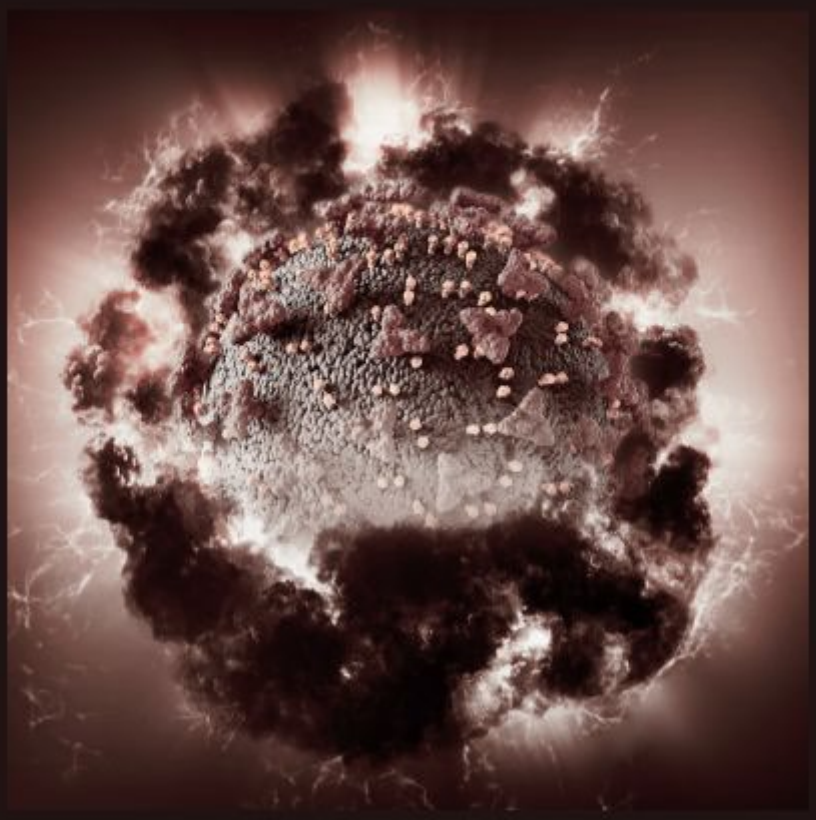

Prevention of Biological Threats in Wartime

Educational manual for advanced training of biosafety and bioethics specialists

Collective of Authors : Igor Khudetskyy et al.

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PREVENTION OF BIOLOGICAL THREATS IN WARTIME

Study aid

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as a study aid for advanced training of biosafety and bioethics specialists

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Study aid “Prevention of biological threats in wartime” provides information on the structure of biological threats in wartime conditions. An analysis of biological threats in the history of wars is provided. An interactive analysis of the features of biothreats and biosecurity in the conditions of modern wars was conducted. Traditional infections as a means of hybrid warfare. The issue of binary and multi-component biological weapons was considered. Focused attention on the so-called infections of war. Study aid “Prevention of biological threats in wartime” is intended for professional development of biosafety and biosecurity specialists.

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CONTENTS

INTRODUCTION

The development of humanity is closely connected with threats and society's response to these threats. Among the threats that humanity typically faces, physical, chemical, and biological threats are distinguished. Countries around the world collectively develop strategies to respond to both existing and potential threats. Measures to counter these threats are implemented both at the international level and by individual countries, taking into account their available economic, material and technical, scientific, informational, human, and other resources. Biological threats hold a significant place in this list and have a number of unique characteristics. Biological hazards differ from all other types of dangers because biological agents can grow and reproduce in a host organism, be transmitted from one object to another, and spread over large areas.

The history of humanity, as a biological species, and the history of *Homo sapiens* are largely connected to the interaction between humans and the biosphere. Humans are an integral part of the biosphere and actively interact with it, constantly influenced by the biosphere while also exerting significant influence on it. Every organism in the environment can simultaneously or sequentially experience the effects of both biotic and abiotic factors, as well as anthropogenic influences.

Biosafety and biosecurity represent a multifaceted, interdisciplinary problem. Study aid addresses important issues related to biosafety and biosecurity, such as current bio-threats, legal aspects of biosecurity and biosafety, interdisciplinary and regional dimensions of biosecurity, and the challenges of detecting bio-threats. These events can undermine achievements in sustainable development and global health due to their potential to cause national and regional instability, global economic consequences, and widespread morbidity and mortality [1].

Significant expansion of the range of biological threats from various pathogens in recent decades is a significant factor that exacerbates the issue of biosecurity. Among the least controlled and most dangerous threats today, the vast majority of experts name bioterrorism and biological warfare. In particular, we are talking about the creation of the latest types of biological weapons, since developments in the field of microbiology are also used for anti-humane purposes. Bioterrorism can be defined as a type of terrorism based on the use or threat of use of pathogenic biological agents. Currently, the existing sequence of terrorist actions with the use of infectious disease agents and bacterial toxins actualizes the problem of biological security, which has acquired a global character and does not depend on state borders. Terrorism, like viruses, is everywhere.

Terrorism has infiltrated everywhere, it follows the system of domination like a shadow, always ready to emerge from the shadows like a double agent. There is no longer a line of demarcation that would make it possible to mark it, terrorism is at the very heart of the culture that fights it, and the visible gap (and hatred) that globally separates the exploited and weakly accused countries from the Western world, secretly from is connected with an internal fault in the dominant system [2].

However, the use of pathogenic biological agents even within the borders of one state can lead to their rapid spread throughout the world (for example, such exotic diseases as Marburg fever, Ebola or various parasitic diseases). All this led to the need to make decisions of a political, economic and philosophical nature, which include the strengthening of international control over work with particularly dangerous pathogens, as well as the development of specific programs to combat bioterrorism.

After all, under modern conditions, humanity is forced to exist, taking into account new technological possibilities, and to find a compromise when solving the problems that arise. All these questions actualize the need for socio-philosophical understanding.

CHAPTER 1. BIOLOGICAL PATHOGENIC AGENTS IN HUMAN HISTORY

1.2. Biological weapons in prehistoric times

Biological pathogenic agents were presumably used by humans as far back as prehistoric times, although this is difficult to confirm or refute. Archaeologists, using modern research methods, believe that poisons were widely employed for fishing, hunting, and warfare in nomadic and primitive tribal societies. Poisons were often obtained from easily accessible plants or animals. Such practices were known among tribes in North America, South America, Sub-Saharan Africa, and Southeast Asia [1-4].

The methods used were highly varied. Some were relatively simple. For example, Melanesian tribes in the territory of present-day Vanuatu would dip arrowheads into the contents of crab burrows, which were contaminated with *Clostridium tetani*, the bacteria responsible for tetanus. Other methods were more complex. The Scythians, in the 7th-3rd centuries BC, used vipers and human blood to produce poison for their arrows [4]. They allowed these components to decompose, and the resulting mixture was applied to arrowheads as a deadly toxin. Some isolated tribes continued to use similar methods even in modern times. In the 14th century BC, the Hittite army sent their enemies sheep infected with tularemia. During the Trojan War (6th century BC), Scythian archers infected their arrows by dipping them into decaying corpses, likely contaminating them with pathogens such as *Clostridium perfringens* and *Clostridium tetani.* Thus, the arrows were likely contaminated with *Clostridium perfringens* and *Clostridium tetani* pathogens.

During the time of Solon (the 6th century BC), in the First Sacred War in Greece, there is an account of the use of non-lethal toxins derived from the hellebore plant to poison the water supply of the city of Kirrha. The leader of this attack was the tyrant Cleisthenes of Sicyon. What happened afterward remains a

matter of debate. The earliest and likely most reliable account comes from the physician and writer Thessalus, who described how the attackers discovered a secret pipeline leading into the city and damaged it. Asclepiades of Nebros advised them to poison the water with hellebore, which soon caused such severe diarrhea among the defenders that they were unable to resist the attack. Asclepiades of Nebros was considered to be an ancestor of Hippocrates, which has led some to wonder whether the guilt of his ancestor's use of poison influenced Hippocrates' decision to establish the Hippocratic Oath [5, 6]. Later historians, such as Frontinus and Polyaenus in the 1st century AD, recounted slightly altered versions of the incident, but the core details align with Thessalus' account. The last major historian to offer a new version of the siege was Pausanias in the 2nd century AD, who attributed the idea and execution of the water poisoning to Solon.

In the 3rd century BC, Carthaginian general Hannibal placed poisonous snakes in clay pots and hurled them into cities and fortresses occupied by enemies.

Other episodes involving the use of biological pathogens in prehistoric times have more historical and archaeological evidence [7-10].

1.2. Biological weapons as a weapon of terror in today's world.

In 1155 Emperor Barbarossa used biological warfare by contaminating wells with human corpses during the war in Tortona, Italy.

During the Hundred Years' War in 1340, Jean, Duke of Normandy, ordered to throw bodies of dead horses over the walls of the besieged Thun-l'Évêque castle, which was held by the English.

One of the most documented cases of using biological pathogenic agents as weapons occurred in 1346 during the Mongol siege of the Genoese city of Caffa (now Feodosia, Crimea, Ukraine). This incident is known from a Genoese chronicle written by Gabriele de' Mussi [11]. Although not an eyewitness, de' Mussi wrote shortly after the events and had access to those who were present, making his account quite reliable. He reported that the Mongols, who controlled

the area around Caffa, laid siege to the city in 1345 but were forced to retreat in 1346 when an outbreak of plague struck their ranks. Before lifting the siege, the Mongols catapulted piles of plague-infected corpses into Caffa, which, according to de' Mussi, caused a plague outbreak within the city. Although the Genoese suffered greatly, they did not surrender Caffa. Some survivors fled the city and returned to their homes in Europe. This is how de' Mussi explained the further spread of the plague. His description aligns with modern knowledge of the transmission of *Yersinia pestis*, the bacterium responsible for the plague. However, research suggests that this was just one of the ways the plague spread to Western Europe.

In 1422 the Lithuanian army, during the siege of Karlštejn (Bohemia), catapulted corpses, dung, and garbage into the city.

The Spanish in 1495 reportedly sold wine mixed with the blood of leprosy patients to their French enemies in Naples (Italy).

In the 1500s Pizarro allegedly offered local South American communities clothing contaminated with smallpox.

In 1650 the Polish army reportedly used “fiery saliva” from rabid dogs against their enemies.

In 1518 the Spanish conquistador Hernán Cortés infected the Aztecs with smallpox. The local population, having no immunity to the disease, was reduced by about half.

In 1710 during the Russo-Swedish War, Russian forces used the bodies of plague victims to trigger an epidemic in the enemy's camp.

In 1767 Sir Jeffrey Amherst, a British general, gave blankets contaminated with smallpox to Native Americans who had supported the French, England's enemies. The resulting epidemic weakened the Native population, allowing Amherst to secure victory in the war.

In 1797 Napoleon flooded the plains around Mantua, Italy, to exacerbate the spread of malaria.

During the American Civil War in 1863, Confederate forces reportedly sold clothing from yellow fever and smallpox victims to Union troops.

In 1915 during World War I, both France and Germany infected horses and cattle with anthrax and glanders, then sent them across enemy lines.

In the 1930s and 1940s Japan conducted extensive biological weapons experiments in China.

Although biological weapons were not officially used during World War II, the Nuremberg Trials revealed that Germany had prepared for large-scale bacteriological warfare. Experiments continued until the war's final days in many German concentration camps, where prisoners were infected with diseases like typhus, anthrax, malaria, dysentery, tuberculosis, and other infectious diseases.

In 1942 British forces conducted an experiment using anthrax pathogens as a biological weapon on a remote island off the coast of Scotland. The anthrax killed sheep on the island, and the contamination was so severe that 15 years later, the island had to be completely decontaminated by burning it with napalm.

In 1943 large-scale efforts to develop biological weapons began in the UK, the Soviet Union, and other countries. In 1979, there was an anthrax outbreak near Sverdlovsk (now Yekaterinburg), resulting in 64 deaths. It is believed that the cause was an accident at a facility involved in biological weapons production, specifically working with anthrax pathogens.

From the 1960s onwards, approximately 70 crimes and 60 terrorist acts involving biological agents were recorded. The most famous incident occurred in 1984, when members of the Rajneeshee sect used *Salmonella typhimurium* as a non-lethal weapon in restaurant salad bars in Dalles County, Oregon, USA, sickening 751 people.

In 1995, the Aum Shinrikyo cult attempted at least 10 biological attacks using aerosols of dangerous pathogens like anthrax, botulinum toxin, and Ebola virus. Fortunately, all these attempts failed.

In September-October 2001 anthrax spores were used in aerosol form in a series of attacks in the United States.

In February 2009 a group of around 40 terrorists in Algeria accidentally infected themselves with an unknown pathogen while attempting a biological terrorist attack, leading to their deaths.

During the Iran-Iraq War (1980-1988) both sides accused each other of using biological weapons.

In 1998 the United States launched a vaccination program against anthrax for its military personnel.

CHAPTER 2. BIOLOGICAL THREATS. THE EVOLUTION OF THE CONCEPT AND ITS MODERN CONTENT

Natural biohazards and human-induced biohazards are considered in the current structure of biological threats. They can cause pandemics, create persistent infection hotspots, and be used as weapons in wars, terrorist acts or sabotages.

The readiness of the world and each country is determined by the Global Health Security Index [2], which consists of 140 questions organized into six categories, 34 indicators and 85 sub-indicators.

The six global categories are:

- prevention (antimicrobial resistance, zoonoses, biosafety, biosecurity, dual-use research, and the principle of responsible research, immunization);
- detection and reporting (laboratory systems, real-time detection and reporting, epidemiological personnel, data exchange among professionals in human health, animal health, and environmental protection);
- rapid response (emergency preparedness planning, implementation of response plans, emergency operational response, coordination of public health and safety authorities, risk communication, access to communication infrastructure, trade and tourism restrictions);
- health system (capabilities to provide medical care in clinics, hospitals, and local health centers, medical countermeasures and personnel deployment, access to healthcare, communication with healthcare workers during public health emergencies, infection control practices, availability of equipment, ability to test and approve new medical countermeasures);
- compliance with international requirements (implementation of the International Health Regulations [12-13] for reporting and reducing the risk of emergencies, cross-border emergency response agreements threatening human and animal health, international commitments, joint external

evaluation and veterinary services performance, funding, sharing genetic and biological data and samples);

- risks (political and security risks, socio-economic resilience, adequacy of infrastructure, environmental risks, vulnerabilities of the public health system).

The index also includes indicators of countries' capabilities to reduce global catastrophic biological risks (GCBR), which are unprecedented-scale biological risks that can cause serious harm to human civilization at the global level, potentially undermining its long-term potential.

It is also necessary to emphasize the role of the human factor in the formation of biosafety. This is the role of the individual in creating biological threats according to their structure, as well as conscious or unconscious mistakes of specialists in the creation and implementation of biosafety systems in all six global categories.

2.1. Non-combat biological threats

Humanity and pandemics. Throughout its history, humanity has experienced at least 17 known pandemics, which, according to various estimates, have caused between 300 to 500 million deaths [3, 4]. These pandemics had a significant impact on the socio-economic state of society, leading to social upheavals, wars, total terror, and the decline of cities, states, and civilizations. The list of biological pathogenic agents (BPAs) that have been or could potentially become causes of pandemics includes the pathogens of plague, cholera, dengue fever, influenza, typhus, smallpox, measles, tuberculosis, malaria, yellow fever, coronaviruses, and many others.

There are various approaches to classifying dangerous biological agents. Currently, the structure of biohazards includes two major categories: natural biohazards and human-induced biohazards [5].

1. *Natural biohazards* include:

- The emergence of antibiotic-resistant bacterial infections (tuberculosis, pneumonia);
- Natural emergent pathogens linked to the industrial or agricultural development of new areas, where new dangerous BPAs arise (monkeypox, Ebola, Lassa fever);
- The spread of zoonoses, i.e., infected animal populations that transmit diseases to humans through direct contact, food or water;
- Toxins, as products of the life activity of certain BPAs (deoxynivalenol, aflatoxins, ochratoxin);
- Outbreaks of human parasitic infections;
- Invasive alien species (plants, animals and microorganisms).

2. Human-induced or human-related biological risks can further be divided into:
   - Intentionally induced risks, such as the use of harmful biological agents for military, terrorist or economic purposes;
   - Biotechnology risks involving the development of uncontrolled dangerous mutations as an undesirable side effect of traditional crossbreeding, selection, and modern industrial and food biotechnologies.

Pathogenic and safe biological agents. The vast majority of biological agents are harmless, and many of them are beneficial. Non-pathogenic microorganisms play a significant role in natural processes – they are an important link in the exchange of substances in ecosystems, serving as decomposers. They participate in the sulfur, iron, and other element cycles, decompose substances of animal and plant origin, ensure the self-purification of water bodies, enrich the soil with nitrogen, and are used by humans in chemical and medical industries, and also in food production. However, hundreds of species of microorganisms that live in colonies have unfortunately been lost to biodiversity in Ukraine due to a lack of protection, which may negatively impact scientific research, the development of agriculture, forestry, pharmaceuticals and environmental protection [6]. Some properties of non-pathogenic biological agents are used in biotechnology to produce metabolites or enzymes. Non-pathogenic BPAs are also used for disease

control (biocontrol and biofertilizers in agriculture, probiotics), for the restoration of contaminated areas (bioremediation), or in food processes (fermentation). However, even beneficial non-pathogenic agents, when influenced by other components of the technological process or by deliberate human transformation to enhance beneficial properties, can acquire pathogenic properties.

Pathogenic microorganisms, although they represent only a small part of the microbial world, pose a significant threat to the health of humans, animals, and agriculture. They can cause diseases with serious consequences for human populations, as well as economic and environmental impacts.

From a practical perspective, pathogenicity or virulence is the ability of some microorganisms to cause diseases. However, microbiologists recognize that pathogenicity is a form of versatility and specialization that allows certain microorganisms to multiply within a specific host (human, animal, or plant) and damage the host's cells or environment. Such transformed environments can be either beneficial or harmful to humans. For example, the fermentation processes of fungi in baking technologies or the spoilage of petroleum products. Although cellular damage is not always clinically evident, a significant portion of infected hosts show signs of disease or eventually die.

Considering that the infection process is a battle between two organisms, the complete death of all infected organisms would result in the extinction of the biological pathogenic agent itself. Therefore, there are no absolutely lethal BPAs in nature that cause 100 % mortality of all infected organisms. It is also true that there are no absolutely safe BPAs. Changes in the surrounding environment activate adaptive processes, which in turn lead to the acquisition of new properties by BPAs. These new properties can be either beneficial or extremely dangerous for humans.

Opportunistic microorganisms. The outcome of an infection depends on the properties of the pathogen (virulence, invasiveness, toxic or allergenic effects) and the immune state of the host organism. From this perspective, pathogens are divided into two main types: primary pathogens, which cause disease in at least

some healthy individuals, and opportunistic microorganisms, which cause disease only in individuals with weakened immune defenses due to specific conditions.

Regional aspect. The level and nature of biohazards are often related to specific territories or climatic zones characterized by certain endemic infectious diseases. In Ukraine, the most relevant vector-borne and natural focal diseases include tularemia, leptospirosis, arboviral infections, ornithosis, Lyme disease (borreliosis), tick-borne encephalitis, and ehrlichiosis, which have varying levels of prevalence among birds, animals, and humans. Ukraine's geographic, climatic, and flora-fauna characteristics are conducive to the formation of ecological complexes involving various species of birds and animals (as reservoirs of pathogens), as well as a wide range of vectors involved in the transmission of infectious agents.

Ukraine is situated within international transcontinental migration corridors for birds, which facilitate the circulation of West Nile fever (WNF) within the country's territory. The existence of natural WNF hotspots in Ukraine has been confirmed in the North-Western Black Sea region (Crimea, Odesa, Mykolaiv, and Kherson regions), as well as in eastern and western regions. A potential threat in Ukraine also comes from over 13.5 thousand locations where anthrax outbreaks could occur, as there are burial sites for animals that died from the disease [7]. Each year, isolated cases of animal and human infection are recorded in the country [8]. Global microclimate changes and migration processes create risks for the formation of new endemic hotspots.

Laboratory-acquired infection (*LAI*): Biological pathogenic agents are pathogenic microorganisms for humans (bacteria, viruses, chlamydia, rickettsia, protozoa, fungi, mycoplasmas), genetically engineered modified microorganisms, biological toxins, helminths, as well as any objects and materials (including field, clinical, and autopsy specimens) suspected of containing the aforementioned agents [9]. They have been widely cultivated and studied since the end of the last century (isolation of pure cultures).

One of the unfortunate consequences of working with etiological agents is infection with laboratory-associated infections. Such cases have been documented

as early as the 1890s, and their number continues to rise. Laboratory workers are at higher risk of contracting certain agents (e.g., *Mycobacterium tuberculosis*, *Brucella*, hepatitis B virus) compared to the general population. At the same time, laboratories and facilities that work with various biological agents, including microorganisms that can act as pathogens, are considered as zones of the highest biohazard risk – both for individuals and humanity as a whole.

However, human history has been relatively free from large-scale catastrophes at BPA (biological pathogenic agent) sites, unlike chemical or radioactive accidents, which have caused numerous casualties and long-term threats to human health and the environment. However, unlike chemical or radioactive accidents, which have resulted in numerous fatalities and long-term dangers to human health and the environment, the history of humanity has been relatively free from large-scale disasters involving BPAs.

