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## PATHOGENIC MICROORGANISMS AND BIOTOXINS AS BIOLOGICAL COMBAT AGENTS FOR TERRORIST AIMS

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**Abstract:** Today's world is facing a great evil that comes from man, from people who for various reasons entered into a fight against the rest of humanity. That evil is terrorism. It represents an organized and systematic application of violence to cause a feeling of fear and personal insecurity among other people, i.e. among citizens, to cause disorganization in the functioning of the state apparatus and to violate the authority of the state in order to achieve certain political goals. At the root of terrorism is terror. It represents a set of gross and frightening violent actions that cause a feeling of fear and insecurity in people. The most frequently applied procedures for the implementation of terror are murders, abductions of persons, diversions against public facilities - shopping centers, school facilities, roads, bridges, telecommunications devices, water installations, means of transport, military facilities and installations. To achieve their dark goals, terrorists use diversions against objects that contain hidden forces - hydroaccumulation dams, chemical factories, oil wells and nuclear power plants. Intelligence data and some developments in recent times say that they are ready for terrorist actions by polluting and infecting water sources, food, clothing, etc., polluting the human environment, causing epidemics of infectious diseases, etc.

Biological warfare agents are used to cause epidemics of infectious diseases in peace and in war. They represent pathogenic microorganisms or toxins specially prepared to cause infectious diseases or intoxication of people, animals or plants for the purpose of their destruction or incapacitation in order to reduce the defensive, i.e. military efficiency of the enemy's forces and thereby create conditions for the realization of the immediate military or political objective of the party using those means.

The paper provides brief reviews of pathogenic microorganisms that can be used as biological warfare agents: bacteria, viruses and rickettsiae. A special review is given to biotoxins, especially the botulinum biotoxin, which is the most dangerous and also the most suitable for use as a biological warfare agent. Due to the limited number of pages, the paper presents the basic microbiological characteristics of pathogenic microorganisms from the point of view of their possible use as biological warfare agents.

**Keywords:** pathogenic microorganisms, biotoxins, biological, combat agents, terrorist,

### 1. INTRODUCTION

Pathogenic microorganisms or toxins that are used as biological warfare agents are collectively called biological agents in military terminology. Biological agents are bacteria, protozoa, rickettsiae, viruses or fungi and their toxins specially prepared for military or terrorist purposes.

The bacteria of some of the dangerous infectious diseases can be grown very easily and therefore are most useful for preparing biological agents intended for terrorist actions. They, as we know, are grown on nutrient media and very easily, efficiently and quickly. For example, beef broth enriched with peptone (bouillon) is an excellent substrate for the cultivation of pathogenic bacteria.

Unlike bacteria, viruses live and multiply only intracellularly. Because they consist of DNA or RNA, they are divided into DNA and RNA viruses. Many viruses have been present in living things for years and do not cause disease. Such is, for example, the herpes simplex I virus that lives in the throat of healthy people and causes disease only when the host's resistance is weakened for any reason. But a large number of viruses are pathogenic and as such are the causative agents of infectious diseases.

Rickettsiae are small microorganisms, similar to bacteria. They are larger than viruses and smaller than bacteria. Their dimensions range from 01 to 03 microns. Like other microorganisms, rickettsiae can be benign and pathogenic.

The use of biological agents is a very convenient way to carry out terrorist actions. This is primarily due to the fact that they are most suitable for secret or covert transmission, and then also to the fact that many of them are relatively easy to prepare and in conditions of modest technological equipment, and even with manual means. It is precisely

because of this that the possibilities for the use of biological warfare agents in conditions of existence of extreme and anarchist-minded terrorists are very great.

Biological agents are the most effective means of achieving terrorist goals by performing sabotage-terrorist actions. It is not necessary to have a developed imagination to imagine and perceive the horror that would be created among the population of a city after the knowledge that an epidemic of plague or cholera has occurred. Even the very threat of terrorists that biological agents can be used leads to high-intensity fear among the citizens of the threatened countries.

## 2. PATHOGENIC BACTERIA

As is known, pathogenic bacteria are the causative agents of dysentery, typhoid, paratyphoid, cholera, plague, tularemia, anthrax, brucellosis, sakagia, etc. All of these bacteria can be used as biological warfare agents for terrorist purposes.

**Dysentery** is an acute, infectious and contagious disease of the colon followed by frequent, bloody-mucous stools. It is caused by a group of bacteria collectively called Shigellae (Todorovic, 1981). These are Gram-positive, non-motile bacteria up to three microns long. Several types of Shigella are known. The most toxic of them is Shigella dysenteriae - type I, which secretes an exotoxin with high enterotropic and neurotropic properties, which is why it causes the most severe, that is, the most toxic forms of the disease (Todorovic, 1981).

