

A REVIEW OF RESEARCH ON COMMON BIOLOGICAL AGENTS AND THEIR IMPACT ON ENVIRONMENT

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Biological agents are unique class of microorganisms which can be used to produce the disease in large populations of humans, animals and plants. If used for hostile purposes, any disease-causing microorganism could be considered a weapon. The use of biological agents is not a new concept and history is replete with examples of biological weapon use. Before the 20th century, biological warfare took on three main forms by deliberate poisoning of food and water with infectious material, use of microorganisms or toxins in some form of weapon system, and use of biologically inoculated fabrics. Four kinds of biological warfare agents are *bacteria*, *viruses*, *rickettsiae*, *fungi*. These are distinguished by being living *organisms*, that reproduce within their *host* victims, who then become contagious with a deadly *multiplier effect*, bacteria, viruses, or fungi or *toxin* found in nature can be used to kill or *injure* people. Biological agents may be used for an isolated assassination, as well as to cause incapacitation or death to thousands. These biological agents represent a dangerous military threat because they are alive, and are therefore unpredictable and uncontrollable once released. The act of bioterrorism can range from a simple hoax to the actual use of biological weapons. Biological agents have the potential to make an environment more dangerous over time. If the environment is contaminated, a long-term threat to the population could be created. This paper discusses common biological agents, their mode of action in living organisms and possible impact on the environment.

Keywords: Biological weapon, Bacteria, Virus, Toxin, Microorganisms, Anthrax, Agents, Terrorism, Military warfare

1. Introduction

Attempts to use biological warfare agents 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. Persian, Greek, and Roman literature from 300 BC quotes examples of dead animals used to contaminate wells and other sources of water. Allegations were made during the American Civil War by both sides, but especially against the Confederate Army, of the attempted use of smallpox to cause disease among enemy forces. Current concerns regarding the use of bio-weapons result from their production for use in the 1991 Gulf war and from the increasing number of countries that are engaged in the proliferation of such weapons i.e. from about four in the mid-1970s to about many today [1, 2, 3]. With the beginning of the 21st century, the specter of the threat of weapons of mass destruction increased manifold and in this regard biological weapons/bioterrorism has gained significant importance.

Biological warfare (BW) is the intentional use of microorganisms, and/or toxins derived from living organisms to produce diseases and/or death in humans, livestock and crops. In Finland, Scandinavian freedom fighters mounted on reindeer placed ampules of anthrax in stables of Russian horses in 1916 [4]. In World War I, German infected enemy horses and cattle with glanders (*Burkholderia mallei*) and anthrax (*Bacillus anthracis*) and grain with fungi. Germany had biological warriors as far away as the United States and Argentina, where operatives working as labours attempted to infect live animals being loaded for export to Europe. There are several biological agents that are considered potential terrorist weapons agents which can be used by military and/or by non-state elements, in latter case it is referred as bioterrorism [5-7]. These agents are preferred by terrorist groups as they are relatively easy to produce and could result in more deaths compared to chemical weapons [8]. The great advantage of biological agents are their low production costs, the easy access to a wide range

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of disease-producing biological agents, their non-detection by routine security systems, their easy transportation from one location to another in addition to the fact that their small quantities can be very effective [9].

Some biological agents, such as anthrax, do not cause infectious /contagious diseases. Others, like the smallpox virus, can result in diseases you can catch from other people. Unlike an explosion, a biological attack may or may not be immediately obvious. Biological weapon is not only used for diseases that attack humans but also many effective biological weapons attack animals (e.g. avian influenza viruses) and crops (e.g. rice blast - *Piricularia oryzae*). Importantly, biological weapons attacks on crops, animals, and other resources have impacts on humans and ecology, making their potential for disruption even greater than that of the diseases themselves.

Many biological agents that do not generally kill organisms can also be used in warfare. Foot and mouth disease (*Aphthovirus spp.*) is an excellent example. In agriculture, a similar approach might be taken with attacks with pathogens that reduce (or spoil) harvests without necessarily killing the cultivated plants.

There are four classifications for how biological agents can be used. These are Biological warfare, Bioterrorism, Biocrime and Bioaccident:

- **Biological Warfare (BW)** is the military use of biological agents, where targets of agents are predominately soldiers and/or resources that might hinder a nation's ability to attack and/or defend itself. Military leaders, however, have learned that, as a military asset, biological agents has some important limitations; it is difficult to employ a bioweapon in a way that only the enemy is affected and not friendly forces.
- **Bioterrorism (BT)** is the threat or use of biological agents that is intended to make political, religious or personal statements to governments and populations through attacks primarily aimed at civilians or resources that affect the civilian economy. States have been the biggest developers of biological warfare programs. In the twentieth century, Japan, Germany, the (former) Soviet Union, Iraq, the United States and Great Britain all had

biological warfare development plans. During the 1970's the Soviet Union maintained a large supply of weapons grade smallpox as a possible weapon of the Cold War. The Soviet military adapted smallpox as a strategic weapon, essentially introducing it into the warheads of missiles, bombs and artillery shells. In 1972, the United Nations proffered the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction (usually called the Biological and Toxin Weapons Convention, BTWC). By November 2001, there were 162 signatories and 144 of these had ratified the convention. Even after that the countries of Laos and Kampuchea come under attack by planes and helicopters that deliver multi-colored aerosols ("Yellow Rain") over the population. Both man and animal are affected and some die. With the defection of Iraqi General Hussein Kamal Hassan, evidence continues to grow that the Iraqi biological warfare program is more advanced than previously believed. The Iraqi authorities acknowledge that at the time of the war they had 100 botulinum toxin, 50 anthrax, and 16 aflatoxin bombs, 13 botulinum toxin, 10 anthrax, and 2 aflatoxin Scud missile warheads, and 122-mm rockets filled with anthrax, botulinum toxin, and aflatoxin.