Microbiological laboratories and production facilities are considered as areas of the highest biohazard risk. Infections among individuals working with microorganisms in laboratories have been noted throughout the entire existence of microbiology and are regarded as undeniable evidence of occupational hazards. The first classic case of laboratory-acquired infection among researchers (with typhoid fever) was documented in 1885, and information about it was published in 1915. R. Pike analyzed 3,921 cases of laboratory infections that occurred between 1930 and 1974 in the USA and several European countries. It was found that laboratory infections were caused by more than 160 species of microorganisms, with bacteria being the most common. The mortality rates from laboratory-acquired infections were high, especially for hepatitis B – 71 %, plague – 40 %, cholera – 33 %, yellow fever – 22 %, Rocky Mountain spotted fever – 18 %, and leptospirosis – 15 %. Infections most commonly occurred during accidents involving microorganisms (17.9 %), handling and autopsies of infected laboratory animals (16.9 %), the release of bacterial aerosols during centrifugation or cell destruction (13.6 %), as well as due to unknown reasons (20.0 %). Among the

recently documented cases of laboratory-acquired infections in the literature [13], the following cases have been reported:

- an outbreak of SARS laboratory-acquired infection occurred in China in March-April 2004. The outbreak was caused by unsuccessful or incomplete inactivation of the SARS coronavirus, resulting in 9 cases of infection. During serological analysis of the laboratory staff, three additional cases with serological conversion were detected;
- an outbreak of foot-and-mouth disease occurred in a village southwest of London in August 2007, spreading to several villages in Surrey County. The outbreak was caused by wastewater contamination in a building where inactivated foot-and-mouth vaccine was produced (by the company "Merial"). Subsequently, soil contaminated with the virus was spread to surrounding villages by truck wheels. The damages amounted to tens of millions of pounds, and the export of meat products from the UK was banned for several months;
- in the United States between 2005 and 2007, five cases of cowpox virus were registered in research laboratories. These occurred after syringe splashes during mouse injections. Additionally, there were two cases of brucellosis in clinical laboratories, which arose after handling the pathogen outside of containment boxes, and 21 cases of salmonellosis in a vaccine production laboratory following the spillage of a highly concentrated microorganism suspension;
- in 2012, the United States reported a case of meningococcal meningitis in a research laboratory involving an unvaccinated employee. The individual tragically died two days after the onset of symptoms;
- some of the most dangerous laboratory-acquired infections occurred with the Ebola virus in 2004 at Fort Detrick, Maryland (USA), where a worker was injured by a needle (the worker recovered), and at the State Research Center for Virology and Biotechnology “Vector” (Koltsovo, Russia), where a needle injury resulted in death;

- outbreaks of smallpox were documented in England (1979) and West Germany (1980); tuberculosis in the USA (1980); and Q fever in the USA and West Germany (1982). Most of these cases were associated with the escape of infectious agents from laboratories, leading to infections in individuals not directly involved in experimental research.

Moreover, secondary associated or non-associated infections can arise in affected biosafety levels (BSLs), leading to fatal outcomes or disabilities. The most well-known are HIV-associated infections [14-16].

To mitigate risks associated with safety and accidents, it is essential to develop and strictly adhere to safety protocols for handling hazardous laboratory pathogens and toxins to prevent their accidental release into the environment and unauthorized access to them.

Examples of BSLs commonly used in research or biomedical laboratories include a full range of microorganisms: bacteria, viruses, fungi, protozoa, and multicellular parasites.

Emergencies. A particular category of biological hazards for human populations, animals, and the environment comprises those that can create emergencies.

The research of pathogenic agents and the presence of laboratory-acquired infections (LAIs) have necessitated the classification of human, animal, and plant pathogens according to the risks they pose to humans. Such risk classification allows for the establishment of an appropriate set of safety measures for risk management.

The development of an emergency situation is closely related to a number of characteristics of the biological agent. First and foremost, it considers the ability to infect and cause disease, virulence, severity of illness, availability of preventive measures, and effective treatment options. In other words, it is characterized by the danger the biological agent poses to humans, its ability to spread, and the scale of its impact. The danger to humans can be direct or indirect – through effects on

agriculture, industry, and the environment, which encompass everything that sustains human life and existence.

One of the most useful tools available for assessing microbiological risk is a list of risk groups associated with microbiological agents. It is based on international classification of microorganisms into risk groups that take into account the danger (novelty and pathogenicity) of the biological agent, sources and routes of infection transmission, availability and proven effectiveness of preventive and therapeutic measures, and the presence of relevant host organisms in the environment.

WHO experts have formulated a set of minimum laboratory safety standards compiled in the Laboratory Biosafety Manual. This manual is periodically reviewed, with the latest version published in 2003 [17]. The manual includes definitions of four risk groups based on the relative danger of infection with microorganisms to laboratory workers, the community, livestock, and the environment. While no list of infectious agents is provided, WHO recommends that each country develop its own classification of agents found within that country based on a range of factors.

First and foremost is the pathogenicity of the agent. Important factors include the mode of transmission and the range of host organisms for the biological agent. These can be influenced by existing levels of immunity, host population density and mobility, the presence of relevant combinations of individual factors and circumstances, hygiene standards in society, and the state of the environment.

Equally important for classification and biosafety systems is the presence of effective preventive measures. Such measures may include vaccination or the use of antiserum; sanitary measures, such as food and water hygiene; control of animal water sources or management of arthropods as part of the epidemic process; mobility of people or animals; and the importation of infected animals or animal products.

The availability of effective treatment must also be considered. This includes passive immunization and post-exposure vaccination, antibiotics, and

chemotherapeutic agents, taking into account the possibility of resistant strains emerging.

Experts from the European Federation of Biotechnology, in addition to the four risk classes for laboratory workers and the general population, have introduced a new group called *Group E*. This group encompasses microorganisms that pose a greater threat to the environment than to humans. It includes plant pathogens and certain animal diseases that may not cause significant economic losses (e.g., foot-and-mouth disease viruses, Ralstonia solanacearum) and thereby indirectly create a danger to humans.

The European Federation of Biotechnology has created classification tables by analyzing various existing lists of pathogenic microorganisms in humans, including those from the European Committee for Standardization and the American Biological Safety Association [17, 18]. Due to the variability of agent characteristics, working conditions (diagnostics, research, or production), host models and systems, and other factors, it is recommended to use these classification tables only as a guideline for comparing relative levels of danger posed by agents.

Like WHO experts, biotechnology specialists believe that simply referring to the four risk groups for each specific case is insufficient for conducting risk assessments. They assert that the following factors should be taken into account as appropriate:

- pathogenicity of the agent and infectious dose;
- potential consequences of infection;
- natural routes of infection transmission;
- other routes of infection caused by laboratory manipulations (parenteral, airborne droplet, foodborne);
- stability of the agent in the environment;
- concentration of the agent and volume of materials to be used in the work;
- presence of an appropriate “host” for the agent (human, animal, or plant);

- available information obtained from animal studies, reports of laboratory infections, or clinical reports;
- planned laboratory activities (ultrasound treatment, aerosolization, centrifugation, etc.);
- any genetic manipulations with the organism that may broaden the range of “hosts” for the agent or alter the agent's sensitivity to known and effective treatment regimens;
- availability of effective preventive and therapeutic intervention measures on site.

Based on the information gathered during the risk assessment, the necessary biosafety level for the planned work is assigned, appropriate personal protective equipment is selected, and a standard operating procedure (SOP) is developed, including other intervention measures aimed at ensuring the safest conduct of the work.

From the above, it can be asserted that the range of dangerous biological agents and biological agents that may be used as biological weapons or means of bioterrorism is continually expanding [19, 20].

On one hand, this is related to their constant presence and evolutionary connections; on the other hand, it is linked to human development as a species. Bio-threats are changing and often trigger emergencies with unpredictable developments. Humanity and biosafety and biosecurity systems regularly face emerging (new) and re-emerging (known agents that have acquired new epidemic potential) pathogens that create emergencies on both regional and global scales [21]. These include, in particular, avian influenza viruses A(H5N1) (1997), A(H9N2) (1999), A(H7N7) (2003), A(H7N3) (2004), A(H7N9), A(H10N8) (2013), the pandemic influenza virus A(H1N1)pdm09 (2009), coronaviruses (SARS virus, 2002; Middle East respiratory syndrome MERS-CoV, 2012; COVID-19), etc.

The emergence of each new pathogen is considered a global emergency, as the consequences of the functioning of this new parasitic system for human health and global stability are unknown. Equally concerning are the infectious agents that

have long been known but previously did not possess such epidemic potential (e.g., enterovirus type 71, Ebola virus) [22] and / or have severe courses with the emergence of new clinical forms, increased lethality, or subsequent disability (e.g., enterovirus type 68). This is associated with changes in microorganisms during their evolution in close interaction with humans, either directly or indirectly through domestic or other animals that humans have constant or regular contact with. The emergence of both new and re-emerging biological agents can be triggered by natural and anthropogenic factors. Global warming and glacial melting annually present humanity with new-old forms of dangerous biological agents that it has not encountered for tens or hundreds of thousands of years. Equally significant threats arise from the exploration of new territories, which were previously closed ecosystems with limited circulation of dangerous pathogens, leading to the breaking of interspecies barriers [23, 24].

A range of bio-threats are human-induced, so the emergence of emergencies, epidemics, and pandemics in some cases is directly caused by the actions of specific individuals or groups of people, whether unintentionally or deliberately (e.g., genetic engineering and genetically modified organisms, biological and chemical weapons, bioterrorism, laboratory incidents, and leaks of biological agents).

Similar negative consequences and impacts, in terms of biosafety in general and the development of the epidemic situation in particular, are influenced by the transmission of infections. Globalization, urbanization, tourism, and the mobility of the workforce, along with advancements in transportation technologies, facilitate the rapid international spread of infections. For instance, air travel accounts for over 2 billion passengers per year, covering all continents [25-28].

Another concerning trend in biosafety issues is the formation of so-called hospital strains of infections resistant to traditional medications.

An important element of biosafety and biosecurity against known, emerging, and re-emerging dangerous biological agents is the ability to detect and identify them in a timely manner.

Despite the complexity and interdisciplinarity of the structure and functioning of the biosafety and biosecurity system, a constant element throughout the entire technological chain – from the emergence of biological threats to the identification of biological agents, and ultimately to treatment and elimination of biological threats – remains the tools and technologies of biomedical engineering.

2.2. Biological Weapons

Biological weapons are a type of weapon of mass destruction. They can cause mass harm to humans, agricultural animals, and plants in a short time over large areas. **Biological weapons** are one of the most terrifying and least predictable military inventions. Although there have been relatively few attempts to use them for purely military purposes, they rank among the significant threats to international stability in the 21st century.

Biological weapons are economically the cheapest type of weapon with the highest impact-to-cost ratio. For this reason, they are often referred to as the "weapon of poor dictators" and terrorists. Despite the fact that attempts to use biological weapons in modern wars have been very limited, this does not reduce the potential danger of their use. Currently, experts believe that 13 to 20 countries around the world possess the knowledge, technologies, and certain stockpiles of biological weapons. In some countries, this is a necessary component of developing defense measures against biological weapons, while in others, it serves as a tool for blackmail or deterrence.

Biological weapons are designed to target military personnel, civilians, agricultural animals, plants, and logistical resources (such as drinking water supplies, fuels, etc.) of the adversary to gain military or economic advantage.

The use of biological agents as weapons has a unique nature, characterized by the ability to spread harm uncontrollably beyond the application site. An artificial epidemic can affect both "enemies" and "friends". Therefore, a necessary

element of a military system using such weapons is the presence of protective measures for military personnel, the population, and the economy.

Biological weapons consist of specialized munitions and combat devices equipped with biological pathogenic agents (pathogenic microorganisms, toxins, herbicides, infected arthropods) and means of delivery.

Biological weapons (BW) are a three-component system:

BW = BPA + ZD + BBp + ZIZ + ZSP

BPA = BR = BPA + St

Where:

- BPA = Biological Pathogenic Agents (BPA) + Stabilizer (St)
- BPA = Specially selected biological pathogenic agents stored and used in the form of a biological recipe (BR).
- ZD = Means of delivery (aircraft, missiles, artillery).
- BBp = Biological munitions, which consist of a container with biological recipe + a device that creates and disperses biological aerosol (generator). The aerosol can be ground-based or airborne, as well as linear or multi-point.
- ZIZ = Individual protective equipment.
- ZSP = Specific preventive measures.
- St = Stabilizer, a special preserving agent or nutrient medium.

Key Factors and Features of Biological Weapon Effects:

- the ability to cause mass damage (disease and intoxication) with subsequent escalation in the area of application;
- the ability for spontaneous progressive spread over large territories beyond the area of application;
- the duration of damages and secondary damages, formation of resistant and endemic foci;
- the possibility of concealed application; BCA have no visible or organoleptic signs of use;
- complex specific indication of BCA;

- selectivity of action and variety of damages;
- complicated protection;
- strong psychogenic impact that can cause panic (uncontrolled, irrational, or harmful actions);
- high contagiousness and infectivity;
- the ability to penetrate non-hermetic spaces;
- dependence of combat effectiveness on meteorological conditions;
- the danger of retroactivity.

Technical Requirements for Biological Pathogenic Agents:

- *High Virulence and Contagiousness*: must be capable of causing severe disease and spreading easily;
- *Controlled Resistance to Environmental Conditions*: should remain effective under various conditions;
- *Controlled Incubation Period:* ability to regulate the time from exposure to disease onset;
- *Suitability for Use in Munitions:* capability to form aerosols and remain stable in munitions over time.

Main methods of using biological weapons:

- *Contamination of Ground Air Layers with BPAs*: aerosolizing BPAs to infect the air.
- *Aerosol Spread of Infected Vectors*: dispersing infected organisms or carriers.
- *Sabotage Method*: deliberate hidden contamination using sabotage materials at locations frequented by people, food supplies, water sources, etc.

Known biological warfare agents that have been researched as biological weapons and were at various stages of readiness for use: plague, anthrax, tularemia, smallpox, hemorrhagic fevers. Classification of Biological Pathogenic Agents by Purpose: biological pathogenic agents that can be used as biological weapons may have the following classifications based on tactical and technical characteristics.

Classification of Biological Pathogenic Agents.

1. **By incubation period and maximum number of affected individuals**:

- fast-acting (within the first day): botulism, plant and animal toxins;
- delayed action (2-5 days): anthrax, plague, yellow fever;
- postponed action (more than 5 days): smallpox, typhus, brucellosis.

2. **By severity of effects**:

- predominantly lethal effects: plague, anthrax, botulism;
- predominantly non-lethal effects with temporary loss of capacity: tularemia, brucellosis, glanders, Q fever.

3. **By contagiousness**:

- highly contagious: plague, smallpox;
- contagious under certain conditions or with vectors: yellow fever, typhus;
- non-contagious: all zoonoses.

4. **By stability in the external environment**:

- low stability: up to 3 hours (plague, botulism, yellow fever);
- relatively stable: from 3 to 24 hours (smallpox, brucellosis, tularemia);
- high stability: over 24 hours (anthrax, Q fever).

5. **By purpose**:

- for affecting only humans: *Plague bacteria*, *Smallpox viruses*, *Hemorrhagic fever viruses*, *Japanese encephalitis viruses*, and others;
- for affecting only agricultural animals: *Bovine plague*, *African swine fever*, *Avian plague*, *Sheep pox*, and others;
- for affecting both humans and agricultural animals: *Anthrax bacteria*, *Toxoplasmosis, Tularemia, Brucellosis, Botulinum* toxin, *Staphylococcal* toxin;
- for damaging agricultural crops: potato late blight, rice blast, rusts of cereal grains, gummosis of sugarcane, cotton, etc.;
- for damaging protective facilities, communication means, equipment, and other material and technical resources: fungi of the genus *Aspergillus* and bacteria of the genus *Methylobacterium* for damaging electrical and radio

insulation, radio-electronic equipment; fungi of the genus *Cladosporium*, *Penicillium, Mucor*, and the proteobacteria *Pseudomonas* for damaging fuel and lubricants; iron bacteria and sulfur bacteria for accelerating the corrosion of metals and alloys in military and other equipment.

2.3. Bioterrorism, biological diversion and criminal use of biological pathogenic agents

Subjects of terrorist activity, trying to resist means struggle by society and law enforcement agencies of various states, improve their methods of illegal activity and are constantly searching other technological means of criminal influence on society. As a result, what, at the beginning of the 21st century, on the positive development of social and political situation in the world, the negative impact of terrorism is constantly increasing activity or the threat of its use by terrorists advanced scientific (chemical, biological, bacteriological) technologies when anonymously carrying out terrorist activities (without making any demands by any persons). Political and legal opposition to similar scenarios of the development of terrorist activity and minimization by application weapons of mass destruction in the world, should be formed on the basis of detailed study of social confrontations in the world and the prerequisites, essence and consequences high-tech terrorist activity.

In many countries, diversionary activities are viewed as a distinct form of armed confrontation during wartime and in the interwar period. During wartime, diversionary actions on enemy territory or behind their lines are aimed at disrupting their activities on the front, disorganizing command, disrupting weapon supply, and creating panic among troops. Diversions are carried out by specially trained sabotage and reconnaissance groups (SRGs) or individual saboteurs. Diversion is one of the main elements of guerrilla warfare strategy.

The primary distinction between diversion and a terrorist act is that the preparation for diversions is backed by the entire scientific, technical, and military-

industrial capability of the state. In contrast, a terrorist act is typically carried out by an illegal group with limited capabilities that is forced to operate secretly.

According to Article 113 of the Criminal Code of Ukraine, diversion is defined as actions aimed at weakening the state, such as explosions, arson, or other acts intended for the mass destruction of people, causing bodily harm or other damage to their health, or for the destruction or damage of objects that have significant economic or defense importance, as well as actions intended for radioactive contamination, mass poisoning, or the spread of epidemics, epizootics, or epiphytotics [14].

Biological diversion is carried out covertly during its execution, and the fact of its conduct and the perpetrators are subsequently concealed. The scale of such criminal acts sharply increases in the pre-war and wartime periods. The very fact of biological diversions constitutes a violation of international law, and states accused of such actions by the international community deny these allegations and meticulously conceal any evidence of their involvement.

Thus, the most dangerous type of modern technological terrorism is precisely biological terrorism, the essence of which is application biological weapons, which act as microorganisms or poisons substances. The danger of this type of terrorism lies in its absence control of the spread, the impossibility of a quick termination of terrorist activity act and elimination of consequences, as well as in a large number of human and material sacrifices. It is also worth noting that bioterrorism directly encroaches on security objects, creating real threats to them. To such objects include: a person, society and the state.

The national legislation of each democratic country and Ukraine in including puts a person, his rights and interests in the first place, i.e defines a person as the main object of security.

Analyzing the security of a specific person, it should be combined with the issue of other people's safety. Since a person belongs to society, there is part of it and is directly interdependent with it. Society's security does not exist by itself, separated from human security relationships, social life, state or international

relations. It is closely related to all components of social life that affect existence and self-preservation of each person, in particular, and society in as a whole That is why, in the case of neglecting the state of society, it is impossible full security of the person belonging to it is issued society.

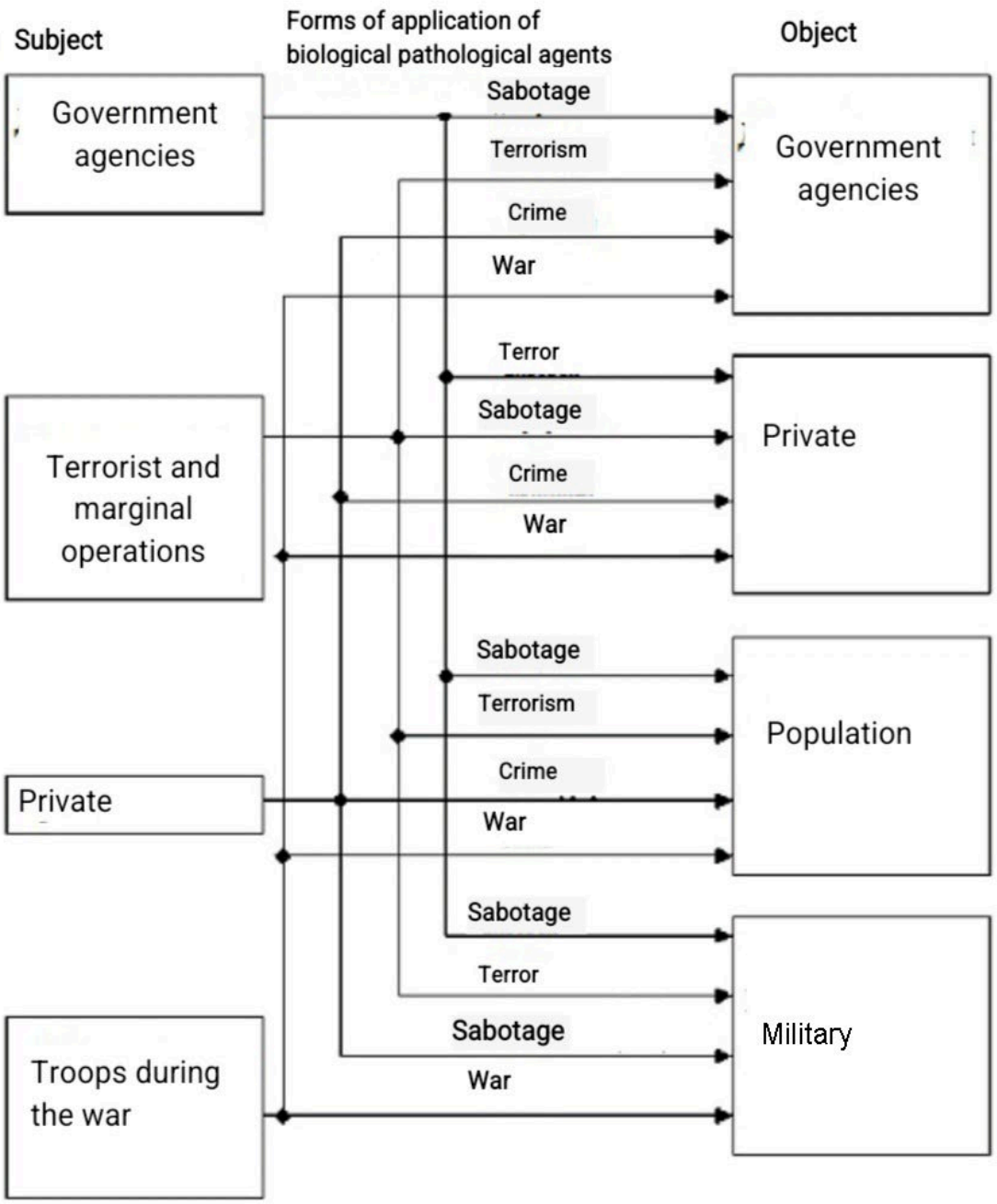

Fig. 2.1. Forms of application of biological weapons

2.4. Binary Biological Weapons

The JASON group, consisting of specialists in bio-threats and biotechnology, which may be used in the development of biological warfare agents, has identified six classes of genetically engineered pathogens that could pose a serious threat to society. These include, but are not limited to, binary biological weapons, genetically modified biological pathogenic agents (BPA), gene therapy as a weapon, stealth viruses, pathogens with altered natural reservoirs (Host Swapping Diseases), genetically created BPA, and personalized BPA. Some of these genetically engineered pathogens, according to historical records, have already been produced and stockpiled. Each of the seven can be created, has already been created, or may even be used as a weapon.