Shigella can be cultivated on simple substrates. But selective substrates (SS and DES) are usually used for their cultivation, because they prevent the development of other germs that are also found in the stool. Suitable for biological contamination of food and water. Due to the possibility of easy cultivation and covert transmission with contaminated food and liquids, they are suitable for use in terrorist actions.

**Typhus abdominalis** is an acute infectious and contagious disease of a septic-toxic nature. Its causative agent is the bacterium Salmonella typhi. It is a gram-negative short, thin and highly motile bacterium, with rounded edges, 2 – 4 microns long and ½ micron wide. It has a toxic effect with the endotoxin it secretes.

This bacterium grows well on all common media, especially if they are slightly alkaline. The most favorable temperature for its propagation on prepared substrates is 37 C. The substrate for its propagation can also be ordinary broth.

The disease is transmitted to healthy people through food and water if they are previously infected with the typhoid bacteria. Salmonella typhi is therefore particularly suitable for use for subversive and terrorist purposes

**Paratyphus** is an acute infectious disease very similar to typhoid fever. Its causative agents are salmonella. Three types of paratyphoid salmonella - Salmonellae paratyphi - have been differentiated. That is why there are three types of paratyphoid – Paratyphus A, B and C.

The disease is transmitted in the same way as typhoid fever, which is why the possibilities for its spread by applying subversive terrorist methods of action are very high.

**Cholera** is a severe, acute, very epidemic toxic-infectious disease with a rapid onset, followed by vomiting, profuse and watery diarrhea, rapid fluid loss and collapse (Milosevic, Guelmino, 1984). The percentage of mortality in this disease among untreated patients is about 50%.

The causative agent of this disease is the choleric vibrio – Vibrio cholerae. It is a gram-negative, aerobic and highly mobile bacterium. It is comma-shaped with a length of about 2 and a width of about 0.5 microns. It can be cultured very easily and quickly from the stool of the infected person in 1% peptone water and on alkaline blood agar.

Cholera is transmitted through infected water (usually in the first wave of the epidemic), and then through food contaminated by unclean hands, dishes and cutlery used by the infected. The transmitters of this disease are also flies that have been in contact with stool or recovered stomach contents of patients.

The characteristics of the causative agent of the disease and the possibilities for its quick and simple cultivation point to the conclusion that this disease can be spread very easily by applying sabotage-terrorist actions.

**Plague (Pestis)** is a highly contagious and fatal acute infectious disease, characterized by severe septicemia, high fever, rapid evolution, with prostration, progressive weakening of the cardiovascular system, obscuration of the sensorium, delirium and coma. It is an anthroozoonosis caused by the bacterium Yersinia pestis. It primarily attacks mice and other rodents. Infected fleas transmit it to humans from them. It occurs in three clinical forms: glandular or bubonic plague which is the most common, pulmonary plague and plague sepsis.

The causative agent of plague, Yersinia pestis, is a small, gram-negative, aerobic bacterium. It multiplies well on blood agar, and deoxycholate agar is a particularly favorable medium for it. The resistance of this bacterium to the effects of the external environment is very low. It is very sensitive to heat. The sun's rays quickly destroy it. But in the saliva of the infected person and in the dried excrement of the flea, it can survive for several days.

Against this background, it has been established that plague hotspots in certain regions can be maintained for a long series of years with occasional outbreaks of the disease among rodents and small insectivores. Fields where the

disease existed remain infected even after being dormant for several years, or even ten years. In Iran, it has been proven that the plague bacteria can survive in the soil for over five years. This changes the hitherto accepted understanding that *Yersinia pestis* is not resistant in the external environment (Todorovic, 1981).

Wild rodents and small insectivores are a natural reservoir of the plague bacteria. From them, the infection can be transmitted to domestic mice in cities and villages, and from them, very easily, to people. Therefore, the immediate source of bubonic plague infection for humans is the infected flea on mice, while for pneumonic plague, it is the mucus droplets expelled from the patient's respiratory organs or his saliva. Infection can also occur through contact with infectious material oozing from infected animals or humans.

Plague can also be transmitted through ship mice or through mice in ship containers with goods, of course if there is an epizootic of the disease or it has been previously caused. In addition, infected ectoparasites and mice can be transferred with the goods in the containers. In this connection, it is important to note that infected ectoparasites (fleas) can remain alive for days and even weeks in favorable conditions of moisture and temperature (Milosevic, Guelmino, 1966). The disease can also be transmitted by infected people during the incubation period or people suffering from mild clinical forms of the disease (Pestis minor, Todorovic, 1981).

