BIOWEAPONS AND BIOTERRORISM: A REVIEW OF HISTORY AND BIOLOGICAL AGENTS

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ABSTRACT

Bioweapons is a thorny issue due to their destructive capabilities, and for the potential to generate panic and terror among the affected people. Used since pre-Christian times, bioweapons have resulted in the decimation of whole populations and have changed the geopolitics of several places. In this paper, a summary of the main wars and terrorist activities carried out using bioweapons over the time is presented. In addition, the main biological warfare agents and related pathologies are considered, as according to the U.S. Centers for Disease Control and Prevention’s (CDC) priority classification. The emergence of potentially more destructive biological agents, due to the widespread introduction of biotechnology, is also analysed.

Keywords: Bioweapons; bioterrorism; biological agents and toxins; fatality rates; biosafety level (BSL).

1. INTRODUCTION

DaSilva (1999) defined biological warfare as the intentional use of microorganisms, and toxins, generally of microbial, plant or animal origin, to produce diseases and deaths among humans, livestock and crops. Biological warfare and bioterrorism are very complex subjects, mainly due to the many agents that can be used as weapons and for the wide range of ways for dissemination into the environment and population. A biological event provides for the presence of at least two actors: one or more pathogens (bacteria, viruses or toxins) and a vehicle for their dissemination. In addition to the high spread capacity and lethality of potential biological agents, their invisibility and extremely difficult short-term detection makes it impossible for immediate diagnosis until the subsequent increase of infections. In fact, most biological weapons (except, for example, toxins and bacterial spores) have a unique quality that other non-conventional weapons (such as chemical and radiological) do
not have; biological agents are able to multiply in the host organism and be transmitted in turn to new hosts, generating in this way with unpredictable effects on the population, both in terms of number of victims and geographical spread (Rotz et al., 2002; Zalini, 2010; Vogel, 2012; Tucker, 2013).

Among the reasons which make bioweapons attractive is their very low cost when compared to both conventional and unconventional weapons. For example, NATO (1996) reported that according to data processed in 1969 by U.S. experts, the costs for an attack on an area of 1 km$^2$ to civilian populations with different weapons are: 1$/km^2$ for bioweapons, 600$/km^2$ for chemical, 800$/km^2$ for nuclear and 2,000$/km^2$ for conventional armaments. Furthermore, recent advances in life science and biotechnology have made it relatively straightforward to produce large quantities of biological agents with facilities and expertise available to everyone, even to terrorist and paramilitary groups (Zalini, 2010; Vogel, 2012; Tucker, 2013).

In this paper, a summary of the main wars and terrorist activities carried out using bioweapons over the time is presented. In addition, the main biological warfare agents and related pathologies are considered, as according to the U.S. Centers for Disease Control and Prevention’s (CDC) priority classification. The emergence of potentially more destructive biological agents, due to the widespread introduction of biotechnology, is also analysed.

2. HISTORY OF BIOLOGICAL WARFARE

2.1 Pre-World Wars

The use of biological agents as war weapons is not a modern era novelty. Although it is not easy to identify a definite time when the use of bioweapons began, ancient evidence reported that in pre-Christian era, around 300 B.C., the Greeks used animal cadavers to contaminate water wells of enemies. This strategy was also used the by the Romans and Persians (SIPRI, 1971a). In a later period, during the battle of Tortona, Italy, in 1155, bodies of dead soldiers and animals were used to contaminate water wells by Emperor Barbarossa’s troops (Clarke, 1968). In the 14th century, during the siege of Kaffa by the Tartars (now Feodosiya, Ukraine, a city near the Black Sea, at that time under the control of the Genoese), among the Tartar army, an epidemic of plague was spread. The besiegers thought to catapult the cadavers of their dead comrades within the walls of the city of Kaffa, resulting in a turning point in the war; the Genoese fled from Kaffa, carrying with them their sick. On the return trip to Genoa, they ported at several ports in the Mediterranean Sea. While some sources believe a possible correlation between the epidemic of plague in Kaffa and the pandemic that decimated most of the population of Europe in the following decades (Black Death), most authors share the view of two events were independent (Wheelis, 2002).
In 1422, during the siege of Carolstein, Lithuanian soldiers catapulted cadavers of dead soldiers and excrements into the city, frightening the population affected and spreading lethal fevers in many cases (Newark, 1988). The next documented use of biological agents as a war weapon occurred more than three centuries later. During the French-Indian War (1754-1767), the British commander, Sir Jeffrey Amherst, ordered the distribution of blankets infected with smallpox to decimate the population of Indian tribes hostile to the British. The distribution of infected blankets occurred in the summer of 1763, and the resurgence of the virus among the indigenous lasted for more than 200 years (Bhalla & Warheit, 2004; Riedel, 2004).

