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Bioterrorism: warfare of the 21st century

Review Article

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Summary

The terrorist attacks on and after September 11, 2001 have drawn attention to the fact that besides the traditional warfare, microorganism-based weapons are playing greater and greater role in military activities, especially in terror attacks. The ongoing war activities worldwide and the continuing international conflicts suggest that despite the international treaties, sooner or later germ warfare must be considered. The relatively easy preparation, storage and application of biologic weapons, despite the strictly regulated access, hold out major advantages when compared to traditional warfare, especially for terrorist activities. Germ warfare, divided into three different categories by the Center for Disease Control and Prevention USA, are based on use of bacteria, toxins and viruses. Besides the causative agents of the traditionally fearful contagious diseases (plague, smallpox, anthrax) other biologic agents (toxin of C. botulinum, hemorrhagic fever viruses) also play an important role in biologic warfare. Cognition of biologic weapons is very important because the infections originate from causative agents existing in the natural environment, but are generated artificially. These infections, whether air or food born, may generate numerous infections. Since the initial clinical features are usually not characteristic for the disease, it is crucial to be aware of the circumstances and clinical symptoms indicating biologic attack when produced en masse. When bio attack is recognized in time, suitable countermeasures may effectively reduce the severeness and expansion of the resulting infection.

I. Introduction

Though only the events on and after 11 September 2001 have drawn the public's attention to the dangers of terrorism and bioterrorism, the use of biologic warfare originates from the ancient times. Even in the Native American wars in 1754-1767 blankets contaminated with smallpox were used in order to infect the Native Americans (Henderson et al, 2002). Widespread research of the production of biological warfare was initiated in the 1930s and during the World War II. "Product development" was based on the traditionally fearful infections (plague, smallpox, anthrax), but other infective agents were also considered (botulinum toxin, tularaemia, hemorrhagic fevers). Moreover, agents not considered very dangerous have also been taken into account (Salmonella, vermin) as biological weapons (Phills et al, 1972; Torok et al, 1977). During the cold war, both the East and the West had been intensively working on the development of biologic warfare until the restriction signed by President Nixon in 1969 and the United Nations treaty signed in 1970 have banned the biological weapon research. Though the treaty has been signed by 140 countries, 17 of them are still possessing biological weapons. Events of the past few years have drawn attention to the fact that bioterrorism is a living threat for all countries, thus we have to be prepared and acceptable countermeasures have to be authorized.

II. General features of biological weapons

Microorganisms suitable for developing biological weapons possess several advantageous features when compared to traditional missiles. Since these microorganisms can be found in nature (in several cases also in laboratories) and are ready-to-use, no money, time

and energy are needed for development. Their easiness of being processed in great quantity, low need for storage space, and delayed effect assuring escape for the assassins make them the first choice for terrorist attack. The high number of induced infections generates fear and panic among the public which facilitates further diffusion of the disease (Hughes, 1999).

Naturally, not all microorganisms are suitable for biological warfare development. According to the classification of the Center for Disease Control and Prevention, USA (CDC), three groups (CDC, 2000) of infective agents can be created based on the virulence, mortality and panic-inducing ability (**Table 1**). In general, the „best" biological weapons are easy-to-access, easy-to-produce, and easy-to-spread; generate widespread infection and serious disease. These requirements are completely fulfilled by the causative agents of plague, smallpox, anthrax, tularaemia, and by the toxin of Clostridium botulinum.

There are several ways to artificially induce and diffuse infections. Early experiments have mainly focused on injecting infectious agents into recipients or on the use of vectors (flies, mosquitoes), but these methods assure only restricted facilitation of spreading infections.

Table 1. Classification of potential biological weapons according to the Center for Disease Control and Prevention, USA.