With the fall of state-supported use of biological agents, in the late 20th century, violent non-state actors began seeking to acquire or develop biological agents to use in attacks on civilians. Small factions and cults have begun to advance their own interests with bioweapons. The rise of guerrilla tactics by non-state actors in the last half of the twentieth century was due to several factors. These included the flowering of ethnic nationalism (e.g. Irish, Basque, Zionist), anti-colonial sentiments in the vast British, French and other empires, and new ideologies such as communism. Terrorist groups with a nationalist agenda have formed in every part of the world. For example, the Irish Republican Army grew from the quest by Irish Catholics to form an independent republic, rather than being part of Great Britain. Similarly, the Kurds, a distinct ethnic and linguistic group in Turkey, Syria, Iran and Iraq, have sought

national autonomy since the beginning of the 20th Century. Religiously motivated terrorism is considered the most alarming terrorist threat today [7]. Groups that justify their violence (bioterrorism) on the basis of their biased religious approach come from almost all religious group including Islam, Christianity, Judaism, Hinduism and other religions have given rise to their own forms of militant extremism. However, there is no Islamic teaching which justifies terrorism. Bioterrorism is an attractive weapon because biological agents are relatively easy and inexpensive to obtain or produce, can be easily disseminated, and can cause widespread fear and panic beyond the actual physical damage they can cause.

- **Biocrime (BC)** is the threat or use of biological agents for individual objectives such as revenge or financial gain.
- **Bioaccident (BA)** defined as the unintentional release of an agent from a laboratory or other facility. Biocrimes and Bioaccidents comprise events that typically have small effects on populations and do not require specific plans for large-scale preparedness and response.

Some reviews provide descriptions of agents, agent types, characteristics, and the medical hazards of specific agents [10,11,12]

2. Biological Attack Delivery System

Biological agents can be dispersed by spraying them into the air, by infecting animals that carry the disease to humans and by contaminating food and water. Biological agents are delivered in wet or dry form. Dry powders composed of very small particles which have better dissemination characteristics, and have advantages in storage. Dried agents require an increased level of technological sophistication to produce, although freeze drying or spray drying technology has been available in industry for a number of years. Delivery methods include:

- **Aerosols** - biological agents are dispersed into the air, forming a fine mist that may drift for miles. Inhaling the agent may cause disease in people or animals.
- **Animals** - some diseases are spread by insects and animals, such as fleas, mice, flies, mosquitoes, and livestock.

- **Food and water contamination** - some pathogenic organisms and toxins may persist in food and water supplies. Most microbes can be killed, and toxins deactivated, by cooking food and boiling water. Most microbes are killed by boiling water for one minute, but some require longer. Follow official instructions.
- **Person-to-person** - spread of a few infectious agents is also possible. Humans have been the source of infection for smallpox, plague, and the Lassa viruses

Other types of delivery systems for biological agents have been designed by various countries. These include bombs or bomblets that release the agent by exploding (generally very inefficient delivery systems), land and sea mines, pipe bombs, and many other special devices (Figure 1). Clandestine means of delivering biological warfare agents are potentially available to terrorists or special forces units; these include devices that penetrate and carry the agent into the body via the percutaneous route, such as pellets or flechettes, or means to contaminate food or water supplies so that the agent would be ingested.

3. Categorization of Biological Agents

The United States Centers for Disease Control & Prevention (CDC) applied the following 4 criteria to assess and characterize potential biological weapon agents that cause infections in humans into Categories A, B, and C [13].

1. Potential public health impact, which is based on an agent's ability to cause illness and death
2. Dissemination potential, which is based on an agent's stability, mass production and/or delivery potential, and the contagiousness, or the degree to which it will spread from person to person
3. Public perception, which is an estimation of how much fear is associated with an agent and how much civil disruption might ensue
4. Special public health preparations required, which is related to the stockpile, surveillance, and diagnostic requirements associated with an agent.

Characteristics and agents falling in different categories are summarized in Table 1.

