DOI 10.56871/RBR.2024.35.28.010
UDC 616.98+579.843.98+579.852.11-036.22-092-07-08-084+619+623.458

ANTHRAX: NEAR AND FAR

© Nadezhda S. Kozlova[1], Dmitry P. Gladin[2], Aleksandr M. Korolyuk[2, 3], Ilya A. Baranov[1]

[1] North-Western State Medical University named after I.I. Mechnikov. Kirochnaya str., 41, Saint Petersburg, Russian Federation, 191015; Piskarevskiy ave., 47, Saint Petersburg, 195067
[2] Saint Petersburg State Pediatric Medical University. Lithuania 2, Saint Petersburg, Russian Federation, 194100
[3] Saint Petersburg Scientific Research Institute of Vaccines and Serums and an enterprise for the production of bacterial preparations. Svobody str., 52, Saint Petersburg, Krasnoye Selo, Russian Federation, 198320

Contact information: Dmitry P. Gladin — Candidate of Medical Sciences, Associate Professor, Acting Head of the Department of Microbiology, Virology and Immunology. E-mail: gladin1975@mail.ru ORCID: https://orcid.org/0000-0003-4957-7110 SPIN: 8149-9885

For citation: Kozlova NS, Gladin DP, Korolyuk AM, Baranov IA. Anthrax: near and far. Russian Biomedical Research. 2024;9(1):88-99. DOI: https://doi.org/10.56871/RBR.2024.35.28.010

Received: 20.12.2023 **Revised: 15.01.2024** **Accepted: 04.03.2024**

Abstract. Anthrax refers to particularly dangerous infections with high mortality, which, with untimely diagnosis and the absence of etiotropic therapy, can reach 90%, and with the pulmonary form — 100%. According to WHO, from two to twenty thousand cases of anthrax in humans are registered annually in the world, including fatal cases, more often in developing countries. In August 2023, an anthrax outbreak was identified in Kazakhstan. In Russia, over the past 15 years, only about 10–30 cases of this disease in humans have been registered annually, while the last two ones were recorded in March 2023 in Chuvashia. At the same time, the risk of anthrax outbreaks in the country exists due to the large number of soil anthrax foci, both registered and not accounted for, spontaneous sanitation of which cannot be expected. The exposure of such foci can be facilitated by the currently increased risks of man-made and natural disasters, as well as stable trends in increasing the temperature regime. This is confirmed by the outbreak of anthrax in the Yamalo-Nenets Autonomous Okrug in July 2016, the provoking factor of which is considered to be an abnormally high temperature (more than 34 °C for several days). The increased relevance of this disease is also associated with a sharp increase in the threat of bioterrorism in modern conditions, the potential agent of which is the causative agent of anthrax. Due to the high resistance of the pathogen spores in the environment, the aerosol mechanism of disease transmission, the possibility of obtaining antibiotic-resistant strains and strains that cause disease in the immune body, the anthrax causative agent is one of the most likely infectious agents that can be used to create biological weapons.

Keywords: anthrax, epidemiology, pathogenesis, clinical manifestations, diagnosis, treatment, prevention, anthrax vaccines, biological weapons

СИБИРСКАЯ ЯЗВА: ДАЛЕКАЯ И БЛИЗКАЯ

© Надежда Сергеевна Козлова[1], Дмитрий Павлович Гладин[2],
Александр Михайлович Королюк[2, 3], Илья Андреевич Баранов[1]

[1] Северо-Западный государственный медицинский университет имени И.И. Мечникова. 191015, Российская Федерация, г. Санкт-Петербург, ул. Кирочная, 41; 195067, г. Санкт-Петербург, Пискаревский пр., 47
[2] Санкт-Петербургский государственный педиатрический медицинский университет. 194100, Российская Федерация, г. Санкт-Петербург, ул. Литовская, 2
[3] Санкт-Петербургский научно-исследовательский институт вакцин и сывороток и предприятие по производству бактериальных препаратов. 198320, г. Санкт-Петербург, Российская Федерация, г. Красное Село, ул. Свободы, 52

Контактная информация: Дмитрий Павлович Гладин — к.м.н., доцент, и.о. заведующего кафедрой микробиологии, вирусологии и иммунологии. E-mail: gladin1975@mail.ru ORCID: https://orcid.org/0000-0003-4957-7110 SPIN: 8149-9885

Для цитирования: Козлова Н.С., Гладин Д.П., Королюк А.М., Баранов И.А. Сибирская язва: далекая и близкая // Российские биомедицинские исследования. 2024. Т. 9. № 1. С. 88–99. DOI: https://doi.org/10.56871/RBR.2024.35.28.010

Поступила: 20.12.2023 **Одобрена: 15.01.2024** **Принята к печати: 04.03.2024**

Резюме. Сибирская язва (антракс) относится к особо опасным инфекциям с высокой летальностью, достигающей при несвоевременной диагностике и отсутствии этиотропной терапии 90%, а при легочной форме — 100%. По данным ВОЗ, ежегодно в мире регистрируется от двух до двадцати тысяч случаев сибирской язвы у людей, в том числе с летальным исходом, чаще в развивающихся странах. В августе 2023 г. была выявлена вспышка сибирской язвы в Казахстане. В России за последние 15 лет ежегодно регистрируются около 10–30 случаев этого заболевания у людей, при этом последние два были зафиксированы в марте 2023 г. в Чувашии. Несмотря на низкую заболеваемость в целом, риск возникновения вспышек сибирской язвы в стране остается высоким из-за большого числа почвенных сибиреязвенных очагов как зарегистрированных, так и неучтенных, самопроизвольной санации которых ожидать не приходится. Вскрытию таких очагов могут способствовать возросшие в настоящее время риски техногенных и природных катастроф, а также устойчивые тенденции в отношении повышения температурного режима. Это подтверждает вспышка сибирской язвы в Ямало-Ненецком автономном округе в июле 2016 г., провоцирующим фактором которой считается аномально высокая температура (более 34 °С в течение нескольких дней). Повышение актуальности данного заболевания связано также с резким возрастанием в современных условиях угрозы биотерроризма, потенциальным агентом которого является возбудитель сибирской язвы. Вследствие высокой устойчивости спор возбудителя во внешней среде, аэрозольного механизма передачи заболевания, возможности получения антибиотикорезистентных штаммов и штаммов, вызывающих заболевание в иммунном организме, возбудитель сибирской язвы является одним из наиболее вероятных инфекционных агентов, которые могут быть использованы для создания биологического оружия.

