DOI: 10.29326/2304-196X-2021-2-37-151-158
UDC 619:616.98:579.852.11

Features of anthrax natural foci and *Bacillus anthracis* ecology

A. P. Rodionov[1], E. A. Artemeva[2], L. A. Melnikova[3], M. A. Kosarev[4], S. V. Ivanova[5]
Federal State Budgetary Scientific Institution "Federal Center for Toxicological, Radiation, and Biological Safety" (FSBSI "FCTRBS-ARRVI"), Republic of Tatarstan, Kazan, Russia
[1] *ORCID 0000-0003-0853-5678, e-mail: alexandrvetspets@gmail.com*
[2] *ORCID 0000-0002-6204-6077, e-mail: artemevaelena21@mail.ru*
[3] *ORCID 0000-0002-0159-3843, e-mail: vnivi@mail.ru*
[4] *ORCID 0000-0002-5577-486X, e-mail: kosarev@vnivi.ru*
[5] *ORCID 0000-0002-4378-8569, e-mail: 9274281396@mail.ru*

SUMMARY
Anthrax remains a global problem, both for veterinary and human medicine, due to the wide spread of its soil foci throughout the world. The ability to sporulate is the main feature of *Bacillus anthracis*, which allows the pathogen to persist in the environment for a long time. Understanding the ecology of *B. anthracis* is essential for successful control of this infection. This review analyzes the data from the global literature, reflecting the modern understanding of the vital functions of the anthrax agent in various ecological niches. As a result of the work, it was revealed that many links in the chain of *B. anthracis* lifecycle in the abiotic environment remain poorly understood. A more in-depth study is required for issues related to the mechanisms, ways of living and evolution of the anthrax causative agent outside the animal body. A separate section of the review describes the problems of anthrax foci in soil. It is shown that today there are no effective and environmentally friendly methods and means of their elimination. In addition, the question of the expediency of their use remains open. According to some researchers, the increasingly emerging initiatives for the elimination or conservation of anthrax burial sites are not only useless, but also harmful, since they exclude the possibility of further predicting the risks associated with soil foci that surround livestock burial sites and cannot be decontaminated. The study and new approaches to solution of the highlighted issues will make a significant contribution to solving the global problem of protecting animals and people from this infection.

Keywords: Anthrax, ecology, *Bacillus anthracis*, soil foci, natural focality, cattle burial site, permanently infected settlement.

For citation: Rodionov A. P., Artemeva E. A., Melnikova L. A., Kosarev M. A., Ivanova S. V. Features of anthrax natural foci and *Bacillus anthracis* ecology. *Veterinary Science Today*. 2021; 2 (37): 151–158. DOI: 10.29326/2304-196X-2021-2-37-151-158.

Conflict of interests: The authors declare no conflict of interest.

For correspondence: Alexander P. Rodionov, Junior Researcher, Laboratory for Collection of Strains of Microorganisms, FSBSI "FCTRBS-ARRVI", 420075, Russia, Republic of Tatarstan, Kazan, Scientific town-2, *e-mail: alexandrvetspets@gmail.com.*

УДК 619:616.98:579.852.11

Особенности природной очаговости сибирской язвы и экологии *Bacillus anthracis*

А. П. Родионов[1], Е. А. Артемьева[2], Л. А. Мельникова[3], М. А. Косарев[4], С. В. Иванова[5]
ФГБНУ «Федеральный центр токсикологической, радиационной и биологической безопасности» (ФГБНУ «ФЦТРБ-ВНИВИ»), Республика Татарстан, г. Казань, Россия
[1] *ORCID 0000-0003-0853-5678, e-mail: alexandrvetspets@gmail.com*
[2] *ORCID 0000-0002-6204-6077, e-mail: artemevaelena21@mail.ru*
[3] *ORCID 0000-0002-0159-3843, e-mail: vnivi@mail.ru*
[4] *ORCID 0000-0002-5577-486X, e-mail: kosarev@vnivi.ru*
[5] *ORCID 0000-0002-4378-8569, e-mail: 9274281396@mail.ru*