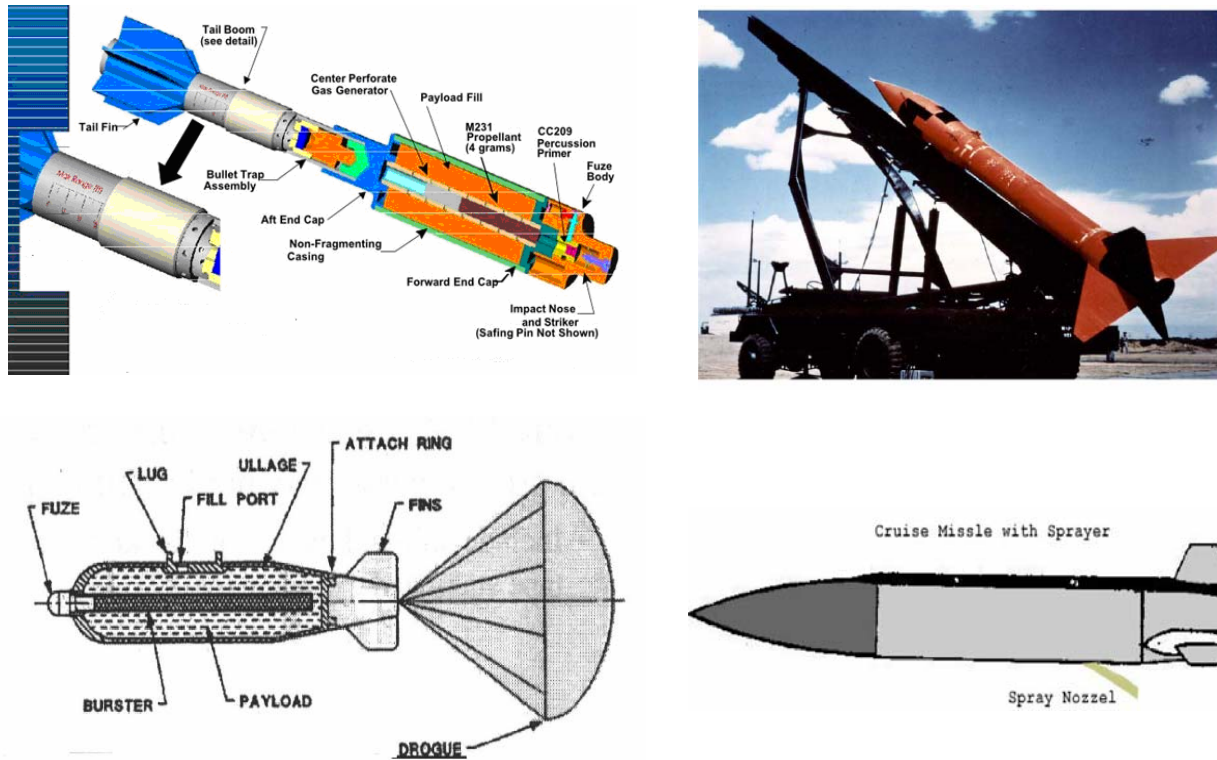


Figure 1. Typical biological delivery weapons.

Table 1. Characteristics of different category biological agents [13].

	Category		
	A	B	C
Biological Agents	Anthrax ,Plague, Tularemia, Botulism, Smallpox, Hemorrhagic Fever , Viruses	Brucella species, Clostridium perfringens, Salmonella, Burkholderia mallei, Burkholderia pseudomallei, Chlamydia psittaci, Q fever, Ricin toxin from Ricinus communis, Staphylococcal enterotoxin B, Typhus Fever, Viral encephalitis, Vibrio cholerae	Includes emerging infectious diseases Like Nipah virus
Characteristics	Easily disseminated or spread person-to-person Highly lethal Serious public health effects May cause great panic and social disruption	Moderately easy to disseminate Moderate morbidity Less lethal than category A agents Require fewer special public health preparations	Potential for wide dissemination and high morbidity, lethality, and major public health effects

4. Commonly Used Biological Agents in Warfare

Among more than 1200 biological agents that could be used to cause illness or death, relatively few possess the necessary characteristics to make them ideal candidates for biological warfare or terrorism agents. The ideal biological agents are relatively easy to acquire, process, and use. Only a small amount would be needed to kill or

incapacitate hundreds of thousands of people in a metropolitan area. Biological warfare agents are easy to hide and difficult to detect or protect against. They are invisible, odorless, tasteless, and can be spread silently.

The two basic groups of biological agents that would likely be used as weapons are live agents and toxins.

a. *Live agents*

Live agents are living organisms like viruses, bacteria, and fungi. They can be delivered directly (artillery or aircraft spray), or through a vector such as a flea or tick. For some agents, only a few organisms are needed to cause infection, especially when inhaled. Live agents are small and light; they can be spread great distances by the wind and can float into unfiltered or non air tight places. These require time after they are ingested to multiply enough to overcome the body's defenses. This incubation period may vary from hours to days or weeks depending on the type of organism. Thus, to be effective, a live agent attack would need to be launched well in advance of a tactical assault. Live agents also have life cycles in which to grow, reproduce, age, and die. While they live, these agents usually require protection and nutrition supplied by another living organism (the host) to survive and grow. Weathering (wind, rain, and sunlight) rapidly reduces their numbers. Some bacterial agents produce spores that can form protective coats and survive longer. However, the hazard from most live agents may only last for one day. Live agents are not detectable by any of the five physical senses; usually the first indication of a biological attack is the ill soldier. The diseases caused by live agents may be difficult to control because they are often easily spread from soldier to soldier, directly or indirectly.

b. *Biological toxins*

Toxins are by-products (poisons) produced by plants, animals, or microorganisms. It is the poisons that harm man, not the organisms which make the toxins. In the past, the only way to deliver toxins on a large scale was by using the organism. With today's technology large quantities of many toxins can be produced; thus, they can be delivered without the accompanying organism. Toxins have several desirable traits. They are poisonous compounds that do not grow, reproduce, or die after they have been dispersed; they are more easily controlled than live organisms. Field monitors capable of providing prompt warning of a toxin attack are not available; therefore, soldiers must learn to quickly recognize signs of attack, such as observing unexplained symptoms of victims. Toxins produce effects similar to those caused by chemical agents; however, the victims will not respond to the first

aid measures that work against chemical agents. Unlike live agents, toxins can penetrate the unbroken skin; when mixed with a skin penetrant such as dimethyl sulfoxide, their speed of penetration is increased. Because the effects on the body are direct, the symptoms of an attack may appear very rapidly. The potency of most toxins is such that very small doses will cause injuries and/or death. Thus, their use by an enemy may be an alternative to chemical agents because it allows the use of fewer resources to cover the same or a larger area. Slight exposure at the edges of an attack area may produce severe symptoms or death from exposure to toxins because of their extreme toxicity. Lethal or injury downwind hazard zones for toxins may be far greater than those of CW agents.

4.1. *Live agent*

Live agents used as weapon are living organisms like bacteria, viruses, and fungi.

4.1.1 *Bacteria*

Anthrax

Anthrax is caused by the bacterium *Bacillus anthracis*, which is an aerobic, gram-positive, spore-forming, non motile bacillus and commonly affects hoofed animals such as sheep and goats [14]. Humans who come into contact with the infected animals can get sick from anthrax, as well. Anthrax spores can remain viable for several decades under suitable environmental conditions. *B. anthracis* spores are indefinitely stable in the environment; they survive for 2 years in pond water [8] and 40 years or more in soil [14,15]. The spores are also heat resistant but are inactivated in water after 25 min. at 95°C [15]. Sporulation occurs only when the organism in the carcass is exposed to air.

