Letter to the Editor

THE EVOLUTION OF BIOLOGICAL AGENTS FOR TERRORISTIC PURPOSES. A NARRATIVE HISTORICAL REVIEW

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ABSTRACT

A terrorist action can take place with traditional weapons and/or with weapons of mass destruction. According to the United Nations Office on Drugs and Crime, it can be divided into conventional and non-conventional weapons; the present investigation aimed to evaluate through a literature overview the evolution of biological agents for terroristic purposes. Conventional weapons are the common firearms such as pistols (semiautomatic, revolvers), light submachine guns, small artillery (machine guns, small mortars, portable missile launchers, mines), and heavy artillery weapons (machine guns of larger caliber, mortars, missiles). The narrative review focused on non-conventional weapons since their effects extend to large quantities of people, over a large territory, and for a prolonged period, also called "weapons of mass destruction", and as such, are subject to complex, restrictive political agreements. The non-conventional weapons include several types of weapons, including nuclear weapons, biological weapons, chemical weapons, and radiogenic weapons. However, terrorism has ancient roots, and the term terrorism was used for the first time during the French Revolution. Many changes over time have occurred, both in the evolution of development and the non-legalized use of the aggressive agents that today are identified with the acronym N.B.C. (Nuclear, Biological, and Chemical).

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INTRODUCTION

Countering the development and production of weapons of mass destruction is, therefore, impossible without states devoting more economic resources to organizational activities and studies in this area. For an effective response against biological attacks, for example, it is necessary to equip the leading hospitals with large quantities of antibacterial drugs and adequate facilities for the treatment of infected and intoxicated people, as well as tools to guarantee prompt investigations into the causes and mechanisms of spread of epidemics (1, 2).

Biological warfare, more commonly known as bacteriological warfare, has ancient origins. The Romans used the dead animals to pollute the water reserves of the besieged enemies and the Tartars; in 1347, during the siege of the Crimean city of Kaffa, the garrison of the Genoese Republic in the Black Sea catapulted corpses of plague victims into the city. Carried by the ships of the fleeing Genoese, the disease landed in Europe, where, in the Middle Ages, it killed over twenty million people in three years. The war of infections continued over the centuries, from the period of colonization (infected smallpox blankets donated to the Indians of Canada or syphilis prostitutes sent to the Maori of New Zealand) until the Second World War, with the Japanese flooding Manchuria with plague, cholera, and leptospirosis. The risk that biological war could entail, above all for the uncontrollable effects that derive from it, had led the first group of countries, including Italy, to sign a "non-use treaty" in Geneva (3) which entered into force on February 8, 1928 and was subsequently signed by over 120 states. These include Iran (1929), Iraq (1930), Pakistan (1960), Syria (1968), the United States (1975) and Afghanistan (1986) (1, 4, 5). However, the 1928 Geneva Protocol did not put any effective control on the acceding States. Even the subsequent Convention on Biological and Toxic Weapons of 1972 (6), which prohibits their development, production, acquisition, storage, and possession, does not contemplate verification procedures, a loophole that allowed many States to continue biological warfare programs for another twenty years (3, 5-13).

Features of biological agents

Biological weapons are more devastating than the atomic bomb because they can cause pandemic diseases. The production of biological weapons is highly accessible without the need for investments and specific technologies, nor do you need any factories and plants; even simple research labs can be quickly converted. Some studies have calculated that hitting a square kilometer of territory would cost 800 USD with nuclear weapons, 600 USD with chemical agents, but only 1 USD with biological agents. A laboratory to produce viruses, bacteria, or any other infectious agent is easily dissimulated: the equipment needed for production is generally on sale and identical to any analysis laboratory. The "raw materials" are readily available from universities, pharmaceutical companies, and research centers. This is why the risk that bacteriological attacks can be carried out in a moment of extremely high tension is particularly topical (1, 14).

Biological weapons are bacteria, viruses, and toxins that cause disease, usually fatal, and are used to affect the military and civilian population. Even the diffusion method is within reach and difficult to predict: each system used has uncontrollable effects. Depending on the type of infection, people are infected and die within hours on most days. Those who are contaminated, in turn, become infectious, spreading the epidemic in continuous progression. Toxins are generally more lethal and faster acting, capable of causing death in a matter of hours, if not minutes. Viruses and bacteria require an incubation period of one day to six weeks before symptoms appear. The reason for the terror induced in the Western world is because, silent and invisible until the moment of use, they produce particularly insidious and difficult-to-recognize symptoms, easily confused with an epidemic of flu. There is a wide variety of possible infectious agents: bacterial agents (carbuncle, lung plague, epidemics among animals), viral agents (smallpox, yellow fever, equine encephalitis, flu), toxins (botulinum, mycotoxins) (1, 15).