Ключевые слова: сибирская язва, эпидемиология, патогенез, клинические проявления, диагностика, лечение, профилактика, вакцины против сибирской язвы, биологическое оружие

ANTHRAX EPIDEMIOLOGY

Anthrax is ubiquitous almost all over the globe and registered everywhere except Alaska, Greenland and the Arctic Ocean islands. According to the World Health Organization (WHO), the global human incidence is estimated at two to twenty thousand cases per year [7, 9]. In recent decades, anthrax remains relevant for developed countries, including the Russian Federation [13], where it occurs in isolated cases [14], potentially possible within certain regions of the country. Thus, in Russia, on average, about 10–30 anthrax cases in humans are reported per year, with the last two recorded in March 2023 in Chuvashia. In 2023, animal anthrax cases were registered in five regions of the Russian Federation: the Chuvash Republic, Tambov, Ryazan, Voronezh oblasts and the Republic of Tyva. According to WHO, there are 250–300 outbreaks of anthrax among animals in the world each year and about one million animals die. Many animals, primarily herbivores — cattle, camels, deers (especially reindeers), horses, donkeys, etc. — are susceptible to anthrax. Animals are most often infected orally by two routes: alimentary, by eating infected food, including bone meal, grass or soil, or by water, by drinking water from water bodies contaminated with effluents from enterprises processing raw materials of animal origin or groundwater communicating with soil anthrax foci. A necessary condition for the oral mechanism is damage of the gastrointestinal tract (GIT) mucosa, which is observed when coarse food is consumed or in inflammatory diseases. Less frequently, a vector-borne mechanism of transmission is carried out. Carriers may be gadflies, fire flies, in whose mouth apparatus the pathogen remains viable for up to 5–7 days, mosquitoes, midges, as well as various species of ticks. Anthrax is characterized by seasonality, the greatest number of outbreaks is registered in the warm season, from May to September, when animals graze on pastures, but isolated outbreaks of anthrax are possible in winter when using infected fodder. Sick animals excrete the pathogen in saliva, urine, feces, and all organs and tissues of an anthrax-dead animal contain huge amounts of bacilli. Thus, 1 ml of blood of such animals contains 109 microbial cellsThe anthrax pathogen enters the environment, primarily the soil, from sick animals and humans, or from the burial of dead animal carcasses. There it forms spores and can persist for a very long time, remaining highly virulent, which determines the stationarity of anthrax. In the Russian Federation, practically every fifth settlement has a territorial connection with anthrax-affected stationary points, where there are burials of corpses of animals killed by anthrax [10].

According to the indicators of the anthrax epizootic process, the territory of Russia is divided into three zones:

1) zone of sporadic occurrence — the territory north of 56° latitude (Murmansk, Leningrad, Pskov, Novgorod

and other regions) and eastern regions of Transbaikal;
2) zone of periodic occurrence — territory between 56 and 52° latitude (Moscow, Smolensk, Nizhny Novgorod, Irkutsk and Kemerovo oblasts, Tatarstan and others);
3) zone of stable occurrence — from 53° latitude to southern borders (Kursk, Voronezh, Rostov, Volgograd and other regions).

In the general structure of anthrax morbidity, diseases among humans account for 1–2%. The mechanisms of human infection are diverse: contact, oral, aerogenic, and vector-borne transmission mechanisms can occur, with the contact mechanism being the main one, accounting for 90–99% of all cases of infection [4, 5]. As a rule, *B. anthracis* gets on human skin by direct contact during the care of sick animals, their slaughter, carcass cutting, as well as by contact with soil, water, raw materials of animal origin and finished products made of fur, leather, wool, bristles. Cases of infection have been described by walking barefoot on contaminated soil, by striking with a pick contaminated with infected soil, by using shaving brushes made of contaminated bristles, by injecting therapeutic drugs with needles contaminated with spores of the pathogen, and by wearing fur, leather and wool products infected with spores. Thus, during the Russo-Japanese War (1904–1905), an outbreak of anthrax (about a thousand patients) was described among the soldiers of the Russian army in the Far East, associated with the supply of sheepskin coats infected with *B. anthracis* spores. As already mentioned, a prerequisite for infection is the violation of the integrity of the skin, the presence of macro- or micro-damage on the skin. Oral mechanism (alimentary route) of transmission is possible through consumption of infected meat and meat products, milk without sufficient thermal treatment. Transmissible mechanism of transmission is realized by the bite of blood-sucking insects. The aerogenic mechanism of transmission, which is realized by air and dust, requires the presence of *B. anthracis* spores aerosol in the air, which is created at enterprises processing raw materials of animal origin, use of organic fertilizers, collection of waste materials, etc. The incidence of anthrax among humans is sporadic with isolated group outbreaks, with humans being an epidemiologic dead end. As a rule, human-to-human infection is not observed, a person is not a source of infection. This may be due to several reasons: the short duration and low intensity of excretion of the pathogen from the patient's body, changes in its properties, and the absence of transmission mechanisms characteristic of the disease between people. Three types of anthrax diseases can be distinguished in humans due to the peculiarities of their labor activity and everyday life: occupational-agricultural, which account for more than 60%, occupational-industrial which account about 20%, and non-occupational (casual) which account about 15% [6]. At the same time, the occupational-agricultural and non-occupational types of the disease are characterized by seasonality: they occur more often in the summer-autumn period and coincide with the corresponding epizootics in domestic animals. The occupational-industrial type does not depend on the time of year. The occupational-agricultural type of anthrax is characteristic of people working in public livestock farming, of the mechanisms of transmission is more often contact, oral (alimentary) route is possible, rarely vector-borne route is possible. Infection occurs, as a rule, by vegetative forms of *B. anthracis*. The latest anthrax outbreak in Yamal in July 2016 [6, 15] belongs to the occupational-agricultural type, as the source of infection was reindeer, and reindeer herders and their family members became ill.

The occupational-industrial type is characteristic of people working in industries that process raw materials of animal origin. This type of anthrax is characterized by contact and aerogenic transmission mechanisms, and infection occurs with spore forms of the pathogen. For the first time this type of anthrax was described in the middle of the XIX century in England at enterprises of the textile industry, it is also known under the names "wool sorters' disease", "rag makers' disease". The latter was common in Russia among collectors of landfill rags contaminated with excreta and animal dung. The unprofessional type is observed: in people who have had contact with a sick animal in the private sector or accidentally; during consumption of infected meat or products contaminated with soil containing spores; during use of fur and other products.

Sanitary and Epidemiological Rules 3.1.7.2629-10 "Anthrax Prevention" provide definitions of the following concepts important for epidemiologic surveillance and antisybriasis measures: epizootic center, epidemic center, stationary unfavorable point, soil hotspot and threatened area:

"Epizootic center is the location of the source or factors of transmission of the infectious agent within the boundaries in which the transmission of the agent to susceptible animals or humans is possible (pasture area, watering hole, livestock house, livestock processing plant and others).

An epidemic center is an area where a case or cases of human disease have been reported.

Stationary unfavorable point is a settlement, livestock farm, pasture, tract, on the territory of which an epizootic focus has been detected, regardless of the period of time of its occurrence.

Soil hotspot is a cattle burial ground, biothermal pit and other places where corpses of animals killed by anthrax are buried.

Threatened area is animal farms, populated areas, administrative districts where is a threat of anthrax cases in animals or people".

Currently, 8 thousand anthrax-infected cattle burial grounds are registered in Russia. In fact, the official statistics figures are greatly underestimated, as there are a large number of unrecorded cattle burial grounds in many areas. In the Russian Federation, there are more than 35,000 stationary anthrax-unfavorable points [4, 8] with soil anthrax foci, most of them located in Siberia and southern Russia. A settlement in which human or animal disease has once occurred is considered to be permanently anthrax-prone. Thus, the main sources of infection for humans are the organism of a sick animal and soil anthrax foci. Mass vaccination of animals is currently underway, so the role of soil anthrax foci in maintaining *B. anthracis* as a species in nature is crucial. In natural conditions they are sanitized extremely slowly, the factors contributing to sanitation are insolation, antagonism of microorganisms, bactericidal action of some plants. In this connection, the study and analysis of sibiriazvirus soil foci depending on soil-bioclimatic and geographical factors and the problem of their decontamination are very important.