Binary Biological Weapons. This type of biological weapon consists of a two-component system with independent elements that can be safely used separately, but when combined or simultaneously introduced into an organism, they create a lethal combination. This may include a primary and auxiliary virus or plasmids of bacterial virulence. An example of such a viral binary system is the hepatitis D virus and its helper, the hepatitis B virus. From the perspective of binary effect, the hepatitis D virus must infect cells simultaneously with the unrelated hepatitis B virus. Both viruses are primarily transmitted through sexual contact, damaged coverings, or blood. The D virus exploits proteins expressed by the larger B virus, significantly increasing the severity of the disease caused by hepatitis B. Hepatitis D infection is not possible on its own. Examples of bacterial virulence plasmids include plague (*Yersinia pestis*), anthrax (*Bacillus anthracis*), dysentery (*Shigella dysenteriae*), and *E. coli* (*Escherichia coli*) [15].

Binary biological weapons already exist. The process of creating this potential biological weapon was revealed by a former defector from the Soviet Union. In 1992, a defector codenamed “Fortune Temple” described his experience working with binary biological weapons. He indicated that the former Soviet Union [29] secretly continued research on a “new and improved super-plague”

(*Yersinia pestis*) despite President Yeltsin's order to halt their offensive biological warfare program. The defector explained that the super-plague “will not only be more resistant to many antibiotics but will also be produced using a special new process. In its initial form, the plague will not be virulent, so it will be safe to handle and store. Russian scientists have found a way to turn this non-toxic plague back into a lethal, antibiotic-resistant form as soon as it is needed for use as a weapon” [29]. Due to its properties and ability to be stored in large quantities for extended periods without causing harm, it is believed that Russia still retains this biological weapon.

It can also be argued that countries with the equipment, materials, resources, and knowledge can very easily produce these genetically engineered pathogens. Binary biological weapons are good candidates for future use because of their benign properties, allowing for easy storage and handling. Since the components are not separately dangerous or hazardous, they can be easily transported, requiring fewer signatures for manufacturers. This also complicates tracking.

Future Applications. The processes for binary biological weapons are already known and will remain relevant. In the wrong hands, biological weapons pose a dangerous threat.

Genetically Modified Pathogens. The successful completion of the Human Genome Project paved the way for understanding the nature and content of complex genetic information that could be used to create new biological forms of life. According to the European Bioinformatics Institute, as of December 2021, scientists have sequenced the genomes of approximately tens of thousands of viruses, plasmids, and bacteria. Information about some of them is available online to both specialists and non-specialists.

To date, around 599 viruses, 205 plasmids found in nature, 31 bacteria, 1 fungus, 2 animals, and 1 genomic sequence from plants are known [21, 31]. This vast amount of information about human genomes can expand life forms using synthetic genes, synthetic viruses, and synthetic organisms [22, 32]. Designer genes have become one of the greatest breakthroughs in biotechnology.

Using a technique called recombinant DNA technology (gene splicing), a single gene is inserted into an organism to alter its genetic properties. This technique involves the insertion of plasmids, which are small fragments of bacterial DNA, into the DNA of other bacteria to enhance the virulence or other pathogenic properties of the host bacteria.

An example is gene splicing for the production of insulin for diabetics. The genes responsible for insulin production are combined with plasmid DNA, which can then infect bacteria. The infected bacteria then multiply, resulting in a large quantity of insulin for therapeutic purposes. Despite the advantages of this biotechnology, one must not underestimate the dangers, as genes can be programmed for infectious states that can easily be transformed into biological weapons.

As biotechnology advances and technologies improve, scientists are exploring complex genetic information to enhance human life and possibly create new forms of organisms. Another method of gene therapy is DNA shuffling.

DNA shuffling – also known as multi-gene shuffling, gene mixing, and directed molecular evolution *in vitr*o – has significantly increased the efficiency with which a wide variety of genetic sequences can be obtained. A quantum leap in the ability to generate new DNA sequences can be used to create large DNA libraries, which can then be screened or selected for a range of desirable traits, such as improved protein function and / or increased protein production [26].

Using this method, there has been an observed increase in the production of antibiotics produced by bacteria. This biotechnology undoubtedly offers great opportunities for medical purposes, but it can also have a significant impact on the production of genetically engineered pathogens that are resistant to drugs or vaccines, and can enhance virulence, making them well-suited for biological warfare.

The state of biological weapons: designer genes as the most lethal form of future biological warfare. Designer genes may become the deadliest form of biological weapons in the future. Countries interested in developing lethal weapons

can openly use genomic sequence databases to select the genes they wish to create. One assessment noted: “The ever-expanding microbial genome databases now contain a list of all potential genes involved in pathogenicity and virulence, adhesion and colonization of host cells, immune evasion, and antibiotic resistance, from which the most lethal combinations can be selected” [25]. With such vast information available, it would be possible to create diseases using synthetic viruses capable of wiping out entire populations.

Imagine the use of synthetic viruses to recreate the 1918 Spanish flu pandemic, which killed 20 million people – the worst in history [26]. Scientific and technological advances in genetic engineering of pathogens have already changed the outlook on the future of biological weapons and their threats. In October 2004, the strain of the 1918 Spanish flu was partially reconstructed by researchers from the University of Wisconsin using reverse engineering methods. The influenza A virus was fully sequenced and characterized the following year. Experts predicted that “while the knowledge, means, and ingenuity to carry out such experiments are not yet within the grasp of most non-experts, this situation is likely to change within the next 5-10 years” [27].

Although this experiment was conducted to prevent the reemergence of a devastating influenza pandemic [28], in the wrong hands, it presents potential offensive capabilities for biological warfare.

Future Applications. This is the type of biological weapon to watch out for in the next 25 years. This technology is highly complex, and only countries or groups with biotechnological capabilities will be able to develop these genetically engineered pathogens. Advancements will continue to grow as the scientific world finds new and innovative ways to manipulate human genetics.

Gene Therapy as a Weapon. Gene therapy involves repairing or replacing an organism’s gene, permanently altering its genetic makeup. By replacing existing genes with harmful ones, this technique could be used to produce biological weapons. Gene therapy might be the silver bullet for treating human genetic diseases. This process involves replacing a faulty gene with a good one to

normalize the recipient's condition. A vector is needed to transfer the "healthy" gene to its target. Typically, "viruses that have been genetically modified to carry normal human DNA" are used, such as "retroviruses, adenoviruses, adeno-associated viruses, and herpes simplex viruses".

There are two classes of gene therapy: germline (reproductive) and somatic (therapeutic). DNA changes in a germline cell allow it to correct faulty genes, enabling the new solution to be passed on to future generations. Somatic cell gene therapy is different in that it only affects the individual receiving the treatment.

Biological Weapon Status. Gene therapy has already been used in both animal research and human clinical trials. Numerous successful applications of gene therapy have been published showing promising results. For example, the University of Michigan and Kansai Medical University in Japan reported that "they used gene therapy to restore hearing in adult deaf animals" [31]. According to the research, "gene therapy can be used to regenerate functional hair cells required for hearing restoration by delivering a 'healthy' gene via an adenovector into nonsensory cells found in the deaf cochlea... and significant hearing improvement was observed after birth". Another example of this technology involved replacing a mutant gene that disrupts the production of an enzyme called "adenosine deaminase (ADA)" [33]. Blood was drawn, treated, and reintroduced into the human system. According to the report, this therapy was relatively successful, although other cases using gene therapy have been less successful.

Despite the significant progress in gene therapy, there are still more questions to answer and techniques to refine before this therapy becomes an effective treatment for many diseases.

Another significant result of gene therapy was the experiment with the mousepox virus in Australia. Researchers accidentally developed a deadly strain of the mousepox virus while attempting to prevent a plague in the mouse population. This genetically modified virus attacked the immune system of the test mice, killing all of them. Researchers also found that sixty percent of previously vaccinated mice died within days of infection [34]. Although this was

unintentionally created, if the same modified virus were added to smallpox, it could be just as lethal to humans.

Future Applications: It is expected that gene therapy will become more popular. It will continue to improve and could undoubtedly be chosen as a biological weapon. The rapid growth of biotechnology may create more opportunities to find new ways to combat diseases or create new ones. Countries willing to work with biotechnology are likely to consider gene therapy a viable biological weapon. Groups or individuals without resources or funding will find it challenging to create such biological weapons.

Stealth Viruses or Stealth Infections. Stealth viruses are viral infections that infiltrate cells and remain dormant for extended periods until triggered by an external factor to cause disease. In the context of warfare, these viruses could spread to large populations, and their activation could be delayed or used as a threat for blackmail. The core concept of this potential biological weapon is to "produce a tightly regulated, enigmatic viral infection that can penetrate and spread within human cells using vectors" (similar to gene therapy) and then remain inactive for a certain period until activated by an internal or external signal. This signal can then prompt the virus to inflict severe damage on the system. Stealth viruses could also be adapted for covertly infecting a target population over a prolonged period, using the threat of activation for coercion.

Status of Biological Weapons. Stealth viruses, like gene therapy, require a vector to be introduced into the body and remain dormant until a trigger mechanism is activated internally or externally. Imagine a virus that causes cancer infiltrating a human cell and lying dormant until an external signal prompts the disease. When the signal activates, the cells become abnormal and can rapidly generate atypical cell growth, leading to tumors and ultimately death. Now apply this concept to a population where an HIV virus spreads among the target demographic. At a specific time chosen by the perpetrator, the signal triggers harm to the entire population simultaneously. Although this bioweapon is futuristic, it is not inconceivable and warrants further study.

Future Applications. Stealth viruses could become a potential biological weapon by 2035. Much remains to be learned about the timing of the trigger mechanism to make this a feasible biological weapon. However, with the rapid advancement of biotechnology, countries capable of conducting research and development can undoubtedly achieve such levels of knowledge. It is unlikely that groups or individuals will possess this biological weapon.

Host swapping pathogens (host swapping diseases). Similar to natural viruses like West Nile and Ebola, animal viruses could potentially be genetically modified and engineered to infect humans as a powerful tactic in biological warfare [2].

State of biological weapons: stealth viruses, like gene therapy, require the introduction of a vector into the body and remain dormant until a trigger mechanism is activated internally or externally. Imagine a cancer-causing virus entering a human cell and lying dormant until an external signal provokes the disease. Once the signal is triggered, the cells become abnormal, quickly generating abnormal cell growth, leading to a tumor and eventually death. Now apply this concept to a population where the HIV virus is spread among a target population. At a specific time chosen by the malicious actor, the signal is activated, causing harm to the entire population simultaneously. While this type of bioweapon may seem futuristic, it is not implausible and deserves further study.

Future Applications. Stealth viruses could become a potential biological weapon by 2035. Much more needs to be learned about the timing of the trigger mechanism to make this a viable bioweapon. However, with the rapid growth of biotechnology, countries with research and development capabilities can certainly reach such a level of knowledge. It is unlikely that groups or individuals would possess such a biological weapon.

Pathogens with altered natural hosts (host swapping diseases). Similar to natural viruses like West Nile and Ebola, animal viruses could potentially be genetically modified and engineered to infect humans as a powerful biological warfare tactic.

Host-Swapping Diseases. Most viruses do not cause diseases and are generally considered parasites. They exist in evolutionary “equilibrium” with their hosts, but if that “balance” is disturbed, one of two things can happen: viruses become either virulent or benign. The “balance” is disturbed when a virus escapes its host's reservoir and crosses into another species, where it can generate a different virus by mutating or accidentally picking up other genes. Animal viruses typically reside in natural conditions within a “reservoir” or specific species of animals and cause little harm to their host. For example, Eastern equine encephalitis uses waterfowl as a reservoir, rodents carry hantavirus, bats host the Ebola virus, and chimpanzees harbor the AIDS virus. When these viruses leave their natural host reservoirs, they can produce extremely lethal pathogens.

State of Biological Weapons. Host-swapping diseases already represent a new threat in biological warfare. The Centers for Disease Control and Prevention (CDC) classify them as a Category A agent, meaning they are of high priority.

Future Applications. It can be argued that host-swapping diseases already exist as biological weapons. Nations, groups, and even individuals could potentially have relatively easy access to these types of biological weapons. With the rapid development of biotechnology and its dual-use nature, these genetically engineered pathogens could be devastating to populations.

Designer biological pathogenic agents (designer diseases). With access to complete genomes and advancements in gene synthesis, scientists will soon be able to design pathogens by creating synthetic genes, synthetic viruses, and perhaps entirely new organisms. Biotechnology can be used to manipulate cellular mechanisms to induce diseases. For example, an agent could be designed to cause cells to proliferate uncontrollably, as in cancer, or to initiate apoptosis, programmed cell death.

The understanding of cellular and molecular biology has progressed to a point where it's conceptually possible to design a disease first and then create a pathogen to achieve the desired effect. These designer diseases could work by attacking the immune system to impair the body's natural disease-fighting abilities

(similar to how HIV causes AIDS) or by reactivating dormant genes to induce cellular destruction (as in the spread of cancer). Alternatively, they could instruct cells to commit suicide, leading to cell death (apoptosis). While apoptosis can be useful for treating diseases like cancer, it could also be exploited to activate "death pathways" that kill all cells simultaneously.

State of Biological Weapons. Designer diseases are undoubtedly a futuristic form of biological weapons, but they are by no means inconceivable. Imagine developing a disease that could wipe out an entire population or a specific ethnic group. This type of biological weapon requires further research and investigation to fully understand its nature, properties, and potential harm.

Future Applications. Designer diseases could be a viable candidate as a potential biological weapon by 2035. This type of weapon deserves further evaluation for future studies. Countries with the resources and capabilities to conduct research and development could certainly acquire the knowledge to make this biological weapon a reality. It is unlikely that groups or individuals will possess this biological weapon.

Personalized biological pathogen agents. In the coming years, it is conceivable to create a pathogen that targets the genome of a specific individual. This agent could spread through populations with minimal or no symptoms, but it would be fatal to the intended target.

2.5. Biohazards and the Human Factor

The majority of scientists advocate for peace and work to prevent their research from being used for war and terror [1]. However, none of the 20th-century biological weapons programs were carried out without an active involvement of biologists, biotechnologists, and medical professionals. This raises concerns and underscores the need for safeguards to prevent scientists from participating in the development of biological weapons.

At certain times, various political and military justifications were developed, prompting the involvement of hundreds or even thousands of scientists in biological weapons development [2]. It is very difficult to find reliable information on why and how individual scientists made the decision to participate in biological weapons programs.

It must be acknowledged that the extremely fine line between BPA (biological protective agents) research for biodefense and the use of this research for the development of biological weapons allowed program leaders and recruitment agencies to often conceal the true objectives of the research. Often, a scientist would only learn the project's final goal after years of work. Other realities of the time, such as global confrontations, the arms race, propaganda, and manipulations of patriotism, as well as state guarantees of legality, encouraged scientists to participate in projects related to weapons of mass destruction in general, and biological and toxic weapons in particular. One frequent justification for the development of strategic biological weapons was the suspicion that an aggressive enemy had already developed similar weapons.

Today, the development, testing, and stockpiling of biological weapons are prohibited by international law, and all major state-funded programs have been discontinued. However, BPA research continues in many countries around the world.

This creates certain risks that new or perceived threats to national security, dubious patriotism, greed for wealth or other economic motives, career ambitions, the desire to gain scientific fame as a pioneer, personal revenge motives, grievances against society, psychological disorders, or some combination of these motives could convince certain specialists to disregard any moral concerns about BPA and use their knowledge to harm humanity [3].

Often, when signing treaties banning biological weapons, certain formulations were used, that created conditions for their potential violation.

In 1925 the signing of the Geneva Protocol banned the use of both chemical and bacteriological weapons. However, France, as a signatory of the treaty and

already having an active biological weapons program, formally reserved an important exception: the right to possess biological weapons for a mirrored response if attacked first. This exception changed the international norm from a complete prohibition to a “no first use” policy, which later allowed other signatories, including the United Kingdom and the Soviet Union, to justify their offensive biological weapons programs in the name of defense.

Although biological weapons programs had a clearly military nature, political leaders maintained full control over them. In the Soviet Union, around 1925, military doctor Yakov Fishman became the head of the new Soviet biological weapons program, which was part of the modernization of the Soviet army promoted by General Mikhail Tukhachevsky [4]. However, when Soviet leader Joseph Stalin came to power, he grew suspicious of both military officials and medical scientists, and the days of this first Soviet biological weapons program were over.

During the purges of 1937, when Stalin consolidated his power by eliminating all potential opposition, Tukhachevsky was executed, and Fishman was imprisoned along with many other microbiologists in both military and civilian fields.

For most of the interwar period, political leaders in Britain and the United States were not concerned about biological weapons as a threat or military advantage. British medical experts were more focused on protecting civilians from German air raids and wartime deprivation, as well as defense against chemical rather than imagined biological attacks. Meanwhile, the USA maintained its course. As the Senate aggressively lobbied for the Army Chemical Corps and industry, the country failed to ratify the 1925 Geneva Protocol, thereby leaving its chemical capabilities open. At that time, American military experts dismissed the practicality of biological weapons, doubting that microbes with their uncertain effects could compete with conventional explosives – a view that persisted for years later, even when the USA biological weapons program was in full swing.

With the approach of war with Germany, Canadian Nobel laureate Frederick Banting, co-discoverer of insulin, became convinced that those controlling the German army were ruthless enough to develop and use biological weapons, and that Britain should prepare for defense and counterattack. Dismissing objections that airborne microbes were too fragile to be infectious, banting envisioned various ways of disseminating microbes, from spraying them from aircraft to distributing dried pathogens through the mail. In 1939, he advocated for his position in London, promoting a plan for research, development, and use of biological weapons alongside civil defense measures. In 1940, under German air attacks, Maurice Hankey, the former Secretary of the Cabinet of Ministers of Britain, who was impressed by Banting's ideas, he convinced the Minister of Supply to establish a biological weapons program at Porton Down, next to the existing chemical factory.

The project leader, microbiologist Paul Fildes, interpreted his mission as fundamentally offensive and led the development and testing of an effective anthrax bomb. His strategic capabilities caught the attention of Prime Minister Winston Churchill, who, with Britain's survival at stake, was looking for new weapons to defeat Nazi Germany.

Like many of his time-including Churchill-Banting embraced the justification of total war, which legitimized attacks on cities and factories as a means to undermine the enemy's economic structure. He wrote, “In the past, war was largely confined to men in uniform, but with the increasing mechanization of armies and the introduction of air forces, there is a growing reliance on the home country, and eight or ten people working in the Home now need to keep one person at the front line. This situation changes the nature of war. It really means one nation is fighting another. Given this, killing or incapacitating ten unarmed workers at home is just as effective as incapacitating a soldier, and if this can be done with less risk, then it would be advantageous to use any means of warfare to achieve this”.

It is possible that new or imagined threats to national security may persuade biologists to set aside any moral doubts regarding secret science. Banting's definition of total war resonates with the bombing of German cities by the Allies, the development and use of atomic weapons by the USA, and the covert search by British, American, and Canadian forces for strategic biological warfare. By the end of 1942, the USA, then at war, provided its significant resources-scientific and technological expertise, laboratories and production facilities, military officers and troops, testing grounds, and repurposed munitions factories-to what soon became the largest biological warfare project in history. A forward-looking report by Columbia University scientists Theodore Rosebury and Alvin Cabot in 1942 outlined potential "candidates" for pathogens, as well as organizational structures and strategies for civil defense. Ira Baldwin, a fermentation expert from the University of Wisconsin, oversaw the mass production of anthrax spores for bomb filling. Hundreds of other scientists, both civilian and military, participated in the biological warfare research, kept secret like the Manhattan Project. However, World War II ended before any biological weapon, including the anthrax bomb, reached a level competitive with nuclear weapons.

While Britain and the United States were actively seeking biological weapons, it was the Japanese military that first used them. In 1934, military doctor General Shiro Ishii established Japan's secret biological warfare program, which lasted until 1945. Its main base was in the Japanese-occupied Manchuria, near the city of Harbin. Over the years, through his connections with medical schools, Ishii was able to recruit hundreds of researchers by promising them a unique opportunity to conduct experiments with infectious diseases on live human subjects, most of whom were Han Chinese.

Although Ishii and his researchers made history as the first to employ microbial weapons, they failed to achieve the technical sophistication of the British and American wartime programs. Ishii's first major anti-civilian campaign from 1940 to 1942 took place in northern China, where fleas infected with the plague

were spread across port cities and villages. Ishii's second major campaign in 1943 employed anthrax and glanders to attack villages southwest of Shanghai, in retaliation for their assistance to American pilots during the Doolittle Raid on Tokyo in 1942, as well as part of Japanese "scorched earth" policy to prevent Allied forces from using airfields in the area.

As early as 1944, the USA military intelligence had a false impression that the Imperial Japanese Army had developed a premier biological warfare program and that information about it should be concealed from the Soviet Union. While the Allies publicly prosecuted Nazi officials in Nuremberg, Germany, for mass murder and inhumane medical experiments, American officials in Tokyo assured former scientists of the Japanese program immunity from prosecution for war crimes in exchange for information about their biological experiments and attacks. the USA General Douglas MacArthur, responsible for the Tokyo War Crimes Tribunal and the reconstruction of Japan, had sufficient authority to broker this deal, which protected Japanese Emperor Hirohito and various members of his family who were likely aware of the program's details.

This secret agreement on immunity and the long-standing denial by the USA, Britain, and Japan kept the public unaware of the consequences of using biological weapons. Such a lack of awareness prevented people from demanding legitimate limitations on arms control, as was the case in response to the spectacle of nuclear bombs in Hiroshima and Nagasaki, even though about 200,000 Chinese civilians died from microbial attacks. From 1945 to 1948, during the Nuremberg Trials, the atrocities committed by the Nazis were front-page news worldwide, while information about the Japanese biological warfare program was actively concealed. Details of these crimes only emerged years later, too late to curb the clandestine proliferation of biological weapons.

One of the courageous voices against biological weapons during this early postwar period was Theodore Rosebury, who was the head of Camp Detrick, the research center for the American program in Maryland. Rosebury left Detrick in 1945, during a period of relative openness that allowed scientists to publish the

results of their wartime defense research regarding, for example, vaccines for poultry and cattle plague, post-exposure therapies for anthrax, tularemia, and glanders, isolation of pure bacterial toxins (botulinum toxin), and airborne plant diseases.