Category A	Variola virus
	Bacillus anthracis
	Francisella tularensis
	Yersinia pestis
	Ebola virus
	Marburg virus
	Lassa virus
	Junin (and related) viruses
	Clostridium botulinum toxin
Category B	Coxiella burnetti
	Brucella species
	Burkholderia mallei
	Salmonella species
	Shigella dysenteriae
	Escherichia coli 0157:H7
	Vibrio cholerae
	Cryptosporidium parvum
	Eastern encephalitis virus
	Western encephalitis virus
	Venezuelai encephalitis virus
	Staphylococcus enterotoxin
	Epsylon enterotoxin (Clostridim perfringens)
	Ricinus
Category C	Nipah virus
	Hantaviruses
	Yellow fever
	Hemorrhagic fever viruses spread by tick bite
	Tick encephalitis viruses
	Mycobacterium tuberculosis (multiresistent)

Moreover, since vectors can not be completely controlled, diffusion of the disease could not be accurately predicted. Later experiments were conducted on generating diseases by infected food and water, but the strict public health restrictions, highly effective filtering of drinking water and heat treatments of food prevent most of the infections. In the past two decades, place have been given to diffusing infections by air (aerosol) and (in case of anthrax) by mail. Since the infective agents cause no change in the colour or smell of air and due to their small size (<5 micrometers) their sedimentation is very slow, they are able to cover great distances without being detected and are able to venenate a great number of people. Diffusion by mail takes advantage of the unusual way of spreading infection and lack of control, and assures the infection of the previously selected persons (Detels et al, 2002).

III. Potential biological weapons

A. Anthrax

The disease caused by the B. anthracis is already mentioned in the Bible, and has been denominated the "black disaster" during the great epidemics in the Middle Ages. Due to the great discoveries in the 19th century, the disease has become rare and only sporadic cases can be found in most countries of Europe.

As biological warfare, B anthracis was used first by the German army during World War I when they infected the livestock marked out for the Allies. After the military occupation of Manchuria by Japan in 1932, prisoners of war were used as experimental subjects for anthrax (Harris, 1994). The British army spread anthrax infection as part of an experiment on the uninhabited island of Gruinard at the Scottish shores in 1942, but due to the dominant direction of wind, the microorganisms reached the Scottish mainland causing numerous infections among cats and sheep. Spores of the bacterium originating from this experiment can be found on the Gruinard island even nowadays.

During the cold war, both the Soviet Union and the USA produced great quantities of missiles filled with B. anthracis, until the production has been banned by the United Nation treaty signed in 1970 and the stocks have been annihilated. According to a simulation conducted by the WHO in the same year, 50 kilograms of B. anthracis spread over a city with 5 million inhabitants would cause 250,000 cases of infection and 100,000 cases of death (Detels et al, 2002).

In the 1990s, an extremist terrorist group attacked the subway system of Tokyo, Japan, but fortunately, no clinical cases resulted from the assault. Analysts think it possible that the bacteria the terrorists had obtained stemmed from an avirulent stock; thus, the attack has been "unsuccessful". After 11 September 2007, five anthrax cases have been proven to be caused by inhalation of the infectious agent among mail service employees with two cases resulting in death. These employees have been infected by anthrax bacteria wrapped in envelopes (Henderson et al, 2002).

B. Smallpox

As already mentioned before, smallpox as biological weapon has been used for the first time in the North American Indian wars between 1754 and 1767. Blankets of patients died of smallpox were given as presents to the Native Americans resulting in the decrease of the native population by fifty percents. Thanks to the discovery of Edward Jenner, with continuous vaccination, smallpox has been eradicated from Earth by 1977; therefore, smallpox vaccination is not compulsory since 1980. All laboratory stocks of smallpox should have been destructed by 1999, but because of sake of research, annulment has been postponed. Smallpox, as biological weapon was mostly developed in the former Soviet Union and they have managed to produce the virus in great quantity (several tons/year). According to certain sources, research is still conducted in Russia in order to generate more virulent and recombinant stocks (Henderson et al, 2002).

C. Plague

Plague was one of the contagious diseases feared the most. Historical descriptions exist already from 541 BC when a pandemic originated from Egypt and spread all over the then-known world. The "black death" in 1346 destructed about one-third of the European population and resulted in significant religious, cultural and political changes.

Plague was used as biological weapon for the first time in World War II by the Japanese military. Flies infected with plague were spread over the highly populated parts of China, causing a severe plague epidemic (Harris, 1994). In the years of the cold war, both world-powers succeeded in spreading plague in aerosol, independently from the precarious vectors. According to the simulation conducted by the WHO in 1970, 50 kilograms of Y. pestis spread over a city of 5 million inhabitants would cause 150,000 clinical cases and 36,000 deaths. From the point of origin, the infection would diffuse in a 10-km-diameter circle, and the panic generated in the population would further exacerbate the situation. It is also worth to mention that lethality of plague caused by inhalation is 4 times of the lethality of the classical clinical case (57% versus 14%); thus, the already dangerous infection would become even more hazardous when caused by inhalation of the infective agent (Henderson et al, 2002).