The products used are obtained with modifications that make them resistant to the action of known antibiotics, at least until the agent causing the infection has been precisely identified, and the antidote or therapy and other has been found (16). For this, it can take many days, not counting the time necessary to isolate all potentially infected people, immunize them, and wait for the epidemic to disappear. For some of these infections, there is a vaccine, which, however, cannot be imposed on an entire country without creating collective panic, especially if we consider that protective drugs do not provide total immunity, often cause side effects of a certain magnitude and of course, each of them is effective only against one of the dozens of germs that can be used in an attack (Table I) (17).

Table I. *Biological agents doses, incubation time, and availability of vaccines.*

Agent	Infective dose	Incubation period (days)	Vaccine
Anthrax	8.000-50.000 spores	1-5	Available
Brucellosis	10-100 organisms	5-60 (sometimes months)	Not available
Plague	100-500 organisms	2-3	Available but ineffective
Q fever	1-10 organisms	10-40	Under study
Tularemia	10-50 organisms	2-10	Under study
Smallpox	10-100 organisms	7-17	Available
Viral encephalitis	10-100 organisms	2-6 (encephalitis equine Venezuelan) 7-14 (Western and Eastern equine encephalitis)	Under study
Viral hemorrhagic fever	1-10 organisms	4-21	Under study
Botulinum toxin	0,001 μg/kg (type A)	1-5	Under study
Staphylococcal enterotoxins	30 ng (debilitating) 1,7 ng (lethal)	1-6 ore	Not available

Finally, for the defense against genetically modified organisms, which are particularly resistant, there are neither protections nor cures nor is there experience of their infectivity. Much information on the spreading of diseases caused by bacteriological weapons comes from accidents or experiments on the unsuspecting population. For example, in 1979, an accidental release of the carbuncle bacillus from the Soviet plant in Sverdlovsk provided many valuable indications of the toxicity of the bacterium. The most Viruses used for war or terrorist purposes and their families are reported in Table II (1, 14).

Table II. *Viruses used for war or terrorist purposes.*

- **Filoviridae** (Ebola and Marburg haemorrhagic fevers virus)
- **Arenaviridae** (Lassa hemorrhagic fever virus; Junin; Machupo)
- **Bunyaviridae** (Congo-Crimea Hemorrhagic Fever Virus)
- **Flaviviridae** (yellow fever virus)
- **Togaviridae** (Venezuelan Encephalitis Alphavirus; eastern equine, western equine)

The spread can occur by air of infected vectors (mosquitoes, ticks) or contamination of materials and objects of everyday use. In general, these viruses are not very resistant to the external environment, whose cycle in nature is maintained by vectors or infection tanks. They are still not identified in the case of Filoviruses. The incubation period depends on the agent involved: 2-21 days for Ebolavirus; 3-9 days for Marburg virus; 7-21 days for Virus Lassa; 7-16 days for Junin and Machupo viruses (Argentine and Bolivian hemorrhagic fevers); 5-15 days for Alphavirus. In forms transmissible by human infection, the patient is contagious if the virus is present in the blood for several months starting from the pre-clinical period.

The clinical features of viral hemorrhagic fevers and viral encephalitis are, at least at the beginning, remarkably similar, non-specific, and flu-like: fever, general malaise, prostration, and bone and joint pain. Within 1-4 days, symptoms that point towards a definitive diagnosis (exanthema, hemorrhagic manifestations, or neurological signs) appear. Lethality is varied: 50-90% for Ebolavirus, 25% for Marburg virus; 15-60% for Virus Lassa; 5-30% for Argentine and Bolivian hemorrhagic fevers; 5-15% for Eastern equine encephalitis, 5-80% for Western equine encephalitis; 2-50% for Congo-Crimea haemorrhagic fever; 20-50% for yellow fever (in jaundiced forms) (1, 17, 18).

Variola major (smallpox)

It is a DNA virus, pathogenic only for humans, and highly diffusible. Before the eradication of the disease (WHO declaration in 1980), it was responsible for extremely high morbidity and mortality, with an attack rate between unvaccinated populations of about 50%. The possible diffusion can occur by dissemination by aircraft, with the formation of colorless, odorless, and invisible aerosols for contamination of materials and objects of everyday use and of different nature (e.g., paper, fabrics, leathers, objects of everyday use, etc.) The incubation period is from 7 to 17 days, usually 10-14 days; commonly, 10-12 days for the first symptoms to appear, then another 2-4 days for the rash to appear. The period of contagiousness begins from the appearance of the first lesions to the fall of all the crusts; the contagiousness is most significant in the first week of illness due to the high concentration of viruses in saliva. At the onset, the symptoms are nonspecific flu-like: fever, general malaise, prostration, bone, and joint pain, which is followed by an eruption that affects mucous membranes and skin within 2-4 days, with progression in subsequent stages of macules, papules, pustules, crusts and with the possibility of successive waves. The lethality of the greater smallpox is 20-40%; smallpox, or alastrim, caused by the variola minor virus (similar clinical form but more attenuated and benign course) is less than 1% (17).

