to Nov. 2001, 23 cases of bio-terrorism occurred in US which mostly involved, postal workers, where letters contaminated with anthrax were handled or opened.

### Definition

Biological weapons or biological warfare agents include microorganisms or biologic toxins that are used to produce death or disease in humans, animals and plants.<sup>5</sup> The ability of infectious agent to cause widespread illness and thus to cause societal disruptions and panic, together with low cost of these agents led to their being called as "Poor Man's Nuclear Arsenal".<sup>8</sup>

Desirable biological weapons are characterized by low visibility, high potency, substantial accessibility and relatively easy delivery (as an aerosol with particle diameter size 1-5µm).

### Classification of Biological Warfare Agents (BWA)

The biological warfare agents can be classified as :

Bacteria	Anthrax, Plague, Brucellosis, Cholera, Clostridium perf toxin, Staph enterotoxin B, Melioidosis, Tularemia
Virus	Congo Crimen Hemorrhagic Fever, Ebola Hemorrhagic Fever, Small Pox, Rift Valley Fever, Venezuelan Equine Encephalitis
Fungus	Trichothecene Mycotoxin
Rickettsia	Q Fever
Misc	Saxitoxin (derived from paralytic shellfish) Ricin (cytotoxin derived from castor bean mesh)

### Biological Warfare Agents - Uses and Consequences

Biological warfare agents are still used as they were before 20th century. The employment of BWA is not limited to war alone, but can occur at anytime, at any place and by anyone. They can be employed as weapons of mass destruction. Aerosols of biological warfare agents may deliver incapacitating or lethal inocula over large geographic areas

and produce mass casualties or as plant pathogens to destroy crops, devastate the food chain and cause famine. Contamination of food and water is another mode of delivery to targeted population. The use of biological warfare agents has far reaching consequences. The threatened use of BW agents can result in fear and panic in a population, whether under attack or being threatened to gain political advantages in political activities. The stress associated with a biological attack could create high numbers of acute and potentially chronic psychiatric casualties.<sup>9</sup> Because of their concealed delivery, easy transportation, difficult identification and easy escape of performer before BW agent release is apparent, they are readily adaptable for terrorist operations. They may also be employed during political events (especially multinational events)<sup>10</sup> to create injury or political disorders.

### Modes of Delivery

BWA are unconventional weapons and can be delivered by unconventional means. The most effective method is aerosol sprays (most likely to be used by terrorists and military groups), because of their particle size (1-5µm) due to which they are most efficiently delivered to their target (air sacs of lung).<sup>11,12</sup> Aerosol generators, generate particle of optimal size and deliver aerosol via point source (fixed aerosol devices with sprayers) or line source (such as moving vehicle, airplane, boat). Other modes of delivery are food and water contamination, conventional explosive munitions and by covert injections.

### Portal of Entry

These BW Agents mainly enter through respiratory tract (following inhalation of aerosolized BWA). Others routes are exposed mucosal surfaces, (nose/mouth/eyes), GIT (through contaminated food and water), intact skin (barrier against most BWA except mycotoxin) and injection (traumatic wounds).

### Environmental Detection

Currently no reliable detection system exists for BWA. Methods being developed are -<sup>13</sup>

*Biological Integrated Detection System (BIDS)*, which is a multi-component system that provides monitoring, sampling detection and presumptive identification. BIDS is vehicle based and must be located in BW aerosol cloud to detect agents. These technologies use components that automatically determine the count/size of particle, determine if particles are living organisms, classify some basic cell characteristics using Ag-Ab analysis for identification.

*A Short Range Biological Standoff Detection System (SRB SDS)*, which employs UV and laser-induced fluorescence to detect aerosol clouds.

*A Long-Range Biological Standoff Detection System (LRB SDS)*, which employs laser system mounted in a helicopter to scan, designated area of interest.

*Portal Shield System*, which consists of network of biological and chemical point detectors, linked to computer/communication control systems.