D. Botulinum toxin

Toxin of C. botulinum was used as biological weapon for the first time in 1932 in Manchuria by the Japanese army (Harris, 1994) and was later, during World War II, under development in the USA. The development program was stopped by the United Nation treaty signed in 1970. During the Gulf War in 1991, 19,000 litres of concentrated botulinum toxin were found, mostly ready-to-use, built in missiles.

The toxin can be spread in aerosol as well as with infected food. In case of a food born epidemic, possibility of biological attack should be considered if the toxin type is unusual (C, D, E, F, G), but in case of toxin type E only if the infected food is not seafood. Further enhances suspicion when the infection is geographically well localised with very similar symptoms but no common food or other source of infection can be identified (Henderson et al, 2002).

Despite of its hazards, botulinum toxin may also be used for curing different symptoms. It is already applied for the treatment of torticollis, blepharospam and strabismus and in cosmetology in anti-aging creams.

E. Tularaemia

Though being less known than the previously discussed infections, F. tularensis is the most virulent pathogenic bacterium; inhalation of only 10 bacteria already results in a clinically manifest infection. The microorganism is prevalent in Eurasia and North America. The disease was nominated "plague of rodents" in 1911, because mostly these animals fall victim to the infection. Humans may be infected by insect bite, infected animal tissues, food, water, soil, inhalation of infected aerosol. Mostly hunters, butchers, agricultural and laboratory employees are affected. No spread from human to human is known yet.

As a biological weapon, tularaemia has been used for the first time during World War II (Harris, 1994). According to the simulation conducted in 1970, 50 kilograms of F. tularensis spread over a city with 5 million inhabitants, 250,000 clinical cases and 19,000 deaths should be considered. It is worth to compare these numbers to the ones mentioned in connection with the plague WHO simulation (Henderson et al, 2002).

F. Haemorrhagic fevers

Haemorrhagic fevers are infectious diseases characterized by fever and severe bleeding, spread by insect bites, infected aerosol, contact with infected animal cadavers. Human-to-human spread (except in bunyaviruses and flaviviruses) was detected by direct contact, infected blood and air born. Ebola virus, Marburg virus and Lassa virus particles were detected by molecular biologic methods (PCR) in the seminal fluid of patients 82, 83 and 90 days after recovery respectively. Furthermore, sexual transmission to the partners of these patients was also detected. Virus of the Argentinean hemorrhagic fever is able to infect sexual partners of recovered patients 7-22 days after disappearance of symptoms (Henderson et al, 2002).

Use of hemorrhagic fever viruses as biological weapons was studied both in the Soviet Union and in the USA. Non-primate monkeys were successfully infected with virus-containing aerosol and inhalation of only several virions is able to generate clinical symptoms of the disease. Since these viruses belong to the category A biological weapons, handling of a possible epidemic would require special countermeasures and would have serious public health impact (Henderson et al, 2002).

G. Other infective agents

Besides the contagious diseases traditionally considered very dangerous, other, relatively harmless microorganisms can also used as biological weapons.

In 1991, four college students were deliberately infected in Toronto, Canada with eggs of Ascaris suum that normally cause vermin infection of pigs. After having been treated at the intensive care unit, all four students were discharged completely recovered from the local hospital (Phills et al, 1972).

Members of the Rajneeshee religious group infected the salads served at the election buffets with Salmonella typhimurium in the USA (Wasco County, Oregon) in 1984 in order to influence the outcome of the elections. The attack resulted in the infection of 751 persons (Torok et al, 1977).

In their confessions to the Commission of People's Rights, members of the apartheid groups admitted that among other means, biological weapons were used as well against the anti-apartheid forces in South Africa (Detels et al, 2002).