РЕЗЮМЕ
Сибирская язва остается глобальной проблемой как для ветеринарной, так и для гуманной медицины в связи с широким распространением ее почвенных очагов во всем мире. Способность к споруляции является главной особенностью *Bacillus anthracis*, позволяющей возбудителю сохраняться в окружающей среде в течение длительного времени. Понимание экологии *B. anthracis* необходимо для успешной борьбы с данной инфекцией. В настоящем обзоре проведен анализ данных мировой литературы, отражающих современное представление о жизнедеятельности возбудителя сибирской язвы в различных экологических нишах. В результате работы выявлено, что многие звенья в цепи жизнедеятельности *B. anthracis* в абиотической среде остаются

малоизученными. Более глубокого изучения требуют вопросы, касающиеся механизмов, способов существования и эволюции возбудителя сибирской язвы вне организма животного. Отдельным разделом обзора представлены проблемы почвенных очагов сибирской язвы. Показано, что на сегодняшний день не существует эффективных и безвредных для окружающей среды методов и средств их ликвидации. Кроме того, остается открытым вопрос о целесообразности их применения. По мнению некоторых исследователей, все чаще возникающие инициативы ликвидации или консервации сибиреязвенных скотомогильников не только бесполезны, но и вредны, так как исключают возможность в дальнейшем прогнозировать риски, связанные с почвенными очагами, которые окружают скотомогильники и не могут быть обезврежены. Изучение и новые подходы к решению освещенных вопросов внесут свой значительный вклад в решение глобальной проблемы защиты животных и людей от данной природно-очаговой инфекции.

Ключевые слова: Сибирская язва, экология, *Bacillus anthracis*, почвенные очаги, природная очаговость, скотомогильник, стационарно неблагополучный пункт.

Для цитирования: Родионов А. П., Артемьева Е. А., Мельникова Л. А., Косарев М. А., Иванова С. В. Особенности природной очаговости сибирской язвы и экологии *Bacillus anthracis*. *Ветеринария сегодня*. 2021; 2 (37): 151–158. DOI: 10.29326/2304-196X-2021-2-37-151-158.

Конфликт интересов: Авторы заявляют об отсутствии конфликта финансовых/нефинансовых интересов, связанных с написанием статьи.

Для корреспонденции: Родионов Александр Павлович, младший научный сотрудник лаборатории коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», 420075, Россия, Республика Татарстан, г. Казань, Научный городок-2, *e-mail: alexandrvetspets@gmail.com.*

INTRODUCTION

The main feature of the causative agent of anthrax (*Bacillus anthracis*) is the ability to form spores that persist in the environment for decades until they get the possibility to penetrate into a susceptible organism. *B. anthracis* in spore form is a perfect infectious agent. To date, there are a large number of works devoted to the processes occurring in *B. anthracis* infected macroorganism [1, 2]. However, aspects concerning *B. anthracis* relationships in soil ecosystems and the environment are still poorly understood. This review analyzes the life cycle of *B. anthracis* in various ecological niches.

Bacillus anthracis spore and sporulation

The spores are formed in the environment or in laboratory conditions when grown on nutrient media, provided there is an access of oxygen, lack of nutrients, high humidity, and temperature of 26–37 °C. One vegetative cell is capable of forming a single spore, which is located in the center or subterminally. At temperatures above 43 °C or below 12 °C, sporulation does not occur.

Sporulation is triggered by the lack of a nutrient substrate. In this case, the *spo0A* gene encoding the protein of the same name is activated. Then, by phosphorylation the Spo0A protein is activated into Spo0A~P, causing the expression of more than 200 genes. These genes are responsible for sporulation. When the endospore formation is complete, the mother cell wall is lysed, releasing the mature spore into the environment [3].

The *B. anthracis* spore consists of the core, surrounded by coats: the cortex, the coat proteins and the exosporium (Fig. 1) [4].

The spore core consists of a chromosome tightly bound to acid-soluble proteins [5]. The interaction between DNA and proteins, high levels of dipicolinic acid, calcium and other ions provide protection from a variety of adverse effects, including elevated temperatures and ultraviolet radiation.

The cortex is the inner part of the spore, surrounded by a membrane and peptidoglycan layer, which in turn are surrounded by several layers of proteins called the coat proteins.

The coat protein surface is distinguished by folds, which extend along the long axis of the spore, and allow the spore to withstand the core growth during its germination [6–10].