From the above it can be concluded without a doubt that the plague bacterium is a very dangerous means of biological warfare easily applicable for sabotage-terrorist actions. Bearing in mind the fact that modern air traffic allows the transfer of passengers from one end of the world to the other in less than a day and night, materials, insects, rodents and people infected with this disease can easily be transferred. The frequent appearance of martyrs - Islamic fanatic suicides points to the thought that the possibility of their application to transmit this disease for terrorist purposes is not excluded.

**Tularemia** is an acute infectious and regularly fatal disease of rodents. It is transmitted from sick animals to humans mostly directly and only rarely through insects. In humans, the disease can last from six days to ten months. The mortality rate in untreated tularatremia ranges from 1 to 7%. (Taktičko tehnički priručnik DSNO, 1964)

This disease is caused by the bacterium *Francisella tularensis*. It is a gram-negative, rod- or bead-shaped aerobic bacterium up to 4 microns in size. It secretes endotoxin which is neurotropic and has necrotizing properties. It can be kept alive in pus for weeks and in water for months. A temperature of 60 C kills it in ten minutes. Coagulated egg yolk or Francis medium consisting of blood, cystine, agar and glucose can serve as a nutrient medium for its cultivation. This allows easy cultivation of this bacterium.

The easy way of cultivation and its ability to be maintained in water for a long time makes this bacterium easily applicable for subversive-terrorist purposes by contaminating drinking water and water pipes.

**Anthrax** is a zoonosis that mainly affects herbivores. Humans are infected with anthrax directly from animals or indirectly - through contact with raw materials, semi-finished products and high-quality processed products of animal origin. If the anthrax infection becomes generalized in the body, it causes anthrax sepsis, often followed by hemorrhagic meningitis, which is characterized by severe toxic symptoms and high mortality.

The causative agent of anthrax is *Bacillus anthracis*. It is a gram positive aerobic bacterium that produces spores under aerobic conditions. The bacterium reproduces very well on ordinary food. On agar, it makes rough colonies that spread quickly and resemble jellyfish.

The transmission of the disease is carried out through the anthrax spores, which are extremely resistant to physical and chemical influences and are therefore long-lived (Todorovic, 1981). They can live up to 60 years and maybe even longer. Because of this, anthrax is suitable for long-term biological contamination of land.

According to the place where the disease is located in the body, there are three forms of the disease: skin, lung and intestinal anthrax.

Cutaneous anthrax occurs when anthrax spores enter the body through minor or major skin injuries when working with infected material.

Pulmonary anthrax occurs as a result of inhaling anthrax spores that are found in aerosols composed of fibers and dust that are created in textile factories where wool is processed.

In this connection, it should be noted that anthrax spores in powder form are suitable for biological air contamination in rooms where people stay. For this purpose, the ventilation installations of those rooms can be used. Intestinal anthrax usually occurs after eating undercooked meat infected with anthrax spores or after eating with unclean hands that have these spores. This points to the conclusion that anthrax spores can biologically contaminate food, especially meat products.

**Brucellosis (Brucellosis)** is an infectious disease from the group of zoonoses as old as the relationship between humans and animals. The causative agents of the disease are bacteria called *Brucella*. There are three types and *Brucella abortus* – in cattle. *Brucella suis* – in pigs and *Brucella melitensis* – in goats. The disease is transmitted from animals to humans. The entry point of the infection is the mucous membrane of the digestive tract and the skin if there are injuries or even micro-injuries. *Brucella* are suitable for biological contamination of milk and milk

products through which they can be transmitted to humans. Therefore, food, especially dairy food, should be protected from sabotage-terrorist activities.

**Malleus** is an infectious disease of domestic animals - horses, donkeys and mules. It rarely occurs in bivalves. It also infects people.

The causative agent of this disease is *Bacillus mallei*. It is a sessile, asporogenous, gram-negative bacterium in the form of a short dash, 2-5 in length and ½ micron in width. It multiplies quite well on alkaline media (agar, bouillon, etc.) at a temperature of 33-37 C. It also multiplies well on potatoes, first in the form of a yellow deposit which then acquires a reddish color. Exposed directly to the sun dies in 2 hours. It is resistant in dried pus and blood. In stables it can be kept for a long time. The bacteria can be kept alive for 12 to 15 days in the pastures where the horses were fed. It has been established that on a favorable nutrient medium and sheltered from light it can be kept alive for several weeks.

The relatively easy cultivation of the pathogen and its good resistance to the external environment allow it to be used for biological contamination of the space where cattle and people reside in order to inflict material and human losses on the opponent.