There are three main routes of anthrax infection:

- **Cutaneous anthrax** occurs when anthrax touches a cut or scrape on the skin.
- **Inhalation anthrax** develops when anthrax spores enter the lungs.
- **Gastrointestinal anthrax** occurs when someone eats anthrax-tainted meat. The disease usually develops within one week, and can affect the mouth, esophagus,

intestines, and colon. The infection can spread to the bloodstream, and may result in death.

Infection occurs predominantly through the cutaneous route and only rarely via the respiratory or gastrointestinal (GI) route. There is no human-to-human transmission of anthrax. Symptoms of anthrax begin after a 1 to 6 day incubation period following exposure. For contact or cutaneous anthrax, itching will occur at the site of exposure followed by the formation of a lesion. Untreated contact anthrax has a fatality rate of 5-20 percent, but with effective antibiotic treatment, few deaths occur. Initial symptoms for inhalational anthrax are generally non-specific: low grade fever, a dry hacking cough, and weakness. The person may briefly improve after 2 to 4 days; however within 24 hours after this brief improvement, respiratory distress occurs with shock and death following shortly thereafter. Almost all cases of inhalational anthrax, in which treatment was begun after patients have exhibited symptoms, have resulted in death, regardless of post-exposure [16].

Inhalation anthrax is the most likely form of disease to follow military or terrorist attack. Such an attack likely will involve the aerosolized delivery of anthrax spores. Ingestion of anthrax infected meat that has not been cooked sufficiently results in Oropharyngeal and gastrointestinal. After an incubation period of 2-5 days, patients with oropharyngeal disease present with severe sore throat or a local oral or tonsillar ulcer, usually associated with fever, toxicity, and swelling of the neck due to cervical or submandibular lymphadenitis or edema. Dysphagia and respiratory distress also may be present. GI anthrax begins with nonspecific symptoms of nausea, vomiting, and fever. These symptoms are followed in most patients by severe abdominal pain. The presenting sign may be an acute abdomen, which may be associated with hematemesis, massive ascites, and diarrhea. Mortality rate in both forms may be as high as 50%, especially in the GI form.

For anthrax to be effective as a covert agent, it must be aerosolized into very small particles. In the largest biological weapons accident known – the anthrax outbreak in former Soviet Union in 1979, sheep became ill with anthrax at a distance of 200 kilometers from the release point of the

organism from a military facility in the southeastern portion of the city (known as Compound 19 and still off limits to visitors today)

Anthrax is the preferred biological warfare agent because of its highly lethal nature [17]. Easy to produce in large quantities, easy to weaponize, its stability and limited detection capability. Anthrax spores can and have been used as a biological warfare weapon. Scandinavian "freedom fighters" (the rebel groups) used anthrax with unknown results against the Imperial Russian Army in Finland in 1916. In 1942 British bioweapons trials severely contaminated Gruinard Island in Scotland with anthrax spores. Five million "cattle cakes" impregnated with anthrax were prepared and stored at Porton Down in 'Operation Vegetarian'—an anti-livestock weapon intended for attacks on Germany by the Royal Air Force. The infected cattle cakes were to be dropped on Germany in 1944. However neither the cakes nor the bomb were used, the cattle cakes were incinerated in late 1945. Concentrated anthrax spores were used for bioterrorism in the 2001 anthrax attacks in the United States, through postal letters containing the spores. Only a few grams of material were used in these attacks and in August 2008 the US Department of Justice announced they believed that Dr. Bruce Ivins, a senior biodefense researcher, was responsible in helping terrorist for these attack [18].

Plague

Plague is a deadly infectious disease caused by the enterobacteria *Yersinia pestis* (*Pasteurella pestis*). It is a zoonotic, carried by rodents and spread to humans via fleas. Although the largest outbreaks of plague have been associated with fly *X cheopis*, all fleas should be considered dangerous in plague-endemic areas. The black rat, *Rattus rattus*, has been most responsible worldwide for the persistence and spread of plague in urban epidemics.

Plague is notorious throughout history, due to the unprecedented scale of death and devastation it brought. Throughout history, the oriental rat flea (*Xenopsylla cheopis*) has been largely responsible for spreading bubonic plague. After the flea ingests a blood meal on a bacteremic animal, bacilli can multiply and essentially block the flea's foregut with a fibrinoid mass of bacteria.

When an infected flea with a blocked foregut attempts to feed again, it regurgitates clotted blood and bacteria into the victim's bloodstream and so passes the infection onto the next victim, whether rat or human. As many as 24,000 organisms may be inoculated into the host. Plague is characterized by the abrupt onset of high fevers, painful lymphadenopathy, and bacteremia. Septicemic plague sometimes can ensue from untreated bubonic plague or, *de novo*, after a fleabite. Patients with the bubonic form of the disease may develop secondary pneumonic plague. This complication can lead to human-to-human spread by the respiratory route and cause primary pneumonic plague. Pneumonic plague is the most severe form of disease and, untreated, has a mortality rate approaching 100%.