2.2 World Wars I and II

Several biological warfare actions carried out during the World War are not sufficiently confirmed in the literature. However, it is frequently reported that the Germans inoculated cattle with *Bacillus anthracis* and *Pseudomonas mallei*, responsible to cause severe diseases such as anthrax and glanders, before sending them into enemy states (SIPRI, 1971a; Poupard and Miller, 1992; Hugh-Jones, 1992). As World War I saw the large-scale use of non-conventional chemical weapons, it was expected that World War II would see more extensive use of biological weapons.

During this war, many countries conducted research programmes on the development of bioweapons; the Japanese programme, conducted under the direction of Lt. Gen. Shiro Ishii, was certainly the most ambitious (1892-1959). The research in this direction started in 1928; during this year, Lt. Gen. Ishii visited many European and American countries to learn useful techniques and information about the possible uses of biological weapons. Upon returning to his homeland, he was provided a substantial grant in order to constitute a massive bioweapons research centre, known as the Unit 731, located at Beiyinhe in Manchuria. The research centre staffed over 3,000 scientists, mainly microbiologists. The experiments were conducted on prisoners of war, principally Koreans, Chinese and Russian soldiers. The prisoners were used to test numerous bioweapons, including *Yersinia pestis*, *Vibrio cholera*, *Neisseria meningitidis* and *Bacillus anthracis* (Leitenberg, 2001). Christopher *et al.* (1997) report that during this research, several thousand prisoners died as a result of the experiments conducted on them. However, the mortality rate around the area of Unit 731 remained very high for several years. If we consider the total count these deaths, we reach the considerable sum of 200,000 deaths as a result of the activities carried out by Lt. Gen. Ishii (Harris, 2002). In 1942, the poor control of the infection spread resulted in the death of 1,700 Japanese soldiers (Sokolski & Ludes, 2001).

Many other nations carried out experiments on potential biological agents, but information reported in the literature is rather limited. It is important to note the experiments conducted in 1942 by the British army on the Island of Gruinard, off the Scotland coast, where anthrax dirty bombs were tested (Manchee *et al.*, 1981).
The island was contaminated and uninhabitable until 1990, when extensive land decontamination was carried out (Aldhous, 1990).

2.3 Post-World Wars

Until World War II, the U.S. remained considerably behind other nations in research on bioweapons. The golden age for both the test and development of bioweapons in the U.S. was immediately after the conclusion of World War II, when it received the results of the experiments performed by the Japanese Unit 731. The U.S. also worked directly with Lt. Gen. Ishii, the former director of Unit 731 (Christopher et al., 1997).

In September 1950, the U.S. Navy conducted an experiment on civilians in order to assess the vulnerability of a large American coastal town to a biological attack; in the San Francisco Bay, a cloud of *Serratia marcescens* (a low pathogenic bacterium mainly responsible for infections of skin and respiratory tract) was spread by boat. The infection struck, as a result of subsequent checks, almost the entire population (1 million people). Even though the bacterium was almost harmless, several individuals showed effects of respiratory diseases and some of them died (Christopher et al., 1997).

A few years later (1956-1958), in Georgia and Florida, swarms of mosquitoes, probably carriers of yellow fever, were released in order to verify vulnerability to an air attack. Even though the documents are still kept top secret, several sources report that some individuals died from the bites of insects. A last large scale experiment which was documented, consists of the dissemination of *Bacillus subtilis* in the New York subway in the summer of 1966. The experiment resulted in the infections, although without consequences, of more than one million people. It demonstrated that the spread of a pathogen in the whole subway network from a single station, due to the displacement of air in the tunnels, was possible (Zygmunt, 2006).

In the 1970s, the USSR conducted an ambitious research programme on bioweapons, but, unlike the U.S. programmes, of which the secrecy has been partially removed, an aura of mystery about Russian research programmes still remains. According to Davis (1999), the USSR, between 1973 and 1974, formed an organisation called the Chief Directorate for Biological Preparation (Biopreparat), with the purpose of developing and producing bioweapons. Although there are no unambiguous data about the number of individuals employed by Biopreparat, it is believed that more than 50,000 people were working in the whole system connected to the structure, including scientists and technicians, who were placed in 52 research and production factories. In these facilities, high amounts of etiologic agents of plague, tularemia, anthrax, glanders, smallpox and Venezuelan equine encephalomyelitis were studied and produced. In addition to biological agents from natural sources, the Soviets also studied and applied technologies of genetic engineering in order to increase the aggressiveness of biological agents through
biotechnology. The aim of this work was the production of a new more dangerous, more easily spread and more difficult to identify combat generation of bioweapons.