*Joint Biological Point Detection System*, which is an automatic air-sampling device and provides visual and audible alarms in presence of biological warfare agents.

*By Examination of Environmental Samples.* Point source munitions will leave environmental residue of BWA near point of release.

### **Management of Biological Warfare Agent Casualties**

Principles of management include :

1. Recognition of biological warfare injuries.
2. Triage
3. EMT (Emergency Medical Treatment)
4. Decontamination
5. Medical evacuation
6. Specific therapy
7. Prevention

Medical personnel must be familiar with signs and symptoms of BWA casualties and must attempt to distinguish between epidemic of natural origin and BW attack.

### **CLINICAL RECOGNITION OR DIAGNOSIS**

Successful management of exposure to BWA relies on early recognition. Medical units should rely on information not only from detectors and intelligence sources, but also from casualties themselves. This applies particularly to BW weapons/agents since at present there are no rapid methods of identification or detection. Some of the problems in recognition and diagnosis of BWA attack are discussed here.

Unlike chemical agents, which typically lead to violent disease syndromes within minutes at site of exposure, disease resulting from biological agents have incubation period of days.<sup>14</sup> This attack may not be apparent until days or even weeks after the attack has occurred. Therefore, the first indication that a BW attack has occurred may be large number of patients simultaneously presenting with a similar disease. Such an event could be confused with naturally occurring epidemic. Early identification of BW attack may be further confounded by difficulties in early clinical diagnosis. Other potential confounding factors are, lack of clinical experience with potential BW agents, and possible difference in clinical presentations from a naturally acquired disease versus an aerosolized agent. Classic, fully differentiated syndromes may not be apparent until late in the clinical course. The nature and timing of symptoms will vary with the route of exposure, nature and dose of agent used. Early recognition of first few cases of disease enable medical personnel to implement BW defensive measures.<sup>14</sup>

Preliminary criteria for suggestive outbreaks of disease that could provide indications of a possible biological weapons event include the following :

- \* Disease (or strain) not endemic
- \* Unusual antibiotic resistance patterns
- \* Atypical clinical presentation
- \* Case distribution geographically and/or temporally

inconsistent (e.g., compressed time course)

- \* Other inconstant elements (e.g., number of cases, mortality and morbidity rates, deviations from disease occurrence baseline)

Indications of possible BW agent attack include the following :

- \* Disease entity that is unusual or that does not occur naturally in a given geographic area
- \* Multiple disease entities in the same patients, indicating that mixed agent have been used in the attack
- \* Large number of both military and civilian casualties when such populations inhabit the same area
- \* Data suggesting a massive point-source outbreak
- \* Apparent aerosol route of infection
- \* High morbidity and mortality rates relative to the number of personnel at risk.
- \* Illness limited to fairly localized or circumscribed geographic areas
- \* Low attack rates in personnel who work in areas with filtered air supplies or closed ventilation systems
- \* Absence of a competent natural vector in the area of outbreak (for a biological agent that is vector-borne in nature)

### **Lab Diagnosis**

1. Most of the attacks are clinically recognized.
2. They are further identified by usual lab tests (microscopy, culture, ELISA, mass spectroscopy, animal inoculation methods, Ab detection (e.g. IgM), PCR and by detection of metabolic products of infections/toxic agents in clinical specimens.

### **Triage**

Triage is done as whether EMT or decontamination requires priority.

1. *Immediate* : Casualties who require life saving care within a short time, when that care is available and of short duration.
2. *Delayed* : Casualties with severe injuries who are in need of major or prolonged surgery or other care and who will require hospitalization, but delay of this care will not adversely affect the outcome of the injury.
3. *Minimal* : Casualties who have minor injuries, can be helped by non-physician medical personnel, will not be evacuated, and will be able to return to duty shortly.