Plague has a long history as a biological weapon. Use of infected animal carcasses, such as cows or horses, and human carcasses, by the Xiongnu/Huns, Mongols, Turks, and other groups, to contaminate enemy water supplies. Han Dynasty General Huo Qubing is recorded to have died of such a contamination while engaging in warfare against the Xiongnu. In 1347, Mongol army sieged Caffa, a great trade emporium on the Crimean peninsula. After a protracted siege during which the Mongol army was reportedly withering from the disease, they decided to use the infected corpses as a biological weapon. The corpses were catapulted over the city walls, infecting the inhabitants [18]. During World War II, the Japanese Army developed weaponised plague, based on the breeding and release of large numbers of fleas. During the Japanese occupation of Manchuria, Unit 731 deliberately infected Chinese, Korean, and Manchurian civilians and prisoners of war with the plague bacterium. These subjects, termed "maruta", or "logs", were then studied by dissection, others by vivisection while still conscious. Members of the unit such as Shiro Ishii were exonerated from the Tokyo tribunal by Douglas MacArthur but twelve of them were prosecuted in the Khabarovsk War Crime Trials in 1949 during which some admitted having spread Bubonic plague within a 36-km radius around the city of Changde [20].

After World War II, both the United States and the Soviet Union developed means of weaponising pneumonic plague. Experiments included various delivery methods, vacuum

drying, sizing the bacterium, developing strains resistant to antibiotics, combining the bacterium with other diseases (such as diphtheria), and genetic engineering. Scientists who worked in USSR bio-weapons programs have stated that the Soviet effort was formidable and that large stocks of weaponised plague bacteria were produced. Information on many of the Soviet projects is largely unavailable. Aerosolized pneumonic plague remains the most significant threat.

Tularemia

Tularemia (also known as "rabbit fever", "deer-fly fever", "Ohara fever" and "Francis disease") is a serious infectious disease caused by the bacterium *Francisella tularensis* which is a small, gram-negative, non-motile coccobacillus. The most important species of this bacterium is *F. tularensis tularensis* (Type A) and is highly virulent for humans and domestic rabbits. *F. tularensis palaeartica* (Type B) occurs mainly in aquatic rodents (beavers, muskrats) and small rodents in northern Eurasia. It is less virulent for humans and rabbits. Typhoidal tularemia produces influenza-like symptoms and may result in death if untreated [21]. The primary vectors are ticks and deer flies, but the disease can also be spread through other arthropods [22]. Under natural conditions, *F. tularensis* may survive for extended periods in a cold, moist environment.

The disease is characterized by fever, localized skin or mucous membrane ulceration, regional lymphadenopathy, and occasionally pneumonia. *Francisella tularensis* has long been considered a potential biological weapon. Japanese studied it as a biological agent between 1932 and 1945, it was also examined for military purposes in the West. It is suspected tularemia outbreaks affecting tens of thousands of Soviet and German soldiers on the eastern European front during World War II may have been the result of intentional use [23]. In the 1950s and 1960s, the US military developed weapons that would disseminate *F. tularensis* aerosols [24].

F. tularensis is considered to be a viable bio weapons agent, and it has been included in the biological warfare programs of the USA, USSR and Japan at various times [25]. In the US, practical research into using tularemia as a bio

weapon took place at Camp Detrick in the 1950s. It was viewed as an attractive agent because it is easy to aerosolize, it is highly infective; it is highly incapacitating to infected persons.

Although *F tularensis* could be used as a weapon in a number of ways, but it is believed that an aerosol release would have the greatest adverse medical and public health consequences.

Release in a densely populated area would be expected to result in an abrupt onset of large numbers of cases of acute, nonspecific febrile illness beginning 3 to 5 days later (incubation range, 1-14 days), with pleuropneumonitis developing in a significant proportion of cases during the ensuing days and weeks.

Q fever

Q fever is a disease caused by infection with a bacterium *Coxiella burnetii*, that affects both humans and animals. *Coxiella burnetii*, a rickettsial [14] or rickettsia-like [26] organism common among domestic farm animals.

This organism is uncommon but may be found in cattle, sheep, goats and other domestic mammals, including cats and dogs. The infection results from inhalation of contaminated particles in the air, and from contact with the vaginal mucus, milk, feces, urine or semen of infected animals. The incubation period is 9-40 days. It is considered possibly the most infectious disease in the world, as a human being can be infected by a single bacterium. Although *C burnetii* is unable to grow or replicate outside host cells, a spore like form of the organism is extremely resistant to heat, pressure, and many antiseptic compounds and thus allows *C burnetii* to persist in the environment for long periods under harsh conditions. In contrast to this high degree of inherent resilience and transmissibility, the acute clinical disease associated with Q fever is usually a benign, although temporarily incapacitating, illness in humans. Even without treatment, most patients recover.

The host range of *C burnetii* is diverse and includes a large number of mammalian species and arthropods. Among these, the human is the only host identified that experiences an illness as a result of infection. A number of different strains of *C burnetii* have been identified worldwide, and different clinical manifestations and complications

may be associated with the various strains. Human infection with *C burnetii* is usually the result of inhalation of infected aerosols; however, it may occur after consumption of unpasteurized dairy products. Humans are the only hosts that commonly develop an illness as a result of the infection. Incubation varies from 2-40 days (mean 15 d).

Q fever has been described as a possible biological weapon [27]. The United States investigated Q fever as a potential biological warfare agent in the 1950s with eventual standardization as agent OU. At Fort Detrick and Dugway Proving Ground human trials were conducted on White coat volunteers to determine the median infective dose and course of infection. Q fever is a category "B" agent [28]. It can be contagious and is very stable in aerosols in a wide range of temperatures. Q fever microorganisms may survive on surfaces up to 60 days.

4.1.2 Viral agents

Smallpox

Variola, the causative agent of smallpox, is poxviruses (family Poxviridae, genus Orthopoxvirus). In 1980, the World Health Organization (WHO) declared endemic smallpox eradicated, with the last occurrence in Somalia in 1977.