Among the countries that developed a massive programme on bioweapons research, in the post-World Wars era, is Iraq. It started its research and development programme in the field of biological warfare in 1974, contextualising it in an organisation called the State Organization for Trade and Industry (Davis, 1999). The programme consisted of the study and production of botulinum toxin, anthrax, aflatoxin and ricin, as well as antiviral agents and viral agents, such as rotavirus, infectious hemorrhagic conjunctivitis and camel pox. The programme involved about 300 scientists, who completed their training in Western European countries (Leitenberg, 2001).

2.4 International Treaties

The first measures against the use of bioweapons were taken in the 19th century during the Hague Conference in 1899, and then confirmed in the same place in 1907, with the document entitled Laws and Customs of War on Land, signed and ratified by 24 countries regarding the prohibition on the use of poisoned arms (Leitenberg, 2001). In 1925, with awareness of the horrors of World War I, especially in regards to the use of chemical weapons, the Geneva Protocol on the Prohibited Use in War of Asphyxiating, Poisonous or Other Gases, and of Bacteriological Methods of Warfare was signed. Although this treaty was signed by a considerable number of nations (even though it was only ratified by the U.S. in the mid-1970s), it only prohibited the use of biological agents as weapons, but not their development and stockpiling (Christopher et al., 1997).

In view of the limited effectiveness of the Geneva Protocol in the control of bioweapons development and proliferation, in 1972, the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction was initiated. Initially signed by over 100 nations, the Convention became effective in 1975. However, this convention, similar to the Geneva Protocol, has several loopholes. First of all, it does not provide guidelines for the protocol on compliance verification. Moreover, it only prohibits the use and development of bioweapons in quantities that have no justification for prophylactic, protective or other peaceful purposes (Riedel, 2004). It is evident how this assertion is open to interpretation, as it does not define the threshold quantities or substantial limitations to the development and production of bioweapons (SIPRI 1971b, 1973). Bioterrorist events that have taken place consequent to the ratification of the Biological Weapons Convention (BWC) in 1972 have confirmed that the convention does not prevent the proliferation of biological weapons.
2.5 The Rise of Bioterrorism

Even after the ratification of the BWC, a large number of countries went on to develop, produce and test biological agents for military purposes. Since the 1980s, terrorist groups have increasingly considered bioweapons as a highly destabilising tool for civil society and economy. The large scale advent of biotechnology and the reduced difficulty in production of genetically modified organisms have made the potential creation of multi-drug resistant pathogens with enhanced virulence factors possible. The use of biological agents in the last decades is mainly attributable to terrorist groups, more or less isolated, who used bioweapons as a strategy to defend extremist religious ideas by striking civilian populations or sensible government targets (Cronin, 2004).

In 1984, in The Dalles, Oregon, U.S., a group of extremist followers of Bhagwan Shree Rajneesh (also known as Osho) contaminated the salad in 10 different salad bars with the pathogen of salmonellosis, *Salmonella thyphimurium*, in order to disable the population. A total of 751 people contracted the disease and several of them were hospitalised. Although there were no fatalities, this terrorist act is considered the largest bioterrorist attack in the history of the U.S. (Török et al., 1997). In the 1990s, the Japanese cult of Aum Shinrikyo tested different bioweapons, including botulin toxin, anthrax, cholera, and Q fever. In 1993, during a humanitarian mission in Africa, it tried to obtain samples of the Ebola virus. Between 1990 and 1995, the cult attempted to carry out several bioterrorist acts in Tokyo using vaporised biological agents, including botulinum toxin and anthrax spores. Fortunately, the attacks were unsuccessful (Olson, 1999).

A significant bioterrorist event occurred in the U.S. contextually to the dramatic attacks to the World Trade Center in New York in September 2001. The release of *Bacillus anthracis* spores through the U.S. postal system was carried out with letters addressed to the press and to government officials. There were 22 confirmed cases of anthrax contamination, consisting of 12 cutaneous and 10 inhalational cases. The 12 cutaneous patients responded positively to antibiotic treatment, while of the 10 inhalational cases, 4 were fatal (McCarthy, 2001). In 2002, in Manchester, U.K., six terrorists were arrested for being found in possession of ricin, and in 2004, traces of the same toxin were found at the Dirksen Senate Office Building in Washington D.C. (Bhalla & Warheit, 2004) It appears evident then that the use of biological agents has moved, in recent times, to terrorist groups. This creates very strong concerns that the use of bioweapons by terrorists can create unexpected scenarios characterised by massive destructive potential.
3. BIOLOGICAL AGENTS

3.1 Categories of Biological Agents

The U.S. Centers for Disease Control and Prevention (CDC) defines a bioterrorism attack as “the deliberate release of viruses, bacteria or other germs (agents) used to cause illness or death in people, animals, or plants” (CDC, 2013). It classifies biological agents into three categories (Table 1):

1. Category A: Agents that can be easily disseminated or transmitted from person to person. They result in high mortality rates and have the potential for major public health impact. They might cause public panic and social disruption, and require special action for public health preparedness.