Nonetheless, the public remained uninformed about the offensive advancements of the USA, such as large-scale production facilities for anthrax, brucellosis, and agents against rice and wheat, as well as the development, production, and testing of biological bombs, including a new cluster bomb. In 1949, Rosebury published a book titled Peace or Plague, explaining why, for the sake of humanity, world powers should abandon biological weapons. By the time his book was released, publications about the American program were becoming increasingly limited, and members of Congress and the press exaggerated the imminent threat of Soviet biological weapons, based on poorly assessed intelligence data. Backed by these claims, American program scientists at the onset of the Cold War sought to make biological weapons competitive with atomic bombs, primarily targeting Soviet cities.

Starting in the 1960s and continuing through the Vietnam War, program scientists had greater freedom to plan biological attacks on nearly any terrain or population, whether rural or urban. During these years, countless biologists and physicians covertly used their skills for military purposes, practically without oversight or high-level scrutiny, both in military or other institutions and from Congress. The program's experiments included nearly a decade of tularemia research on volunteer servicemen from the Seventh-day Adventist Church, who were infected through aerosols and then treated with antibiotics. Participants were unaware that the goal of the research was to standardize bomb dissemination of tularemia for anti-civilian attacks, just as the USA ambitiously conducted high-altitude dispersal of pathogens using jet aircraft to cover hundreds of square miles.

The Vietnam War era also marked the closure of the program, in which civilian scientists played an influential role. The widespread use of chemicals, riot control agents, and herbicides in Vietnam provoked international criticism and

brought public attention to the lesser-known U.S. biological weapons program. In 1966, 5,000 scientists signed a letter of concern to President Lyndon Johnson not against the war per se, but aimed at reviewing U.S. policy regarding chemical and biological weapons. Under pressure from the Joint Chiefs of Staff, Johnson did not offer a public response. The task was passed on to the next president, Richard Nixon, who approached it head-on.

In 1969, Harvard University biologist Matthew Meselson argued that biological warfare research in the USA created a model that could easily be emulated by other, less powerful countries, ultimately jeopardizing the USA security. In November of that year, in an unprecedented move in the USA history, Nixon promptly renounced biological weapons on behalf of the United States. Britain and France, both of which had become nuclear states, had already retreated from their offensive research and shifted to defensive efforts. In addition to scaling back the use of genetic and molecular biology advancements by the USA military forces, Nixon's decision paved the way for the 1972 Biological and Toxin Weapons Convention (BTWC), which required signatories to prohibit any activities related to the development of biological weapons. Unfortunately, due to the Cold War, the BTWC was not afforded aggressive transparency measures, such as on-site inspections, which would have made it a more effective limitation on proliferation.

In 1975, the Soviet Union exploited this loophole and launched a vast offensive biological warfare program that incorporated both advanced biology and new military delivery systems. Although this clearly violated the BTWC, the suspicion that the USA was secretly supporting its program served as justification for the Soviet leadership to embark on this massive undertaking. The growing militarization of the Soviet Union and the totalitarian secrecy that characterized its government and society allowed for unrestrained, industrial-scale pursuits of biological weapons, engaging tens of thousands of scientists and technicians. According to two high-ranking Soviet scientists – Ken Alibek and Igor Domaradskiy – the routine bureaucratic pressure of the program, inter-laboratory competition, and Kremlin policy focused them on specific technical tasks. These

included creating antibiotic-resistant strains of tularemia and achieving high production targets for anthrax suspensions – on the order of tons. The conditions of their work, centered on loyalty to the state, allowed them to disregard the suffering and deaths of civilian populations.

Now that the Cold War has ended and global communication and travel have become the norm, there is a heightened sense of shared risk. This phenomenon is perhaps most acutely felt in the realm of new and emerging infectious diseases, which spread rapidly and require international solutions. At the same time, new shifts in the doctrine of total war continue to trouble the world -such as genocides, wars, and terrorist acts where civilian lives are politically expendable – and the consequences of such conflicts ricochet globally.

When it comes to biological weapons, the pertinent question is whether microbiologists will ever use their talents to serve malicious rather than beneficial functions of medical science, thereby increasing the risks of dangerous diseases for vulnerable population groups. History shows that biologists, like anyone else, can succumb to political schemes to the extent that they lose their moral compass. The problem of malicious science, and thus its resolution, lies within self-reproducing political systems that cater to specific interests but can be influenced by civil society.

What can professionals dedicated to the life sciences do in response to political interpretations of national security risks that seek to create biological weapons programs? History offers three important lessons. First, any government advancement of secret research on dangerous pathogens should be met with skepticism. As in the past, secrecy unjustifiably increases the risks of disastrous disease outbreaks. During any unusual disease outbreak, accurate information is the best defense for vulnerable populations. Whether discussing the anthrax outbreak in Sverdlovsk, Soviet Union, in 1979, when anthrax spores were accidentally released from a secret military facility, or the SARS epidemic in 2003, state secrecy caused panic and cost lives.

Those who protest against strengthening the BTWC remain stuck in the Cold War, when state secrecy was equated with national security.

Secondly, any claims that an adversary has developed or is developing microbial weapons should be carefully assessed. Imaginary threats have often been used to justify increases in military funding and research, and most frequently have turned out to be unfounded. Just recently, the anonymous anthrax letters of 2001 in the USA provoked a radical shift in biosafety priorities at the National Institutes of Health (Bethesda, MD, USA) and the dissemination of numerous federal anti-bioterrorism projects, which are now in urgent need of evaluation. Throughout 2002, fear scenarios regarding biological weapons – particularly the alleged threat of smallpox from Saddam Hussein – manipulated the American public to support the invasion of Iraq in 2003.

While the threat of biological weapons may be real, the third approach is to use all available legal means to prevent and punish abuses of biological and medical research. The world needs guarantees of transparency in government and other institutions that may perform dual-use functions, or, as in high-containment laboratories, engage in biosafety research that could threaten public health. For this, the BTWC needs to be updated and strengthened at the organizational level, in conjunction with the Chemical Weapons Convention of 1993.

Those who protest against strengthening the BTWC remain stuck in the Cold War, when state secrecy was equated with national security. The world has changed and continues to change, accelerating toward new ways of sharing information, new scientific breakthroughs, and new sources of conflict and competition. Enhanced state and transnational oversight, established through collaborative networks, is needed and possible. Furthermore, individuals involved in the development, production, trade, or use of biological weapons should be recognized internationally as criminals and denied safe haven anywhere in the world. In addition to existing measures, such an agenda offers hope that in the future, the application of biological sciences will remain dedicated solely to improving health.

CHAPTER 3. BIOLOGICAL AND MEDICAL CHARACTERRISTICS OF HUMAN INFECTIOUS DISEASE AGENT THAT CAN BE USED FOR THE DEVELOPMENT OF BIOLOGICAL WEAPONS

The revolution in biology at the turn of the 20th and 21st centuries not only led to the development of biotechnology and new achievements in medicine but also created scientific and technological prerequisites for the development of advanced biological weapons of mass destruction. In the list of the least controlled and most dangerous threats to humanity, the majority of experts name bioaggression, bioterrorism, and “ecological wars”.

A bioterrorist attack is difficult to predict, making rapid response, recognition, and management of consequences critical. Medical professionals play a key role in responding to bioterrorism because they can help with the timely detection of events and reduce the number of affected individuals. It is important to be familiar with the characteristics of agents that can be used to develop biological weapons, as well as methods for their detection and control.

Some biological agents can pose a health risk to the population in the event of a bioterrorist attack or biological warfare.

The use of biological weapons is not a new practice. As early as the 6th century BC, it was reported that the Assyrians contaminated water supplies with the fungus *Claviceps purpurea*. A well-known historical case is when the Tatar army catapulted the bodies of plague victims over the walls of the city of Kaffa in 1346, and the British spread smallpox through infected blankets to Native Americans in 1767. Mycotoxins were used in Laos, Cambodia, and Afghanistan in the form of so-called "yellow rain." The rise in religious cults and extremist political groups also increases the threat of bioterrorism today. The most significant biological attack in the United States occurred when a religious cult contaminated restaurants with salmonella in Oregon in 1984.

In September 2001, letters containing anthrax spores were sent to the USA senators and several media outlets. The Centers for Disease Control and Prevention identified 22 cases of anthrax during this attack: 11 patients with pulmonary anthrax, 5 of whom died, and 11 with cutaneous anthrax, all of whom survived.

Multiple viral agents have been classified by the CDC as potential weapons of mass destruction or agents for biologic terrorism. Agents such as smallpox, viral hemorrhagic fever viruses, agents of viral encephalitis, and others are of concern because they are highly infectious and relatively easy to produce. Although dispersion might be difficult, the risk is magnified by the fact that large populations are susceptible to these agents and only limited treatment and vaccination strategies exist. Although the risk of large-scale bioterrorism using viral agents is small, public health programs and health care providers must be prepared for this potentially devastating impact on public health.

European soldiers used smallpox as bioweapons when variola-contaminated clothing and blankets were delivered to South American natives in the 15th century. During the French and Indian Wars (1754-1767), British soldiers used variola-contaminated blankets to initiate an outbreak of smallpox among American Indians sympathetic to Americans. The epidemic that ensued killed more than half of the affected tribes. With the cessation of routine smallpox vaccinations in the early 1980s, the deliberate release of smallpox would be potentially catastrophic. Over the last several decades, there has been increasing concern about the use of viral agents as weapons of mass destruction, especially because they are potentially highly contagious in a susceptible population, carry a high case fatality ratio and, except for smallpox, there are limited vaccination strategies. Potential viral agents include smallpox, hemorrhagic fever viruses, Nipah virus, Venezuelan equine encephalitis virus, and hantaviruses. Others, like influenza, are also potential agents. It is the intent of this review to discuss some of the viral agents that pose a threat if used as biological agents of mass destruction.

Biological warfare agents are biological agents such as viruses, bacteria, fungi, protozoa, or toxins produced by them, which, when intentionally spread, cause diseases in humans, animals, or plants (Table 3.1).

Table 3.1. Agents that can be used as biological weapons

	Agents	**Diseases**	**Mode of infection**	**Possible release method**
Bacteria	*Bac. anthracis*	Anthrax	Aerosol	Spores
	Y. pestis	Plague	Aerosol	Vegetative cells
	Br. melitensis	Brucellosis	Aerosol	Vegetative cells
	Br. abortus			
Bacteria	*B. mallei*	Sap	Aerosol	Vegetative cells
	B. pseudomallei	Melioidosis	Aerosol	Vegetative cells
Viruses	Smallpox virus	Smallpox	Aerosol	Viral particles
	Ebola virus	Ebola fever	Aerosol	Viral particles
	Marburg virus	Marburg hemorrhagic fever	Aerosol	Viral particles
Toxins	*C. botulinum*	Botulism	Food / water	Toxin
	S. aureus	Staphylococcal enterotoxin B	Food / water	Toxin
	Ricin (plant-derived)	Ricin toxin	Food / water	Toxin
	Trichothecene (fungal-derived)	Trichothecin T2 toxin	Food / water	Toxin

The use of biological agents as weapons has some unique characteristics. The effects of these agents are not immediate and may take several hours or even weeks before symptoms appear in the affected population. Such attacks require the release of a small amount of viable material, which can then self-replicate. Viruses can only replicate inside living cells and are pathogenic to humans, animals, and plants. They consist of proteins and nucleic acids (DNA and RNA) and can reproduce and spread much more quickly. Bacteria are single-celled prokaryotic organisms with a defined cell wall. Fungi are single-celled or multicellular eukaryotic organisms. It is known that several types of fungi cause diseases in plants, and some can also affect humans. Toxins are secondary metabolites

produced by bacteria, fungi, and other organisms, which can act in very low concentrations, affecting cell functions.

The Centers for Disease Control and Prevention (CDC) has classified biological warfare agents into three categories (A, B, and C) based on their potential threat to society (Table 3.2).

Table 3.2. Classification of agents of bioterrorism / biological weapons

Category A	**Category B**	**Category C**
High impact agents: – easily spread; – cause high mortality; – cause public panic and social unrest; – require special health care measures.	Second-priority agents: – spread at a moderate rate; – cause moderate morbidity; – demand increased disease surveillance and diagnostic capacity of the health care system.	Third-priority agents: – can be created for mass dissemination in the future; – there is a potential for high morbidity, mortality and significant impact on health.
Pathogens		
Bacillus anthracis (anthrax) *Clostridium botulinum toxin (botulism)* *Francisella tularensis (tularemia)* *Variola major (smallpox)* *Yersinia pestis (plague)* *Filoviruses* *Ebola virus (Ebola hemorrhagic fever)* *Marburg virus (Marburg hemorrhagic fever)* *Arenaviruses* *Hunin virus (Argentine hemorrhagic fever) and related viruses* *Lassa virus (Lassa fever)*	Alpha viruses Viruses of eastern and western equine encephalomyelitis Venezuelan equine encephalomyelitis virus Types of brucella (brucellosis) Burkholderia mallei (sap) Coxiella burnetii (Q fever) Clostridium perfringens epsilon toxin Ricin toxin from Ricinus communis Staphylococcal enterotoxin B Cryptosporidium parvum *E. coli* O157: H7 Salmonella species Shigella dysenteriae Cholera vibrio	Hantaviruses Multi-resistant tuberculosis Nipah virus Viruses of tick-borne encephalitis Viruses of tick-borne hemorrhagic fever Yellow fever

In the event of an unconfirmed (undeclared) biological attack, the early symptoms of infection are likely to be non-specific. Indicators of a biological weapon attack may include a large number of patients presenting similar symptoms simultaneously, as well as an increase in morbidity and mortality compared to more common diseases. Other signs include a significant number of geographically linked patients (those living or working in the same area, attending events, etc.).

The use of agents that cause severe illness and high mortality can quickly overwhelm healthcare systems and resources, which is another important risk factor.

Some pathogens can be treated with medications or their spread can be prevented by immunization or prophylactic drugs, while others can only be managed through supportive care. Early detection of a biological attack, identifying the agents used, and determining the population at risk are crucial for both incident management and patient care.

3.1. Viruses as infectious disease agents that can be used in biological weapons

The epidemic that ensued killed more than half of the affected tribes. With the cessation of routine smallpox vaccinations in the early 1980s, the deliberate release of smallpox would be potentially catastrophic. Over the last several decades, there has been increasing concern about the use of viral agents as weapons of mass destruction, especially because they are potentially highly contagious in a susceptible population, carry a high case fatality ratio and, except for smallpox, there are limited vaccination strategies. Potential viral agents in clude smallpox, hemorrhagic fever viruses, Nipah virus, Venezuelan equine encephalitis virus, and hantaviruses. Others, like influenza, are also potential agents. It is the intent of this review to discuss some of the viral agents that pose a threat if used as biological agents of mass destruction.

3.1.1. Viruses causing hemorrhagic fever

Viruses causing hemorrhagic fever refers to a group of viral agents that share a number of characteristics. They are all lipid-enveloped RNA viruses and re-quire an animal or insect host reservoir. They are geographically restricted to specific regions of the world where they create enzootic infection. Human disease is

sporadic and usually follows accidental exposure to contaminated saliva, urine, or feces of infected animals, insect bites, or occasionally from human to human due to exposure to contaminated tissue or body fluids. Agents of VHF include arena-viruses [Lassa fever and South American hemorrhagic fever viruses (SAHF)], bunyaviruses [Hanta-virus and Crimean-Congo hemorrhagic fever virus (CCHF)], flaviviruses [Dengue hemorrhagic fever (DHF), tick-borne encephalitis, and yellow fever viruses], and filoviruses (Ebola and Marburg viruses). For the most part, there are only limited treatment and vaccination options for persons either infected with or exposed to these agents. Although there is no overt evidence that these agents have been weaponized, it is possible that they could be disseminated by aerosolization and used as weapons of mass destruction.

Transmission to humans after contact with contaminated medical equipment has been described with Ebola virus, and human-to-human transmission occurs with Ebola / Marburg, CCHF, Lassa fever, and Junin viruses.

As a group, agents of VHF spread hematogenously to multiple organs, where they target the vascular bed, causing microvascular damage and marked changes in vascular permeability. Severe manifestations of infection result from the interplay of vascular permeability, release of proinflammatory cytokines, cytotoxic factors, and autoantibodies, complement activation and systemic coagulopathy. Clinically, VHF commonly presents with fever, myalgias, headaches, and prostration. In-cubation periods are usually brief and range from 2 to 19 days. Most have abrupt fever accompanied by severe headache and severe myalgias. Pain in the chest, back and abdomen occur in DHF, Ebola / Marburg, and Lassa fever; conjunctival injection is commonly observed in Lassa fever, CCHF, and SAHF. Renal insufficiency is seen in Lassa fever and Rift Valley fever, and significant hepatic injury is observed in DHF, Ebola / Marburg, and CCHF. The presence of proteinuria helps to predict Lassa fever and SAHF. With the exception of Lassa fever, significant thrombocytopenia and leukopenia are com-mon and overt hemorrhage can be observed.

Diagnosis of VHF can be confirmed using viral antigen detection in CCHF and Rift Valley fever, by polymerase chain reaction in DHF and Ebola, serologically (immunofluorescence or ELISA) in Ebola / Marburg, Rift Valley fever, Lassa fever, or SAHF, and by viral identification (electron microscopy) or culture. With the exception of DHF, specialized microbiologic containment (biosafety level 4) is required for safe handling of these agents. Medical treatment requires intensive care in those most severely ill with monitoring of the patient's hemodynamic, hematologic, neurologic, and renal status. Although supportive care is the mainstay of treatment of DHF, the antiviral drug ribavirin has been shown to be useful in those with CCHF and Lassa fever and is possibly effective in Rift Valley fever and Argentine hemorrhagic fever. Convalescent plasma containing neutralizing antibodies has been shown to improve survival in those infected with certain forms of SAHF (Bolivian and Venezuelan hemorrhagic fever) and possibly those with Ebola.

With the exception of DHF, the blood and secretions of patients with VHF contain large quantities of virus; hence, these patients pose significant hospital infection control problems. Guidelines for management and control of VHF have been reviewed elsewhere. Despite aggressive treatment, mortality for patients with VHF remains high, ranging from 1 % with uncomplicated Dengue to 90 % with Ebola. Long term sequelae may include eighth nerve deafness and alopecia in Lassa fever and retinitis in Rift Valley fever.

Of greater concern is the absence of approved vaccines or definitive chemoprophylaxis regimens for prevention of infection. An effective attenuated live viral vaccine for Argentine hemorrhagic fever has recently been described in humans and in animals and may have cross-protective efficacy for other forms of SAHF. Protective efficacy of a vaccinia-virus expressing Lassa fever virus structural proteins in a macaque model. Compared with nonimmunized control animals, those that received the vaccinia expressing glycoproteins G1 and G2 were significantly.

The agents that cause hemorrhagic fever include:

- Arenaviruses (Lassa fever, South American hemorrhagic fever viruses)
- Bunyaviruses (Hantavirus, Crimean-Congo hemorrhagic fever virus)
- Flaviviruses (Dengue hemorrhagic fever, tick-borne encephalitis, yellow fever virus)
- Filoviruses (Ebola virus, Marburg virus)

These viruses cause microvascular damage and changes in vascular permeability. Severe manifestations of the disease are associated with changes in vascular permeability, release of pro-inflammatory cytokines, cytotoxic factors and autoantibodies, activation of complement and systemic coagulopathy.

Symptoms of infection with these viruses include fever, myalgia, headache, and prostration (Table 3.3).

Table 3.3. Medical characteristics of viruses that cause hemorrhagic fever

Pathogen	**Incubation period (days)**	**Clinical manifestations**	**Mortality, %**
Dengue virus	4-7	Fever, headache, abdominal pain, back pain, ecchymosis, shock thrombocytopenia, leukopenia, increased liver function tests	1-50
Ebola virus/ Marburg	2-19	Fever, headache, abdominal pain, cough, myalgia, adenopathy, jaundice, bleeding, maculopapular rash, thrombocytopenia, leukopenia, increased liver function tests	25-90
Crimea-Congo hemorrhagic fever virus	3-12	Fever, headache, myalgia, flushing, conjunctival injection, bleeding, leukopenia, thrombocytopenia, diffuse intravascular coagulation, elevated liver function test	15-30
Rift Valley fever	2-5	Fever, headache, encephalopathy, retinitis, jaundice, increased creatinine level, bleeding, thrombocytopenia	≤ 50

Lassa virus	5-16	Fever, pharyngitis, back pain, facial swelling, conjunctivitis, shock, bleeding, increased creatinine level, proteinuria	15-25
South American hemorrhagic fever virus	7-14	Fever, headache, photophobia, conjunctival injection, adenopathy, petechiae, shock, bleeding, leukopenia, thrombocytopenia, increased creatinine	15-30

Appropriate treatment of individuals with suspected viral hemorrhagic fever includes early diagnosis.

When infected with the Lassa virus, ribavirin is indicated. It improves the results of treatment if it is received at the beginning of the disease. Newer agents such as favipiravir and LASV-specific monoclonal antibodies are also currently being evaluated. Currently, there are no effective vaccines against Lassa fever. When infected with the Crimean-Congo hemorrhagic fever virus, the treatment is mainly supportive. Ribavirin has demonstrated antiviral activity against this virus *in vitro*. There is currently no effective vaccine for humans.

In case of infection with the Ebola and Marburg viruses, supportive treatment is provided. There are currently no vaccines available against Marburg virus. There is one FDA-approved Zaire Ebola virus vaccine.

To date, there are no effective antiviral regimens for the treatment of dengue fever, so treatment involves supportive care. One vaccine is currently available in Latin America and Southeast Asia. However, the World Health Organization recommends vaccination only for individuals with a history of dengue fever.

3.1.2. Smallpox virus

The smallpox virus is the largest of the animal viruses. The virus contains double-stranded DNA and has a complex structure. The nucleoid is embedded in an ellipsoidal body, forming the thick center of the virion, and a double membrane surrounds the virus particle. The virus is very virulent, with a mortality rate of

30 % in unvaccinated individuals. The virus belongs to the genus *Orthopoxviridae*, family *Poxviridae*.

The smallpox virus comes in two strains: *Variola major*, which causes severe smallpox, and *Variola minor*, which causes smallpox. Both of these strains cannot be differentiated based on immunological methods and differ only in clinical manifestations.