### **Emergency Medical Treatment (EMT)**

EMT and decontamination may be performed in rapid sequence. Treatment follows the universally accepted algorithm of first ensuring the adequacy of airway breathing and circulation.<sup>16</sup>

### **Decontamination**

Decontamination is the physical process of removing residual chemicals from persons, equipment and the

**Table 1 : Summary of Biological Warfare Agents**

Disease (Causative Agent)	Incubation Period	Early Symptoms/ Prodrome	Highly Suggestive Signs/Clinical Syndrome	Diagnostic Assay (Characteristic Findings)
Inhalational Anthrax (Bacillus anthracis)	1-6 days (up to 42 days reported in literature)	*Non-specific: fever, malaise, cough, dyspnoea, headache, chills, weakness, vomiting, abdominal and chest pain	*Widened mediastinum on chest X-ray in a previously healthy febrile person *Brief (0-3 d) improvement after prodrome, then rapid onset of severe resp. distress, stridor, resp. failure (due to hemorrhagic mediastinitis and thoracic lymphadenitis) shock, and death within 24-36 hrs. *Parenchymal infiltrates unusual. *Hemorrhagic meningitis may also occur.	*Gram stain (can be done on unspun blood) or Wright stain culture (positive w/in 6-24 hrs) *Antigen detection (DFA, ELISA, and PCR in ref labs only). (Large gram positive encapsulated bacilli non-motile)
Smallpox (Variola virus) Note: If smallpox is suspected, report case immediately - before obtaining diagnostic samples.	7-17 days (avg 12-14 days)	*Non-specific: fever, malaise, headache, prostration, rigors, vomiting, severe backache	*Centrifugal, synchronous rash (all lesions at same developmental stage) Maculopapular, vesicular, then pustular, begins on face, mucus membranes, hands and forearms, may include palms and soles, spreads to lower extremities and then to trunk; lesions deeply seated in dermis. *Death in - 35%.	*PCR *Viral isolation, *Electron or light microscopy, *Serology (200nm brick-shaped DNA virus [orthopoxvirus])
Pneumonic Plague (Yersinia pestis)	1-6 days (avg 2-4 days)	-Non-specific high fever, cough, chills, dyspnoea, headache, common	*Fulminant pneumonia, often with hemoptysis, *Rapid progression of respiratory failure, septicemia and shock. *Pneumonic consolidation on x-ray and hemoptysis distinguish plague from inhalational anthrax	*Gram, wright, Giemsa, Wayson or FA stain, *Culture *Rapid assays (ELISA, DFA, PCR) in ref. Labs (Gram negative coccobacilli. "safety- pin- bipolar staining)
Botulism (Clostridium botulinum toxins)	2 hours 8 days (avg 1- 3 days) *Foodborne 12-36 hrs *Inhalational 24-48 hrs	*Usually none. If foodborne, possibly nausea, vomiting, abdominal cramps or diarrhoea	*Acute, afebrile, alert pt. *Symmetrical cranial nerve palsies and descending paresis/flaccid paralysis *Bulbar symptoms - ptosis, diplopia, dysarthria, dysphonia, dysphagia.	*Clinical diagnosis. *Mouse bioassay for toxin (takes 1-2 days) Available at NYS Clinical Bacteriology Laboratory only Ag. - ELISA
Q Fever (Coxiella burnetii)	10-40 days	*Chills, Sweats, Headache, Cough, Myalgias	*CXR-Patchy Infiltrates *Leucocytosis	*Serology - ELISA - IFA
Staphylococcal	1-5 days	*Fever, Headache, Myalgia, Cough,	*Elevated transaminases *Pulmonary edema *High gr Fever, Cough Ab-ELISA	*Ag-Elisa *Serology
Enterotoxin-b	Chest Pain, nausea,	(last for upto 4 weeks) vomiting diarrhea	*Conjunctival injection	
Brucellosis	5-60 days	*Fever, Chills, sweat, headache myalgia, anorexia Chest Pain.	*Pallor, arthritis, spinal tenderness, lymphadenopathy hepatosplenomegaly, meningitis, pneumonia	*Serology agglutination *Culture

environment. Every person arriving at Medical Treatment Facility (MTF) from biological warfare contaminated area is considered contaminated unless there is positive proof to contrary.<sup>17,18</sup>

- (a) Initial decontamination involves removal from the contaminated environment, removal of all contaminated clothes and copious irrigation with water.
- (b) Exposed person is revised with dilute household bleach solution.