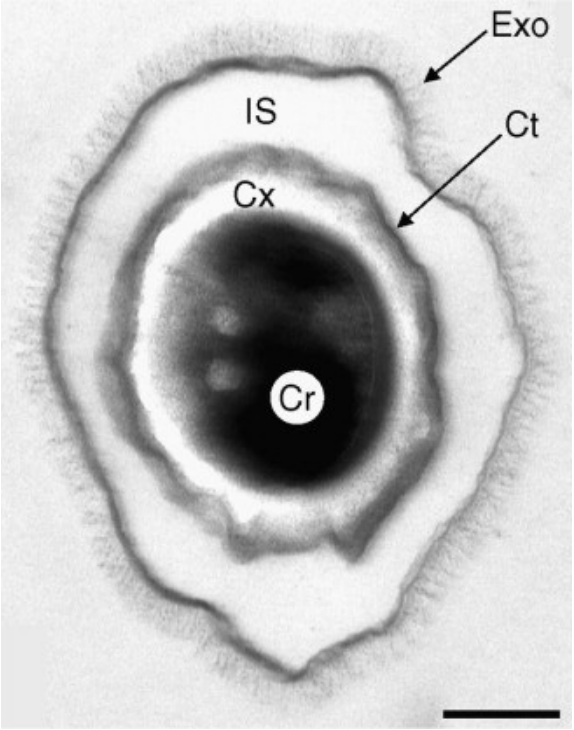

Fig. 1. Thin-section electron micrograph of a Bacillus anthracis spore (Sterne strain). Core (Cr), cortex (Cx), coat (Ct), interspace (IS) and exosporium (Exo) are indicated [4]

Рис. 1. Строение споры возбудителя сибирской язвы: Cr – ядро, Cx – кортекс, Ct – белковая оболочка, IS – промежуток, Exo – экзоспориум [4]

