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## BIOSAFETY AND BIOLOGICAL FACTORS

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### Introduction

Biological threats go beyond infectious disease epidemics. A biological threat should be construed as the effects of deployment of biological warfare or the occurrence of biological events of natural origin, which can have significant impact on the breach of security in the military, social, ecological and biological context [1]. Biological factors are often recognized as an important and increasingly appreciated problem of widely understood public health. Epidemiological data indicates that, worldwide, at least several hundred million people are exposed to them. A fallacious conviction prevails that biological factors generally affect health care workers who are exposed to them as part of their daily professional routine [2]. However, we rarely realize that biological factors affect us as part of many types of work, also in public spaces. Exposure to biological factors in the workplace and out of work has become increasingly frequent, leading to many adverse health effects. Health care authorities should be sensitive to the emergence of disease foci caused by biological factors (bioterrorism), which requires their quick reaction [3,4]. It is also important to guarantee the security of personnel in all employment areas where contact with these factors is expected. It is estimated that the frequency of use of weapons of mass destruction is directly proportional to the availability of these means and their production costs, as well as their production capacity. These are the main reasons that indicate that a growing number of terrorist organizations can

start using them [5]. The purpose of the study is to present the current knowledge on the preparedness of government bodies (administration and health care) to fight biological threats of various origin.

## 1. Bioterrorism

There are more than 100 definitions which characterize bioterrorism [6]. Particularly following the events of September 11, 2001, the notion of terrorism entered the common discourse all over the world [4]. It is not a new phenomenon, but has been continuously changing, developing in multiple layers. In this sense, the forms, means and objectives of terrorists have transformed from unidentified IEDs (*Improvised Explosive Device*), through chemical means, to chemical warfare [4,6]. Depending on the motives, the objective and the method of attack, we can distinguish various types of terrorism, e.g. radiological terrorism, nuclear terrorism, chemical terrorism and biological terrorism [7].

The progress of civilization, and particularly scientific and technical development, have significant impact on the development of the bioterrorism phenomenon. In their attacks, terrorists employ state of the art technologies, including those derived from biological sciences. In the recent years, terrorism has become an extraordinary threat to global security, as it encompasses a broad selection of measures, from unidentified explosive charges, through chemicals (e.g. Novichok), to biological warfare (castor oil, *Bacillus anthracis*, multi-resistant bacteria strains) [3,6,8].

### The “ideal” biological factor

Biological warfare can be used to perform an assault on a single person, an army division, as well as on a civilian population. It is perceived as an attractive means for terrorists, as it is easy to produce and cheap [9]. Many potentially useful factors in biological warfare cause contagious diseases among people and animals, but few of them can be successfully used to produce effective biological warfare. [10] Microorganisms which can be used as biological warfare are relatively easy to culture, since they can be obtained from diseased animals and from the environment [11].

The features of biological means which determine their effectiveness are [9,12-15]:

- ease of spreading;
- ease of concealing and transporting;
- small weight of particles which can be dispersed (1-5 mm) in the form of aerosol;
- invisibility during attack;
- low detection rate at the initial stage of the attack;
- no effective treatment;
- no vaccine;
- high mortality rate;
- possibility of infection through direct contact;
- high immunity to external factors;
- ease of production, including mass production.

Analyzing the assault methods utilizing biological combat warfare, we can distinguish two types of attack: concealed and open [12]. An attack with the use of biological warfare can be conducted in several ways, among others by spreading pathogens, invading one's body with the pathogen, or taking advantage of the pathogens' multiplication capacity [13].

The following can be used to spread pathogens [12,14]:

- biological aerosols (used to introduce pathogens to ventilation and air conditioning);

- natural germ carriers (insects, rats);
- water and food contamination;
- contaminated items and parcels, abandoned and sent.

Pathogens can invade one's body through the airway, through skin, or through the alimentary tract. In the event of a bio-terrorist attack, pathogenic microorganisms can be spread by the wind. Dispersed in the form of aerosol, they can be transported at large distances.

Vectors can also include carriers (lice, fleas, mice, rats), from which pathogenic microorganisms can be transferred directly onto humans or into water or food [12].