(c) Patient is placed in PPW (Patient Protective Wrap) for protection from BWA and should be isolated in designed tents.<sup>19</sup>

**Medical Evacuation**

After the identification and decontamination of casualties, measures must be taken to prevent contamination of ambulance and air evacuation assets. Many BWA casualties may be safely evacuated using basic infection control guidelines.<sup>20</sup> The United States Army Medical Research

**Table 1 : Summary of Biological Warfare Agents (contd)**

Infection Control/Isolation	Chemotherapy	Chemoprophylaxis	Vaccine availability
*No person to person transmission *Isolation not required. *Standard precautions. *Decontaminate accidental spills of potentially contaminated material using disinfectant (5% hypochlorite).	Penicillin-resistant or unknown sensitivity: *Ciprofloxacin 400 mg IV 12 q *Doxycycline 200 mg IV then 100 mg IV q 12 Known penicillin- sensitive: *Penicillin G 4 million U IV q 4; *Amoxicillin 500 mg IV q 8 *Streptomycin 30mg/kg IM qd Duration: 60 days unless vaccinated.	Penicillin-resistant or unknown sensitivity: *Ciprofloxacin 500 mg PO bid Known penicillin sensitive: *Amoxicillin 500 mg PO tid or *Doxycycline 100 mg bid Duration: 60 days (if vaccinated, then 30 days)	Michigan Biological Institute Vaccine (Licensed) 0.5 ml SC at 0,2,4 weeks and 6,12,18 Mo. then annual boosters.
*Highly transmissible *Isolation required. (negative pressure, HEPA filtration) *Contact and airborne precautions for 17 days following exposure *Pt. most infectious for the 7-10 d following onset or rash	*Supportive care antibiotics as indicated to treat secondary infection *Cidofovir (effective in Vitro)	*VIG (0.6 ml/kg IM within 3 days) for serious complications of smallpox vaccination. Note: neither smallpox vaccine nor VIG are commercially available. Would only be released by CDC if smallpox case(s) confirmed.	*Wyeth Calf Lymph Vaccinia Vaccine *DOD cell-culture derived vaccinia vaccine (IND) *Vaccination within 4 days of exposure
*Highly transmissible. *Resp isolation until pt. has been treated with antibiotics for 48-72 hrs. *Droplet precautions until patient treated for) 3 days.	*Streptomycin 3 mg/kg IM qd in 2divided doses or 1 gm IM BID *Gentamicin 5 mg/kg IM or IV q 24 or 2mg/kg loading dose followed by 17 mg/kg IM or IV q 8 In mass casualty situation: *Doxycycline 100 mg PO bid: *Ciprofloxacin 500 mg PO bid *Chloramphenicol 1 g IV q6H Duration: 10-14 d days	*Doxycycline 100 mg PO bid. *Ciprofloxacin 500 mg PO bid *Tetracycline 500 mg PO QID Duration: 7 days	*Greer Inactivated vaccine (licensed) 1 ML, than 0 2 ml boost at 1-3 & 3 - 6 MO
*Standard Precautions	*Supportive care (long term ventilation may be needed.) *CDC trivalent equine antitoxin for serotypes A, B, E. *DOD heptavalent antitoxin for serotypes A-G.	*None	*DOD Pentavalent Toxoid For Serotypes A-E (IND) SC at 0,2 & 12 week than yearly boosters
*Standard precautions	*Tetracycline 500 mg PO q 6h *Doxycycline 100 mg PO q 12 Duration : 5-7 days	*Tetracycline, Doxycycline (start 8 -12 d post-exposure) Duration : 5 days	* (IND) 610 Inactivated Vaccine as single 0.5 ml SC
*Standard Precautions	*Ventilatory support and supportive care	*Not available (NA)	*NA
*Standard Precautions	*Doxycycline 200mg/d PO + *Rcin 600-900 mg/d PO Duration : 6 weeks	*Doxycycline and Rcin x 3 weeks (for inadvertently inoculated lesions)	*NA