IV. Genetic engineering in bioweapon development

The ever-developing possibilities of creating genetically modified microorganisms increases the probability of new strains being used as bioweapons – strains which may use virulence factors other than those targeted in traditional vaccines (Ales and Katial, 2004). However, the idea of developing biologic warfare by genetic engineering is not completely new. Cold-era scientists in the former Soviet Union developed a form of plague resistant to 16 different antibiotics (Niiler, 2002), as well as a more virulent strain of smallpox (Henderson et al, 2002) and a C botulinum strain expressing recombinant toxin (Henderson et al, 2002). Other sources hinted that Russia is in possession of a modified Ebola virus weapon (Niiler, 2002). According to Greenfield and Bronze, all possible bioweapon agents share the potential of naturally occurring or genetically engineered resistance to the currently available antimicrobial therapies (Greenfield and Bronze, 2003).

Recently, synthetic biologists are increasingly able to alter large parts of genomes at once and assemble new ones from scratch (Kaplan and Magnus, 2003; Service, 2006). The technique, called directed molecular evolution, has been developed by companies such as Maxygen in Redwood City, CA (Dennis, 2001; Niiler, 2002). Theoretically, it might be possible to build novel microorganisms from a set of different component parts, though most experts don't think it as a realistic scenario yet (Dennis, 2001). But making subtle genetic alterations to existing pathogens to increase their virulence or durability in the environment, or to make them harder to detect or to treat with drugs is well within the limits of today's technology (Dennis, 2001).

Plasmids carrying antibiotic resistance can be moved between bacteria and genes carried on plasmids can be incorporated into the genome, thus creating resistant strains. For example, anthrax, which is usually treated with penicillin, can be made resistant to treatment by introducing a gene coding the enzyme beta-lactamase. The gene coding the botulinum toxin could be introduced into ubiquitous bacteria such as Escheria coli.

DNA shuffling is one of the most powerful methods for unnatural selection of microorganisms. Multiple copies of a given gene are first shattered into fragments, then reassembled using a variation of polymerase chain reaction. This procedure creates a range of "daughter" genes with the fragments linked together in subtly different ways. The enzymes used in the procedure are prone to errors themselves, which introduce point mutations and thus further increase the genetic diversity. These "daughter" genes can then be reintroduced to bacteria which are selected to identify those with the desired traits (Dennis, 2001). The refined version of the technique reassembles fragments taken from families of related genes from different bacteria.

Other approaches that might be used to develop a bioweapon include the deliberate hybridization of related virus strains. Though most crosses of viruses are less potent that the parent strains, sometimes virulence increases – some virulent strains of flue arise as the naturally occurring recombinants of different influenza viruses.

One disturbing possibility is that knowledge of pathogen genomics could be combined with insights to human genetics to target particular ethnic groups.

V. Countermeasures

A. Criminal law and public health: a new working relationship

Events of 2001 have made it clear that in case of a bioterrorist attack countermeasures can not be restricted to public health measures. Follow-up of the clinical cases and collection of evidence consider criminal law employees as well. Public health experts are normally not qualified to handle infectious samples as evidence of law. Furthermore, at least in theory, it is possible that the experts themselves may contaminate the samples collected for analysis. Therefore, the real challenge is to identify the point where public health investigation is transformed into criminal investigation. Criminal investigation against persons performing bioterrorist attack must include public health experts, since investigations consider biological samples and infectious diseases. Besides, countermeasures have to be initiated at local level, by the public health authorities (Detels et al, 2002). Hence, relationship between public health and criminal law should be strengthened.

B. Improvement of public health control and of the infrastructure of public health laboratories

Identification of bioterrorist attack as early as possible is a crucial point in performing the suitable countermeasures and in decrease of the number of casualties. Basis of this identification could be created by the enlargement of online databases and their being opened to the public, by educating the public health experts and by the thorough and continuous control of events bearing the risk of being attacked by biological weapons (e.g. political events, Olympics).

Acquisition of the suitable laboratory equipments and reagents, besides the set-up of a central laboratory may ensure the early diagnosis and identification of the infection and thus the decrease of the number of cases by applying early adequate therapy and prevention.

The first agency of the WHO educating experts for identifying and handling bioterrorist attacks has been set up in Lyon, France in 2001 (Detels et al, 2002). As a consequence, the quality level of the public health laboratories should be improved and in case of a biological attack these laboratories should be able to identify the infective source and the population at risk within a limited time. Among others, countermeasures may contain isolation, set-up of a quarantine, disinfection and decontamination.