Using immunodiffusion techniques, at least seven different major varicella virus antigens can be isolated, and 17 polypeptide chains can be identified. Hemagglutinating, complement-fixing and neutralizing antibodies can be produced in response to smallpox virus antigens. Neutralizing antibodies are directed against two antigens in the surface membrane of virus particles. Complement-fixing antibodies react with an antigen common to each subgroup of unclassified poxviruses.

The incubation period is 12 days, and the clinical manifestation is fever and headache. The infection causes pus-filled blisters all over the body, and the death rate is about 30 % in the unimmunized group, 3 % in the immunized group. Vaccination against smallpox is carried out with the smallpox vaccine virus, which has many antigenic structures in common with the smallpox virus.

Today, smallpox is considered a serious threat for several reasons, the most important of which is that most of the human population is not immunized against the disease. Secondly, smallpox spreads quickly, and the aerosol method of infection allows for mass infection.

Smallpox is an attractive bioterrorism agent be-cause of a mortality rate that can approach 30 % for nonimmune persons, absence of an efficacious antiviral therapy, and person-to-person airborne transmission could rapidly enlarge an outbreak. Few infectious diseases have the potential to cause a widespread disaster like smallpox. These agents are considered “Category A” critical biological agents by the CDC and include smallpox, anthrax, plague, botulism, tularemia, and VHFs. Effective responses to an intentional release of smallpox require broad-based public health preparedness.

Smallpox is caused by variola virus and known to infect only humans.38 This double-stranded DNA virus is a member of the *Orthopoxvirus genus* in the *Poxviridae* family. Additional *Orthopoxvirus species* include cowpox, monkeypox, and vaccinia, the basis of smallpox vaccination programs. These viruses are large, asymmetric, brick-shaped virions that encode unique enzymes that allow viral replication in the cytoplasm of infected cells. With electron micros-copy, orthopoxviruses can be distinguished from other agents of vesiculating rashes, such as varicella zoster virus and herpes simplex virus.

When the disease was common, the diagnosis was confirmed by the typical appearance of the skin lesions. Confusion might have occurred in the 10% of patients who presented with either hemorrhagic or malignant form of variola major. Patients with hemorrhagic disease developed severe prostration, high fever, abdominal pain, and a dusky erythroderma followed by petechiae and cutaneous ecchymoses. Death uniformly occurred within 1 week of rash onset. In the malignant form of variola major, there was a sudden onset of severe malaise and fever, followed by slowly developing confluent lesions that failed to progress to a pustular stage and, unlike typical smallpox, the lesions remained soft, flat, and velvety to the touch. A majority of these patients also died.

Because so few practitioners in the United States have seen smallpox, the initial presentation after a terrorist release will quite probably be misdiagnosed. The differential diagnosis includes varicella, disseminated herpes zoster or herpes simplex virus, impetigo, erythema multiforme, bullous pemphigoid, insect or mite bites, secondary syphilis, or hand, foot, and mouth disease. Among these, the disease most likely to be confused with the rash of smallpox is varicella; however, it has several features that should allow clinicians to distinguish it from smallpox. For example, smallpox has a distinctive viral prodrome that occurs before the onset of a rash. In contrast, chickenpox usually presents without a prodrome or a prodrome that lasts at most then 1 day. The vesicles of chickenpox are superficial, appear in multiple crops, have a centripetal distribution, evolve rapidly over 24 hours, and typically spare the palms and soles. Persons with varicella will often

lack a reliable history of varicella infection or vaccination. Finally, 50 to 80% of these cases will recall recent exposure to someone with varicella.

The side effects of smallpox vaccination are infrequent but well characterized. Among primary vaccinees, the 2 most common complications are inadvertent mucocutaneous autoinoculation from a site of vaccination (529 cases per million doses) and generalized vaccinia (241 cases per million doses). These conditions typically resolve without specific therapy. For treatment of severe cases, a limited supply of type-specific immunoglobulin derived from plasma of persons vaccinated with vaccinia virus is available through the CDC. Other moderate-to-severe complications of vaccinia administration include eczema vaccinatum, progressive vaccinia, and postvaccinial encephalitis. These occur in 40 cases per million doses among primary vaccinees, and are 10-fold less common among revaccinates. Approximately 1 death occurs per million vaccine doses. Contraindications to smallpox vaccination include pregnancy, acute or chronic skin conditions including eczema, immunosuppression, and allergies to vaccine components. No contraindication to vaccination is likely to be recognized during an outbreak of smallpox.

Mathematical modeling of prior smallpox epidemics predicts a rapid increase in secondary cases after reintroduction of smallpox into susceptible populations. A large outbreak would be stemmed only by the rapid implementation of effective public health interventions. Based on prior epidemics, the average number of secondary smallpox cases per primary case is 3.5 to 6.45 Outbreaks in Europe during the early part of the last century suggested 50 % of secondary cases occurred among hospital staff and patients before outbreaks were recognized and control measures undertaken. Current models of an intentional smallpox release predict chaos during the initial recognition and early response, followed by complex coordination of multiple health and law enforcement agencies. Theoretical scenarios suggest that quarantine or vaccination could eventually stop a smallpox outbreak, however both interventions work synergistically when implemented during the early phase of an outbreak.

3.1.3. Hantaviruses

Hantaviruses are zoonotic viruses that cause two human diseases: cardiopulmonary syndrome caused by hantaviruses of the Western Hemisphere; and hemorrhagic fever with renal syndrome caused by Eastern Hemisphere hantaviruses.

The genus Hantavirus is part of the *Bunyavirus* family and consists of well-defined serotypes, each of which is associated with a specific species of primary rodent carrier. Viral particles have a lipid bilayer containing glycoprotein spikes of Gn and Gc glycoproteins. The genome inside the bilayer is bound to RNA-dependent RNA polymerase. The hantavirus genome consists of three segments of single-stranded RNA. The three segments are ordered by size and are designated as large (L), medium (M), and small (S) segments because their lengths are typically 6.6, 3.7, and 2.1 kb for L, M, and S segments respectively.

Pathophysiological sign of hantavirus infection is vascular dysfunction. Patients with cardiopulmonary syndrome caused by hantaviruses typically present with fever, headache, myalgias, and chills, as well as leukocytosis and thrombocytopenia, which rapidly progresses to more severe respiratory disease. After 4–10 days, hantavirus-infected individuals develop flu-like illnesses accompanied by rapidly progressive pulmonary edema. Cardiopulmonary syndrome, unlike other respiratory diseases, occurs in young, healthy adults. The fatality rate can be from 50 to 60 %. Clinical manifestations of hemorrhagic fever with renal syndrome include fever, headache, myalgias, and eye pain associated with hypotension, renal failure with proteinuria, and hemorrhage. Mortality varies by agent, with a mortality rate of 5 to 10 %.

Hantaviruses are potential biological weapons agents because they have a high mortality rate and a rapid disease course with severe cardiopulmonary symptoms.

Currently, there are live attenuated vaccines (Hantavax), DNA vaccines, and subunit vaccines that show varying degrees of efficacy. There are also treatment

strategies for these infections: taking antiviral drugs and ribavirin, which blocks replication by affecting the RNA-dependent RNA polymerase of the virus. However, ribavirin has some limitations as it is toxic to humans and animals at high doses and causes anemia.

Ring vaccination efforts and quarantine may encounter unexpected problems when applied to the aftermath of an intentional smallpox release. Highly mobile, industrialized societies are likely to meet social and legal impediments when trying to identify and quarantine patients and contacts during an outbreak. Media coverage will be intense and encourage widespread demand for a relatively safe vaccine but may also incite fear about a severe, contagious illness for which there is no approved antiviral therapy. Conservative estimates indicate that at least 40 million doses of vaccine would be needed in the United States during a smallpox out-break. Steps are underway to stockpile sufficient vaccine for each person in the United States. Such large quantities could potentially erode a ring vaccination strategy as health care workers, military and law enforcement personnel, and large numbers of other citizens at significantly lower risk for smallpox seek protection through vaccination. Finally, hospitalization of many extremely ill patients with smallpox is likely to rapidly overwhelm existing health care facility infrastructures and further intensify difficulties posed by quarantine.

3.1.4. Nipah virus

Nipah virus (NiV) is an RNA virus of the genus *Henipavirus* of the *Paramyxoviridae* family. Like other paramyxoviruses, NiV virus particles are pleomorphic, spherical to filamentous, and range in size from 40 to 1900 nm. Among the NiVs that cause disease in humans, there are two main genetic lineages, NiV Malaysian (NiV-MY) and NiV Bangladesh (NiV-BD). The NiV-MY genome is 18.246 nucleotides long, while the NiV-BD genome is 18.252 nucleotides long.

Fruit bats of the genus Pteropus, family Pteropodidae, are the main vectors of NiV. The virus does not cause disease in bats, regardless of whether they are infected naturally or experimentally.

Paramyxoviruses have a limited host range, and interspecies transmission is rare. In contrast, NiV exhibits a very broad species tropism – NiV naturally infects pigs, horses, dogs, cats and humans. NiV has also been shown to experimentally infect guinea pigs, hamsters, ferrets, squirrel monkeys, and African green monkeys. This wide range of species tropism is partly due to the fact that NiV uses Ephrin-B2 / B3 molecules as entry receptors, which are highly conserved among all mammals.

The incubation period in humans ranges from 4 days to 2 months. Patients have fever, headache, dizziness and vomiting, severe encephalitis. Many patients have visible signs of brainstem dysfunction, including an abnormal pupillary reflex, vasomotor changes, and convulsions.

A unique and interesting feature of NiV infection is the development of relapse and late-onset encephalitis, some of which have occurred months or years after the acute illness. The longest delay in the onset of late-onset encephalitis was 11 years. A characteristic sign of infection is also neuropsychological changes – depression, personality changes, attention deficit.

The main treatment strategy is ribavirin. Due to its high virulence, spread between animals and humans, and significant morbidity and mortality, NiV meets some criteria to be considered a potential bioterrorism agent.

3.1.5. Venezuelan Equine Encephalitis Virus Complex

Venezuelan equine encephalitis virus complex (VEE) refers to mosquito-borne α-viruses that cause human disease in Central America, Mexico, and occasionally in the United States. Equines serve as the amplifying host and source for mosquito infection. Infection with Western equine encephalitis or Eastern equine encephalitis virus is clinically indistinguishable from VEE; however, VEE

is more likely to be a candidate for biowarfare because of the lower human infective dose. Human infection is episodic; the last major outbreak occurred in Venezuela and Columbia in 1995, during which nearly 100,000 people were infected, implying a high degree of susceptibility within the population. Because VEE is equine-virulent, infection can create major losses in horse and donkey populations. Natural infections may result in 30 to 90 % mortality in equines.

Although human-to-human transmission is not thought to occur, VEE has been studied as an effective bioweapon because of its low human infective dose, easy production, and potential distribution by intentional aerosolization or by release of infected mosquitoes. In the 1950s and 1960s, several countries, including the United States, conducted studies on VEE as a bioweapon; U.S. stockpiles, however, were destroyed in 1969. The potential of VEE to be an effective bioweapon after aerosolization is augmented by the knowledge that some forms are highly infectious and can easily gain direct access to the central nervous system via the olfactory tract.

Natural VEE infection usually is a self-limited illness consisting of fever, chills, malaise, and severe headache with less than 1 % of adults and 4 % of children progressing to severe encephalitis. Ataxia, convulsions, paralysis, and coma may com-plicate the encephalitis and mortality may approach 20 %. Infection during pregnancy may cause fetal neuroanatomical defects or spontaneous abortion. The diagnosis is confirmed by detecting virus in the cerebrospinal fluid by either culture or polymerase chain reaction. Samples should be handled in a biosafety level 3 laboratory. Although the sera of patients with encephalitis may be negative, VEE type-specific antibodies in sera or cerebrospinal fluid specimens also suggest the diagnosis.

There are no definitive antiviral agents for VEE; thus, treatment is largely supportive. Treatment of VEE-infected mice with pegylated IFN-α significantly improved survival from either an aerosolized or subcutaneous challenge infection. Quarantine is not required, but mosquito control methods should be employed if the outbreak is mosquito-borne. Limited studies have shown the protective efficacy

in laboratory animals of a formalin-inactivated vaccine and more recently of a live attenuated viral vaccine and a vaccinia virus recombinant vaccine encoding the VEE structural gene region.

3.2. Rickettsiae as pathogens of human infectious diseases that can be used for the development of biological weapons

The family *Rickettsiaceae* includes a group of microorganisms that phylogenetically occupy an intermediate position between bacteria and viruses. Rickettsiae are small, gram-negative, pleomorphic coccobacilli without flagella, adapted to obligate intracellular parasitism, and transmitted by arthropod vectors. They are primary parasites of arthropods, such as lice, fleas, and ticks, where they are carried in the digestive tract. In vertebrates, including humans, they affect the vascular endothelium and reticuloendothelial cells. The family *Rickettsiaceae* consists of three genera: *Rickettsia*, *Orientia*, and *Ehrlichia*. Former members of this family, *Coxiella burnetii*, which causes Q fever, and *Rochalimaea quintana*, which causes trench fever, were excluded as the former is not transmitted by arthropods, and the latter is not an obligate intracellular parasite.

Species of the genus *Rickettsia* can be divided into two groups: the typhus group (including two species: epidemic flea-borne typhus caused by *R. typhi* and epidemic louse-borne typhus caused by *R. prowazekii*) and the spotted fever group. The genus *Orientia* contains only two species: *O. tsutsugamushi* and *O. chuto*. The genus *Ehrlichia* includes *Ehrlichia chaffeensis*, which causes human monocytic ehrlichiosis, and *Ehrlichia ewingii*, which causes *Ehrlichia ewingii* ehrlichiosis.

As obligate intracellular parasites, these organisms do not grow on cell-free media and require tissue culture or laboratory animals for isolation. Diseases caused by rickettsiae are widespread around the world and are difficult to diagnose. Clinical manifestations of rickettsial diseases can range from mild to very severe, with mortality rates from highly virulent rickettsiae ranging from 2 % to 30 %. The severity of rickettsial disease is associated with the virulence of the pathogen and

host-related factors (e.g., age, timing of diagnosis, liver and kidney dysfunction, CNS impairment, and lung condition). Despite variability in their clinical presentation, pathogenic rickettsiae cause debilitating diseases, and any of the highly virulent rickettsial species listed in Table 3.4 could potentially be used as a biological weapon. Classic epidemic typhus is the most severe rickettsial disease, characterized by high fever (~ 42 °C), severe muscle and joint pain, and the appearance of cerebral disturbances 10 days after infection. Thrombosis of small vessels in the extremities can lead to gangrene and necrosis. The mortality rate among untreated patients is ~ 20 %, and in severe outbreaks, mortality can often reach 40 %.

Table 3.4. Epidemiological Characteristics of Highly Pathogenic Rickettsiae

Rickettsia species	**Disease**	**Vector**	**Host**
R. prowazekii	Epidemic camp fever	Body louse	Human
	Sporadic camp fever	-	Human
	Sporadic camp fever	Lice, fleas	Flying squirrels
R. typhi	Epidemic flea-borne typhus	Fleas	Rodents, opossums
R. reckettsii	American tick-borne rickettsiosis	Ticks	Small mammals, birds
R. conorii	South African tick-borne rickettsiosis	Ticks	Rodents, dogs
R. sibirica	Siberian tick-borne rickettsiosis	Ticks	Rodents

Due to their unique biological characteristics (environmental stability, small size, aerosol spread, low infectious dose, high morbidity, and significant mortality), *R. prowazekii* is most likely to be used as a bioterrorism agent. According to estimates by the World Health Organization, if 50 kg of *R. prowazekii* aerosol were released during a terrorist attack, the result would be over 100,000 casualties (19,000 deaths and 85,000 incapacitated individuals).

Currently, most rickettsial infections are diagnosed based on serological reactions, such as IgG and IgM to *R. rickettsiae*. Although rickettsiae can be

cultured in microbiological laboratories, this approach is not often used for clinical diagnosis as it is complex and requires a high level of biosafety. Other diagnostic options include molecular tests such as PCR and skin biopsies. In addition to suggestive or positive serological tests, patients with rickettsial infections may exhibit thrombocytopenia, hyponatremia, and pleocytosis of the cerebrospinal fluid.

The drug of choice for treating rickettsiosis is doxycycline. In cases of allergy or severe disease, chloramphenicol is used. For mild disease, macrolides such as clarithromycin may also be considered.

3.3. Bacteria as human infectious disease pathogens that can be used for the development of biological weapons bacillus anthracis – the causative agent of anthrax

Bacillus anthracis is a gram-positive, facultatively anaerobic, rod-shaped bacterium that is the causative agent of anthrax. It was discovered by the German physician Robert Koch in 1876, and it became the first bacterium to be experimentally demonstrated as the causative agent of a disease. This discovery also provided the first scientific proof of the germ theory of diseases.

Considering the biological aspects of this pathogen, *B. anthracis* ranges in size from 3 to 5 micrometers and has a genome consisting of 5,227,293 base pairs in a single circular DNA strand. This pathogen has two extrachromosomal DNA plasmids, pXO1 and pXO2, which are responsible for its pathogenicity. Moreover, the bacterium is capable of forming a protective layer called an endospore, which allows it to remain dormant for many years and suddenly cause infection when environmental conditions are favorable. Due to the resilience of the endospore, *B. anthracis* is one of the most commonly considered biological weapons.

When grown in culture, *B. anthracis* typically forms long chains of bacteria. On agar plates, they form large colonies several millimeters wide, usually white or creamy in color. The presence of a protein capsule gives the colonies a slimy

appearance. The capsule, primarily composed of poly-D-gamma-glutamic acid, is crucial for evading the host immune response. While most bacteria are surrounded by a polysaccharide capsule, *B. anthracis* capsule gives it an evolutionary advantage. Polysaccharides are associated with binding to defensins secreted by neutrophils, which inactivate and break down bacteria. By not having this macromolecule in its capsule, *B. anthracis* avoids neutrophil attack and continues to spread the infection. The difference in capsule composition is also significant, as poly-gamma-D-glutamic acid is believed to create a negative charge, which protects the vegetative phase of the bacteria from phagocytosis by macrophages. All these listed indicators show advantages for the possible use of this pathogen as a biological weapon.

Turning to the consideration of the medical aspect of anthrax, it becomes clear why this agent is so dangerous for the human body. This bacterium produces three plasmid-encoded exotoxins: a swelling factor, a calmodulin-dependent adenylate cyclase that causes an increase in intracellular cAMP and is responsible for the severe swelling commonly seen in *B. anthracis* infections, a lethal toxin responsible for tissue necrosis, and a protective antigen named for its use in the production of protective vaccines against anthrax, which mediates the entry of swelling factor cells and lethal toxin. Symptoms of anthrax depend on the type of infection and can take from 1 day to more than 2 months. All types of anthrax can, if left untreated, spread throughout the body and cause severe illness and even death.

Four forms of human anthrax are recognized based on their site of entry.

1. Cutaneous, the most common form (95 %), causes a localized, inflammatory, black, necrotic lesion (eschar). Most often, the sore appears on the face, neck, arms or hands. Development may occur within 1-7 days of exposure.

2. Inhalation, a rare but extremely fatal form, is characterized by flu-like symptoms, chest discomfort, diaphoresis, and body aches. Development usually occurs within a week of exposure, but may take up to two months.

3. Gastrointestinal, a rare but also fatal case (causes death with a probability of up to 25 %), is the result of ingestion of spores. Symptoms include: fever and chills, swelling of the neck, painful swallowing, hoarseness, nausea and vomiting (especially vomiting blood), diarrhea, flushing and redness of the eyes, and abdominal swelling.

4. Injection ulcer symptoms are similar to cutaneous anthrax, but injection anthrax can spread more quickly throughout the body and is more difficult to recognize and treat than cutaneous anthrax. Symptoms include fever, chills, and a cluster of small bumps or blisters that may itch where the drug was injected. A painless sore with a black center that appears after blisters or bumps and swelling around the sore. Abscesses deep under the skin or in the muscle where the drug was injected.

Anthrax spores have indeed been used as biological weapons. The first modern case occurred in 1916 when northern insurgents, supplied by the German General Staff, used anthrax with unknown results against the Imperial Russian Army in Finland. Anthrax was later tested as a biological weapon by Japan's Unit 731 in Manchuria during the 1930s, where many of these tests involved the deliberate infection of prisoners of war, leading to thousands of deaths. The history of biological weapons research in this area is extensive.

In 1942, British biological weapons trials heavily contaminated Gruinard Island in Scotland with anthrax spores (Volum-14578 strain), rendering it off-limits until it was decontaminated in 1990. The U.S. included anthrax in its biological weapons stockpile until 1972, when the country signed the Biological Weapons Convention. President Nixon had already ordered the cancellation of American biological warfare programs in 1969 and the destruction of all existing stockpiles.

In 1978–79, the Rhodesian government used anthrax against livestock and people during its campaign against insurgents. The Soviet Union, during the Cold War, developed and stored between 100 and 200 tons of anthrax spores in Kantubek on Vozrozhdeniya Island, which were abandoned in 1992 and destroyed in 2002.

3.3.1. Pathogen of Plague – *Yersinia pestis*

Yersinia pestis is a Gram-negative, non-motile, rod-shaped bacterium that does not form spores. It is a facultative anaerobic organism capable of infecting humans through the oriental rat flea (*Xenopsylla cheopis*). This bacterium is responsible for the disease known as plague, which has three main forms: pneumonic, septicemic, and bubonic. *Y. pestis* was discovered in 1894 by Alexandre Yersin, a Swiss / French physician and bacteriologist from the Pasteur Institute, during a plague outbreak in Hong Kong.

Y. pestis is non-motile, rod-shaped, facultative anaerobic bacterium with bipolar staining, giving it a "safety pin" appearance, and it produces an anti-phagocytic mucous layer. Like other Yersinia species, it tests negative for urease, lactose fermentation, and indole production. Its closest relative is the gastrointestinal pathogen *Yersinia pseudotuberculosis*, while a more distant relative is *Yersinia enterocolitica.*

Similar to other pathogenic strains, this bacterium exhibits signs of functional loss mutations. The chromosome of the KIM strain contains 4,600,755 base pairs, while the CO92 strain has 4,653,728 base pairs. Like *Y. pseudotuberculosis* and *Y. enterocolitica, Y. pestis* possesses the plasmid pCD1. It also contains two other plasmids, pPCP1 (also known as pPla or pPst) and pMT1 (also known as pFra), which are not found in other Yersinia species. The pFra plasmid encodes phospholipase D, which is crucial for the ability of Y. pestis to be transmitted by fleas, while the pPla plasmid encodes the protease Pla, which activates plasmin in the human body and is a significant virulence factor for pneumonic plague. Together, these plasmids and a pathogenicity island known as HPI encode several proteins that contribute to the pathogenesis for which *Y. pestis* is known. Thus, similar to the anthrax pathogen, the primary danger posed by this pathogen lies in its genetic code.