Various chemical preparations are used for decontamination of soil outbreaks, the most effective ones being dry chlorine lime mixed with soil in the ratio of 1:10 and then moistened with water, and 5% formaldehyde in double treatment (Gruinard Island). There are prospects for application of biological methods of soil disinfection. Anthrax antagonist microbes can be used for this purpose. These include actinomycetes, *B. subtilis, B. mesentericus, B. mycoides.* Specific anthrax bacteriophages can be used, but *B. anthracis* strains resistant to them are found in nature. The All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology has developed a method of decontamination of anthrax cattle burial grounds by burning, which is widely used in Canada [6, 8].

ANTHRAX PATHOGENESIS

The entry gate for anthrax is most often microdamage to the skin; less frequently, the pathogen can enter the body through damaged GI mucosa or through the epithelium of the upper respiratory tract (Fig. 1). An important factor in the development of infection is the form of the pathogen that entered the organism (spore or vegetative). For some time after penetration into the organism, spores behave as inert bodies (in particular, they are not capable of adhesion), at the same time they are taken up by macrophages and delivered by them to regional lymph nodes, where spores can be detected as early as 4–5 hours after infection. Then the process of spore germination into vegetative cells begins, which can occur both at the site of introduction and in regional lymph nodes. In macrophages vegetative cells divide, they form capsules, which promotes their rapid exit from phagocytes with subsequent multiplication in the lymphatic system, while the capsule prevents phagocytosis of vegetative forms. Multiplication of the pathogen in the area of the entrance gate and regional lymph nodes and its production of exotoxin are the cause of impaired vascular permeability, impaired microcirculation, local serous-hemorrhagic edema, inflammation, necrosis and loss of sensitivity in the gate of infection.

In the most common cutaneous form, a focus of hemorrhagic-necrotic inflammation with brown pigment (hemosiderin) is formed in the deep layers of the dermis and regional lymphadenitis develops. In the alimentary route of infection, the introduction of *B. anthracis* is possible throughout the GI tract, more often it occurs in the small intestine, and an important factor is the presence of micro-damage to the intestinal epithelium as a result of inflammatory diseases. In the aerogenic mechanism of infection, spores are taken up by alveolar macrophages, which carry them to tracheobronchial and mediastinal lymph nodes, where they germinate within 1–3 days (orpersist in alveoli or lymph nodes for up to 60 days), break their barrier function and penetrate into the bloodstream. As a result of exotoxin production, edema and necrotic changes, hemorrhagic mediastinitis and pleuritis develop, followed by generalization of the process and the emergence of secondary hemorrhagic sybilliform pneumonia with further fatal outcome. In all forms of infection, the generalization of the process may lead to the development of anthrax sepsis, which may be primary or secondary. The formation of septic foci in various organs and tissues with acute serous-hemorrhagic, hemorrhagic, less often fibrinous-hemorrhagic inflammation, replacement of lymphoid tissue in the spleen and lymph nodes by macrophages and incomplete phagocytosis of the pathogen is characteristic. Increasing toxinemia leads to the synthesis of a large number of proinflammatory cytokines, primarily tumor necrosis factor (TNF), interleukin-1 (IL-1) and others, and causes increased vascular permeability, the development of hemorrhagic manifestations, edema and hemostasis in organs and tissues. All this may eventually lead to the development of infectious toxic shock, Disseminated intravascular coagulation (DIC) and death of the patient.

Thus, the greatest role in the pathogenesis of anthrax belongs to the action of the *B. anthracis* toxin, and the septic course may occur either as a result of primary generalization or as a complication of the local form with the development of secondary generalization.

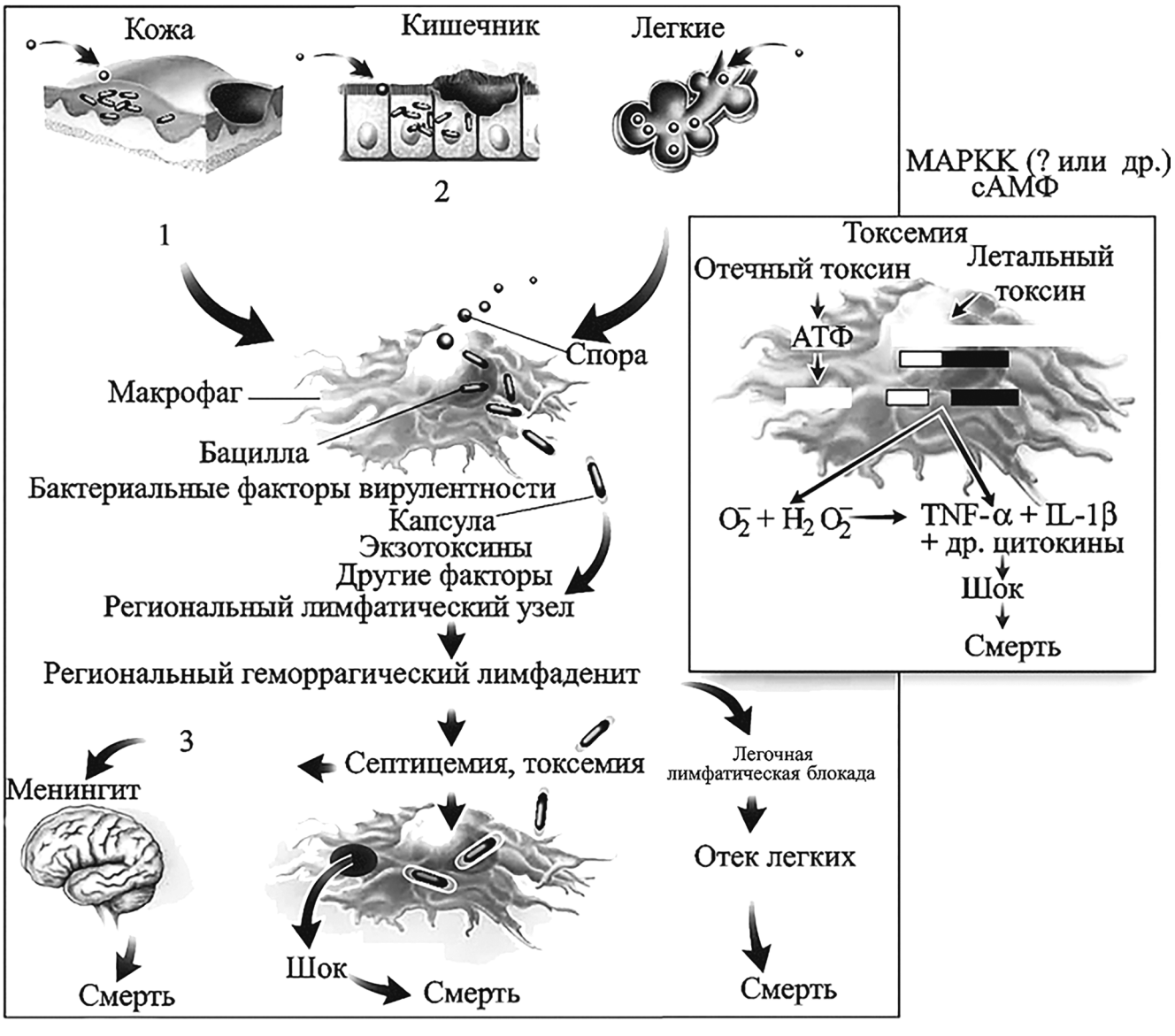

Рис. 1. Патогенез сибиреязвенной инфекции у млекопитающих (Супотницкий, http://supotnitskiy.ru/book/book4-2-2.htm): 1 — низкоуровневое прорастание и рост в участке инфицирования ведут к локальному отеку и некротическому поражению кожи; 2 — низкоуровневое прорастание и рост в участке инфицирования ведут к массивному выпоту, отеку слизистой и некротическому поражению кишечника; 3 — лимфогенное и гематогенное распространение *B. anthracis*. MAPKK (mitogen-activated protein kinase kinase) — митоген-активированный белок киназы киназы; TNF — фактор некроза опухолей; IL — интерлейкин (Dixon T.D. et al., 1999)