### 3. PATHOGENIC VIRUSES

From the point of view of military, that is, sabotage-terrorist actions, the most significant are the viruses of Variola, Yellow Fever, Influenza, Contagious Hepatitis, etc.

**Variola major or Smallpox** is a dangerous infectious disease with a high mortality rate of over 20% of those infected. The causative agent of this disease belongs to DNA viruses. It is a representative of the group of pox viruses and is one of the largest viruses of this group. (250 to 300 millimicrons).

The varicella virus is maintained in the external environment under ordinary conditions and in a dry state for months, preserving its infectivity. Its resistance to physical and chemical agents is very high.

The infection can spread in various ways. But in practice, it usually spreads through the air, the droplets of the sick person or aerosol dust infected with the virus of the disease. The entrance door to the infection in the human body is usually the respiratory tract, and in rarer cases it can also come through the skin or mucous membrane (Todorovic, 1981)

The high resistance to the external environment, the high infectivity and the possibility of being transmitted through contaminated objects and dust aerosols allow the variola virus to be used as a very effective biological agent in sabotage-terrorist actions.

**Yellow fever (Febris flava)** is an acute infectious anthroponosis that affects people in three areas. Its reservoir is primates, especially rhesus, from which it is transmitted to humans by mosquitoes of the genus *Culicinaea*.

The causative agent of this disease is the ARBO virus from group B. It belongs to the RNA viruses. It is sensitive to temperatures of 65 C, as well as to weak solutions of formalin. At low temperatures of -70 C and in an airless space it can be maintained indefinitely. It is cultured in the tissues of the chick embryo in a fertilized chicken egg

From the aspect of usability for sabotage-terrorist actions, the most dangerous is the classic yellow fever. It is an epidemic disease that affects large settlements - cities. Its causative agent is transmitted by the female mosquito *Aedes aegypti*. Mortality from this disease is high and affects about 20% of those affected (Milosevic, Guelmino, 1966)

**Acute viral hepatitis (Hepatitis virosa acuta)** is a contagious viral inflammation of the liver (black liver). It is caused by two viruses: virus A and virus B. That is why the disease appears as Hepatitis virosa A and Hepatitis virosa B.

Both viruses are very resistant to high temperatures. Virus A can remain alive at a temperature of up to 100 C for up to 30 minutes, and at a temperature of -15 C it can be preserved with maintained infectivity for up to two years. The B virus is even more resistant than the A virus. It is destroyed if exposed to temperatures in dry air of 160 C for one hour. In the frozen environment, both viruses can survive for several years. They are also very resistant to ultraviolet rays. Because of these properties, these viruses are extremely useful for military purposes, that is, sabotage-terrorist purposes.

Hepatitis A is significant from the point of view of usability for subversive terrorist purposes. The most common route of infection with this hepatitis is oral, which is why it is also called the disease of dirty hands. The virus is found in the patient's blood and is also eliminated through his stool. The elimination of the virus through the stool usually lasts up to four weeks. The infection can be transmitted directly - through contact with the patient and indirectly through infected objects from the patient's environment (dishes, clothes, toys, books, etc.). But the most common cause of infection is food infected with the virus (milk, vegetables, fruit). The virus can also contaminate water in wells and water pipes, causing explosive epidemics, which makes this hepatitis very dangerous in terms of its applicability in bioterrorism.

**The flu (Grippe, Influenza)** is an acute infectious and highly contagious disease of viral origin. The causative agent of this disease is a virus from the myxovirus group. There are three groups of this virus - A, B and C. A basic characteristic of all three groups is that they are prone to rapid mutation, which is why new strains often appear, some of which are very dangerous because they cause infections with high mortality that spread rapidly as epidemics and pandemics. (Spanish flu, Asian flu, Covid-19).

Reservoir of the influenza virus among separate epidemics is man. It is assumed that animals, especially pigs and horses, can play the role of a reservoir of this virus. The infection is particularly affected by cold, wet and foggy weather in the winter months, late autumn and early spring. The infection is carried out through the droplets released by the patient when coughing and speaking. All people are very susceptible to the virus. Natural immunity is rarely observed.

Influenza is interesting from the point of view of bioterrorist actions because of its high contagiousness. Particularly interesting are the dangerous mutants of the disease that can be easily transmitted by letting previously infected tourists into the areas where the epidemic is intended to spread.