Bacillus anthracis

The *Bacillus anthracis* is a gram-positive bacterium that forms spores that contain the DNA of the microorganism in the event of adverse environmental changes for life. Very resistant to heat, radiation, and extreme pH; when the environmental conditions are favorable again, the spores become living bacteria. It is a disease that has existed for hundreds of years and still affects both animals and humans in many parts of the world today, including Asia, southern Europe, sub-Saharan Africa, and parts of Australia. In its most common natural form, it creates dark wounds on the skin, from which the name derives: anthrax in Greek means coal.

If the spores are inhaled, they penetrate into the lungs, where they multiply rapidly, producing toxins that spread throughout the body through the blood vessels. A billionth of a gram can cause the death of a person. There are three types of anthrax, each has different symptoms (1, 17):

a) Cutaneous or skin carbuncle is the most common form. Usually, it contracts when a person has a wound or abrasion on the skin and comes into direct contact with the anthrax spores. The resulting itchy skin swelling quickly develops into a black wound. Within 2-6 days, the skin lesion passes from the papule state to that of a necrotic eschar. Some people manifest symptoms such as headache, muscle pain, fever, and vomiting. Cutaneous anthrax must be treated quickly.

b) Gastrointestinal carbuncle transmitted by eating meat from infected animals. Initially, it causes symptoms similar to those found in food poisoning, but these can worsen, causing severe abdominal pain, vomiting of blood, and severe diarrhea.

c) The lung carbuncle is the most severe form of human anthrax, and although it is the rarest form, it is the one that is now causing the most concerns. This form of disease occurs when a person is directly exposed to large quantities of anthrax spores in the air, breathing them. Lethality varies according to the forms and fluctuates, in untreated cases, from 5% to 90%.

Yersinia pestis (plague)

Gram-negative germ, non-sporogenic, aerobic, optionally anaerobic, sensitive to the action of common chemical and physical disinfectants; in nature, the infection cycle is maintained by reservoirs (rodents) and vectors (fleas). Diffusion can occur by disseminating bacilli of the plague by aerosol, contamination of materials and objects of everyday use (indirect transmission), and introduction of infected vectors and tanks. It can be manifested as bubonic form (from puncture of infected fleas), lung form (from inhalation of aerosols containing secretions from sick people), or septicemic form (as such or as a complication of bubonic or pulmonary forms). Fleas (disease carriers) remain infected for months in favorable conditions; the spread of the lung form is favored in crowded environments.

The incubation period ranges from 1 to 7 days (can be slightly longer in vaccinated people); in primary lung plague, it is shorter (2 to 4 days). Using *Yersinia pestis* as a biological weapon, disseminated using aerosol, the first cases of lung plague could appear within 2 days (17, 19).

Clostridium botulinum (botulism)

Sporogenous bacillus gram-positive, anaerobic, produces botulinum toxin; 7 antigenic types of botulinum toxin are known (A, B, C, D, E, F, G). Diffusion can occur by contamination of food or by dispersion as an aerosol. The contamination of water resources seems more problematic due to the need for vast quantities of toxin and the inactivation

of this with standard treatments for drinking water (are sufficient for 3-6 days). The spores of *Clostridium botulinum* produced in conditions of absence of oxygen can resist up to 3-5 hours at a temperature of 100°C while at temperatures of 121°C, they are destroyed after 180 seconds; heat resistance is decreased in an acid environment, and the presence of high salt and sugar concentrations. The botulinum toxin is thermolabile and is destroyed by exposure to temperatures above 80 ° C for at least 10 min.

The neurological symptoms of intoxication appear in usually 12-36 hours after ingestion, but it can reach 8 days: the duration of the incubation period is dose-dependent; the prognosis is all the more serious, the shorter the incubation; lethality in the absence of treatment can reach 70-80%. In the case of inhalation, the symptoms appear after 12 hours. It is not transmissible from person to person (1, 17).

CONCLUSIONS

From antiquity to date, we have known about several local and international conflicts. Usually, the soldiers and the armies must fight single battles and wars directly facing the enemies. Still, army officials and politicians always ask their national scientists to search for special weapons that should be invisible, effective, and highly destructive. Several weapons have been developed, sometimes adapting existing substances or creating instruments that subsequently have had peaceful and civilian applications (i.e., biological agents).

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