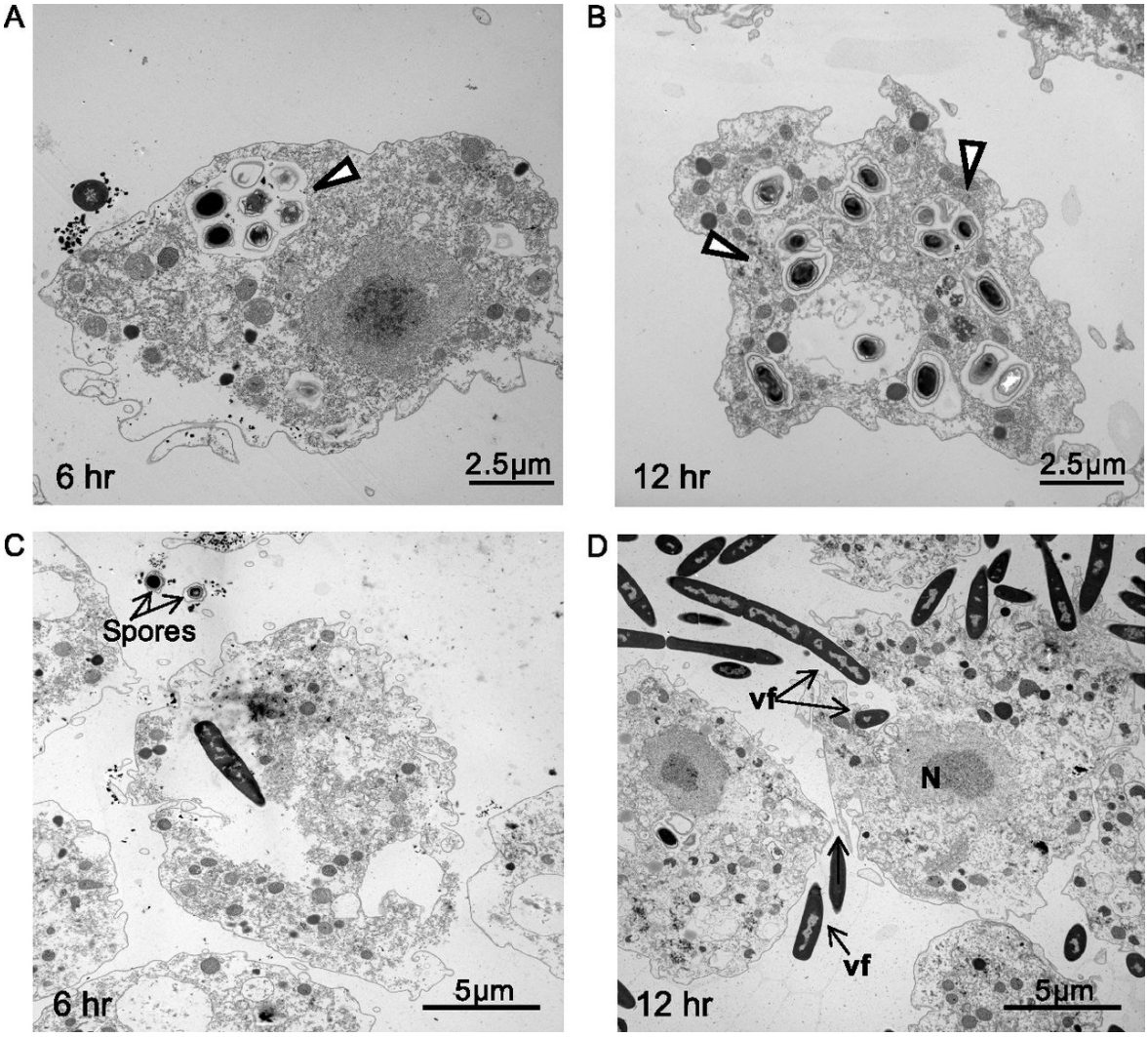

Fig. 2. Transmission electron microscopy analysis of B. anthracis-Acanthamoeba castellanii interactions [27]: *A and B – Micrographs show spores of strain 9131 contained in A. castellanii phagosomes (open arrowheads) after 6 (A) and 12 h (B) of coculture at 37 °C, respectively; C – A vegetative Sterne spore within an A. castellanii trophozoite in a phagosome after 6 h of coculture; D – Vegetative forms of Sterne inside and outside amoebas after 12 h of infection (black arrows). N – nucleus; vf – vegetative form*

Рис. 2. Просвечивающая электронная микроскопия взаимодействия B. anthracis с почвенной амебой [27]: *A и B – находящиеся внутри амебы Acanthamoeba castellanii споры штамма 9131 B. anthracis и начало их прорастания через 6 (A) и 12 ч (B) совместного культивирования при 37 °C; C – прорастание штамма Sterne B. anthracis внутри амебы; D – вегетативные формы штамма Sterne B. anthracis внутри и снаружи амебы через 12 ч после совместного культивирования (черные стрелки). Обозначения: N – ядро; vf – вегетативная форма*

These coat proteins perform a number of important functions:

1) prevent the penetration of large molecules and toxic substances;

2) protect against the aggressive action of other microorganisms [11–13].

In general, the protective functions of these structures allow spores to remain dormant for many years [14, 15].

The exosporium is the outer-most structure of the spore, in most *Bacillus* species it is separated from the underlying structure by interspace, the composition and functional purpose of which remains a mystery. The exosporium consists of a basal membrane surrounded by hair-like projections. The collagen-like glycoprotein BCLA is the main component of these projections. Thanks to the hair-like projections of the outer structure, the spores can adhere to the soil fragments, which allows them to stay on the surface and enter the body of animals during grazing. In recent years, the BCLA protein has been given special attention as a possible antigen for vaccine development [16, 17]. The BCLA protein, composing the exosporium, interacts with the phagocytes of the host organism, thereby promoting the penetration of the pathogen into the cell and its subsequent germination – the process by which the spores stop being dormant [4].

Germination is initiated by presence of essential nutrients, which is detected by receptors in the inner membrane of the spore. The binding of the receptors leads to a cascade of successive reactions, including the influx of water, the release of cations and dipicolinic acid, the pH rises to 7.7, and the glycopeptide cortex is hydrolyzed. When pH changes, intracellular enzymes are activated,

the spore coats are destroyed, and vegetative metabolism reactivates, including the production of powerful virulence factors [18].

Ecology of *B. anthracis* in soil

To date, there are several different theories of the *B. anthracis* ecology in soil. The first was put forth in 1941 [19]. According to this theory, the pathogen is able to germinate in certain «incubator areas», that is, in soils rich in organic matter, calcium, with a pH greater than 6.0 and an ambient temperature above 15.5 °C. Sporadic outbreaks of anthrax occur as a result of the pathogen germination in the surface soils under certain climatic and environmental conditions, contributing to accumulation of high concentrations, able to infect grazing animals.