2. Category B: Agents that are moderately easy to disseminate. They result in moderate morbidity rates and low mortality, and require specific enhanced diagnostic capacity and disease surveillance.

3. Category C: Emerging agents that could be engineered for mass dissemination in the future because of their availability. They are easy to produce and disseminate. They are potentially linked to high morbidity and mortality rates, and major health impact.

Generally, biological agents (including those used as bioweapons) can be further classified according to certain characteristics that define the hazard to health (NATO, 1996):

a. Infectivity: The aptitude of an agent to penetrate and multiply in the host.

b. Pathogenicity: The ability of the agent to cause a disease after penetrating into the body.

c. Transmissibility: The ability of the agent to be transmitted from an infected individual to a healthy one.

d. Ability to neutralise: Its means to have preventive tools and / or therapeutic purposes.

Biological agents can be transmitted through one or more ways. The transmission modes are the following (La Placa, 2010):

a. Parenteral: Agents that are transmitted through body fluids or blood.

b. Airway (by droplets): Agents that are emitted by infected people, which can then be inhaled by surrounding people.

c. Contact: Through which the agents present on the surface of the infected organism can infect another organism.

d. Oral-faecal route: Through objects, foods or other items contaminated with the faeces of infected patients, or through sexual contact.
Table 1: Major biological agents that are possible to be used as bioweapons (CDC, 2013).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diseases</th>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Anthrax</td>
<td><em>Bacillus anthracis</em></td>
</tr>
<tr>
<td></td>
<td>Botulism</td>
<td><em>Clostridium botulinum</em> toxin</td>
</tr>
<tr>
<td></td>
<td>Plague</td>
<td><em>Yersinia pestis</em></td>
</tr>
<tr>
<td></td>
<td>Smallpox</td>
<td><em>Variola major</em></td>
</tr>
<tr>
<td></td>
<td>Tularemia</td>
<td><em>Francisella tularensis</em></td>
</tr>
<tr>
<td></td>
<td>Viral hemorrhagic fevers</td>
<td><em>Filo-viruses and Arenaviruses</em></td>
</tr>
<tr>
<td>B</td>
<td>Brucellosis</td>
<td><em>Brucella spp.</em></td>
</tr>
<tr>
<td></td>
<td>Epsilon toxin</td>
<td><em>Clostridium perfringens</em></td>
</tr>
<tr>
<td></td>
<td>Food safety threats</td>
<td><em>Salmonella spp., E.coli O157:H7, Shigella</em></td>
</tr>
<tr>
<td></td>
<td>Glanders</td>
<td><em>Burkholderia mallei</em></td>
</tr>
<tr>
<td></td>
<td>Melioidosis</td>
<td><em>Burkholderia pseudomallei</em></td>
</tr>
<tr>
<td></td>
<td>Psittacosis</td>
<td><em>Chlamydia psittaci</em></td>
</tr>
<tr>
<td></td>
<td>Q fever</td>
<td><em>Coxiella burnetii</em></td>
</tr>
<tr>
<td></td>
<td>Ricin toxin</td>
<td><em>Ricinus communis</em></td>
</tr>
<tr>
<td></td>
<td>Staphylococcal enterotoxin B</td>
<td><em>Staphylococcus spp.</em></td>
</tr>
<tr>
<td></td>
<td>Typhus fever</td>
<td><em>Rickettsia prowazekii</em></td>
</tr>
<tr>
<td></td>
<td>Viral encephalitis</td>
<td><em>Alphavirus</em></td>
</tr>
<tr>
<td></td>
<td>Water safety threats</td>
<td><em>Vibrio cholerae, Cryptosporidium parvum</em></td>
</tr>
<tr>
<td>C</td>
<td>Emerging infectious diseases</td>
<td><em>Nipahvirus and Hantavirus</em></td>
</tr>
</tbody>
</table>

3.2 Biological Agents That Can Be Used as Bioweapons

While there are numerous pathogens (bacteria, viruses and toxins) that cause diseases in humans, animals and plants, only very few possess the characteristics to be a bioweapon. Eitzen (1997) described the characteristics that make a biological agent a potential bioweapon. Ideally, a bioweapon should be easy to find or produce. In order to develop a biological attack towards sensitive targets or the population, large amounts of biological agents are in fact required; it must be considered that it is necessary to have quite a number of biological agents (or a certain amount of toxin) to generate a disease in a target. The ideal bioweapon also must have a high capacity to incapacitate the affected or, alternatively, be highly lethal. It is appropriate to choose an agent with an incubation period depending on whether immediate or delayed effects are required. Other important characteristics for a biological weapon are the route of transmission, and hence, the ease of dissemination with an appropriate method of delivery. Finally, the stability of the agent must be assessed, especially when large quantities must be stored for indefinite periods (Kortepeter & Parker, 1999).
In the following sub-sections, the key features of the most relevant biological agents (included in category A by the CDC) are reported and categorised according to biological origin. The fatality rates of these agents are shown in Table 2, while the biosafety levels (BSL) required to work with the respective agents are shown in Table 3.