Natural infection occurs following implantation of the virus on the oropharyngeal or respiratory mucosa. The infectious dose is unknown but is believed to be only a few virions. After the migration of virus to and multiplication in regional lymph nodes, an asymptomatic viremia develops on about the third or fourth day, followed by multiplication of virus in the spleen, bone marrow, and lymph nodes. The virus, contained in leukocytes, then localizes in small blood vessels of the dermis and beneath the oral and pharyngeal mucosa and subsequently infects adjacent cells. At the end of the 12 to 14 day incubation period (range, 7-17 days), the patient typically experiences high fever, malaise, and prostration with headache and backache. A maculopapular rash then appears on the mucosa of the mouth and pharynx, face, and forearms, and spreads to the trunk and legs. Within 1 to 2 days, the rash becomes vesicular and, later, pustular. The lesions that first appear in the mouth

and pharynx ulcerate quickly because of the absence of a stratum corneum, releasing large amounts of virus into the saliva. Except for the lesions in the skin and mucous membranes and reticulum cell hyperplasia, other organs are seldom involved. Secondary bacterial infection is not common, and death, which usually occurs during the second week of illness, most likely results from the toxemia associated with circulating immune complexes and soluble variola antigens. Encephalitis sometimes ensues that is indistinguishable from the acute perivascular demyelination observed as a complication of infection due to vaccinia, measles, or varicella [29]. The variola virus is resistant to drying [30] viable variola virus has been recovered from scabs 13 years after collection

Variola represents a significant threat as a BW agent. Variola is highly infectious and is associated with a high mortality rate and secondary spread. The infective dose by aerosol is assumed low (10-100 organisms) or is very small [31]. Smallpox probably was first used as a biological weapon during the French and Indian Wars (1754-1767) by British forces in North America [32]. Soldiers distributed blankets that had been used by smallpox patients with the intent of initiating outbreaks among American Indians. Epidemics occurred, killing more than 50% of many affected tribes. With Edward Jenner's demonstration in 1796 that an infection caused by cowpox protected against smallpox and the rapid diffusion worldwide of the practice of cowpox inoculation (i.e., vaccination), the potential threat of smallpox as a bioweapon was greatly diminished.

Viral Encephalitides

Acute viral encephalitis (enkephalos + -itis, meaning brain inflammation) is often an unusual manifestation of common viral infections. Venezuelan equine encephalomyelitis (VEE) has been weaponized in aerosol form [33, 34]. Most viral infections of the central nervous system (CNS) either involve the meninges, leading to aseptic meningitis, or cause mild meningoencephalitis rather than encephalitis [35]

In general, viral encephalitides can be divided into 4 separate categories based on the cause and pathogenesis of the following complications: acute viral encephalitis; post infectious

encephalomyelitis; slow viral infections of CNS; and chronic degenerative diseases of the central nervous system, which are presumed to be of viral origin.

Although natural infections with these viruses occur following bites from mosquitos, the viruses also are highly infectious by aerosol. Alphaviruses replicate readily to very high titers and are relatively stable. These viruses could be replicated either in a wet or dried form inexpensively in large amounts.. The intentional release as a small-particle aerosol may be expected to infect a high percentage of individuals within an area of at least 10,000 km. The virus could also be purposefully spread by infected mosquitos.

Susceptibility in humans is 90-100%, and virtually 100% of those infected will become ill. After exposure to these viruses, the tissues of the CNS and reticuloendothelial and/or lymphoid systems most commonly are affected in both humans and animals. An acute incapacitating systemic viral febrile syndrome characterizes most infections. The severity of response is highly dependent upon host and viral factors, including the species and immune response of the host, route of infection, and strain and dose of the virus. VEE virus has the capacity to produce large human epidemics. Outcomes are significantly worse for young and elderly patients

Viral hemorrhagic fevers

Viral hemorrhagic fevers are caused by 4 families of viruses [29], which include the Arenaviridae (Lassa, Argentine, Bolivian, Brazilian, Venezuelan hemorrhagic fevers), Bunyaviridae (Rift Valley, Crimean-Congo, Hantavirus), Filoviridae (Marburg, Ebola hemorrhagic fevers), and Flaviviridae (yellow fever, dengue, KyasanurForest, Omsk hemorrhagic fevers). Filoviridae are spread from human to human by infected blood, secretions, organs, or semen. Congo-Crimean hemorrhagic fever is tick-borne but also may be spread by body fluid or the meat of infected animals. Hantavirus is rodent-borne, while Rift Valley fever and yellow fever are spread by mosquitos.

These viruses are characterized by an acute generalized febrile illness that includes malaise,

prostration, increased vascular permeability, and abnormalities of circulatory regulation. All agents are highly infectious via the aerosol route, most are stable as respiratory aerosols, and they can be replicated in cell culture. Thus, they possess characteristics ideal for use by terrorists.

All viral hemorrhagic fevers primarily target vascular beds. They produce microvascular damage and enhance vascular permeability. Clinical manifestations can be diverse and include fever, myalgia, headache, prostration, conjunctival infection, mild hypotension to severe shock, and mucosal and petechial hemorrhages. Neurologic, hematopoietic, hepatic, and pulmonary involvement can be found with more severe disease. Rift Valley fever virus is primarily hepatotropic; a small portion of patients demonstrates hemorrhagic signs. Crimean-Congo virus commonly is associated with severe Disseminated Intravascular Coagulation (DIC) and the most severe hemorrhaging among the viral hemorrhagic fevers. Hantaan virus is associated with pulmonary and renal failure. A sunburn flush on the head, neck, and upper back is somewhat characteristic.