The majority of biological warfare preparations are prepared in the form of aerosols. They are the fastest-working and most effective in this form, compared to water dispersions to when added to food [11,16]. Air contamination (e.g. through dispersion of biological aerosol) is a likely variant of bioterrorism. The effectiveness of this type of attack is high, considering respiratory track surfaces and dosage levels penetrating the body this way. The objects of this attack will most likely be efficient air conditioning systems, public buildings, metro stations, and, most of all, places where people gather: railway stations, airports, shopping centers, sports and cultural centers, government and public buildings, army concentration sites, mass event venues [4]. In the face of these premises, airborne contaminant levels should be constantly monitored. Specific ventilation and air conditioning security systems were developed and implemented in the US. These recommendations generally refer to public buildings and should be taken into account at design stage, as part of risk assessment [17,18].

At this point, an airborne biological factor detection system is not viable, however, using various air filtering options (preceded by a thorough analysis of the building's susceptibility to various threats), combined with other safeguards, can significantly reduce the likelihood of death or threat to human life and health in buildings. Food, water or soil contamination can be another attack method. Collective food consumption spots or potable water intakes are the most exposed to such attacks [4].

### **Description of the most important pathogens as potential bioterrorist factors**

Although many pathogenic factors and toxins cause diseases and poisonings, only some of them can serve as effective factors which could be potentially used in bioterrorist attacks. It should be stressed that terrorists could focus their biological attacks not only on humans, but also on animal breeding facilities and crop farms.

The military aspect of anthrax as a potential biological weapon and anthrax bacilli which are consistently listed number one among the factors of this weapon, as well as increasingly signaled bioterrorism speak for the prevalent threat posed by this pathogen. It is currently believed that anthrax spores are the most likely to be used as biological warfare [14].

Anthrax is an infectious disease caused by *Bacillus anthracis*, a gram-positive bacillus forming spores (which display extraordinary immunity to adverse factors: drying, high temperature). The disease is animal-borne (zoonosis) and progresses in various forms:

- skin form (the most frequent, can be manifested by a black skin lesion),
- inhalational form (severe, with high fever, cough and hemoptysis),
- intestinal form (from the ingestion of raw or uncooked meat from infested animals).

Anthrax is common to sheep and goats which infect one another by contacting soil contaminated with spores. The bacteria form spores in the soil, which not only stay vital in favorable conditions for dozens of years, but also multiply in their vegetative state. Anthrax spores are difficult to destroy, which is why they are the excellent as biological warfare. 2 g of

dried spores of this bacteria, spread evenly in the form of powder in the urban environment of 500 000 people could cause the death or severe disease of 200 000 people [19].

People are infected through wounds, by inhaling the bacteria or ingesting them. Collected susceptibility data (for a risk assessment) indicates that humans are moderately immune to anthrax. The number of anthrax instances depends on the level of exposure to the germ.

Throughout the years, anthrax epidemics have been also recorded in humans, such as the Zimbabwe epidemic which started in 1979 and relapsed in 1984-1985 (a large percentage of the infected and a low mortality rate). The mortality rate is sometimes high, as in the case of the Sverdlovsk incident in Russia in 1979 [12,14,19-21].

Plague (*Pestis*) is an acute infectious diseases caused by bacteria transmitted by rodents and small mammals, as well as humans. The development of plague is caused by non-sporing *Yersinia pestis* bacteria which are transferred from animals onto humans by fleas, through direct contact with sick animals, and seldom through inhalation from another human. There are three forms of the disease

- bubonic plague (*pestis bubonica*);
- pneumonic plague (*pestis pneumonica*);
- septicemic plague (*pestis septica*).

When plague is suspected, a laboratory examination must be carried out [22]. This bacterium is sensitive to heat, disinfectants and UV radiation. Plague bacteria are included in the classic biological warfare arsenal. Their importance stems from the rare occurrence of plague in developed countries, its relatively high mortality rate and the common sensitivity of the general population.

Smallpox is an infectious disease caused by a virus, and its acute course is caused by the *Variola Major* virus. Smallpox was one of the largest plagues of humanity, as it occurred on all continents. The spread of the epidemic was facilitated by the high infectivity of the virus. Infection is usually caused by droplets, which is why the epidemic can spread so fast. In December 1979, WHO announced that smallpox was eradicated all over the world. After smallpox was eradicated and compulsory vaccinations were discontinued, the virus samples were placed in two reference laboratories: at the Virology Institute in Novosibirsk and the Center for Disease Control and Prevention in Atlanta. One of the biggest medical accomplishments – the eradication of smallpox and the liquidation of compulsory vaccinations paradoxically contributed to the potential use of this virus as biological warfare [12].