A competing hypothesis suggests that these local accumulations emerge from the physical pooling of spores in rainwater depressions because of the spores hydrophobic surface character [20–22]. Furthermore, vegetative cells of *B. anthracis* were suggested to be unable to successfully compete with resident soil microbiota and have never been found in natural environments. Also the clonal genetic character of this microorganism, isolated from infected animals, argues against frequent episodes of soil proliferation. This statement is inconsistent with the fact that probe sequencing of soils contaminated with *B. anthracis* spores showed the presence of isolates that lack one or both virulence plasmids [23, 24]. The latter is indicative of the active metabolism of the pathogen in the environment, but its further fate in the soil is disputable.

Over time, an increasing number of laboratory results contradicted the established opinion that *B. anthracis* is an obligate pathogen and is able to reproduce exclusively in susceptible animals. For example, other members of the genetically homogeneous group *B. cereus sensu lato* were discovered as common inhabitants of the invertebrate gut [25], and as saprophytes in the rhizosphere of plants [26]. This gave rise to the assumption that the *B. anthracis* germination is not limited to the animal body.

After study of closely related species, similar studies were conducted in the laboratory conditions, which confirmed the capacity of *B. anthracis* to germinate in the rhizosphere of some plants [26] and inside soil amoebas (Fig. 2) [27], which significantly expanded knowledge about its life cycle and the capacity to dictribute in the environment.

In addition, domestic researchers have found that *B. anthracis* spores can persist and spread in the soil with earthworms. It was found that 50–70% of spores retain their properties and virulence in the worm gut for 30 days (the study period) [28].

No less interesting is the life cycle of *B. anthracis*, interacting with bacteriophages that mediate phenotypic changes and cause the appearance of lysogenic variants of the pathogen with a pronounced improvement in survival.

In the course of long-term studies, various *B. anthracis* phages were identified (Fig. 3) [29], including for vaccine strains with low virulence, such as Sterne, Pasteur, and Vollum [30]. As for field strains, soil isolates of *B. anthracis* often contain phage plaques when cultured [26]. In addition, studies of more than 160 natural *B. anthracis* isolates recovered from the environment and from diseased animals showed that more than 20% of them were infected with various phages. Free, infective phages for *B. anthracis* are also found in many environments, including sewage, tannery effluent, animal hair, soil and water at or near anthrax carcasses, as well as soil at non-endemic areas [29].

As an example of the bacteriophage-mediated variability of *B. anthracis*, the results of studies published in the early 21st century can be cited. The strains of bacilli isolated from wild apes in African countries: Cameroon and Côte d'Ivoire are described in the papers. The studied bacteria were characterized by the motility, resistance to penicillin and diagnostic gamma phage, the ability to form a capsule not only after induction by CO_2 and bicarbonate, the secretion of protective antigen and lethal factor. These strains had both the toxin and the capsule plasmid pBCXO1 and pBCXO2, with sizes corresponding to the *B. anthracis* viru-