Returning to the disease and pathogenicity aspect, it is important to note that this disease is primarily spread by rodents, especially in urban areas, where the

brown rat serves as a carrier. In forested areas, the wild rat becomes the carrier, with transmission to humans occurring directly through bites from infected fleas. If the disease progresses to the pneumonic form, humans can spread the bacteria to others through coughing, vomiting, and possibly sneezing.

The pathogenesis caused by the infection of mammalian hosts by *Y. pestis* is determined by several factors, including the bacteria's ability to suppress and evade normal immune system responses, such as phagocytosis and antibody production. Flea bites allow the bacteria to bypass the skin barrier. *Y. pestis* expresses a plasminogen activator, which is an important virulence factor for pneumonic plague and can degrade blood clots, facilitating systemic invasion. Many bacterial virulence factors have anti-phagocytic properties. Two key anti-phagocytic antigens, termed F1 (fraction 1) and V or LcrV, are crucial for virulence. These antigens are produced by the bacteria at normal human body temperature. Additionally, *Y. pestis* survives and produces antigens F1 and V while residing within white blood cells, such as monocytes, but not in neutrophils. Natural or induced immunity is achieved through the production of specific opsonic antibodies against the F1 and V antigens; antibodies against F1 and V induce phagocytosis by neutrophils. *Y. pestis* proliferates within lymph nodes, where it can avoid destruction by immune system cells like macrophages. The ability of *Y. pestis* to inhibit phagocytosis allows it to grow in lymph nodes and cause lymphadenopathy.

Depending on the form of plague that a person contracts, the disease manifests differently; however, plague generally affects the host cell's ability to communicate with the immune system, preventing the body from mobilizing phagocytic cells to the site of infection. Overall, *Y. pestis* is a universal killer. Besides rodents and humans, it is known to kill camels, chickens, and pigs. Domestic dogs and cats are also susceptible to the plague, with cats being more frequently affected than dogs. In both cases, symptoms are similar to those experienced by humans and can be fatal for the animal. Humans can be exposed to

infected animals (dead or alive) or by inhaling infectious droplets that a sick dog or cat may cough into the air.

3.3.2. The causative agent of thrush is *Burkholderia mallei*

Burkholderia mallei is a gram-negative, bipolar, aerobic bacterium, the causative agent of humans and animals of the genus Burkholderia, which causes phlegm; the Latin name of this disease (malleus) gave the name of the species that causes it. It is closely related to *B. pseudomallei* and is a subspecies of *B. pseudomallei* using the multilocus sequence set. *B. mallei* evolved from *B. pseudomallei* by selective restoration and deletions from the *B. pseudomallei* genome. Unlike *B. pseudomallei* and other members of the genus, *B. mallei* is not motile; its shape is coccobacillary, measuring approximately 1.5-3.0 μm in length and 0.5-1.0 μm in diameter with rounded ends. Most Burkholderiaceae organisms live in the soil; however, *B. mallei* does not. Because this bacterium is an obligate mammalian pathogen, it must infect a mammalian host in order to live and be transmitted from one host to another.

B. mallei is very closely related to *B. pseudomallei*, being 99 % identical in conserved genes to *B. pseudomallei*. It is possible that *B. mallei* actually evolved from a strain of *B. pseudomallei* after the animal was infected. The bacterium would lose genes that were not necessary for life in the host animal. This suggestion is supported by studies comparing strains of *B. mallei* with *B. pseudomallei* and indicating that their two respective genomes are very similar. It is likely that the genes that allowed the bacteria to survive in the soil environment are the genes that gave *B. mallei* the ability to defend itself against bactericides, antibiotics and antifungal drugs. Thus, the reason *B. mallei* is not found outside the host is because it lacks the genes it needs to survive in soil. Genome comparisons also indicate that *B. mallei* is still evolving and adapting to an intracellular lifestyle.

This bacterium can be cultivated in the laboratory using nutrient agar as a medium. When grown in culture, *B. mallei* grows in smooth, gray translucent colonies. In 18 hours at 37 °C, a colony of *B. mallei* can grow to approximately 0.5-1.0 mm in diameter.

B. mallei is responsible for causing foot-and-mouth disease, which historically mainly affected animals such as horses, mules, and donkeys, and rarely humans. Horses are considered a natural host of the infection and are very susceptible to it. *B. mallei* infects and gains access to its host cell by lysis of the entry vacuole, has bacteria-dependent actin-based protein motility, and enters the cell. It is also able to initiate host cell fusion, resulting in multinucleated giant cells (MNGCs). The effect of MNGC is yet to be determined, but it may allow the bacteria to spread to different cells, evade the immune system of the infected host, or allow the bacteria to persist longer in the host. *B. mallei* is able to survive inside the host's cells thanks to its ability to disrupt the cell's bacteria-killing functions. It leaves vacuoles early, which allows bacteria to reproduce effectively inside the cell. Early cell exit also prevents bacteria from being killed by lysosomal defensins and other pathogen-killing agents. MNGCs can help protect bacteria from immune responses. The ability of *B. mallei* to live inside the host cell makes the development of a vaccine against it difficult and complex.

Human infection with *B. mallei* is rare, although it does occasionally occur among laboratory workers who handle the bacteria or those who are often around infected animals. Bacteria usually infect a person through contact with the eyes, nose, mouth or skin cuts. As soon as people are infected, they develop a fever. They eventually develop pneumonia, abscesses, and abscesses that are fatal within a week to 10 days if not treated with antibiotics. The way the bacteria are infected also affects the type of symptoms that will occur. If the bacteria enter through the skin, local skin infection can occur, and inhalation of *B. mallei* can cause septicemia or lung, muscle, liver, or splenic infection. If left untreated, *B. mallei* infection has a 95 % mortality rate, and 50 % mortality among individuals receiving antibiotics.

In general, *B. mallei* and *B. pseudomallei* already had a history of being listed as potential warfare agents. The Centers for Disease Control and Prevention classifies *B. mallei* as an important biological agent category B. As a result, research on *B. mallei* can only be conducted in biosafety level 3 facilities internationally. Although it is so highly infectious and a potential bioweapon, little research has been done on this bacterium.

3.3.3. The causative agent of Q fever is *Coxiella burnetiid*

Coxiella burnetii is an obligate intracellular bacterial pathogen that causes Q fever. The genus Coxiella is morphologically similar to Rickettsia, but with various genetic and physiological differences. *C. burnetii* is a small gram-negative coccobacillary bacterium that is highly resistant to environmental stresses such as high temperature, osmotic pressure, and ultraviolet light. These characteristics are attributed to the small-cell variant form of the organism, which is part of a biphasic developmental cycle, including the more metabolically and replicatively active large-cell variant form. It can survive standard disinfectants and is resistant to many other environmental changes, such as those presented in the phagolysosome. In general, Coxiella was difficult to study because it could not be reproduced outside of the host. However, in 2009, scientists reported a technique that allows bacteria to grow in axenic culture, and suggested that the technique could be useful for studying other pathogens.

Today, morphologically, it becomes possible to distinguish spore and vegetative forms of coxiels. When applying electron microscopy to vegetative forms, i.e. forms of active growth and reproduction, it becomes clear that vegetative forms have a three-layer shell, to which a layer of granular cytoplasm with lipopolysaccharide, bounded by a plasma membrane, adjoins from the inside. When the infectious process develops, with the depletion of opportunities for further growth and reproduction of the infected cell culture, cocciella enter the spore form, which is characterized by compaction of all elements of the pathogen,

as well as thickening of the shell and folding of the genetic material. The spore forms then change their host environment from the destroyed host cells to the surrounding environment. In the future, they can be phagocytosed again and start their cycle of intracellular development again. Cultivation of this pathogen is carried out only on cell media (most often – in yolk sacs of developing chicken embryos). Laboratory animals such as guinea pigs are most affected by Coxiella. There are 6 strains of *S. burnetii*: Hamilton, Vacca, Rasche, Biotzere, Corazon, Dod, however, the antigenic activity of Burnet's cocciella is not a stable value.

Unlike many other infections, in the case of Q fever, the way a person is infected is very important to the course of the disease, just as in the case of plague or anthrax. The mechanisms of infection of people with coxiellosis are different: transmissible, alimentary, aerogenous, contact. Aerogenic occurs more often due to the fact that it is possible due to inhalation of dust, since cocciella are stored for a long time in a dried state, and can be in dust when working with infected materials. The contact mechanism is most often realized during the calving of animals, since there are a lot of coxiels in the amniotic fluid-1 g of droppings contains up to a billion bacteria. Although the transmission mechanism is rare when infecting people, it can be implemented with the participation of ixodid ticks. The alimentary mechanism of transmission of infection is also considered rare, it occurs through feces-contaminated hands of veterinarians, workers of poultry farms, animal farms, as well as when consuming raw milk, cheese, kefir, meat, water contaminated with the pathogen.

So, it becomes clear that the pathogen can enter the human body in different ways. In the future, the pathogen enters the lymphatic system and stays there for a certain time, after which the pathogens are spread throughout the body, being selectively fixed in the cells of the mononuclear phagocyte system (MPS). The largest number of coxiels settles in the tissues of the liver, spleen, and bone marrow. Coxiella do not penetrate the endothelium, epithelium, nerve and muscle tissue and, as a rule, do not damage them. Next, there is a phase of secondary dissemination, or large coxielemia, when the pathogen reproduces in the Simple

Machines Forum (SMF), and due to toxemia, partial damage to the autonomic nervous system occurs – primarily, the cervical part of the sympathetic trunk and solar plexus. In general, it is possible to prolong the manifestations of the disease for several months. In the formation of a chronic course of coxiellosis, the development of endocarditis with vegetations on the leaflets of the aortic valve most often occurs, but this is possible only in a certain part of patients with background heart damage (acute rheumatic fever, artificial valves, etc.), in drug addicts. A chronic course is possible with the appearance of low fever, weakness, arthralgias, damage to various parenchymal organs during pregnancy, when using immunosuppressants, in recipients of tissue and organ transplants, and HIV infection. It is often possible to manifest productive manifestations of the chronic course many months and even years after the early phase.

3.4. Chemical, biological and medical characteristics of toxins that can be used for the development of toxin weapons

The possibility of using biological weapons for terrorism is a real threat that can not only cause one-time losses of the population, but also spread uncontrollably until its complete control. Biological weapons include pathogens and toxins. Toxins are poisons produced by living organisms. Toxins relevant to bioterrorism include ricin, *Clostridium* and *Botulinum* neurotoxins, conotoxins, shigatoxins, saxitoxins, tetrodotoxins, and mycotoxins.

Toxins have properties of biological and chemical weapons. Unlike pathogenic microorganisms, toxins do not cause infection. Ricin causes polyorgan toxicity by blocking protein synthesis. Botulinum toxin blocks acetylcholine in the peripheral nervous system, resulting in muscle paralysis. Clostridium toxin damages cell membranes. Conotoxins block potassium and sodium channels in neurons. Shigatoxins inhibit protein synthesis and induce apoptosis. Saxitoxin and tetrodotoxin inhibit sodium channels in neurons. Mycotoxins include aflatoxins,

which are carcinogens, and trichothecenes, which inhibit protein and nucleic acid synthesis.

Currently, biological weapons play only a minor role due to their unpredictable effects, difficulties in use, and finally due to ethical reasons. However, it should not be forgotten that terrorist groups can threaten the population with toxins, causing panic and possibly death. This illustrates the ubiquitous threat posed by biological weapons and the need for research to develop effective countermeasures.

3.4.1 Trichothecene mycotoxins

Trichothecenes are a large family of chemically related mycotoxins produced by various species of *Fusarium, Myrothecium, Trichoderma, Trichothecium, Cephalosporium, Verticimonosporium* and *Stachybotrys*. They are produced on various grains such as wheat, oats or maize by *Fusarium* species such as *F. graminearum, F. sporotrichioides, F. poae and F. Equiseti*.

Chemical characteristics. Trichothecenes are a group of more than 150 chemically related mycotoxins. Each trichothecene has a core structure consisting of one six-membered ring containing one oxygen atom surrounded by two carbon rings. This central ring structure contains an epoxide or tricyclic ether at the 12, 13 carbon positions, as well as a double bond at 9, 10.

These two functional groups are primarily responsible for the ability to inhibit protein synthesis and cause general cytotoxic effects. Notably, this core structure is amphipathic, containing both polar and nonpolar parts. All trichothecenes are related by this common structure, but each also has a unique pattern of substitution of oxygen-containing functional groups at possible positions on 3, 4, 7, 8, and 15 carbon atoms. These functional groups determine the properties and also serve as the basis for the most commonly used classification system for this family of toxins, which divides them into four groups: Types A, B, C, and D:

Type A – trichothecenes have substituted hydroxyl, ester groups or do not contain functional groups around the ring structure of the core. For example, neosolaniol with hydroxyl substitution at carbon 8 and T-2 toxin with ester substitution at carbon 8.

Type B – classified by the presence of carbonyl functional groups substituted around the main ring structure. For example, nivalenol and trichothecene, both of which have a ketone functional group at carbon 8.

Type C – have an additional carbon 7, an epoxy group of carbon 8. For example, crotocin.

Type D – have an additional ring between carbon 4 and carbon 15. For example, roridin A and satratoxin H.

Although the individual functional groups of these classification types give each trichothecene unique chemical properties, their classification type does not clearly indicate their relative toxicity. While type D trichothecenes are considered the most toxic, types A and B have relatively mixed toxicity.

Molecules are resistant to heat and cannot be destroyed by ultraviolet light. However, under the influence of sodium hydroxide, the epoxide is hydrolyzed, and the toxins become inactive.

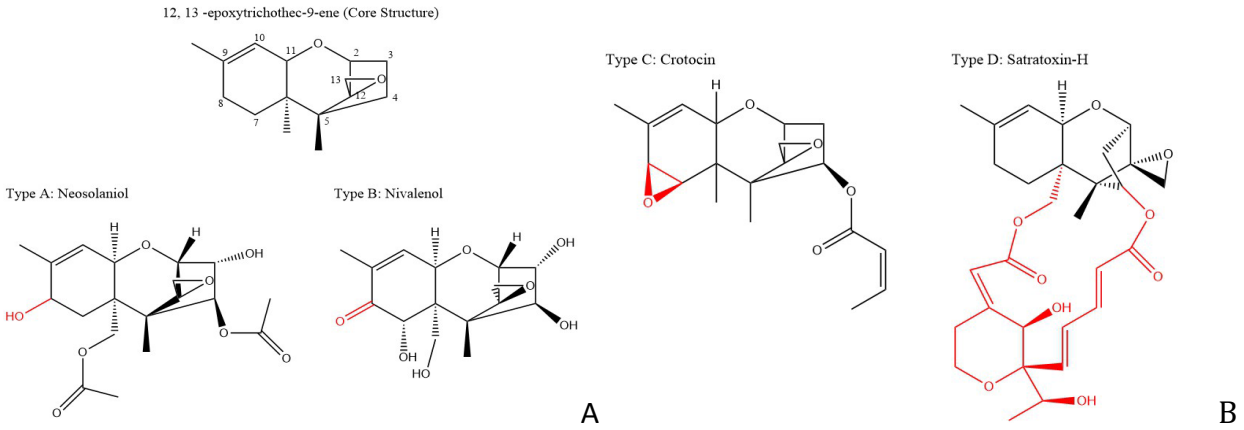

Figure 3.1: The primary structure of all four main types of trichothecenes, with functional groups highlighted in red based on their classification type.

Mechanism of Action: The toxicity of trichothecenes primarily results from their ability to act as inhibitors of protein synthesis. This inhibition occurs at the ribosome during all three stages of protein synthesis: initiation, elongation, and termination.

During initiation, trichothecenes may either inhibit the association of the two ribosomal subunits or block the function of the mature ribosome by preventing the first tRNA from binding to the start codon.

During elongation, they inhibit **peptidyl transferase**, an enzyme that catalyzes the formation of peptide bonds on the 60S ribosomal subunit.

During termination, trichothecenes can inhibit peptidyl transferase or prevent the hydrolysis necessary for completing the protein synthesis process.

In addition to disrupting protein synthesis, trichothecenes affect general cellular enzyme function by targeting thiol groups, which can attack the 12, 13 carbon epoxide ring of trichothecenes. These inhibitory effects are most pronounced in rapidly proliferating cells, such as those in the gastrointestinal tract or bone marrow.

Protein synthesis occurs both in the cell's cytoplasm and mitochondria. This process generates highly oxidized molecules, such as reactive oxygen species (ROS) like hydrogen peroxide, which can damage critical cellular components, including membranes, proteins, and DNA. The inhibition of mitochondrial protein synthesis by trichothecenes leads to an accumulation of ROS, resulting in oxidative stress and the induction of programmed cell death, or apoptosis.

Medical Profile. The specific toxicity of trichothecene mycotoxins varies depending on the toxin, but the **route of exposure** plays a significant role in determining lethality. The severity of poisoning depends on:

- the **concentration** of exposure;
- the **duration** of exposure;
- the **route** of toxin entry into the body.

Highly concentrated solutions or large amounts of gaseous toxin are more likely to cause severe consequences, including death.

Trichothecene mycotoxins can be absorbed through the skin, orally, or by inhalation. They are highly toxic at the subcellular, cellular, and systemic levels. Unlike many other potential chemical weapons, trichothecenes can act through the skin, which is attributed to their amphipathic and lipophilic properties, allowing them to cross cellular membranes easily and interact with various organelles. Their lipophilic nature enables easy absorption through the skin, as well as the mucous membranes of the lungs and intestines. Direct application on the skin or ingestion leads to rapid irritation of the skin or intestinal mucosa. The body's reaction to mycotoxin occurs a few days after consumption, in four stages. The first stage includes inflammation of the mucous membrane of the stomach and intestines. The second stage is characterized by leukopenia, granulopenia and progressive lymphocytosis. The third stage is characterized by the appearance of red rashes on the skin of the body, as well as hemorrhages of the skin and mucous membranes. In severe cases, aphonia and death from suffocation may occur. In the fourth stage, cells in the lymphoid organs and erythropoiesis in the bone marrow and spleen are depleted, and the immune response declines. Infection can be caused by an injury as minor as a cut or scratch.

The following symptoms appear:

- severe itching and redness of the skin, ulcers, peeling of the skin;
- distortion of any of the sense organs, loss of ability to coordinate muscle movements;
- nausea, vomiting and diarrhea;
- pain in the nose and throat, discharge from the nose, itching and sneezing;
- cough, difficulty breathing, wheezing, chest pain and vomiting blood;
- increased body temperature;
- temporary disorders of blood coagulation.

3.4.2 Ricin

Ricin is an AB toxin, where the active (A) part is an enzyme, and the binding (B) part directs the holotoxin (RTAB) from the extracellular compartment into the cytosol, where the substrates are located. Ricin, widely available from the seeds of the castor plant (Ricinus communis), is synthesized as a single polypeptide chain.

Chemical Characteristics. The quaternary structure of ricin is a globular glycosylated heterodimer of approximately 60-65 kDa. The ricin toxin A chain and the ricin toxin B chain have similar molecular weights, 32 kDa and 34 kDa, respectively.

The ricin toxin A chain (RTA) is an N-glycoside hydrolase composed of 267 amino acids. It consists of three structural domains, with approximately 50 % of the polypeptide arranged in alpha-helices and beta-sheets. These three domains form a prominent cleft, which serves as the active site of RTA.

The ricin toxin B chain (RTB) is a lectin composed of 262 amino acids, capable of binding terminal galactose residues on the cell surface. RTB forms a two-lobed structure, resembling a dumbbell, without alpha-helices or beta-sheets. Each lobe contains three subdomains. At least one of the three subdomains in each homologous lobe has a sugar-binding pocket, which gives RTB its functional properties.

While other plants contain protein chains similar to those in ricin, both protein chains must be present to produce toxic effects. For example (fig. 3.2), plants containing only the A protein chain, such as barley, are non-toxic because without the B protein chain, the A chain cannot enter the cell and damage the ribosomes.

The B-chain (RTB), responsible for binding, is an N-glycosylated homodimer shaped like a dumbbell. The A-chain (RTA), the catalytically active part, is connected via a disulfide bridge and crystallizes as a sandwich.