Lassa fever has emerged as a worldwide concern among public health officials because of the ability of Lassa virus to spread from person to person, risk of its importation by international travel, and renewed threats about the potential use of HF viruses for biological warfare

4.2 Biological toxins

Staphylococcal enterotoxin B

Staphylococcal enterotoxin B (SEB) is an exotoxin excreted by the bacterium *Staphylococcus aureus*. SEB is the toxin most commonly associated with classic food poisoning. *Staphylococcus* species thrive and produce toxins in unrefrigerated meats, dairy, bakery products. Nausea, vomiting, and diarrhea normally occur following ingestion of contaminated foodstuffs [36].

SEB has been studied as a potential biological warfare agent because it can easily be aerosolized; it is very stable; and it can cause widespread systemic damage, multiorgan system failure, and even shock and death when inhaled at very high dosages. The toxin causes a

markedly different clinical syndrome when exposure is through a nonenteric route. In a Biological War or terrorist situation, the toxin is likely to be acquired through inhalation of an SEB aerosol. SEB is stable as an aerosol, and the inhaled dose necessary to incapacitate individuals is small (0.004 mcg/kg). Within 24 hours of inhalation of SEB toxin, exposed individuals are likely to be incapacitated by systemic illness created by the toxin. SEB could also be used to contaminate food or small-volume water supplies [37].

Signs and symptoms begin in 2-12 hours after inhalation and 2-10 hours after ingestion. Mild-to-moderate inhalation exposure to SEB produces nonspecific systemic illness that is characterized by fever, chills, headache, nausea, vomiting, dyspnea, chest pain, myalgias, and a nonproductive cough. In mild-to-moderate cases, the physical examination is typically unremarkable. In severe instances, rales are common from pulmonary edema. Depending on the severity of exposure, duration of illness varies from 3-10 days. Coughing may last up to 1 month. Severe exposures can lead to a toxic shock like picture and even death. Oral exposure results in predominately nausea, vomiting, and diarrhea, but fever, chills and myalgias may also be present. Ocular exposure may result in conjunctivitis.

Ricin

Ricin, a plant protein toxin derived from the beans of the castor plant (*Ricinus communis*), is one of the most toxic and easily produced of the plant toxins. Although the lethal toxicity of ricin is approximately 1000-fold less than botulinum toxin, the worldwide ready availability of castor beans and the ease with which toxin can be produced give it significant potential as a biological weapon. The U.S. Centers for Disease Control (CDC) gives a possible minimum figure of 500 micrograms (about the size of a grain of salt) for the lethal dose of ricin in humans if exposure is from injection or inhalation [38].

Following inhalation exposure of ricin, toxicity begins within 4-8 hours and is characterized by the sudden onset of nasal and throat congestion, nausea and vomiting, itching of the eyes, urticaria, and tightness in the chest, cough, and dyspnea. If exposure is significant, pulmonary manifestations occur 12-24 hours and include airway lesions,

alveolar flooding, and severe respiratory distress [39].

In animal studies, death occurs 36-48 hours after severe exposure. Ingestion of ricin is generally less toxic because of its poor absorption and enzymatic degradation in the digestive tract. Out of 751 ingestions recorded, only 14 resulted in a fatality. Clinical manifestations occur rapidly and are characterized by nausea, vomiting, abdominal pain and cramping, diarrhea, fever and chills, hematochezia, and eventually, shock and vascular collapse. Autopsy findings have revealed significant hepatic, splenic, and renal necrosis. At low doses, intramuscular exposures produce flulike symptoms, myalgias, nausea, vomiting, and localized pain and swelling at the injection site. Severe intoxication results in local lymphoid necrosis and GI hemorrhage, as well as diffuse hepatic, splenic, and renal necrosis.

The United States investigated ricin for its military potential during the First World War. During the Second World War the United States and Canada undertook studying ricin in cluster bombs. Though there were plans for mass production and several field trials with different bomblet concepts, the end conclusion was that it was no more economical than using phosgene. The former Soviet Union also had ricin. There were speculations that KGB even used it outside of the Soviet bloc; however, this was never proven.

Botulinum toxin

Botulinum toxins (abbreviated either as BTX or BoNT) are produced by anaerobic, spore-forming, gram-positive bacillus *Clostridium botulinum*. Botulinum toxins are highly lethal [40]. The estimated lethal dose to 50% of the exposed population (LD50) is 0.001 mcg/kg in humans. Since botulinum toxin is so lethal and easy to manufacture and weaponize, it represents a credible threat as a BW agent. When used as a BW or terrorist agent, exposure is likely to occur following inhalation of aerosolized toxin or ingestion of food contaminated with the preformed toxin or microbial spores. Recently, Iraq admitted to active research on the offensive use of botulinum toxins and to weaponizing and deploying more than 100 munitions with botulinum toxin in 1995.

Botulinum toxins are unusual in that they are more toxic when ingested than when inhaled [14]. They are also less toxic than when injected. Symptoms may be experienced within 24-36 hr. A progressive paralysis from head to toe follows, usually seen first in drooping eyelids [13, 41]. Symptom onset is slower after inhalation exposure. Botulinum toxins bind to the presynaptic nerve terminal at the neuromuscular junction and cholinergic autonomic sites. This prevents the presynaptic release of acetylcholine and blocks neurotransmission. Interruption of neurotransmission produces muscular weakness and paralysis. Recovery requires months as the neurons develop new axons.

Mycotoxins (Aflatoxins)

The mycotoxins are highly toxic compounds produced by certain species of filamentous fungi (*Fusarium*, *Myrothecium*, *Cephalosporium*, *Trichoderma*, *Verticimonosporium*, *Stachybotrys* species). These toxins are nonvolatile, low molecular weight compounds that are highly soluble in acetone, ethyl acetate, chloroform, ethanol, methanol, and propylene glycol. The trichothecenes vaporize when heated in organic solvents. After exposure to the mycotoxins, early symptoms begin within minutes. Cutaneous manifestations include burning, tender erythema, edema, and blistering with progression to dermal necrosis and sloughing of large skin areas in lethal cases. Respiratory exposure results in nasal itching, pain, sneezing, epistaxis, rhinorrhea, dyspnea, wheezing, cough, and blood-tinged saliva and sputum. Death may occur within minutes to days depending on the dose and route of exposure [42].