CLINICAL MANIFESTATIONS OF ANTHRAX

The incubation period for anthrax can last from a few hours to 14 days (more often 2–3 days). For vegetative forms the incubation period is usually short; for spore forms it is longer. In contact transmission and cutaneous form of the disease, the incubation period is 2–14 days, while in case of aerogenic and alimentary infection it may be reduced to a few hours. Cutaneous, inhalational (pulmonary), gastrointestinal and septic forms of anthrax are distinguished. The Iseptic form may be primary or secondary (Fig. 2). The International Classification of Diseases (ICD-10) includes cutaneous (A22.0), pulmonary (A22.1), gastrointestinal (A22.2), anthrax sepsis (A22.7), other forms of anthrax (A22.8), and anthrax unspecified (A22.9).

Cutaneous anthrax is the most common form, accounting for 95-99% of all anthrax cases. The skin of the upper limbs (about 50% of all cases) and head (20-30% of all cases) is mostly affected, while the trunk (3–6%) and legs (1-2%) are less frequently affected, with the exposed skin mostly affected. The cutaneous form is usually subdivided into

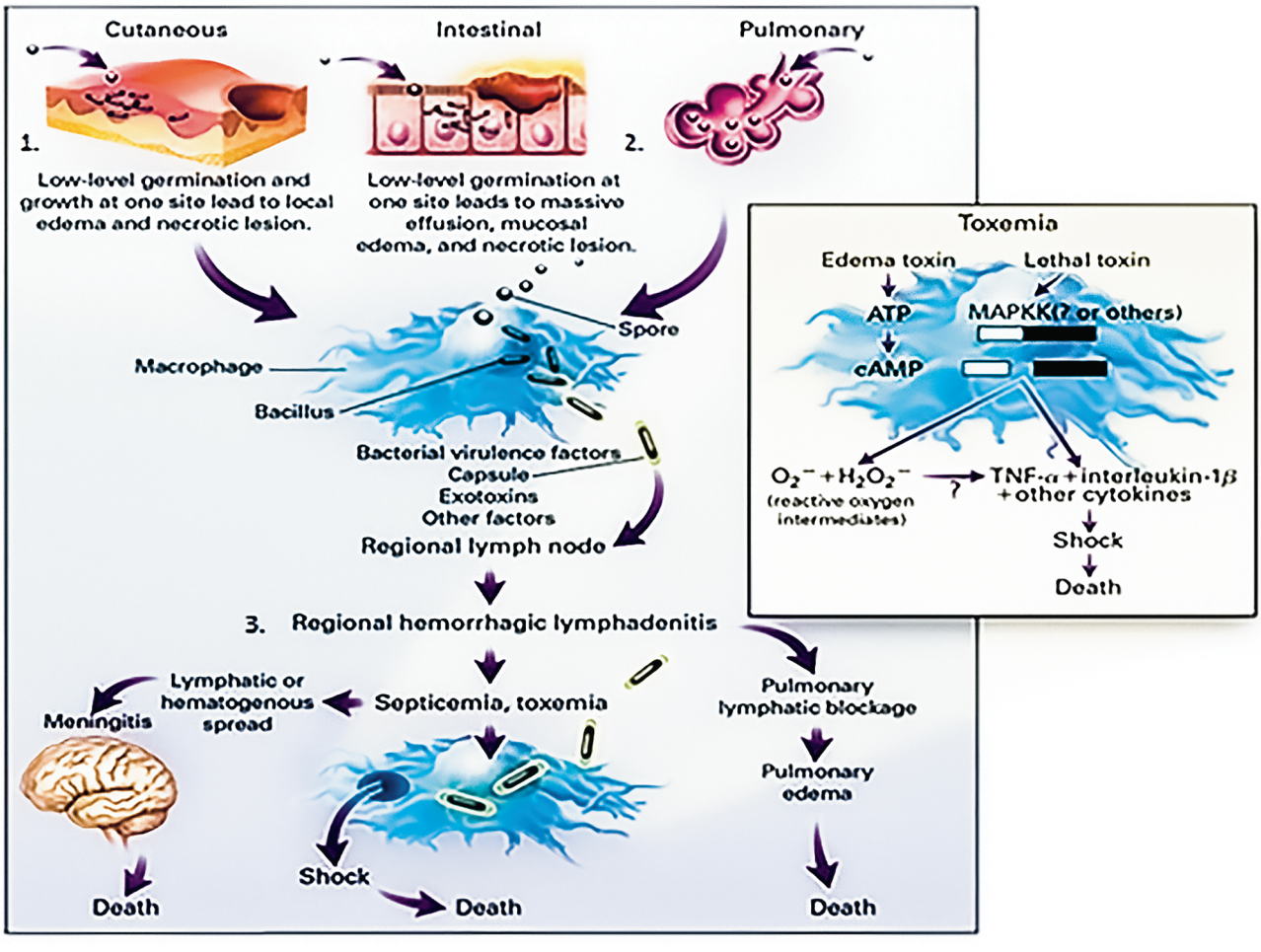

Fig. 1. **Pathogenesis of anthrax infection in mammals (Supotnitskiy, http://supotnitskiy.ru/book/book4-2-2.htm): 1 — low-level germination and growth at the site of infection lead to local swelling and necrotic skin lesions; 2 — low-level germination and growth in the site of infection lead to massive effusion, swelling of the mucous membrane and necrotic lesions of the intestine; 3 — lymphogenous and hematogenous spread of *B. anthracis*. MAPKK — mitogen-activated protein kinase kinase; TNF — tumor necrosis factor; IL — interleukin (Dixon T.D., et al., 1999)**

carbunculous (accounting for 99.1% of cutaneous manifestations), edematous (0.4%), bullous (0.4%), erysipeloid or rust-like (0.1%). Already by the end of the first day develops a pronounced intoxication syndrome that lasts 5–7 days: fever with a rise in temperature to 38–40 °C, chills, headache, weakness, sleep disorders, decreased appetite. At first, a reddish itchy spot similar to an insect bite is formed at the site of introduction of the pathogen (Fig. 3). After a few hours, the spot turns into a papule, then into a vesicle 2–3 mm in diameter, containing serous, then bloody fluid. The vesicle either by scratching or spontaneously opens, thus forming an ulcer with a dark brown or black bottom and raised edges, surrounded by a corolla of secondary pustules, due to which it increases. The skin around the ulcer is edematous and hyperemic.