Tularemia is an acute infectious disease caused by *Francisella tularensis* bacteria transmitted by animals and humans. This bacterium is a small, aerobic gram-positive bacillus. Tularemia is the disease of the northern hemisphere. In Europe, the majority of instances were claimed in Scandinavian countries. Although the bacteria are highly contagious, the human-to-human transmission mechanism has not been proven.

According to the WHO, spreading 50 kg of the poisonous *F.tularensis* on an area inhabited by 5 million people would cause 250 000 disease instances, out of which 19 000 would be fatal [15,22].

Not all microorganisms or their products share the same toxic effect when used in bioterrorist attacks or during wars [16]. At least several dozen pathogens can be used in a possible bioterrorist attack. There are various classifications of toxic microorganisms. In 1999, the US Center for Disease Control and Prevention classified biological agents according to: their pathogenic effect, the possibility of their used in large scale, the difficulty in recognizing diseases and identifying the microorganisms which could be used as biological warfare. This classification can be helpful when building a system of quick diagnostics and treatment, starting with the factors that create the largest and most significant threat [23]. The list contains the 80 most dangerous pathogens: 43 human-derived, 18 animal-derived and 19 plant-derived [8,10,23].

The main groups of biological factors marked with subsequent letters of the alphabet were distinguished:

#### **Category A**

The most dangerous pathogenic factors which spread fast, are very poisonous and cause a high mortality rate. They are also easy to emit and maintain in the environment. They are a grave threat to public health and require constant monitoring. Infection is direct [8,10,12,15].

This category includes:

- *Yersinia pestis* (plague),
- *Bacillus anthracis* (anthrax),
- *Francisella tularensis* (tularemia),
- *Clostridium botulinum* – toksyna (botulinum toxin),
- *Orthopoxvirus* (smallpox),
- *Filioviridae* (Ebola, Marburg – viral hemorrhagic fever),
- *Arenaviridae* (Lassa, Junin virus – viral hemorrhagic fevers).

#### **Category B**

These factors cause moderate pathogenic, mortality and spreading rate. These pathogens are easy to spread. Infection can be spread through water or food. They require special diagnostic methods and increased, very thorough monitoring of disease progress [8,10,12,15].

This group includes [14]:

- *Brucella sp.* (brucellosis),
- *Coxiella burnetti* (Q fever),
- *Rickettsia prowazekii* (typhus),
- *Alphavira* (viral encephalitis),
- rycyna (castor oil poisoning),
- food contamination (*Salmonella sp.* – salmonellosis, *Shigella dysenteriae* - dysentery, *Escherichia coli* O157:H7 infection) or water contamination (*Vibrio cholerae* - cholera, *Cryptosporidium parvum* - cryptosporidiosis).

#### **Category C**

Pathogens and newly developed means which can become the objects of genetic engineering endeavors in the pursuit of easier production (genetic manipulation) and terrorist fight methods thanks to their increased accessibility. They are sources of relatively high pathogenic and mortality rates, and their use can be important from the point of view of public health [8,10,12,15]. Epidemiologists fear these biological warfare factors the most, especially those which were genetically modified, hence their identification and treatment of their effects can be very difficult, if not impossible [14].

This group includes:

- *Flavivirus* (yellow fever),
- *Mycobacterium tuberculosis* XDR (extensively drug-resistant tuberculosis),
- *Nipah virus* (Nipah disease),
- *Hantavirus* (hantavirus).

#### **Category D**

This group is sometimes listed, as it contains pathogens which are very unlikely to be used as biological warfare (HIV) [15].

### **Biosafety in the proliferation of weapons of mass destruction**

Control and safety of use of biological factors in a time of peace (time P) is an important element of preventing their use in potential bioterrorist activities. Ensuring the logistical

security of public complexes, technologies used in the workplace, or dual use materials and intermediate products against unauthorized interception and intentional use is referred to as *biodecurity* [24,25].