Institute of Infectious Diseases maintains an aero-medical isolation team (AIT) which is a rapid response team with worldwide aircraft capability. It is designed to safely evacuate and manage patients with potentially lethal communicable disease. It offers portable containment lab and limited environmental decontamination and specialized consultant expertise. Indications for deployment include cases of highly contagious, lethal or unidentified disease including cases from suspected BW attack.

**Specific Therapy**

Specific Therapy is given according to specific agent given

in Table 1.

Some of the therapies recommended vary from those found in standard references because :

1. BW exposure (aerosol) may produce a disease with clinical features different from naturally occurring disease eg. inhalation (BW) versus cutaneous (endemic) anthrax.<sup>21</sup>
2. An adversary (enemy/opponent) may develop BWA resistant to standard antibiotic therapy.

## Prevention

Prevention is done by active immunization, chemoprophylaxis and personal protective equipment. Chemoprophylaxis and vaccinations are discussed in Table 1.

The primary responsibility of those who treat victims of BWAs is to protect themselves by wearing adequate protective equipment.<sup>15</sup>

Protective equipment includes -

- \* Military protective mask.
- \* Battle dress over garments.
- \* Joint service light weight integrated suit technology.
- \* Protective gloves.
- \* Overboots.
- \* HEPA filter (High Efficiency Particulate Air) masks.
- \* Double layer of battle dress uniform T-shirt.

## CONCLUSION

Biological weapons have recently attracted the attention and the resources of the nation. The terrorist activities will continue to involve bombs and firearms, also include weapons of mass destruction, including biological agents. Discerning the nature of the threat of bioweapons as well as appropriate responses to them requires greater attention to the biological characteristics of these instruments of war and terror.<sup>22</sup> Media communications, planning for war quarantine and decontamination and the role of community leaders are important in the migration of psychological consequences.<sup>23</sup> Now 140 nations have participated in the Biological and Toxin Weapons Convention (BWC) which prohibits the acquisition of biological materials for hostile purpose and armed conflict.<sup>24</sup> Emergency services must build and maintain their ability to manage large scale biological weapon attacks and that requires continued education, training and forethought.

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The biological warfare threat. Lester C. caudle III, M.D., M.t.M. & h.\* Introduction evidence of a soviet biological warfare program.Â Biological warfare agents may be more potent than the most lethal chemical warfare agents and provide a broader area of coverage per pound of payload than any other weapons system. The pro-liferation of technology and the scientific progress in biochemistry and biotechnology have simplified production requirements and provided the oppor-tunity for creation of exotic agents.1. Citation: Madad SS (2014) Bioterrorism: An Emerging Global Health Threat. J Bioterror Biodef 5:129. doi: 10.4172/2157-2526.1000129. Copyright: Â© 2014 Madad SS.Â The United States later admitted it had a biological warfare program, but denied using them. Many countries mentioned above continued their biological weapons research program well into the late 19th century. In 1972, the Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological and Toxin Weapons and on Their Destruction (known as BWC) was developed and ratified among 103 nations [6]. Despite the agreement of BWC, many countries continued with their offensive biological research program. The term 'biological warfare' is well-known. In this article, we delve into the details of its history, current status, and potential future.Â An early example takes us back more than 2 and a half millennia: Assyrians infected their enemy's wells with a rye ergot fungus, which contains chemicals related to LSD. Consuming the tainted water produced a confused mental state, hallucinations, and, in some cases, death. In the 1300s, Tartar (Mongol) warriors besieged the Crimean city of Kaffa. During the siege, many Tartars died at the hands of plague, and their lifeless, infected bodies were hurled over the city walls . Some researchers believe that this tactic may have been responsible for the spread of Black Death plague into Europ