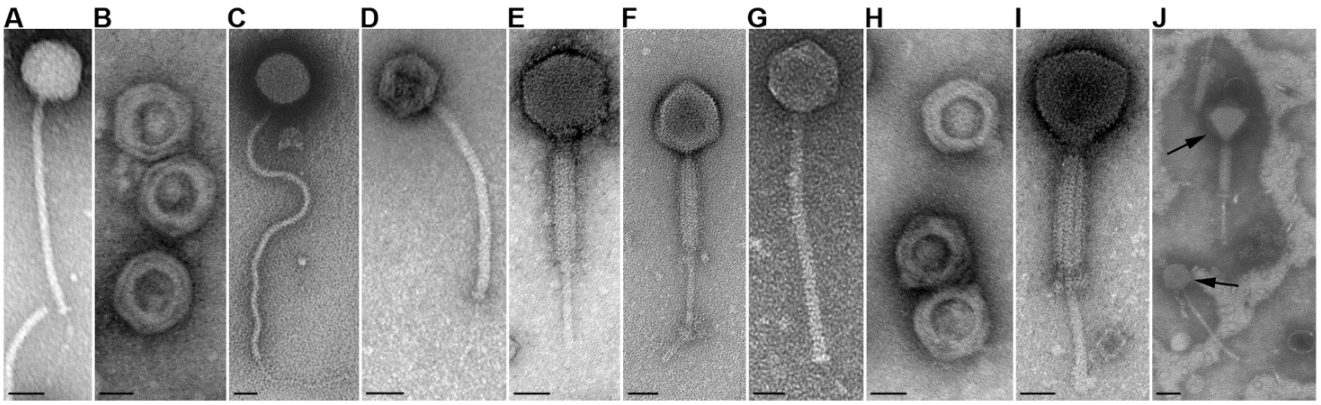

Fig. 3. Transmission electron micrographs of bacteriophages negatively stained with 2% uranyl acetate [29]. *The bacteriophages infecting B. anthracis include, (A) Wβ, (B) Wip1, (C) Wip2, (D) Wip4, (E) Wip5, (F) Frp1, (G) Frp2, (H) Htp1, and (I) Bcp1. An extract from the gut of the earthworm Eisenia fetida is shown (J) with two distinct and uncharacterized phages indicated by arrows*

Рис. 3. Просвечивающая электронная микроскопия бактериофагов, выделенных от B. anthracis, отрицательно окрашенных 2%-м раствором уранилацетата [29]. *Бактериофаги, инфицирующие B. anthracis, включают: (A) Wβ, (B) Wip1, (C) Wip2, (D) Wip4, (E) Wip5, (F) Frp1, (G) Frp2, (H) Htp1 и(I) Bcp1. J – экстракт из кишечника дождевого червя Eisenia fetida (стрелками обозначены два неидентифицированных фага)*

lence plasmids pXO1 and pXO2. Genetic analysis of these strains revealed a close relationship with both classic *B. anthracis* strains and two uncommonly virulent *B. cereus* and *B. thuringiensis* isolates. The authors of the study proposed that the newly discovered strains share a common ancestor with *B. anthracis* or that they emerged recently by transfer of the *B. anthracis* plasmids to a strain of the *B. cereus* group [31–34]. These strains were designated *B. cereus* biovar *anthracis*. These strains were as virulent for mice and guinea pigs as wild-type *B. anthracis* and remained virulent after removal of the plasmid encoding capsule synthesis. In addition to the poly-D-glutamate capsule, these strains were found to produce a hyaluronic acid capsule encoded by the pBXO1 plasmid. Such phenotypic changes enabled systemic dissemination, thus providing a clear evolutionary advantage [35]. In this regard, it is relevant to further study the life of these *B. anthracis* strains in the environment and organisms of susceptible animals as pathogens of potentially new infectious diseases.

Ecology of *B. anthracis* in the environment

More than 50 species of animals belonging to 8 orders and 23 families are susceptible to the *B. anthracis*, which explains the reason for the wide geographical spread of this infection around the world [20]. However, birds are not susceptible to this pathogen, nevertheless, they play a significant role in the epizootology and epidemiology of anthrax, contributing to the spread of spores to new territories [36].

It is known that the condition for *B. anthracis* circulation in nature is the contamination of the soil with spores after the death of a diseased animal. However, if the integrity of the carcass is preserved, the bacilli do not sporulate and die [37]. Therefore, the activity of scavenger birds has a significant impact on the circulation of the pathogen: carcass consumption contributes to spore formation, and dispersing the remains leads to widespread contamination of the soil with spores.

Experimental studies of other authors have shown that birds, consuming meat of infected animals, can secrete spores of the pathogen with excrement for a long time and transfer them in the beak and on the paws. Flying over long distances, birds can spread spores in areas where this disease has not been previously recorded [38]. In our country, scavenger birds consuming reindeer carcasses are actively involved in the spread of *B. anthracis* spores. Synanthropic birds are also dangerous. For example, studies were conducted in the UK to study the role of house sparrows *Passer domesticus* in the spread of *B. anthracis* spores. Scientists have found that 2% of these birds are carriers of *B. anthracis* spores. The researchers suggest that in countries with a high incidence of anthrax, the percentage of infected sparrows must be higher [36].

One of the indicators of anthrax prevalence in nature is the circulation of the pathogen among various rodent species. Reports on the isolation of *B. anthracis* from field rodents in the Russian Federation regions and the former Soviet Union countries evidence that anthrax is latent in naturally infected mouse-like rodents. *B. anthracis* cultures were repeatedly isolated from clinically healthy field mice showing no post-mortem changes in organs and tissues, which reflects the possibility of unhindered spore dissemination with these animal species.