C. Medical arrangements

The most important action is to think of the possibility of biological attack during certain circumstances. To provide the population with effective defence, medical students should already be familiarised with the infective agents that may potentially be used as biological weapon and with the means of their identification. Adequate infrastructure should be built in order to treat and isolate the infected persons, and to set quarantine up. Stocks of antibiotics and vaccines against the possible infections must be accumulated. Pre-exposure vaccination of the naïve population and post-exposure vaccination of the already exposed population may play a crucial role in the prevention of a widespread epidemic (Detels et al, 2002).

D. Mass media

Panic generated by a bioterrorist attack may multiply the number of induced cases and may prohibit the early installation of the effective countermeasures. To avoid panic, to quickly provide the public with accurate information is crucial.

Events of September, 2001 in the USA have drawn attention to the fact that keeping certain pieces of information from the public opens wide area for guessing, exaggerating and hysteria generated by the inappropriate information. In case the government is not willing to share all information with the public (e.g. to facilitate criminal investigation later), then appropriate quantity of data should be provided for the media, and pieces of information must not be controversial. Keeping information secret and leaking them later may shock the trust of the public and may generate further panic. Information that may be disclosed should be recorded and provided for the responsible authorities beforehand, thus coverage given at the time of attack will be unambiguous and trustworthy. The local public health authorities should continuously be kept updated including the treatment and prevention of the diseases generated by the biological attack, thus the public can later be provided with this information without further delay.

VI. Prevention

In prevention of infections resulting from biological attack, defence of water supply, food, air and mail service employees is crucial.

Water supply networks are usually safe against bioterrorist attack. Built-in filtering, cleaning and controlling systems against naturally generated infections are also effective against biological attacks. Due to the high dilution effect in the water networks of huge towns, toxins are very unlikely to generate infection, even if injected in a great quantity. Bacteria are another question, since they are able to multiply even in the cleaned water; therefore, continuous monitoring of the water supply networks is a must (Detels et al, 2002).

Food may be contaminated with microorganisms at several steps during the processing. Plants and animals serving as source of nutrition must be protected by the responsible government all the time. During food processing, the producer is obliged to ensure the purity of the product.

In the industrial societies, widespread distribution of ready-cooked, canned food and ingredients creates possibility for infection and/or contamination even after processing. Fortunately, since the most virulent infective agents are sensitive to heat, and most of the possibly contaminable food is treated with heat prior to consuming, infections deliberately generated by infected food are rare. However, in case of a food born biological attack, strict cooperation of the local, regional and public health authorities, as well as increased efficacy and frequency of food control is needed for the identification of source (Detels et al, 2002).

It is proven that to spread infection deliberately is easiest by contaminating air. On the other hand, infective agents may enter buildings by some other means as well, therefore, prior to re-constructing air circulating and conditioning systems, risk of the building and the persons usually abiding in it should be considered versus the benefit of the re-construction. As a temporary solution, effective air filtering system combined with frequent air sampling and analysis may be installed. In case such a system detects an infective agent, it would automatically shut the air circulation down, and thus would decrease the speed of disseminating infection (Detels et al, 2002).

Mail is not considered the most probable way of using biological weapons, but infections generated by bacteria posted in envelopes have drawn the attention to the necessity of defending mail service employees against such events. Prevention basically means the use of gloves, face masks and respirators in the short term, and neutralisation of the infective agents by irradiating the mail in the long term (Detels et al, 2002).

Such as in any other case of infection, diseases generated by biological attack could be prevented the simplest, most effective and cheapest way by using primary prevention. According to Horton, terrorism is the consequence of widespread changes in politics and social status, most frequently in countries that fail functioning as an independent state (Horton 2001). Moreover, terrorist attacks may also be induced by national politics, religious and/or political persuasions and inequalities considered

unchangeable. Terrorists feel being victimized and are convinced that the only option they have is executing aggressive attacks. Victims' first reaction to terrorist attacks, whether individuals, groups or nations, is to take vengeance. However, this can not be the solution. In order to abolish bioterrorism, its triggering causes should be first ceased. Therefore, even during a terrorist attack, it is important to analyze and understand the national, local and individual difficulties lying in the background. The necessary measures would most probably be unpopular, but the national leaders and the international committees are responsible for identifying and executing the adequate actions (Detels et al, 2002).

Though temper has been evened after 11 September 2001 and no other biological attack of such a great dimension has been committed, the possibility of bioterrorism should always be considered and necessary countermeasures should immediately be taken when the suspicion of such action rises.

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