The notion of *dual use* refers to the fear of the results of potentially sensitive studies carried out in civil institutions falling into the wrong hands. The primary concern is therefore to secure their possible use for safety purposes only. The advantages derived from studies developed in widely understood public health surpass the possible threats they could cause. Despite this, there is legitimate fear that terrorist groups could seize the expert dual use knowledge and certain technologies, which could potentially allow them to conduct attacks with the use of biological factors. An attempt at restricting access to scientific publications, particularly those concerning: genetic modification of pathogens which increase their poisonous effect, pathogen modifications causing them to go undetected by contemporary detection systems, works aiming to improve pathogen spreading devices and systems, studies devoted to the behavior of pathogens after they are released to food and water, disclosure of nucleotide sequences for particularly dangerous and lethal pathogens, could be used to synthesize their genomes without the need to obtain them directly [26].

On the other hand, unexpected diseases occurring, laboratory accidents or other unintended releases of pathogenic factors and pathogens are a hazard which could affect the safety of our societies and threaten many branches of the industry. This has direct impact on the security of personnel and population, as described in OHS rules. These are the two important and complementary areas which affect the perception of the Biological and Toxin Weapon Convention (BTWC) and preventing the bioterrorism phenomenon [27-29].

The BTWC convention which bans any studies, production and accumulation of bacteriological and toxic weapon stocks and stipulating their destruction is the point of departure for creating and implementing laws which could regulate issues related to:

- export and import of dual use goods and technology;
- safety of laboratories, particularly those which apply genetic engineering techniques;
- supervision of microbial and toxin safety.

### **Harmful biological factors in the workplace**

The problem of workplace exposure to biological factors requires an interdisciplinary approach assuming the application of knowledge from various sciences, environments. It also requires us to change our general approach to the issue by raising the rank of preventive measures, superseding rectification [2].

Harmful biological factors in the workplace include those microorganisms (viruses, bacteria, fungi) and the structures and substances they produce, which have negative impact on the human body at work and which can cause occupational diseases [30,31].

Biological factors have a specific effect, since, no dependence between their concentration and contact time and body reaction has been indicated yet for them [32]. This incessant variability of pathogenic microorganisms is the reason why safe exposure levels cannot be determined for the majority of biological factors, i.e. a level, below which no negative health effects are observed. It is therefore impossible to determine their permissible occupational exposure values [30].

Contrary to the majority of chemical and physical factors, there are no commonly acceptable criteria for assessing workplace exposure to biological factors worldwide. Neither are there any normative (reference) values in the workplace, or methodological guidelines. No generally recognized threshold limit values have been adopted yet either [3,32].

Exposure to biological factors can often lead to the development of many adverse health effects in those exposed. Development of normative and reference values for airborne biological health effects is therefore the precondition for preserving a balanced environment in and out of work, as well as for controlling and assessing this environment [3]. Therefore,

according to the regulation, a risk assessment related to exposure to biological factors is a qualitative assessment. This state generally stems from [3]:

- the lack of suitable epidemiological data describing the biological factor – health effect correlation;
- individual susceptibility of each organism exposed to a specific factor;
- small amount of measurement data concerning environmental bio-aerosols;
- the absence of standardization of measurement and experimental methods.

The criteria for performing occupational risk assessments under the said regulation consider [32]:

- the classification and list of harmful biological factors;
- the type of activities performed by the worker, the time and level of exposure to the harmful biological factor;
- potential toxic or allergic effects of the harmful biological factor;
- the possibility of developing a disease in the outcome of the work performed;
- claiming a disease which is directly linked to the work performed;
- recommendation of Sanitary Inspectorate, National Labor Inspectorate bodies and occupational medicine units.

In justified cases (e.g. health care units, veterinary clinics), risk assessment criteria should additionally contain information concerning the potential occurrence of a harmful biological factor in a patient/animal, as well as in materials (samples) collected from them. They should also inform about the threat posed by the harmful biological factors which is known to be present or suspected to be present [32].

The basis for classifying biological factors is their impact on personnel health. Additional criteria for individual groups are:

- the pathogenic capacity for humans and disease severity;
- the possibility of spreading to the population;
- the possibility of implementing adequate prevention and treatment.

The level of exposure to these factors depends on the virulence of microorganisms, i.e. on [30]:

- the severity of the disease they can cause in the worker;
- persistence in the environment;
- infectious dose and transfer paths;
- epidemiological situation in the country;
- individual reaction of the worker and access to prevention and treatment.