A day later, the ulcer reaches the size of 8–15 mm. At the same time as the size of the ulcer increases,

Fig. 2. **Forms of the anthrax (compiled by the authors)**
Рис. 2. **Формы сибирской язвы (составлено авторами)**

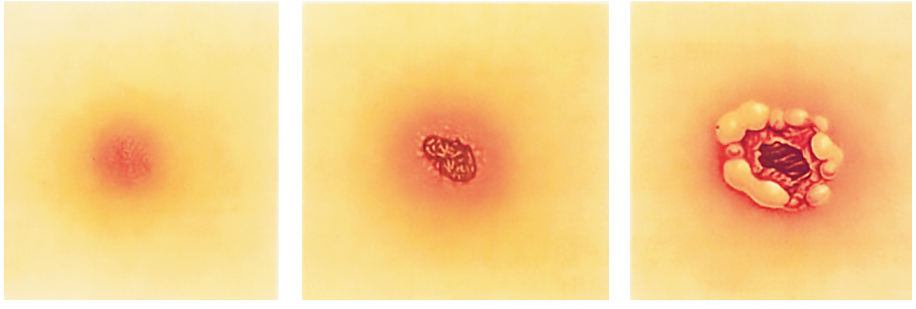

Fig. 3. Anthrax carbuncle formation. (URL: redkie-bolezni.com)

Рис. 3. Образование сибиреязвенного карбункула. (URL: redkie-bolezni.com)

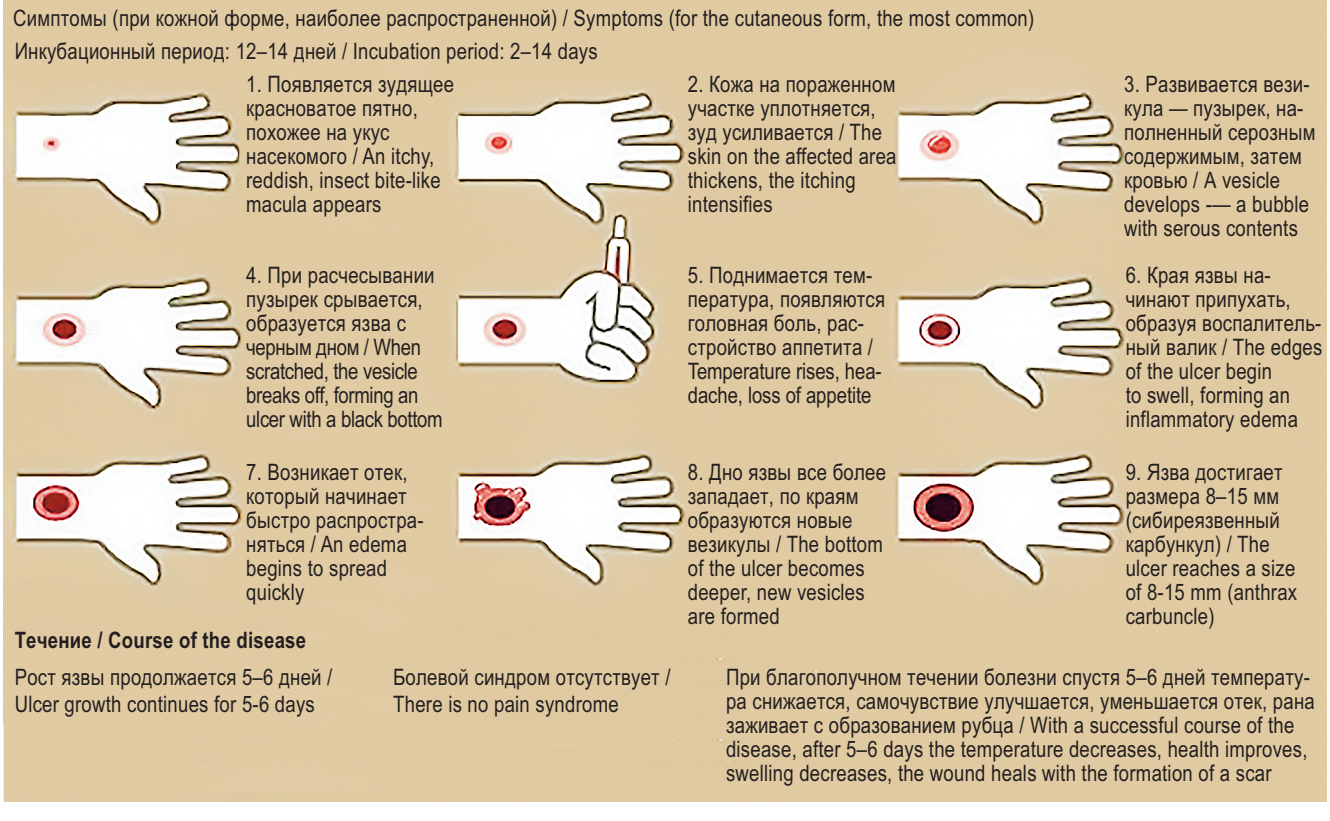

Fig. 4. Development of clinical manifestations in cutaneous anthrax

Рис. 4. Развитие симптомов при кожной форме сибирской язвы

regional lymph nodes become enlarged and thickened, but remain mobile and painless. After 2–3 weeks, due to necrosis, the central part of the ulcer turns into a painless dense scab, which quickly turns black and increases in size (Fig. 4). The scab rises above the skin surface, is surrounded by a pronounced zone of hyperemia and looks like a black coal on a red background ("coal on fire"), which is the basis for the name of the disease — anthrax (coal). There is a yellowish border between the black scab and the red area, making the ulcer tricolor (Fig. 5). By the fourth week, the scab is detached and a crater-shaped ulcer with a granulating floor and purulent discharge is formed; it subsequently undergoes secondary scarring.

Usually one carbuncle is formed, but there can be several, sometimes the number of them can reach 10–20 and more, the size of carbuncles can vary from a few millimeters to ten centimeters in diameter. The main distinguishing feature of the anthrax carbuncle is the absence of pain in the area of necrosis, practically painless is also the area of edema. The edematous form is characterized by the development of extensive edema without visible carbuncle. In the bullous form blisters with hemorrhagic content are formed immediately. In the erysipeloid form erythema with

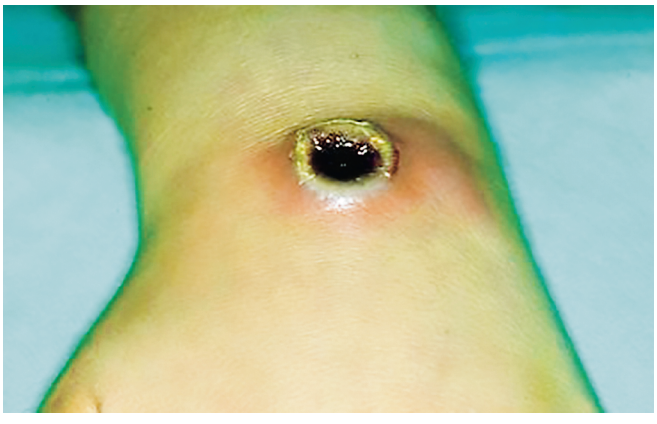

Fig. 5. Anthrax carbuncle. (URL: www.sibmedport.ru)

Рис. 5. Сибиреязвенный карбункул. (URL: www.sibmedport.ru)

whitish blisters develops, after opening of which shallow quickly drying ulcers are formed. Lethality in the cutaneous form, if untreated, may reach 20%, while treatment reduces to 2–3%. Intestinal anthrax is characterized by general intoxication, sharp cutting pain in the epigastric region, vomiting and diarrhea with an admixture of blood. The tongue is dry and covered with white plaque. Pulmonary anthrax is very severe. Against a background of high fever there is pain when breathing, cyanosis, dyspnea, wheezing, cough with frothy bloody sputum. The increase in peribronchial lymph nodes prevents the outflow of lymph and provokes pulmonary edema. Characteristic is a very rapid deterioration of the patient's condition and increasing changes in the lungs. Mortality, even with treatment, is up to 90% of cases. Septic or generalized form is rare. It is characterized by a decrease in body temperature, the development of infectious toxic shock, hypoxia, acidosis, multiorgan failure and disseminated intravascular coagulation. The death occurs on the 2–3rd day with the phenomena of acute collapse.