### Table 2: Fatality rates of Category A biological agents.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Biological Agent</th>
<th>Fatality rate (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Bacillus anthracis</td>
<td>Cutaneous: &lt;1%</td>
<td>CDC, 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Respiratory: 75%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gastrointestinal: 25%-60%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clostridium botulinum</td>
<td>Foodborne: 3-5%</td>
<td>Arnon et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wound and intestinal: 15%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yersinia pestis</td>
<td>8-10%</td>
<td>WHO, 2004</td>
</tr>
<tr>
<td></td>
<td>Francisella tularensis</td>
<td>Subspecies tularensis: 2%</td>
<td>WHO, 2007; Dennis et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subspecies holarctica: fatal cases are rare</td>
<td>WHO, 2007</td>
</tr>
<tr>
<td>Virus</td>
<td>Variola major</td>
<td>30%</td>
<td>CDC, 2013</td>
</tr>
<tr>
<td></td>
<td>Filoviridae</td>
<td>90%</td>
<td>Warfield et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Arenaviridae</td>
<td>15-30%</td>
<td>Briease et al., 2009</td>
</tr>
</tbody>
</table>

### Table 3: Biosafety levels (BSL) required to work with Category A biological agents.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Biological Agent</th>
<th>BSL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Bacillus anthracis</td>
<td>3</td>
<td>WHO, 2004</td>
</tr>
<tr>
<td></td>
<td>Clostridium botulinum</td>
<td>3</td>
<td>Arnon et al., 2001</td>
</tr>
<tr>
<td></td>
<td>Yersinia pestis</td>
<td>2-3</td>
<td>WHO, 2004</td>
</tr>
<tr>
<td></td>
<td>Francisella tularensis</td>
<td>3</td>
<td>Bhalla &amp; Warheit, 2004</td>
</tr>
<tr>
<td>Virus</td>
<td>Variola major</td>
<td>4</td>
<td>DHHS, 2009</td>
</tr>
<tr>
<td></td>
<td>Filoviridae</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arenaviridae</td>
<td>2-3</td>
<td></td>
</tr>
</tbody>
</table>

### 3.2.1 Bacteria

#### 3.2.1.1 Bacillus anthracis

*Bacillus anthracis* is a Gram-positive, non-motile, facultative anaerobic endospore forming bacteria, usually surrounded by a capsule. It is the etiological agent of anthrax, which occurs most frequently when an epizootic or enzootic of herbivores becomes infected after acquiring spores from direct contact with contaminated soil. In humans, the disease can occur when exposed to infected animals, tissue from infected animals or high concentrations of anthrax spores. Anthrax endospores have no measurable metabolism, do not divide, and are resistant to drying, heat,
ultraviolet and ionising radiation, chemical disinfectant, and other forms of stress, remaining in the environment for years (Bhalla & Warheit, 2004), with survival in soil for up 200 years being reported (Yuen, 2001).

The disease is caused by the action of a toxin produced by the vegetative bacillus, which consists of three components; protective antigen (PA), edema factor (EF) and lethal factor (LF). PA binds to cell receptors, mediating the entry of EF and LF into the cell. Another anthrax virulence factor is the D-glutamic acid polypeptide capsule of the vegetative form (WHO, 2004). Three types of anthrax infections can occur; cutaneous, inhalation and gastrointestinal. The cutaneous form is the most common and is characterised by dermal ulcers, painless, non-scarring, pruritic papule progressing to a black depressed eschar with swelling of adjacent lymph glands and oedema (WHO, 2004). Local lymphadenitis and fever can occur, but septicaemia is rare (Moquin & Moquin, 2002). Untreated cutaneous anthrax can become systemic and it is fatal in 5-20% of cases. Gastro-intestinal and inhalation forms are less common. The inhalation form starts with influenza-like symptoms that include fever, fatigue, chills, non-productive cough, vomiting, sweats, myalgia, dyspnoea, confusion, headache and chest and / or abdominal pain, followed by the development of cyanosis, shock, coma and death. The gastro-intestinal form is characterised by fever, nausea, vomiting, abdominal pain and bloody stools. Oropharyngeal infection, on the other hand, is accompanied by oedematous swelling of the neck, often followed by fever and lymphoid involvement (WHO, 2004).