Knowledge of the biological factors which occur or are likely to occur in the activities performed by the worker is an essential element of risk assessment in the case of activities involving the use of biological factors. Identifying the health hazards is simple, as we already know the microorganism the worker is in contact with. In the latter situation, it is necessary to carry out microbiological tests to identify the microorganism [2].

The predominant situation is that the worker is dealing with an unintentional presence of biological factors in the workplace. The types of activities performed determine the responsibilities of the employer, such as providing suitable airtight sealing conditions. It is the more important in the event of a failure causing significant work environment contamination with biological factors. According to the provisions of Directive no. 2000/54/EC, the employer is obligated to counteract the effects of a failure which could potentially lead to the release of harmful biological factors to the environment and the ultimate worker infections

(microbiological risk). These situations often occur in laboratories and biotechnological facilities when a container with infectious microorganism cultures are damaged, ventilation systems break down or toxins are spilled to the environment [31].

Laboratory and industrial works are characterized by their use of infectious biological factors, which carries a threat to the personnel.

Microbiological risk is closely related to biorisk, i.e. the likelihood of occurrence of an unfavorable event, which could potentially lead to the release of a microorganism to the environment in a random or intentional manner, and cause human infection. Biorisk assessments are carried out by analyzing biological safety risks in the laboratory, as related to unauthorized access, loss, burglary, abuse of biological material [30].

Biological safety is a notion which describes control principles, technologies and practices implemented to prevent unintentional exposure of laboratory staff to contact with pathogens and toxins, and their accidental release.

To achieve an adequately high level of biological safety in the laboratory, a suitable strategy should be adopted – one of such possibilities was presented in WHO document titled *Biorisk Management: Laboratory Biosecurity Guidance*. The document presents a concept for minimizing or prevent the occurrence and rectify the effects of human error in the laboratory by defining an approach to biorisk management, the counterparts of which are: biological security and laboratory security [32].

According to the law, all intentional activities carried out in laboratories (including diagnostic laboratories) and in rooms for laboratory animals which were intentionally infected with harmful biological factors classified as threat groups 2 – 4 or which are suspected of being infected with these factors, as well as industrial processes utilizing harmful biological factors classified as threat groups 2 – 4 must be carried out in airtight conditions:

- second airtight level – for a harmful biological factor classified as threat group 2;
- third airtight level – for a harmful biological factor classified as threat group 3;
- fourth airtight level – for a harmful biological factor classified as threat group 4.

As regarding so-called accidental activities in diagnostic laboratories and in rooms for laboratory animals, where works are carried out with the use of materials, for which it is not certain whether they contain harmful biological factors which could possibly cause diseases in humans or not, the second airtight level is applied. In turn, the third and fourth airtight level is applied in the event of a justified necessity [2,30,33].

## Summary and conclusions

An effective system of epidemiological monitoring assumes: threat recognition, transfer of information and implementation of adequate measures. A limited diagnostic base is the weak link of every epidemiological monitoring system. It is therefore justified to ensure the ongoing development of this base, as to facilitate works involving dangerous pathogens which can appear on our territories at any time because of the lack of country borders (unobstructed migration) or when intentionally used in bioterrorist attacks. Especially in the latter case, we have to be prepared to handle unknown pathogens, including modified or genetically engineered ones. It is therefore extremely important to keep the rescue service and health care staff properly trained and equipped, working according to strictly developed procedures. Since time is of essence in infectious diseases, laboratories should not be situated too far from one another, and effective procedures should enable effective response to an identified threat.



To ensure constant preparedness to handle biological threats, it is important to take measures in various areas, including to improve the system of monitoring and detecting diseases, intensify cross-border cooperation and communication, to facilitate international laboratory cooperation and to develop procedures for implementing common international medical remedies.

The aspect of education remains one of the most important factors in building the country's biological safety. Its purpose is to prepare the civilians to possible bioterrorist attacks and to ensure the correct reaction to the mass media, funeral homes, or general practitioners, who could undergo suitable training as part of their medical education.

The location, time and effects of a successful bioterrorist attack are impossible to foresee. A general awareness should be therefore developed in the society to protect humanity from this threat in a more effective manner.

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