Because of their antipersonnel properties, ease of large-scale production, and amenability to dispersal by various methods (dusts, droplets, aerosols, smoke, rockets, artillery mines, portable sprays), mycotoxins have an excellent potential for weaponization [43]. Strong evidence suggests that trichothecenes ("yellow rain") have been used as a BW agent in Southwest Asia and Afghanistan. From 1974-1981, numerous attacks resulted in a minimum of 6310 deaths in Laos, 981 deaths in Cambodia, and 3042 deaths in Afghanistan.

5. Impact of Biological Agents on the Environment

Although the deliberate use of disease as a weapon, against humans, animals or plants, is totally prohibited by the Biological and Toxin Weapons Convention which entered into force in 1975, the danger that biological weapons may be used by rogue States or terrorist groups is increasingly and recognized as presenting the greatest danger of all weapons of mass destruction. The use of chemical or biological agents cause severe environmental damage in addition to their devastating effects on humans. As biological agents (other than toxins) multiply in the infected target population, the quantities needed to cause infection are very much smaller than the amounts of chemical agent needed to cause harm - a few biological micro-organisms may suffice. Consequently, biological weapons have a significantly larger potential area of effect than have chemical weapons and hence the potential impact of biological weapons on environment approaches that of nuclear weapons and can have strategic effects.

The effects and efficiency of biological weapons depends what type is used, its dispersion, upon the weather condition, determining the exposure and lethality for the combatants. Although toxins cannot reproduce within their targets like micro-organisms can, they are generally more lethal. Toxins can kill within minutes or hours whereas micro-organisms can be present for days or weeks before the symptoms appear. This means that a biological attack can occur before anyone realises, or even without being noticed at all as it could be taken as an outbreak of disease. Such an attack can have an impact long after it takes place. It also depends on what form such an attack takes. Biological weapons can be delivered in a missile warhead or in the form of a bomb. However, the most effective way is probably as an aerosol dispersal much like a crop spray. This type of delivery has a greater chance to infect environment.

Biological weapons are capable of causing casualties among living beings - people, other animals and plants - on a giant scale. US field trials carried out in the Pacific Ocean some years ago showed that a single-seater aircraft could establish disease-causing dosages of microbial aerosol at sea level over thousands of square

kilometers. Biological attacks would have incalculable environmental impacts beyond the immediate vicinity of the attacks, and in addition to fearsome public health consequences. The use of biological weapons is not only a threat to humans, it is also a serious threat to agricultural ecosystems, wildlife faunas and their habitats.

A bio-terrorist attack on a nation's livestock would have a devastating effect on that nation's agricultural industry in terms of economic loss. Moreover, it could have harmful spill-over effects on other susceptible wildlife species. Introduced diseases affecting domesticated animals or humans could be particularly harmful for native species that are naturally rare, and species whose numbers have been depleted due to habitat degradation. Foot And Mouth disease alone is known to have an impact on more than 60 wildlife species. Adding to this, a population of wildlife species infected with a human or livestock disease would most likely be culled in order to control the spread of the disease. Despite research showing that it might not be necessary, populations of native wildlife in many areas are already put through rigorous culling or destruction to try and control the transmission of their diseases to domestic animals.

There are reports that the some nations are now developing fungi and viruses that will kill opium poppy, marijuana, and coca plants. These are designed to have a high plant kill rate and to be deliberately sprayed in crop eradication programmes. The US is pressuring some countries like Colombia and Burma with such illicit crops to use these pathogenic fungi to forcibly eradicate them. This strategy carries great dangers of undermining international prohibitions on biological weapons, presenting risks to human health and posing dangers to the environment. Like any other biological agent, the fungi would be very difficult to control after release: they are infectious agents that spread uncontrollably beyond the target area.

While many of the chemicals used in war break down relatively quickly, biological weapons pose an even greater threat than chemical weapons because it may be impossible to reverse the effects of an organism that has been unleashed into the environment. Biological warfare agents present complex psychosocial

challenges for civilian populations. As the incubation periods for bacteria and viruses are long and the biological weapon attacks are not detected immediately, casualties will appear in days to weeks after an actual attack and may be widely dispersed. A bioterrorist attack would require distribution of prophylactic medications and vaccination. This would place extraordinary demands on medical and public institutions and their staffs. The nature of this type of traumatic stressor and the risk groups for psychological sequelae is different from those seen following attacks using chemical or conventional weapons that produce immediate effects.

The fear of an attack from biological weapons is greater than any other attack because all weapons of mass destruction, are the easiest and cheapest to produce. At the most basic level, bioterrorist attacks against people only require access to natural diseases that can cause disastrous epidemics – laboratory cultures or specialized disease strains are not necessary. The very nature of biological weapons would almost certainly lead to panic and terror in the civilian population in the event of an attack, or even a suspected attack. In the US in 2001, anthrax was sent through the post to addresses such as senators and newspapers. Although this did indeed lead to the deaths of five people, the resulting panic throughout the country showed just how quickly an attack could destabilize a nation.

Biological warfare has the potential to pollute the environment and devastate non-human species. As genetic strands of DNA can mutate, it is possible for disease meant only to affect humans to change into something that can kill plants or animals, thus posing a long lasting effects on environment. Future generations can be affected via genetic mutation caused by biological warfare agents. Genetic mutation can manifest itself in many different forms. Some of the people will also be affected through genetic mutation ultimately leading to dystrophic changes or birth defects in newborns.

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