There is no evidence of direct person-to-person spread (Yuen, 2001). After exposure, the incubation period is reported to range from 1 to 7 days, possibly extending up to several weeks. Some vaccines are administered to prevent the disease, such as live spore vaccines based on attenuated strains, and cell-free vaccines based on anthrax PA (WHO, 2004). Regarding therapy, there are three types of antibiotics that are effective against B. anthracis; ciprofloxacin, tetracyclines and penicillins (Bhalla & Warheit, 2004). For laboratory diagnosis and research, manipulations involving clinical specimens, Biosafety Level 2 (BSL-2) practices are recommended, while for manipulations involving activities with a significant aerosol production, Biosafety Level 3 (BSL-3) practices are advised (WHO, 2004).

### 3.2.1.2 Clostridium botulinum

*Clostridium botulinum* is a spore forming and obligate anaerobe, etiological agent of botulism, which can be isolated from the soil, its natural habitat. Four species of *C. botulinum* are known, characterised by different genomes and their common botulinum toxin. In addition, seven distinct antigenic types of botulinum toxin (A-G) are defined by the absence of cross-neutralisation. The toxin is responsible for the disease and is a dichain polypeptide: a heavy chain of 100 KDa is joined by a single disulfide bond to a 50 KDa light chain, which is zinc containing endopeptidase that blocks acetylcholine-containing vesicles from fusing with the terminal membrane of
the motor neuron, resulting in flaccid muscle paralysis (Arnon et al., 2001). Botulinum toxin is the most lethal toxin known and all seven types act in similar ways. Death often occurs as a result of paralysis of pharyngeal and diaphragmatic muscles, followed by respiratory arrest (Bhalla & Warheit, 2004).

Three forms of human botulism exist: food-borne, wound and intestinal. All forms of botulism are caused by absorption of botulinum toxin into the circulation from a wound or mucosal surface; after infection, the incubation period depends on the rate and amount of toxin absorption: from two hours to eight days. Patients affected by botulism are febrile and present symmetric, descending flaccid paralysis with prominent bulbar palsies. Therapy consists of passive immunisation with equine antitoxin, accompanied by supportive care. Botulism can be prevented by administration of a pentavalent (ABCDE) botulinum toxoid, which a recombinant vaccine is in development. BSL-2 practices are recommended for manipulations in laboratory, while BSL-3 practices are suggested for activities with high potential for aerosol or droplet production (Arnon et al., 2001).

3.2.1.3 Yersinia pestis

*Yersinia pestis* is a Gram-negative non-motile, non-spore forming coccobacillus that grows both in aerobic and anaerobic conditions. It can remain viable for days in moist soil or water, but it is killed by direct exposure to sunlight (WHO, 2004). The bacterium is the etiological agent of plague, a disease that can affect humans and animals (La Placa, 2010). Wild rodents are the pathogen carriers and transmission to other animals occurs through fleas, infected animal tissues, contaminated soil or respiratory droplet exposures. In endemic rural areas, persons who come in contact with wild rodent hosts of *Y. pestis* can be affected by the plague, which exists in two forms; bubonic and pneumonic plagues (WHO, 2004).

Bubonic plague occurs if fleas are used as carriers of disease, in which the incubation period is 2-6 days after exposure. Swelling of the lymph nodes occurs (bubones) occurs, associated with onset of fever, chills, headache, followed by nausea and vomiting. Untreated bubonic plague causes septicemia. Pneumonic plague can occur from inhaling organisms or from exposure to infected blood. Productive cough with blood-tinged sputum is a typical symptom of pneumonic plague, which can spread from person to person by coughing (La Placa, 2010).

If started soon after infection, antimicrobial therapy is effective. It consists of administration of streptomycin or gentamicin. Alternative antimicrobial substances are tetracyclines, doxycyclines, chloramphenicol, fluoroquinolones, ciprofloxacin and sulfonamides. Plague vaccine is advised only for high-risk groups, such as laboratory personnel. Vaccination with killed or live attenuated *Y. pestis* is effective against bubonic plague but not against pneumonic plague. BSL-2 practices are recommended for activities involving infective materials and cultures, while BSL-3
may be used in the case of high production of infectious aerosol or direct contact with infected fleas (WHO, 2004).