ANTHRAX AS A BIOLOGICAL WEAPON

Terrorism is currently one of the most acute and urgent problems, acquiring a global, international character in the modern world [6, 17]. It is very tempting for numerous terrorists to use biological weapons, which are no less dangerous than other types of weapons of mass destruction. The concept of bioterrorism has emerged, i.e. the threat of using means of mass destruction of biological (bacteriological) nature for terrorist purposes. Currently, there are at least 40 infectious agents that can be used as biological weapons. Among them, several pathogens, including anthrax, pose the greatest threat.

The latter fulfills most of the requirements for a potential biological weapon agent: it affects humans and animals, has a rapid effect, is highly virulent, extremely stable in the external environment, penetrates the organism by various routes, is easily cultivated in laboratories, and the selection of antibiotic-resistant strains is possible. The most probable way to use such a weapon is to atomize an aerosol containing spores, which would lead to a predominantly pulmonary form of the disease with high lethality. According to certain calculations, if an area of 20 km² is sprayed with sybillivorous spores for 2 hours over a city with a population of five million people, 500 thousand people will be exposed to the risk of infection, and the predicted number of people who fall ill may be 250 thousand, of whom 125 thousand are fatal [6].

The first serious use of *B. anthracis* as a biological weapon was carried out by nationalists against the aborigines of South Africa and Rhodesia (Zimbabwe) to suppress the liberation movement in 1978–1980. The major outbreak of anthrax in April 1979 in the Sverdlovsk region was connected, according to the main version, with the accidental release of pathogen spores from the laboratory of the military camp (Sverdlovsk-19) through damaged filters. According to another version it was made with enemy sabotage. In favor of the latter is the fact that foreign radio stations reported an anthrax epidemic on the 5th of April, while the first diagnosis was made on the 10th of April [16]. According to official data, 64 people died during the entire epidemic, while according to unofficial data there were more than a thousand deaths.

In 1993, the Japanese sect Aum Shinrikyo attempted bioterrorism by spreading *B. anthracis* in offices, which fortunately was unsuccessful. As of 2001, at least 17 countries already had bacteriological weapons at their disposal. New genetically modified strains of *B. anthracis* with increased virulence, polyantibiotic resistance, and the ability to cause disease in the immune system (such cultures are called vaccine-resistant) are constantly being developed. Work is under way to insert genes encoding the synthesis of anthrax toxin into the genome of other microorganisms, such as *B. cereus* and *B. thuringiensis*, for which there are no effective vaccines.

In Septeber 2001, an act of bio-terrorism was attempted in the USA by mailing mail containing anthrax spores, resulting in 22 people falling ill (11 of them felt ill with cutaneous anthrax and 11 felt ill with inhalational anthrax) and 5 deaths. This made obvious the potential danger of *B. anthracis* as an agent of bioterrorism and drew increased attention to the problem, resulting in the development of effective methods of treatment, prophylaxis and rapid diagnosis of anthrax. In 2001, only for the study of the genome of *B. anthracis* one of the scientific centers of the United States was allocated 200 thousand dollars. However, not

only acts of bioterrorism are dangerous, but also negligence when working with especially dangerous pathogens in specialized laboratories. After the events of 2001, under the pretext of combating bioterrorism, numerous biological research centers — laboratories for prevention of bioterrorism — were established in the USA and other countries. As of 2006, there were officially more than 400 such institutions in the U.S. alone, carrying out secret Pentagon programs. In June 2014, at the State Bioterrorism Prevention Laboratory in Atlanta, USA, B. antrhacis was handled in a routine laboratory not designed to handle highly dangerous pathogens, and the samples tested were not neutralized, exposing 75 people in the laboratory to the threat of infection. In May 2015, as a result of criminal negligence, live spores of *B. antrhacis* (68 parcels in total) were sent **by regular post** from a military laboratory at the Utah Proving Ground, USA, to 24 laboratories in 11 states and 5 countries (South Korea, Australia, Canada, United Kingdom, Japan).

Thus, such laboratories can become a source of new threats for spreading the pathogen worldwide.

PRINCIPLES OF LABORATORY DIAGNOSTICS

Effective anthrax therapy requires a diagnosis as soon as possible, but physicians rarely encounter this infection, making them less alert to it. In 10–40% of cutaneous infections, patients are diagnosed as “carbuncle”, “furuncle”, “insect bite” and other similar cases and sent for surgical treatment. A significant difficulty is the recognition of generalized anthrax. At the slightest suspicion of anthrax it is necessary to conduct laboratory diagnostics [1, 3, 12], which is carried out in strict accordance with the current instructions and rules (guidelines 4.2.2413-08, 4.2.2941-11). Microscopic, bacteriologic, biological methods, serodiagnosis, allergodiagnosis, and molecular biological methods [11], in particular molecular typing methods [2, 5], are used. These microbiological methods make it possible to confirm the etiology of the disease, while biochemical methods make it possible to assess its severity. For direct anthrax diagnostic methods (microscopic, bacteriological, biological and rapid diagnostic methods), the material to be examined is the content of vesicles, carbuncles, scabs, sputum, feces, blood, cerebrospinal fluid and sectional material. Materials from cadavers should be taken and examined as soon as possible after death, as extraneous microflora develops rapidly, making it difficult to isolate a pure culture. Clinical material is collected in medical and preventive institutions in protective clothing at the admission of the patient before the start of antibiotic therapy according to guidliness 4.2.2941-11. For this purpose a special kit is used — “universal stack for the collection of material from people and from environmental objects for the study of especially dangerous infectious diseases”. Laboratory personnel shall be provided with protective clothing and personal protective equipment for work with microorganisms of pathogenicity group II.

ANTHRAX TREATMENT AND PREVENTION

Complex therapy of anthrax patients includes two main directions: etiotropic antimicrobial and specific antitoxic therapy and nonspecific symptomatic and antishock therapy. The following antibacterial drugs are used for the treatment of anthrax in Russia: beta-lactams (benzylpenicillin, ampicillin), tetracyclines (tetracycline, doxycycline), fluoroquinolones (ciprofloxacin, pefloxacin, ofloxacin), and rifampicin. Until recently, penicillin was the main drug for the treatment of anthrax, but nowadays, due to the emergence of strains producing beta-lactamases, it should be used only if sensitivity to it has been confirmed. Reserve drugs include aminoglycosides (gentamicin, amikacin, sizomycin), since resistance to antibiotics of this group develops slowly. It is possible to use both individual drugs and their combinations, such as penicillin and tetracycline, ciprofloxacin and rifampicin. Antibiotics are most effective in cutaneous anthrax, while combinations are recommended in septic anthrax. In all cases, treatment should be started as early as possible from the onset of the first clinical symptoms. In the development of sepsis and toxinemia, the use of antimicrobials may be ineffective and even worsen the patient’s condition due to the death of microbes and the release of a large amount of exotoxin that has not yet had time to leave the cells. Neutralization of the toxin in moderate and severe anthrax requires administration of large doses of antisybriasis equine immunoglobulin, which contains active gamma- and beta-globulin fractions isolated from the blood serum of hyperimmunized horses. In addition to neutralizing the toxin, the drug inhibits spore germination and capsule formation (inhibition of glutamine polypeptide synthesis). Recently, the use of sibiriazoon immunoglobulin has been discontinued due to frequently developing allergic reactions. Anti-sybriasis human immunoglobulin, immunoglobulin based on Fab-fragments, preparations based on monoclonal antibodies to the protective antigen, lethal factor and polyglutamine capsule of *B. anthracis,* inhibitors of cell receptors of the protective antigen and others are being developed.