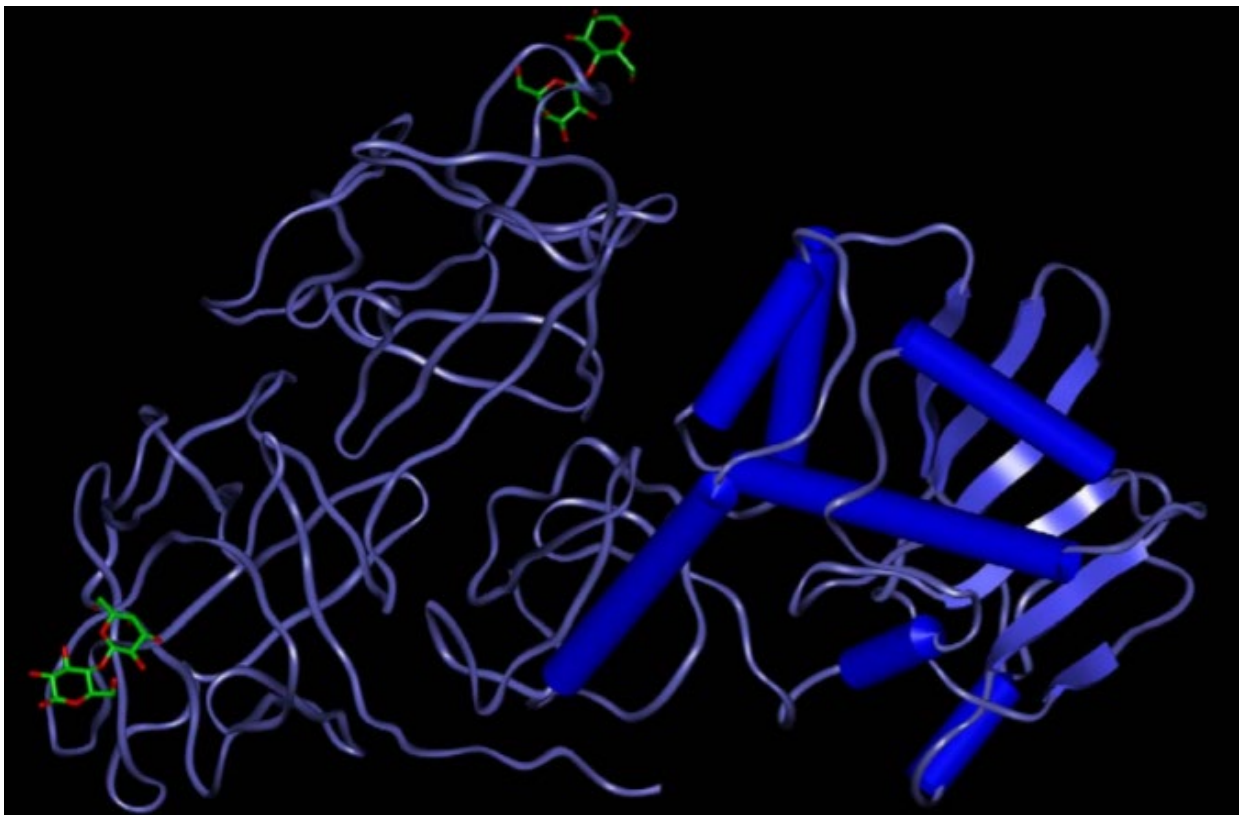

Fig. 3.2. Crystalline Structure of Ricin AB-Toxin

Mechanism of Action. Ricin is highly toxic when inhaled, injected, or ingested. It can also be harmful if dust gets into the eyes or if it is absorbed through damaged skin. Ricin acts as a toxin by inhibiting protein synthesis. It prevents cells from assembling amino acids into proteins according to the instructions they receive from mRNA, disrupting the basic metabolic processes necessary for all living cells. Ricin is resistant to digestion by peptidases, though not entirely immune. When ingested, ricin's pathology is largely confined to the gastrointestinal tract, where it can damage the mucosal lining. With appropriate treatment, most patients recover.

Entry into the Cytoplasm. Ricin’s B-chain binds to complex carbohydrates on the surface of eukaryotic cells, specifically those containing terminal N-acetylgalactosamine or beta-1,4-linked galactose residues. Additionally, ricin's mannose-type glycans can bind to cells that express mannose receptors. RTB binds to the cell surface at a concentration of approximately 10^6-10^8 ricin molecules per cell.

Intracellular vesicles transport ricin to endosomes, which are then delivered to the Golgi apparatus. Since ricin is stable across a wide pH range, degradation in endosomes or lysosomes offers little protection against it. Ricin molecules further penetrate the endoplasmic reticulum (ER).

For ricin to exert its cytotoxic effects, RTA must be reductively separated from RTB to release the steric block at RTA's active site. This process is catalyzed by the protein disulfide isomerase (PDI), located in the ER. Once free, RTA in the ER partially unfolds and penetrates the ER membrane, mimicking a misfolded membrane-bound protein. In the cytoplasm of mammalian cells, RTA is then sorted by cytosolic molecular chaperones Hsc70 and Hsp90, along with their co-chaperones, and a subunit of the proteasome (RPT5), which helps fold RTA into its catalytic conformation. This depurinates the ribosomes, halting protein synthesis.

Ribosome Inactivation. RTA possesses rRNA N-glycosylase activity, responsible for cleaving the glycosidic bond within the large 60S subunit of eukaryotic ribosomal rRNA. RTA specifically and irreversibly hydrolyzes the N-glycosidic bond of the adenine residue at position 4324 (A4324) within 28S rRNA, while leaving the RNA phosphodiester backbone intact. Ricin targets A4324, which is found in a highly conserved 12-nucleotide sequence present across eukaryotic ribosomes. This sequence, 5'-AGUACGAGAGGA-3', called the sarcin-ricin loop, is crucial for binding elongation factors during protein synthesis. The depurination event rapidly and completely inactivates ribosomes, leading to toxicity by inhibiting protein synthesis. A single RTA molecule in the cytoplasm can depurinate approximately 1,500 ribosomes per minute.

Medical Characteristics. Since the symptoms are caused by the inability to produce protein, they may take several hours to days to manifest, depending on the route of exposure and the dose. Ingestion can trigger gastrointestinal symptoms within six hours; within two to five days after exposure, ricin's effects on the central nervous system, adrenal glands, kidneys, and liver become apparent.

Ingestion of ricin causes pain, inflammation, and bleeding in the mucous membranes of the gastrointestinal tract. Gastrointestinal symptoms rapidly

progress to severe nausea, vomiting, diarrhea, and difficulty swallowing (dysphagia). Hemorrhaging results in bloody stools and vomiting blood. Loss of fluid from the gastrointestinal tract can lead to organ failure of the pancreas, kidneys, liver, and gastrointestinal system, and progress to shock. Signs of shock and organ failure include disorientation, stupor, weakness, drowsiness, excessive thirst (polydipsia), low urine output, and blood in the urine (hematuria).

Symptoms from inhaling ricin include coughing and fever. Contact with skin or inhalation of ricin can also trigger allergic reactions, manifesting as eye and lip swelling, asthma, bronchial irritation, dry or sore throat, congestion, skin redness (erythema), blisters, wheezing, itchy and watery eyes, chest tightness, and skin irritation.

The biopharmaceutical company Soligenix, Inc. has licensed a ricin vaccine called RiVax. The vaccine has been found to be safe and immunogenic in mice, rabbits, and humans. Two successful clinical trials have been completed. Soligenix has obtained a U.S. patent for RiVax. The ricin vaccine candidate received orphan drug status in the U.S. and EU, and as of 2019, it was undergoing clinical trials in the USA.

3.4.3 Shiga Toxin (Shiga toxin)

Shiga toxins are a family of related toxins divided into two main groups, Stx1 and Stx2, which are expressed by genes thought to be part of the lambdoid prophage genome. The toxins are named after Kiyoshi Shiga, who first described the bacterial cause of dysentery by *Shigella dysenteriae*.

Chemical Characteristics. The toxin consists of two subunits – labeled A (molecular mass 32,000 Da) and B (molecular mass 7,700 Da) – and belongs to the AB5 toxin group. AB toxins are bipartite protein complexes: the “A” component is typically the “active” part, while the “B” component is the “bindin” part. The designation AB5 indicates that the toxin has one active subunit and five binding subunits. The B subunit forms a pentamer that binds to specific glycolipids of the

host cell, particularly globotriaosylceramide (Gb3). After binding, the A subunit is internalized and cleaved into two parts. The A1 component then binds to the ribosome, disrupting protein synthesis. It has been established that Stx-2 is approximately 400 times more toxic than Stx-1.

Gb3 is present in higher quantities in renal epithelial tissues, which may explain the renal toxicity of Shiga toxin. Gb3 is also found in neurons of the central nervous system and endothelial cells, which can lead to neurotoxicity. Additionally, Stx-2 is known to increase the expression of its receptor GB3 and cause neuronal dysfunction.

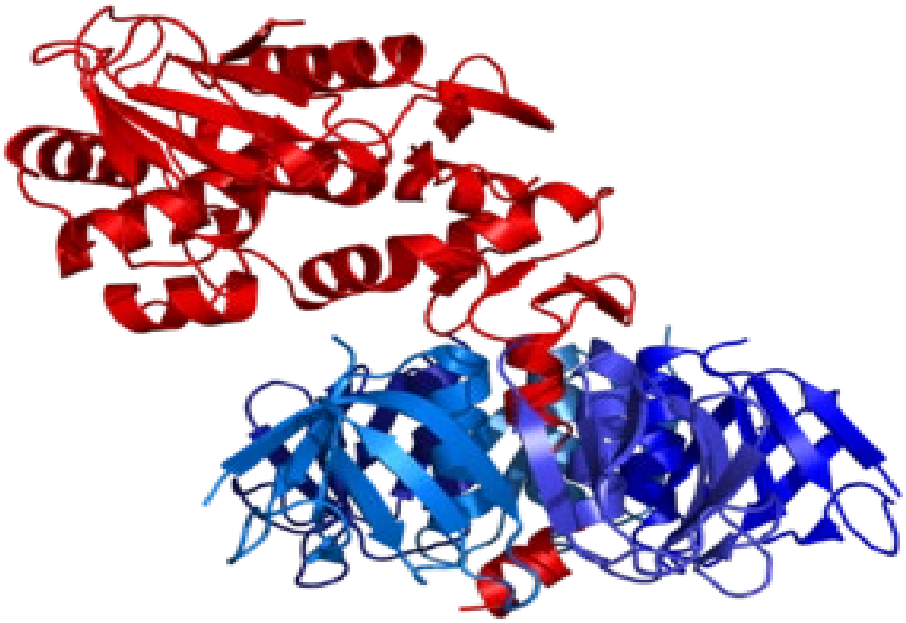

Figure 3.3. SLT2 from *E. coli* O157: H7. The A subunit is shown in red (top), while the B subunits, forming a pentamer, are depicted in various shades of blue (bottom).

Mechanism of Action. The B subunits of Shiga toxin bind to a component of the cell membrane known as the glycolipid globotriaosylceramide (Gb3). This binding induces the formation of narrow tubular membrane invaginations, which lead to the creation of internal membrane channels for bacterial uptake into the cell. Shiga toxin is transported into the cytosol through the Golgi apparatus and the endoplasmic reticulum (ER). The toxins inhibit protein synthesis in target cells by

a mechanism similar to that of the plant toxin ricin. Once inside the cell via macropinosomes, the A subunit cleaves a specific adenine base from 28S rRNA of the 60S ribosomal subunit, thereby halting protein synthesis.

Medical Characteristics. Shiga toxin requires highly specific receptors on the cell surface for attachment and entry into the cell. Species such as cattle, pigs, and deer, which do not carry these receptors, can harbor toxigenic bacteria without suffering negative effects, shedding them in their feces, from which they can spread to humans.

Symptoms of Shiga toxin ingestion include abdominal pain and watery diarrhea. In severe, life-threatening cases, hemorrhagic colitis (HC) is characteristic. The toxin primarily targets small blood vessels, such as those in the digestive tract, kidneys, and lungs, but not larger vessels like arteries or major veins. A specific target of the toxin is the glomerular endothelial cells, which are crucial for kidney function. Destruction of these structures leads to kidney failure and the development of the often fatal and debilitating hemolytic uremic syndrome (HUS). Food poisoning from Shiga toxin often also affects the lungs and nervous system.

3.4.4. Botulinum Toxins (BoNT)

Botulinum toxin (BoNT) is a neurotoxic protein produced by the bacterium *Clostridium botulinum* and related species. It prevents the release of the neurotransmitter acetylcholine from axon terminals at the neuromuscular junction, causing flaccid paralysis. The toxin leads to the disease known as botulism.

Chemical Characteristics. There are seven serologically distinct BoNTs, labeled A–G, belonging to the clostridial neurotoxins (CNT) group, and they are produced by various strains of *C. botulinum*, *C. baratii*, and *C. butyricum*. These toxins, along with the closely related tetanus neurotoxin (TeNT) synthesized by *C. tetani*, are initially produced as single-chain proteins of approximately 150 kDa. They are subsequently cleaved by endogenous proteases into a heavy chain (HC)

of ~ 100 kDa and a light chain (LC) of ~ 50 kDa. These chains remain linked by a disulfide bridge and non-covalent interactions. Therefore, CNTs are classified as AB toxins.

Crystallographic studies of BoNT/A and BoNT/B have revealed that all CNTs consist of three functionally independent domains, each performing distinct roles in the multi-step intoxication process (fig. 3.4).

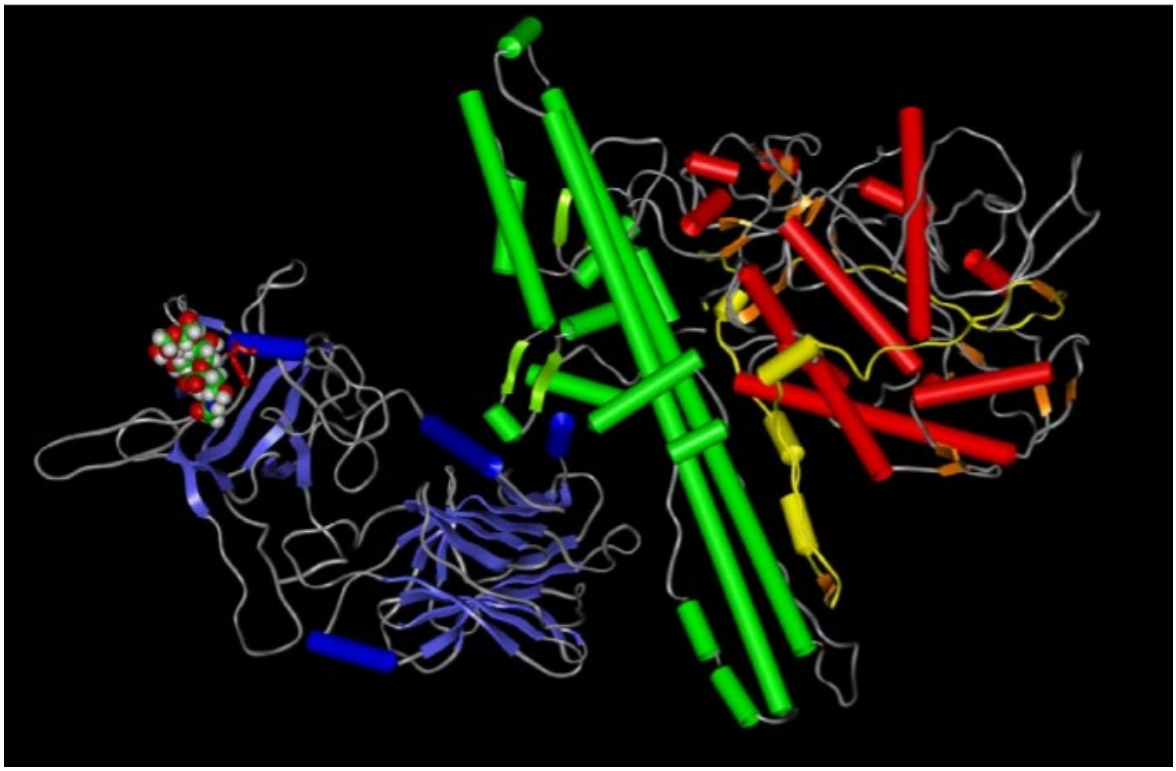

Figure 3.4. Crystal structure of BoNT/B and sialyllactose.

The 50 kDa HC fragment is responsible for neuro-specific binding (far-left domain). It contains two subdomains, with the C-terminal half possessing a ganglioside-binding pocket, as indicated by the binding of a sialyllactose molecule (space-filling structure). The 50 kDa translocation domain (HN, middle domain) is highly helical and contains the 50 kDa light chain (LC, far-right domain), which acts as a Zn^{2+} – dependent endoprotease. The LC is connected by a disulfide bridge and a polypeptide chain.

Mechanism of Action. *Botulinum toxin* works by cleaving key proteins essential for nerve activation. First, the toxin specifically binds to nerves that

utilize the neurotransmitter acetylcholine. Once bound to the nerve terminal, the neuron internalizes the toxin in a vesicle through receptor-mediated endocytosis. As the vesicle moves inward, it acidifies, activating the part of the toxin that enables it to push through the vesicle membrane into the cell's cytoplasm. Once inside, the toxin cleaves SNARE proteins (which mediate vesicle fusion with their target membrane compartments), preventing acetylcholine vesicles from binding to the intracellular membrane. This blockade stops the release of acetylcholine, halting nerve signaling and resulting in paralysis.

Medical Characteristics. Botulinum neurotoxins (BoNTs), which cause three forms of natural botulism in humans (foodborne, wound, and intestinal), are among the most potent agents known. The estimated median lethal dose (MLD) of BoNT/A for humans is approximately 0.3 ng/kg when administered intravenously, 20 ng/(min/m^3) via inhalation, and 1 g/kg orally. Experiments with primates have shown increased toxicity when BoNT is introduced into the body through the respiratory route.

The lethal dose is highly dependent on the degree of purity. The purer the neurotoxin, the less toxic it is when taken orally. For instance, pure BoNT/A is nearly 100,000 times less toxic than the BoNT/A complex when ingested. This paradoxical behavior is rooted in the complex composition of the neurotoxin complex. In the case of foodborne infection, BoNT, being a protein, must withstand the low pH in the stomach and the attack of pancreatic enzymes in the upper part of the small intestine before being absorbed in the lower intestinal tract.

Safe passage through the hostile environment of the gastrointestinal tract is ensured by accompanying proteins, which include various hemagglutinins and a non-toxic non-hemagglutinating protein (NTNH) weighing 120 kDa. Together with BoNT, they form various complexes, also known as precursor toxins, that are resistant to proteases and acid but break down immediately at physiological pH. Hemagglutinin acts as an adhesin, allowing the precursor toxin to bind to intestinal epithelial cells and red blood cells. However, when administered orally, the pure neurotoxin loses much of its toxicity as it is nearly completely degraded before

absorption. The same holds true for inhalation of the neurotoxin, as the surface of the lung mucosa is rich in proteolytic activity. Nevertheless, pure neurotoxin does not lose its toxicity when administered parenterally, for example, intraperitoneally, as protease protection is unnecessary in this case.

3.5. Epizootics and epiphytotics: biological and epidemiological characteristics of pathogens

Epidemic processes have a significant impact not only directly on the health of disease carriers but also on the economy, demographic indicators, quality of life, biological diversity, and even on social and cultural aspects of life. For the survival of humanity, it is necessary to control the agents of epidemic processes to mitigate the risks of negative effects on the stable development of quality of life in the world. In particular, such control is essential for the agents of epiphytotics and epizootics, as they are factors in global catastrophes such as famine, climate change, and economic crises. To adequately control the risk of outbreaks of epidemic processes, it is necessary to provide a characterization of potential agents involved. This characterization should include data on the biological origin of the pathogen, its natural reservoir, modes of transmission within populations, pathogenesis, existing methods for combating the disease, and for epizootics, whether the disease is zoonotic.

Epiphytotics. Rice blast, potato late blight, cereal rusts and Panama disease of bananas are among the most influential epiphytotics today. They negatively impact the yield of major agricultural crops, which can lead to malnutrition, famine, and economic crises in some countries that depend on the export of these crops.

3.5.1. Biological and Epidemiological Characteristics of Pathogens

Rice blast. Rice blast is caused by the filamentous ascomycete fungus *Magnaporthe oryzae*, which has recently been identified as a new species distinct from *Magnaporthe grisea* based on multilocus genealogy and mating experiments. It is noted that the M. oryzae strains infecting rice originated from a single point and then spread worldwide as rice cultivation expanded, which began in the Middle Yangtze Valley in China around 7000 years ago. To invade rice cells, the fungus *Magnaporthe oryzae* forms special cells known as appressoria, which generate pressure and physical force to rupture the leaf cuticle. The formation of the appressorium is regulated by the progression of the cell cycle with the emergence of a germ tube from a three-celled conidium followed by the migration of one nucleus into the developing germ tube where it undergoes mitosis approximately 4-6 hours after germination. After mitosis, one daughter nucleus enters the developing appressorium, while the other returns to the conidium. Subsequently, the three nuclei in the conidium break down along with the remaining contents of the spore, leaving a single nucleus in the mature appressorium. Later, the nucleus of the appressorium migrates into the penetrating peg, where it undergoes further rounds of mitosis as invasive hyphae develop (see fig. 3.5.).

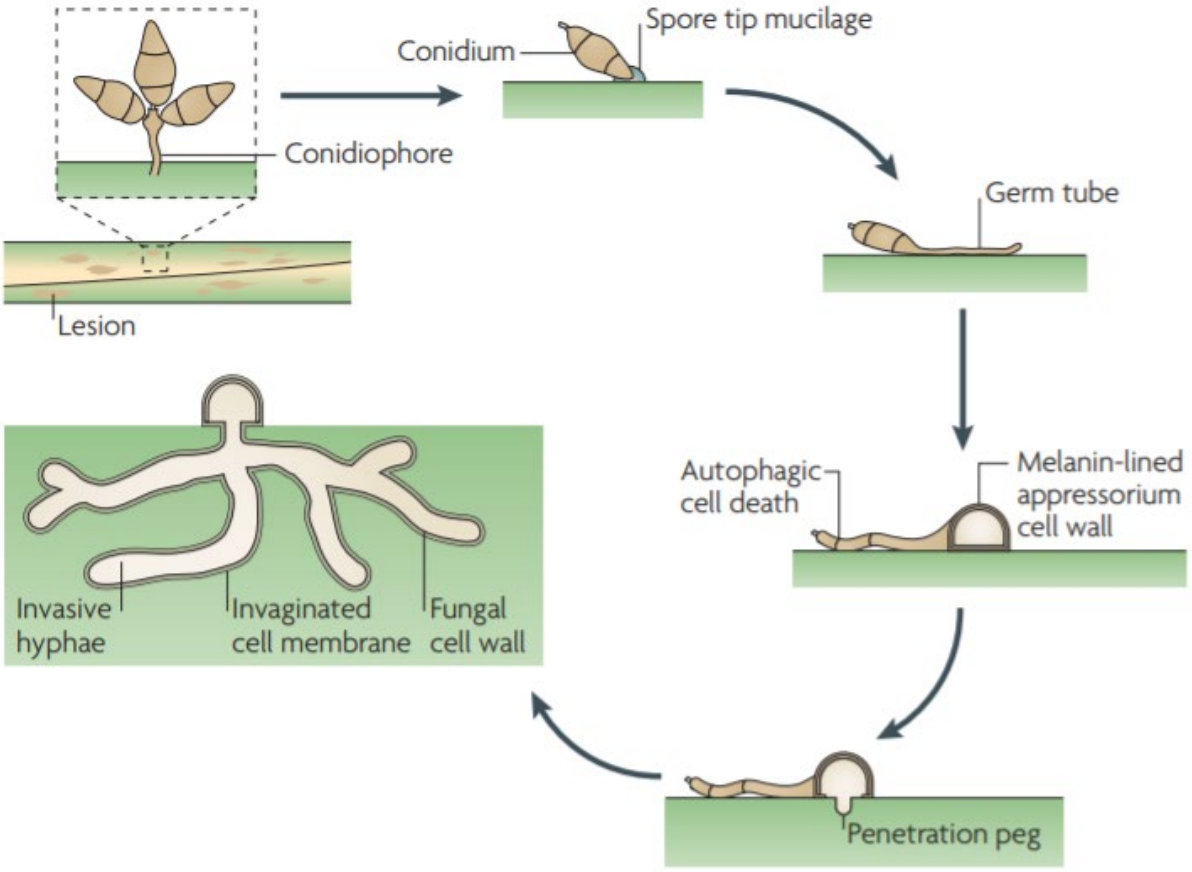

Fig. 3.5. Life Cycle of *M. oryzae*

The appressorium of *M. oryzae* is well known for generating sufficient turgor and physical force to penetrate the cuticle. Understanding how this is achieved in the absence of external nutrients is an intriguing physiological question. Hydrostatic turgor in the appressorium is generated by the accumulation of glycerol, which is obtained from compounds stored in the spore, such as mannitol, glycogen, lipids, and trehalose. Lipid bodies are mobilized during the formation of the appressorium and enter vacuoles, where they undergo rapid lipolysis. The transfer of lipid bodies depends on the Pmk1 MAPK genes, and lipolysis occurs under the action of up to seven intracellular triacylglycerol lipases. Lipase activity releases glycerol directly from fatty acid residues, but the catabolism of fatty acids through β-oxidation is also important for appressorium function, indicating that β-oxidation of peroxisomal fatty acids, which produces

acetyl-CoA, is vital for appressorium function. During the fungal infection process, acetyl-CoA derived from fatty acids can be utilized, for example, in secondary metabolic pathways, such as the biosynthesis of melanin and polyketides. The maturation of the appressorium in *M. oryzae* involves the deposition of melanin and chitin within the inner side of the cell wall. Melanin plays a structural role in reinforcing the appressorium and forms an impermeable layer to prevent the leakage of osmolytes, which generate significant internal turgor (up to 8.0 MPa) necessary for penetrating the cuticle. Melanin biosynthesis mutants cannot generate sufficient internal turgor due to leakage from the appressorium, and therefore are non-pathogenic.