### 3.2.1.4 Francisella tularensis

*Francisella tularensis* is a small, Gram-negative, non-motile, facultative intracellular, aerobic coccobacillus. It is responsible of tularemia, which is a zoonotic disease. Two bacterium sub-species exist; *F. tularensis tularensis* (Type A) and *F. tularensis palaearctica* (Type B). Type A is more virulent than Type B (WHO, 2004). The organism can survive for up to several weeks in soil, water, straw and soil. Many wild animals (rabbits, beavers, muskrats, hares, voles) are the pathogen carriers. Humans can be infected when bitten by arthropods, by ingestion of contaminated food and water, and inhalation of contaminated aerosols. Direct contact with infected animals is also dangerous for humans, but person-to-person transmission has not been observed (Bhalla & Warheit, 2004).

After infection, the incubation period is generally 3-5 days, but it can extend up to 14 days. Symptoms of the disease depend on the virulence of the infectious agent. Two different clinical manifestations exist; ulceroglandular (75% of cases) and typhoidal (25% of cases) tularemia. The first is characterised by indolent ulcer at the site of entry and painful swelling of local lymph glands; the expression “typhoidal tularemia” indicates systemic illness without apparent site of primary infection. Painful pharyngitis and cervical lymphadenitis are caused by infection through ingestion of contaminated food or water (Bhalla & Warheit, 2004).

Treatment consists of administration of intramuscular streptomycin. Parenteral gentamicin can be used as an alternative drug, while for pre-exposure prophylaxis, an live, attenuated vaccine is available. However, for antimicrobial prophylaxis, oral administration of doxycycline or ciprofloxacin is advised for a 14-day period following the last day of exposure. BSL-2 practices are recommended for routine manipulations of clinical specimens from human and animals, while BSL-3 practices are recommended for manipulations including risk of infectious aerosol production (Bhalla & Warheit, 2004).

### 3.2.2 Virus

#### 3.3.2.1 Variola major and Poxviridae

*Poxviridae* comprise a family of genetically related, large, enveloped, DNA viruses that replicate exclusively within the cytoplasm of vertebrate or invertebrate cells. Only the member of the genus *Orthopoxvirus*, which includes *smallpox*, *monkeypox*, *vaccinia*, and *cowpox* can infect humans. Of these, only smallpox is readily transmitted from person to person via saliva or nasal secretion droplets and contaminated objects (Moss, 2007).
The most common clinicopathologic presentation of smallpox was a systemically virulent form of the disease known as variola major with a case mortality rate of up to 30 to 40%. Saliva or nasal secretion droplets from infected individuals are responsible for inter-human transmission. After oropharyngeal or respiratory mucosa infection, and the asymptomatic, non-infectious period of incubation (7-17 days), many patients present high fever and the malaise of prodromal illness. Maculopapular rashes then appears on the mucosa of the mouth and pharynx, face, and forearms, and spreads to the trunk and legs. This is the most contagious stage because of the high viral titers present in the oropharyngeal tissues. Within 1-2 days, the rash becomes vesicular and later pustular. Scabs subsequently develop that, if the person survives, leave pitted scars called pocks from which the word pox has been derived (Knipe et al., 2001).

A more severe but much less common manifestation of variola major, known as malignant or hemorrhagic smallpox, is associated with a near 100% case fatality rate. Humans are the only known hosts of the virus, facilitating the global Variola eradication, by the World Health Organization (WHO) in 1980 after a successful global vaccination campaign, which was subsequently discontinued (Fenner et al., 2007). The cessation of vaccination not only exposed populations to the risk of a bioterrorist attacks, but also increasing prevalence of zoonotic poxvirus such as monkeypox (Rimoin et al., 2010).

Currently, there are no available treatments for smallpox infection and the therapy involves supportive care as antipyretic and anti-inflammatory treatments to relieve pain and fever. Antibiotics are prescribed for eventual bacterial super-infections (Knipe et al., 2001; Bhalla & Warheit, 2004). All experiments using live variola virus are to be done within WHO approved Biosafety Level 4 (BSL-4) laboratories; one is at the CDC in Atlanta, U.S., while the other one is at the State Research Center of Virology and Biotechnology in Koltsovo, Russia (DHHS, 2009).

### 3.3.2.2 Filoviridae.

The Filoviridae family (from the Latin term *filum*, referring to the shape of the virion), consists of enveloped, negative-stranded, RNA viruses that cause severe zoonotic hemorrhagic fever in humans and non-human primates. The family includes two distinct genera; *Marburgvirus* and *Ebolavirus*. The genus *Marburgvirus* includes a single species, *Marburg marburgvirus*, which has two members, Marburg (MARV) and Ravn (RAVV) viruses. The genus *Ebolavirus* includes five species, each of which has a single member; *Zaire ebolavirus* (EBOV), *Sudan ebolavirus* (SUDV), *Taï Forest ebolavirus* (TAFV), *Bundibugyo ebolavirus* (BDBV) and *Reston ebolavirus* (RESTV) (Adams & Carstens, 2012).