Nonspecific prophylaxis of anthrax in humans includes a set of veterinary and medical-sanitary measures (Sanitary and Epidemiologic Rules 3.1.7.2629-10). These include:

- vaccination of susceptible animals, which is effective, but does not ensure complete elimination of

the pathogen due to its prolonged persistence in the soil;
- identification, accounting and elimination of sybiraemic foci;
- sanitary and epidemiological control in anthrax-affected areas, as well as during the procurement, processing, transportation and storage of raw materials of animal origin;
- prophylactic, current and final disinfection, incineration rather than burial of corpses of infected animals and raw materials,
- sanitary and educational work with the population, etc.

Emergency prophylaxis is carried out when anthrax cases appear among animals or people, as well as when there is a threat of aerosol contamination in case of bioterrorism. It is carried out in the area of an active anthrax focus using antibiotics of different groups no later than five days after possible contamination — contact with infected animals or livestock products. Rifampicin, doxycycline, ampicillin, oxacillin, ciprofloxacin orally; gentamicin intramuscularly in maximum doses for five days are recommended for use. Equine anti-anthrax immunoglobulin can also be used: adults in a dose of 20–25 ml, adolescents from 14 to 17 years can get 12 ml, children can get 5 ml (methodological recommendations 0100/3556-04-34).

The optimal duration of emergency prophylaxis for suspected aerogenic anthrax infection has not been precisely determined. In 2001 in the USA, people at risk of such infection were given emergency prophylaxis with a course of an antimicrobial therapy (ciprofloxacin, doxycycline or amoxicillin) for 60 days, using 3.75 million tablets. The need for prolonged preventive antibiotic therapy is due to the effect of delayed spore germination in the lungs. Spores can persist in the alveoli for several weeks (up to 8 weeks or more), while antibiotics are active only against vegetative cells and germinating spores.

Vaccines are used for early anthrax prophylaxis (they create immunity lasting up to one year). Two vaccines are currently registered and used in Russia:

1) anthrax live dry vaccine for subcutaneous and scarification, containing live spores of the vaccine strain STI (after the name of the Sanitary and Technical Institute where the vaccine was developed);
2) combined liquid anthrax vaccine for subcutaneous administration, contains a mixture of live spores of vaccine strain STI-1 and purified concentrated protective anthrax antigen adsorbed on aluminum hydroxide.

An aerosolized vaccine has also been developed.

Risk contingents subject to prophylactic vaccination include:

- animal handlers and other persons professionally engaged in the pre-slaughter housing of livestock, as well as slaughtering, skinning and cutting of carcasses;
- persons engaged in the collection, storage, transportation and primary processing of raw materials of animal origin;
- laboratory personnel working with material suspected of being infected with anthrax;
- persons performing certain work in anthrax-enzootic areas (agricultural, agro- and hydromeliorative, construction and other work related to excavation and movement of soil; procurement, field, geological, survey, expeditionary);
- military personnel in the presence of epidemiological indications.

Foreign countries use chemical vaccines based on a protective antigen produced by a capsule-free nonproteolytic avirulent strain of *B. anthracis* adsorbed on aluminum hydroxide or alum. The best known vaccines from this group are the American AVA — Anthrax Vaccine Adsorbed (capsule-free strain V770-NP1-R) and the British AVP — Anthrax Vaccine Precipitated (avirulent strain Sterne 34F2) with a 30% increased amount of lethal factor.

Despite their efficacy, the existing vaccines have a number of drawbacks, which necessitates additional work on their improvement aimed at increasing immunogenicity and reducing reactogenicity. To increase the immunogenicity of recombinant vaccines, it is proposed to use *B. subtilis*, *B. brevis* and others synthesizing capsular polypeptides of *B. anthracis*. Vaccines based on recombinant DNA, polyvalent vaccines, including those against anthrax, etc., are being developed. Most researchers agree that a full-fledged chemical vaccine should contain antigens aimed at the production of anti-spore, anti-capsule and anti-toxic immunity in the body.

CONCLUSION

In modern conditions, anthrax maintains a global nosoareal and continues to be an urgent problem for many countries, including the Russian Federation. Vaccination of susceptible animals does not ensure elimination of the pathogen from environmental objects. The unpredictable long-term survival of *B. anthracis* spores in soil allows the pathogen to retain not only viability but also virulence. Hyperendemic foci remain on the territory of the country, the activation of which can occur as a result of natural disasters and climatic changes, as

well as anthropogenic impact and man-made disasters. We should not forget about the threat of importation of infected raw materials of animal origin into the territory of the country from neighboring countries, as well as the increasing threat of bioterrorism. The emergence of not only antibiotic-resistant but also vaccine-resistant strains of *B. anthracis* in nature is alarming. All this indicates the need to create a comprehensive anthrax control program aimed at improving the methods of its diagnosis, treatment and prevention, as well as the identification and sanitation of soil anthrax foci.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

Funding source. This study was not supported by any external sources of funding.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