The mechanical nature of fungal penetration into the host plant contradicts the fact that after breaching the leaf cuticle, *M. oryzae* develops a close interaction with living plant cells and asymptomatically, biotrophically proliferates during the first 72 hours of infection. How *M. oryzae* is able to evade or suppress the plant's defense forces is unknown, although the process likely involves the active modulation of the plant defense response by molecules derived from the fungus. The genome sequence of *M. oryzae* suggests a large number of secreted proteins that could be used in this capacity as effectors, but it remains unclear whether they are secreted into the apoplastic space between the fungal cell wall and the invaginated plant plasma membrane, or delivered directly into the plant cytoplasm.

Recently, *M. oryzae* has gained status as a model organism for studying plant diseases caused by fungi, largely due to its economic importance and the experimental capabilities of the fungus. Importantly, *M. oryzae* shares many characteristics related to other significant pathogens of cereal crops, such as appressorium formation and invasion of intracellular tissue. This opens up the possibility of searching for common processes and disease determinants that can be targeted for intervene in a wide range of crop diseases.

3.5.2. Late Blight of Potatoes

Phytophthora infestans is an oomycete or water mold, a fungus-like microorganism that causes a serious disease in potatoes and tomatoes known as late blight or potato blight. Early blight, caused by *Alternaria solani*, is also often referred to as “potato blight”. Late blight was the main culprit in the European famines of the 1840s, particularly the Irish Potato Famine from 1845 to 1852. This organism can also infect some other members of the nightshade family. The pathogen thrives in moist, cool environments: sporulation is optimal at 12-18 °C in water-saturated or nearly saturated conditions, while zoospores are favored at temperatures below 15 °C. The rate of lesion growth is generally optimal at slightly warmer temperatures ranging from 20 to 24 °C.

The mycelium of the oomycete *Phytophthora infestans* produces haustoria, which grow in the intercellular space of the plant and do not penetrate the cell cytoplasm as they remain protected by the cell membrane. The haustorium produces effectors, proteins that the potato plant recognizes or fails to recognize as they pass through the cell membrane. The success of *P. infestans* as a pathogen stem from its effective reproduction in both asexual and sexual forms. In its asexual form, *P. infestans* produces thousands of sporangia for each lesion on sporangia-producing structures, which are indefinite structures that facilitate the distribution of sporangia in the air through passive movement by wind, rain, or wind-driven currents. Sporangia can germinate directly at temperatures above 15 °C and rapidly develop mycelial growth and subsequent sporangia on the tissues of leaves, stems, and fruits. At lower temperatures, sporangia refrain from growing mycelium, directly forming and releasing zoospores (asexual spores), which then germinate and cause new infections at even faster rates. Depending on environmental conditions, the regeneration time can be short, with the entire cycle repeating every 5 to 7 days.

The presence of both mating types allows for sexual recombination and the production of new, potentially more aggressive isolates. This has also made disease

management efforts even more complicated. Therefore, control of late blight is mandatory. Common methods used to combat infection include cultural practices, fungicide sprays, and the use of resistant varieties. Managing late blight solely through cultural practices can be very challenging, and *P. infestans* can be particularly destructive in areas where both tomatoes and potatoes are grown year-round, such as in the highland tropics of Africa, South America, Asia, and Europe. In recent years, the popularity of chemical control has increased, and two main groups of compounds are regularly used, including protective agents (e.g., chlorothalonil, dithiocarbamates, and tri phenyl tin hydroxide) and systemic fungicides (e.g., phenylamides such as metalaxyl/mefenoxam, nitrogen aliphatic fungicides such as cymoxanil, and morpholine fungicides such as dimethomorph). Chemical treatment can also be ineffective, especially when environmental conditions are highly favorable for disease development. Moreover, improper application of phenylamides has created selective pressure on the pathogen, leading to the emergence of phenylamide-resistant isolates of *P. infestans* in many countries. These issues, along with considerations of economic and environmental safety, necessitate careful adoption of effective and sustainable disease management strategies, including the development and integration of commercially acceptable varieties with genetic resistance to *late blight*.

3.5.3. Cereal Rust

Striped rust and stem rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst) and *P. graminis* f. sp. *tritici* (Pgt), respectively, are among the most significant wheat diseases worldwide, posing a major threat to global wheat production. Devastating epiphytotics can occur over vast areas within weeks if susceptible varieties are widely grown and weather conditions are favorable for rust. Recently, striped rust has become one of the most destructive diseases in wheat. In recent years, this disease has emerged as one of the largest biotic constraints on wheat production, threatening global food supply. Since 1999, five destructive

epiphytotics of striped rust have occurred in Central Asia, with outbreaks particularly severe in Uzbekistan, Turkey, and Iran. In 2009 and 2010, serious outbreaks occurred in Central and Western Asia and North Africa, indicating that the disease requires international attention.

Both striped rust and stem rust pathogens are macrocyclic heteroecious fungi with a complete life cycle consisting of five spore stages. They have wheat and other cereal crops as primary hosts, while grasses serve as alternate hosts for infection with urediniospores and aeciospores and for the production of teliospores and basidiospores. Barberry (*Berberis* spp.) and mahonia (*Mahonia* spp.) serve as alternative hosts for the infection of basidiospores and the production of pycniospores and aeciospores.

Several studies on the molecular characterization of Pgt populations using single wheat samples have shown that sexual recombination occurs in some Asian countries, such as China, Nepal, Pakistan, and Turkey. Currently, natural infection of barberry by Pgt has only been detected at low frequencies in China. However, in Europe and North America, barberry plants have not been found infected with Pgt, but are heavily infected with Pgt. In the Pacific Northwest of the USA, Pgt basidiospores can infect barberry, providing aeciospores for the infection and epidemic of wheat and barley stem rust. However, Pgt cannot infect barberry due to the degradation of teliospores and the unfavorable phenology of barberry. Thus, weather factors play an important role in reducing infection of barberry by both pathogens. The occurrence of rust in wheat, other cereals, and grasses, as well as the presence of barberry plants in various geographical regions, particularly in Asia and Europe, is well known. The role of barberry in stem rust epidemics has been established for a long time. However, the importance of barberry species as alternative hosts for Pgt and potential habitats for barberry that serve for sexual reproduction, as well as their role in disease recurrence, have yet to be determined.

Rust epidemics are heavily influenced by weather conditions and cropping systems. Weather affects not only survival, infection, growth, and asexual reproduction but also impacts various stages throughout the complete sexual cycle,

particularly the survival of teliospores, germination, and infection of basidiospores on barberry. However, synchronization between the susceptible stage of alternate hosts and viable teliospores, along with favorable weather conditions, is critical for infection of alternate hosts by basidiospores and subsequent infection of cereals by aeciospores and urediniospores.

While the susceptibility of many barberry species has been demonstrated under controlled conditions, natural infection relies on several additional factors. First, teliospores must germinate when susceptible tissues of alternate hosts are present and must come into contact with germinating basidiospores under favorable weather conditions. Second, susceptible wheat plants must be available within the dispersal range of aeciospores during the seedling formation on alternate hosts. Third, weather conditions must be suitable for the infection of cereal crops by aeciospores. Therefore, predicting or assessing the infection of barberry by basidiospores based on weather conditions is vital for determining the role of alternate hosts in rust epidemics.

However, to date most studies have focused on urediniospore infection without linking it to barberry infection. To predict basidiospore infection, in addition to optimal temperatures, the duration of leaf wetness (32 hours) is a crucial criterion for infection by barberry.

3.5.4. Panama Disease of Bananas (Fusariosis)

Fusarium wilt (fusariosis) is one of the most destructive diseases of bananas. The pathogen likely originated in Southeast Asia, but the disease was first recognized in other locations. The initial description by Bancroft (1876) from Australia was accompanied by reports from tropical America (Costa Rica and Panama in 1890). A sharp increase in new records occurred in the early 1900s, most of which described damage to export plantations. Currently, the disease is found virtually everywhere bananas are cultivated. Fusarium wilt is caused by

Fusarium oxysporum f. sp. *cubense* (Foc) (tropical race 4), a fungus that affects a wide range of hosts of any age.

Symptoms of fusariosis begin with the yellowing and wilting of older leaves, progressing to younger leaves until the entire plant dies. Internally, plants with advanced infections show discoloration of the rhizome and necrosis of the xylem vessels in the pseudostem. Foc is a soil-borne pathogen that produces chlamydospores, allowing the fungus to persist in the soil in the absence of a host. Once the soil is infected, susceptible varieties cannot be successfully replanted for 30 years. As a result, fusarium wilt destroyed the banana industry based on the Gros Michel variety in Central America in the mid-20th century, forcing trade to shift to resistant varieties of the Cavendish subgroup. Cavendish varieties addressed the issues of banana export trade from Latin America, where tropical race 4 (TR4) is absent, but not in Asian countries where TR4 is present. Thus, fusarium wilt remains a limitation for susceptible varieties and continues to be considered a major threat to banana production, as, unlike black leaf streak disease, it cannot be controlled with fungicides.

Control strategies for TR4 are based on visual monitoring for early symptom emergence, eradication of infected plants, and isolation of affected areas to reduce the spread of pathogens. However, these strategies are often impractical and therefore not implemented. Additionally, identification is complicated by the aforementioned concept of race, which does not adequately account for genetic variations. As a result, alternative characterization strategies have been introduced. Analysis of the vegetative compatibility group (VCG) and phylogenetic studies based on molecular data have revealed greater genetic variations in Foc. Thus, VCG testing is useful for diagnosing TR4 but requires labor-intensive formation and characterization of mutants and the availability of testers.

3.5.4. Epizootics

The most influential epizootics currently include diseases such as Newcastle disease, foot-and-mouth disease, rabies, and leptospirosis. The first two diseases have a global impact on the food supply for humanity and can therefore cause hunger and economic crises. The latter two diseases are severely debilitating and are managed with palliative therapy. Both diseases are zoonotic, unlike foot-and-mouth disease and Newcastle disease, making it quite challenging to develop an effective vaccine that provides long-lasting active immunity. Therefore, these two diseases pose a significant risk to the healthcare system in the event of large-scale epizootics that could escalate into epidemics.

3.5.5. Newcastle Disease (ND)

Newcastle Disease (ND) is one of the most serious infectious diseases affecting birds, and virulent outbreaks of ND require reporting to the World Organisation for Animal Health (OIE) by member countries. The etiological agent, Newcastle Disease virus (NDV), belongs to the genus *Avulavirus* in the *Paramyxoviridae* family within the *Mononegavirales* order, and is referred to as avian paramyxovirus 1 (APMV-1), one of the nine identified serotypes of APMV. The enveloped virus has a negatively-sensed single-stranded RNA genome that encodes six proteins, including the nucleocapsid, phosphoprotein, matrix (M), fusion (F), hemagglutinin-neuraminidase, and RNA-dependent RNA polymerase proteins. Currently, many genetically diverse strains of NDV circulate worldwide. Chickens are highly susceptible to virulent APMV-1, while ducks and geese can be infected but show few or no clinical signs. Human contact with infected birds (e.g., in poultry farms) can result in mild conjunctivitis and flu-like symptoms, but Newcastle virus poses no threat to human health.

Symptoms of the disease include (fig. 3.6):

- sudden death;

- lack of energy and appetite;
- swelling around the eyes and neck;
- decreased activity, tremors, drooping wings, twisting of the head and neck, dizziness, complete stiffness;
- greenish, watery diarrhea;
- decreased egg production or soft eggs, deformed eggs;
- sneezing, shortness of breath, runny nose, cough.

Figure 3.6. Symptoms of Newcastle Disease in Chickens

First observed in Java, Indonesia, in 1926, NDV has since spread worldwide, and outbreaks continue to occur and persist in domestic poultry. There are five pathotypes that classify different virus strains: viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic, and asymptomatic enteric. Virulent strains can lead to up to 100 % mortality in unvaccinated NDV-infected chickens, while vaccination can help induce cross-protective antibodies. However, limited access to vaccines and the lack of proper infrastructure in low-income countries restrict the protection that vaccines can provide to prevent ND outbreaks. These limitations underscore the increasing need for a better understanding of the

fundamental molecular mechanisms of host response to NDV infection, particularly in the respiratory system, where NDV replicates, to develop new therapeutic strategies and improve current prevention methods against NDV.

Local mucosal immunity in the respiratory tract plays a crucial role in defending against respiratory pathogens. The trachea is one of the essential tissues/organs due to its role in the early replication of viruses and immunity against various pathogens, such as NDV, avian influenza, and mycobacteria. Additionally, epithelial cells in the respiratory tract are one of the primary sites of NDV replication, and the transport of infected NDV epithelial cells is a significant factor in presenting viral antigens to antigen-presenting cells.

3.5.6. Rabies

Rabies is an acute, progressive, incurable viral encephalitis. The causative agents are neurotropic RNA viruses from the family Rhabdoviridae, genus Lyssavirus. Reservoirs among mammals include carnivores and bats, but rabid dogs still pose the greatest threat worldwide. The viral transmission occurs primarily through animal bites, and once the virus settles in peripheral wounds, it undergoes centripetal passage to the central nervous system. After virus replication, there is centrifugal spread to the primary exit portals, the salivary glands. The epidemiological significance of any rabies reservoir state remains highly speculative. Although incubation periods average 1 to 3 months, the disease has been reported to appear days or years after exposure.

Rabies should be suspected in patients with a history of animal bites and traditional clinical manifestations, but the absence of such clues makes the diagnosis postmortem a challenge. The pathogenetic mechanisms remain inadequately studied, and current assistance involves only palliative measures.

Rabies is an unpredictable disease, with the only characteristic feature being its non-specificity in presentation. For practical purposes, regardless of the species, the primary cardinal signs are similar and may include: subfebrile temperature,

lack of appetite, paresthesia, ataxia, agitation, altered mentation, and, inevitably, paralysis, coma, and death. It is believed that specific symptoms such as hydrophobia and aerophobia are limited to humans. However, this belief may reflect subjective interpretation allowed by single-species communication.

Modern medical attention largely focuses on preventing exposure and intervening before clinical onset. Prevention encompasses careful wound management, vaccination, and the administration of rabies immunoglobulin. While rabies is a significant zoonosis, canine rabies can be eradicated, and the application of new vaccine technologies allows for substantial disease control among wildlife species. Nevertheless, despite significant technical advances in the past century, rabies remains a disease of negligence and represents a contemporary puzzle for public health.

3.5.7. Leptospirosis

Leptospirosis is a blood infection caused by bacteria of the genus *Leptospira*. It is a widespread and potentially fatal zoonosis that is endemic in many tropical regions and can cause large epidemics following heavy rainfall and flooding. Infection occurs through direct or indirect contact with infected reservoir host animals that carry the pathogen in their renal tubules and shed pathogenic leptospires in their urine. While many wild and domestic animals can serve as reservoir hosts, the brown rat (*Rattus norvegicus*) is the most significant source of human infection.

Individuals living in urban slums characterized by inadequate sanitation and poor housing are at a high risk of exposure to rats and leptospirosis. The global burden of leptospirosis is expected to increase due to demographic shifts leading to more impoverished cities in tropical regions, exacerbated by worsening storms and urban flooding due to climate change. Data from prospective epidemiological studies indicate that most human leptospiral infections in endemic areas are mild or

asymptomatic. The development of more severe outcomes likely depends on three factors: epidemiological conditions, host susceptibility, and pathogen virulence.

Leptospirosis typically presents as a non-specific acute febrile illness characterized by fever, myalgia, and headache, which can be confused with other diseases such as influenza and dengue fever. New diagnostic methods facilitate early diagnosis and treatment with antibiotics. Mortality remains significant, related both to delays in diagnosis due to a lack of infrastructure and adequate clinical suspicion, as well as other poorly understood reasons, which may include inherent pathogenicity of certain *Leptospira* strains or genetically determined immunopathological responses in the host. Pulmonary hemorrhage is increasingly recognized as a major, often fatal manifestation of leptospirosis, the pathogenesis of which remains unclear.

The completion of genome sequencing for *Leptospira interrogans* serovar *lai* and other ongoing projects in leptospiral genome sequencing promise to guide further work on the disease. Current mainstays of treatment include tetracyclines and β-lactams/cephalosporins. There is no vaccine available. Prevention largely depends on sanitary measures, which can be difficult to implement, especially in developing countries.

3.5.8. Foot-and-Mouth Disease (FMD)

Foot-and-mouth disease (FMD) is a highly contagious disease affecting cloven-hoofed animals. The disease was first described in the 16th century and was the first animal pathogen identified as a virus. The etiological agent is the foot-and-mouth disease virus (FMDV), which belongs to the family *Picornaviridae*. FMD is rarely zoonotic; most humans can become mechanical carriers of the virus for up to 36 hours but do not become viremic. However, about 40 cases of human infection have been reported, all in individuals who had close contact with infected livestock. Clinical signs in humans included fever, malaise, and oral vesicles, which typically resolved within a week. Due to the rarity of human infection, FMD

does not raise significant public health concerns and is not classified as a zoonotic disease.

There is a clear difference in susceptibility to FMD depending on the host species and viral serotype. Among susceptible species, differences in the severity of infection can exist based on the viral load at inoculation, the involved serotype, the affected species, and the individual animal's immunity. A wide range of clinical symptoms can manifest, from subclinical infections, as commonly observed in African buffaloes, to acute lethal infections with significant pancreatic pathology, as seen in mountain gazelles.

FMD virus is epithelial-trophic, and typical lesions include vesicles that rupture, leaving erosions or ulcers, leading to lameness or difficulty in feeding. Lesions often occur in the oral cavity (tongue, dental pad) and in the coronary bands of bovines and interdigital spaces in suids and cervids. Lesions may also occur on the snout or knees in wild boar. Animals typically recover from acute infection within 7-14 days; however, carrier states can persist in some species. Additionally, a chronic post-viremia syndrome has been described, which may include secondary skin infections, hoof development defects, decreased milk production, and heat intolerance.

Transmission usually occurs via aerosolization of the virus. Although mucosal abrasions may also lead to infection, approximately 10,000 times more virus is required to establish infection through this route. Aerosol spread is often associated with wildlife, but the precise transmission routes are uncertain, as it is challenging to determine the contribution of other potential transmission methods (e.g., fomites or waterborne animals). The virus has been isolated from milk, semen, urine, and feces.

Replication occurs rapidly, with many experimentally infected species demonstrating the virus in respiratory secretions within 24 hours post-infection and in epithelial cells of lesions within 72 hours. The incubation period ranges from 2 to 14 days, depending on the infection dose and transmission route. In domestic pigs, infection typically occurs through feeding contaminated pig feed or direct

contact with infected animals or fomites. Pigs are less susceptible to aerosol transmission than cattle; however, they shed the highest amount of aerosolized virus.

The impact of foot-and-mouth disease is not uniform across the world. The losses from foot-and-mouth disease in production have a significant impact on the poorest regions of the world, where more and more people are directly dependent on livestock. Foot-and-mouth disease reduces herd fertility, leading to less efficient herd structures and discouraging the use of high-yield breeds that are sensitive to the disease. Overall, direct losses limit livestock productivity, which affects food security.

In countries where control programs are constantly implemented, managing and controlling foot-and-mouth disease incurs substantial costs. These control programs are often difficult to discontinue due to the risk of new outbreaks. The presence or even the threat of foot-and-mouth disease hinders access to profitable international markets. In countries free from foot-and-mouth disease, outbreaks occur periodically, and the costs of restoring disease-free status are enormous.

The modern world is filled with various risks of epidemic processes emerging, influenced by many factors such as climate change, the development of resistance of pathogens to herbicides/fungicides, the inadequate effectiveness of vaccines, viral mutagenicity, and more. However, with proper monitoring of these indicators and establishing connections between these factors, it is possible to prevent epidemic outbreaks in advance. Additionally, preventive methods for combating such diseases can be controlled through genetic engineering techniques.

The biological and epidemiological characteristics of the pathogens of the epiphytotics and epizootics described above allow biosafety experts to analyze the existing situation and understand how a particular disease might impact society, enabling timely preventive measures. These characteristics also determine the scope for further work by sustainable development specialists and researchers who handle large volumes of information to analyze gaps in the health sector.

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