The natural carrier hosts of these viruses have not yet been identified. However, Ebola virus RNA has been detected in terrestrial mammals in Central Africa. Evidence is emerging that African, Asian and possibly also European bats are...
natural carriers of filoviruses and these animals could transmit the virus directly to humans or via intermediate hosts, including gorillas and swine. Following transmission to humans, spread of the virus between individuals is the result of direct contact with blood or other body fluids from infected patients. Filoviruses exhibit different virulence in humans; EBOV and MARV infection is associated with case-fatality rates of up to 90% while RESTV seems to be apathogenic (Sanchez et al., 2007; Kuhn et al., 2011).

In infected individuals, after the incubation period, ranging from 2 to 21 days, the onset of illness begins with generic flu-like symptoms characterised by high fever, severe headache and malaise followed by gastrointestinal symptoms including abdominal pain, severe nausea, vomiting and watery diarrhea.

The majority of patients also present clear hemorrhagic manifestations, such as ecchymoses, mucosal bleeding and hematemesis. Fatalities typically occur 8–16 days following the onset of symptoms, with death usually caused by severe diffuse coagulopathy, multiorgan failure, shock and coma (Brauburger et al., 2012). There is no specific therapy against filoviral infections and supportive care is provided to limit the symptoms (Clark et al., 2012). Due to the lack of approved therapeutics or vaccines along with the high lethality and infectivity, work with Filoviridae is restricted to high-containment BSL-4 laboratories (DHHS, 2009).

3.2.2.3 Arenaviridae

Arenaviridae family consists of enveloped, negative-stranded, bi-partite RNA viruses that cause chronic infections in rodents (animals) and zoonotically acquired disease in humans (Salvato et al., 2011). The genus Arenavirus includes 22 viral species which, based on genetic and geographical data are divided into two groups; Old World (OW) and New World (NW) complexes. The OW complex includes the worldwide distributed Lymphocytic choriomeningitis virus (LCMV), which causes acute aseptic meningoencephalitis in humans, and other viruses endemic to the African continent, including Lassa (LASV) and Lujo (LUJV) viruses, which cause hemorrhagic fever (HF). The larger group of NW arenavirus is further divided into three clades; A, B and C. Clade B is the more relevant in term of human pathology, since it contains most of HF-causing arenaviruses in South America (Charrel & de Lamballerie, 2003).

Virus transmission occurs usually through human contact with excretions or materials contaminated with the excretions of an infected rodent, while secondary person-to-person transmission can occur with some arenaviruses, such as Lassa, Machupo and Lujo viruses (Weber & Rutala, 2001). After 1-2 weeks of incubation period, HF infection produces a wide range of symptoms and pathology, including headache, cough and sore throat, nausea, vomiting, and diarrhea. Several complications can arise, including pleural effusions, neurological complications, facial edema and bleeding from mucosal surface. Advanced stages of disease are
often associated with shock and death (Schattner et al., 2013). No licensed vaccines, prophylactic or therapeutic treatments are available against arenavirus infection. Currently, therapy consists of ribavirin administration, accompanied by supportive care (Vela, 2012). BSL-4 containment is required for all pathogenic hemorrhagic fever-causing arenaviruses while BSL-2 / 3 laboratory environment is advised for handling of other arenaviruses (DHHS, 2009).

4. CONCLUSION

The use of biological agents as bioweapons has its roots in ancient times, when the concepts of bacteria, toxin or virus were not known yet. Over 2,000 years ago, rudimentary techniques of biological warfare resolved the first disputes among people. Hand by hand with the evolution of modern science (especially in the 18th century), the possibility of using biological agents as bioweapons has been refined. In the last few decades, the development of innovative biotechnology techniques has provided the knowledge to create more aggressive bioweapons. These new organisms cause great concern, because they can produce devastating and completely unexpected effects, of the same level or even higher than the most dangerous wild type biological agents.

Although international conventions prohibit the use of biological agents for offensive purposes, it is known that many terrorist groups continue their research about the possible use of biological agents as bioweapons. The concerns related to biological agents are aroused, as well as the effects in terms of victims, both from the objective difficulties in the detection of a potential attack. A release of biological agents is difficult to detect with current technology, especially when it comes to a stand-off revelation compared to point detection. Biological agents have a unique feature when compared to other non-conventional weapons (chemical or radiological); with the exception of toxins, they are able to multiply in the host and in turn be transmitted to other individuals. Hence, immediate identification of a biological attack is essential, in order to take appropriate containment measures to contain further dissemination. Therefore, there is a clear need to develop new technologies to detect biological agents from long-range, in order to take immediate action in the event of both intentional and unintentional biological agents releases.

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