#### **4. PATHOGENIC RICKETTSIA**

Some of the pathogenic rickettsia, due to their infectious characteristics and the possibility of easy reproduction, are suitable for the preparation of biological agents that can then be used for subversive-terrorist actions. The best-known and most easily applicable rickettsia for this type of combat action is the causative agent of typhus typhus

**Typhus typhus (Typhus exanthematicus)** or simply freckle is a dangerous and severe infectious disease. It often occurs in the form of large epidemics, especially in winter and spring, and especially in extraordinary circumstances (wars, earthquakes, floods, etc.).

The causative agent of freckles is *Rickettsia prowazeki*. It is a gram-negative, small rickettsia that is at the limit of visibility with an ordinary microscope. It cannot be cultivated on ordinary nutrient medium. It is cultivated only on living tissues, namely on the chorioallantoic membrane of a fertilized chicken egg, on the lungs of a mouse, etc. It cannot be sustained in the outside world. In dried lice excrement, this rickettsia can be maintained with preserved virulence for up to a year. It can only be found in lice and in humans. Only lice transmit it from person to person, and that is the white louse, which is an exclusively human parasite. Infection can also occur through the mucous membrane of the eyes if they are rubbed with hands infected with lice feces or with dust containing rickettsiae. Humans can also become infected through the respiratory tract, by inhaling dust containing rickettsiae originating from the excrement of lice.

The disease can be easily transmitted by contaminated lice, or clothing contaminated with feces from infected lice, or through dust containing particles of the feces of such lice. Due to its high contagiousness, the causative agent of freckles is easily applicable for bioterrorist purposes.

#### **5. BIOTOXINS**

Biotoxins are products of pathogenic microorganisms with high toxicity, which makes them usable for sabotage-terrorist actions. The most toxic biotoxin is the toxin secreted by the bacterium *Clostridium botulinum*. The natural reservoir of this bacterium is the digestive tract of many animals and fish. It easily reproduces in canned meat, cured meat products (sausages, hams, dried or smoked meat), then in canned vegetables, especially if it is canned at home, as well as in canned fish. The toxin produced by this bacterium is a protein exotoxin with enormous toxicity. It is one of the strongest toxins known to medicine. One milligram of this toxin can kill 20,000,000 mice. The lethal dose for humans is hundredths of a gram. Theoretically, one gram of this toxin can kill about 10,000,000 people.

There are 6 types of botulinum biotoxin. A, B, C, D, E and F. The most dangerous of them is type E. All of them can be prepared in crystalline form and as such can be used as biological warfare agents. Due to the enormous toxicity, they can be prepared in very small quantities that can be easily hidden and transferred from one place to another. This makes them easy to use for bioterrorist purposes.

#### **6. CONCLUSION**

The modern world is facing a great evil that threatens humanity in a variety of ways, creating fear among millions of innocent people. That evil is terrorism. One of the possible ways of terrorist threat to people is the use of biological warfare agents. Many of the pathogenic microorganisms are easily produced and even more easily transferred from one place to another. They can be transmitted in various ampoules, in the form of sprays, powders, pens, deodorant packaging, by contamination of food, clothes, by releasing infected animals (insects, birds, mammals, etc.). Infections of dangerous infectious diseases can also be transmitted by fanatical infected people who, until the stage of severe debilitation from the disease, can appear in public places and infect healthy people.



Three possible ways of applying biological warfare agents are generally accepted: as aerosols, through infected animals and through infected vectors (insects, ticks).

The application of biological warfare agents by means of aerosols is the most likely way of their dispersal. This method has the following advantages: the respiratory tract is a susceptible place for the entry of a large number of potential biological agents; with this method of application, large areas could be covered with only one attack; usual regular hygiene measures are not effective for protection against aerosol attack.

The application of biological agents through infected animals and vectors is less likely due to the uncertainty of the outcome in terms of adaptation of infected animals (most often wild rodents). However, this method of dispersal has an advantage in that it provides maximum protection of biological agents from the action of external environmental factors (wind, rain, sunlight, etc.).

Developed traffic, especially air traffic, allows bioterrorists to easily move from one end of the world to another. The great possibilities for the production of biological agents and in relatively modest technological conditions and the enormous possibilities for their covert transfer contribute to the danger of bioterrorism hanging like a sword of Damocles over modern humanity. Only the fact that infectious diseases know no borders and spread easily, so in a short time, as epidemics and pandemics can threaten the social groups to which the bioterrorists belong, without a doubt deters them from using biological agents in terrorist acts. But anarchist-minded and fanatical terrorists have always existed, exist and will exist. It is not excluded the possibility that they, in order to achieve their dark goals, also reach for pathogenic microorganisms and their toxins. Because of this, bioterrorism is one of the biggest dangers facing the modern world. The removal or reduction of this danger represents a great challenge for members of all professions who work in the service of humanity, and especially for members of the medical profession.

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