REFERENCES

1. Alekseev V.V., Alipov A. N., Andreev V. A. i dr. Medicinskie laboratornye tekhnologii. [Medical laboratory technologies]. Tom 2. Moskva: GEOTAR-Media Publ.; 2013. (in Russian).
2. Goncharova Yu.O., Bahteeva I.V., Mironova R.I. i dr. Mul'tilokusnoe sikvens-tipirovanie shtammov sibiriyazvennogo mikroba, vydelennyh na territorii Rossii i sopredel'nyh gosudarstv. [Multilocus sequence typing of anthrax microbe strains isolated on the territory of Russia and neighboring countries]. Problemy osobo opasnyh infekcij. 2021; 1: 95–102. (in Russian).
3. Danilova L.A., Basharina O.B., Krasnikova E.N. i dr. Spravochnik po laboratornym metodam issledovaniya. [Handbook of laboratory research methods]. Moskva: Piter Publ.; 2003. (in Russian).
4. Dugarzhapova Z.F., Chesnokova M.V., Ivanova T.A. i dr. Sovershenstvovanie metodicheskih podhodov k obsledovaniyu sibireyazvennyh zahoronenij i skotomogil'nikov. [Improving methodological approaches to the examination of anthrax burials and cattle burial grounds]. Problemy osobo opasnyh infekcij. 2019; 4: 41–7. (in Russian).
5. Eremenko E.I., Ryazanova A.G., Pisarenko S.V. i dr. Novye geneticheskie markery dlya molekulyarnogo tipirovaniya shtammov Vacillus anthracis. [New genetic markers for molecular typing of Bacillus anthracis strains]. Problemy osobo opasnyh infekcii. 2019; 3: 43–50. (in Russian).
6. Kozlova N.S. Zoonozy: sibirskaya yazva. [Zoonoses: anthrax]. Sankt-Peterburg; 2017. (in Russian).
7. Kozlova N.S., Gladin D.P., Korolyuk A.M., Baranov I.A. Sibirskaya yazva: biologicheskie osobennosti vozbuditelya. [Anthrax: biological features of the pathogen]. Russian Biomedical Research. 2023; 8(2): 112–8. (in Russian).
8. Marinin L.I., Onishchenko G.G., Kravchenko T.B. i dr. Sibirskaya yazva cheloveka: epidemiologiya, profilaktika, diagnostika, lechenie. [Human anthrax: epidemiology, prevention, diagnosis, treatment]. Obolensk; 2008. (in Russian).
9. Logvin F.V., Kondratenko T.A., Vodyanickaya S.Yu. Sibirskaya yazva v mire, stranah SNG i Rossijskoj Federacii. [Anthrax in the world, CIS countries and the Russian Federation]. Medicinskij vestnik yuga Rossii. 2017; 8(3): 17–22. (in Russian).
10. Logvin F.V., Kondratenko T.A, Vodyanickaya S.Yu. i dr. Rezul'taty kompleksnoj ocenki territorii Rostovskoj oblasti po sibirskoj yazve po stepeni epizootologo-epidemiologicheskoj opasnosti. [Results of a comprehensive assessment of the territory of the Rostov region for anthrax according to the degree of epizootological and epidemiological danger]. Medicinskij vestnik yuga Rossii. 2017; 8(4): 93–8. (in Russian).
11. Nizkorodova A.S., Mal'ceva E.R., Berdygulova Zh.A. i dr. Detekciya Vacillus anthracis po genam profaga lambda_Ba03 posredstvom PCR v real'nom vremeni. [Detection of Bacillus anthracis by the genes of the lambda_Ba03 prophage using real-time PCR]. Problemy osobo opasnyh infekcii. 2022; 3: 170–2. (in Russian).
12. Poryadok organizacii i provedeniya laboratornoj diagnostiki sibirskoj yazvy dlya laboratorij territorial'nogo, regional'nogo i federal'nogo urovnej. [The procedure for organizing and conducting laboratory diagnostics of anthrax for laboratories at the territorial, regional and federal levels]. MUK 4.2.2941—11. Moskva; 2011. (in Russian).
13. Raichich S.R., Saburova S.A., Shabejkin A.A. i dr. Ocenka situacii po sibirskoj yazve na osnove ranzhirovaniya territorij po stepeni riska. [Assessment of the anthrax situation based on ranking territories by risk level]. Problemy osobo opasnyh infekcii. 2020; 4: 125–32. (in Russian).
14. Ryazanova A.G., Skudareva O.N., Gerasimenko D.K. i dr. Epidemiologicheskaya i epizootologicheskaya obstanovka po sibirskoj yazve v mire v 2021 g., prognoz na 2022 g. v Rossijskoj federacii.

[Epidemiological and epizootological situation regarding anthrax in the world in 2021, forecast for 2022 in the Russian Federation]. Problemy osobo opasnyh infekcii. 2022; 1: 64–70. (in Russian).
15. Savickaya T.A., Trifonov V.A., Milova I.V. i dr. Sibirskaya yazva v respublike Tatarstan (1920–2020 gg.). [Anthrax in the Republic of Tatarstan (1920–2020)]. Problemy osobo opasnyh infekcii. 2022; 3: 129–36. (in Russian).
16. Supotnickij M.V. Biologicheskaya vojna. [Biological warfare]. Moskva: Russkaya panorama, Kafedra Publ.; 2013. (in Russian).
17. Wagar E. Bioterrorism and the Role of the Clinical Microbiology Laboratory. Clin Microbiol Rev. 2016; 29(1): 175–89. DOI:10.1128/CMR.00033.

ЛИТЕРАТУРА

1. Алексеев В.В., Алипов А.Н., Андреев В.А. и др. Медицинские лабораторные технологии. Том 2. М.: ГЭОТАР-Медиа; 2013.
2. Гончарова Ю.О., Бахтеева И.В., Миронова Р.И. и др. Мультилокусное сиквенс-типирование штаммов сибиреязвенного микроба, выделенных на территории России и сопредельных государств. Проблемы особо опасных инфекций. 2021; 1: 95-102.
3. Данилова Л.А., Башарина О.Б., Красникова Е.Н. и др. Справочник по лабораторным методам исследования. М.: Питер; 2003.
4. Дугаржапова З.Ф., Чеснокова М.В., Иванова Т.А. и др. Совершенствование методических подходов к обследованию сибиреязвенных захоронений и скотомогильников. Проблемы особо опасных инфекций. 2019; 4: 41–7.
5. Еременко Е.И., Рязанова А.Г., Писаренко С.В. и др. Новые генетические маркеры для молекулярного типирования штаммов Bacillus anthracis. Проблемы особо опасных инфекции. 2019; 3: 43–50.
6. Козлова Н.С. Зоонозы: сибирская язва. СПб.; 2017.
7. Козлова Н.С., Гладин Д.П., Королюк А.М., Баранов И.А. Сибирская язва: биологические особенности возбудителя. Russian Biomedical Research. 2023; 8(2): 112–8.
8. Маринин Л.И., Онищенко Г.Г., Кравченко Т.Б. и др. Сибирская язва человека: эпидемиология, профилактика, диагностика, лечение. Оболенск; 2008.
9. Логвин Ф.В., Кондратенко Т.А., Водяницкая С.Ю. Сибирская язва в мире, странах СНГ и Российской Федерации. Медицинский вестник юга России. 2017; 8(3): 17–22.
10. Логвин Ф.В., Кондратенко Т.А, Водяницкая С.Ю. и др. Результаты комплексной оценки территории Ростовской области по сибирской язве по степени эпизоотолого-эпидемиологической опасности. Медицинский вестник юга России. 2017; 8(4): 93–8.
11. Низкородова А.С., Мальцева Э.Р., Бердыгулова Ж.А. и др. Детекция Bacillus anthracis по генам профага lambda_Ba03 посредством ПЦР в реальном времени. Проблемы особо опасных инфекции. 2022; 3: 170–2.
12. Порядок организации и проведения лабораторной диагностики сибирской язвы для лабораторий территориального, регионального и федерального уровней. МУК 4.2.2941—11. М.; 2011.
13. Раичич С.Р., Сабурова С.А., Шабейкин А.А. и др. Оценка ситуации по сибирской язве на основе ранжирования территорий по степени риска. Проблемы особо опасных инфекции. 2020; 4: 125–32.
14. Рязанова А.Г., Скударева О.Н., Герасименко Д.К. и др. Эпидемиологическая и эпизоотологическая обстановка по сибирской язве в мире в 2021 г., прогноз на 2022 г. в Российской федерации. Проблемы особо опасных инфекции. 2022; 1: 64–70.
15. Савицкая Т.А., Трифонов В.А., Милова И.В. и др. Сибирская язва в республике Татарстан (1920–2020 гг.). Проблемы особо опасных инфекции. 2022; 3: 129–36.
16. Супотницкий М.В. Биологическая война. М.: Русская панорама, Кафедра; 2013.
17. Wagar E. Bioterrorism and the Role of the Clinical Microbiology Laboratory. Clin Microbiol Rev. 2016; 29(1): 175–89. doi:10.1128/CMR.00033-15.