The ability to transfer *B. anthracis* spores with blood-sucking insects is also very important. Flies, horseflies, ticks, and mosquitoes feed on the blood of infected animals. Then, moving and biting a healthy animal, they introduce the pathogen into a new susceptible organism. In addition, it was found that, flying into the adjacent vegetation, they secrete both spores of the pathogen and vegetative cells. The researchers noted that *B. anthracis* spores were found on the leaves of plants at a distance of 1–3 meters from the dead animal carcass [20].

Thus, in addition to susceptible animals, there are a large number of species that contribute to the maintenance and spread of the pathogen in the environment, which, in turn, makes it difficult to control this infection and requires strict measures for specific prevention of anthrax.

Problems of anthrax soil foci

One of the main reservoirs of *B. anthracis* is the soil, which is considered the second source of the disease after infected animals. Infection with *B. anthracis* spore form was reported after contacts with spore-contaminated soil in 3–14% out of total number of cases [39]. Spore-contaminated soil can remain a source of infection for many decades. To date, it has been established that anthrax bacillus spores can persist for up to 200 years. However, the exact period of possible presence in the soil and the ability to infect living organisms with *B. anthracis* spores has not yet been established [20].

A retrospective analysis of data on anthrax incidence in Russia in the 18th and 19th centuries shows that it was one of the most common diseases. During this period, more than 100,000 cases were officially recorded in the country. In the 20th century, 69,827 anthrax outbreaks occurred on the territory of our country [40]. Many carcasses were buried without prompt control, which led to a wide spread of soil foci and an increase in the number of anthrax animal burial sites on the territory of Russia.

In the Russian Federation today, there are more than 35,000 permanently anthrax infected settlements, 14,109 animal burial sites, of which 3,193 are anthrax burial sites [41]. Abandoned animal burial sites and animal burial sites with unknown geographical coordinates are particularly dangerous. Many soil foci are not marked on maps or on the terrain. Initially, these burials were under the control of local veterinary services, but over many decades, as a result of numerous reorganizations and the transfer of control functions over animal burial sites from one agency to another, the archived data on these animal burial sites were lost in most cases. As a result, on the territory of our country there are a large number of anthrax soil foci, both known and uncontrolled, which pose a great danger of potential spread and infection with this highly dangerous infection.

To date, Russian scientists have developed a number of methods for the disinfection of anthrax soil foci, but there are no effective and environmentally friendly methods among them. It should also be noted that it is impossible to accurately determine the effectiveness of disinfection of anthrax soil focus, since, according to researchers, the possibility of detecting *B. anthracis* and isolating it from the soil is no more than 1.5% [42]. In this regard, all existing anthrax burials have a potential danger to a greater or lesser extent [43].

Recently, due to the use of previously abandoned land, increase in residential development, there is a need for a detailed study of this danger, which, according to

researchers, persists because of violated conditions for burial site maintenance [44]. According to available data, on the national scale, on average, 37% of biological waste disposal sites are in poor veterinary and sanitary condition [40]. The situation related to the maintenance of burial sites in infected regions poses a potential danger and requires constant monitoring of the state of these sites.

Currently, specialists are developing methods for studying the epizootological and epidemiological danger of anthrax burials, aimed at assessing the risks of their possible impact on outbreak occurrence and the spread of infection in order to prevent it [42].

According to the researchers, the increasingly emerging initiatives for the elimination or conservation of anthrax burial sites are not only useless, but also harmful, since they exclude the possibility of further predicting the risks associated with the soil foci that surround the burial sites and cannot be decontaminated [42, 45]. In addition, local disinfection of known soil foci is not able to provide its complete elimination. Due to the fact that several dozen species of wild animals are susceptible to anthrax, which are potential carriers of it, it can be assumed that there are many other foci in the wild, and with each new animal that becomes diseased, their number increases.

CONCLUSION

One hundred and fifty years of studying the ecology of *B. anthracis* has allowed us to shed light on many aspects of the pathogen's existence in the environment, to establish its connection and interaction with various species of living organisms. However, many links in the life chain of *B. anthracis* in the abiotic environment remain poorly understood. Questions concerning the mechanisms, ways of existence and evolution of anthrax causative agent outside the animal's body also require an in-depth study, which will make a significant contribution to solving the global problem of protecting animals and people from this natural focal infection.

REFERENCES

1. Ivanova S. V., Melnikova L. A., Rodionov A. P. Dynamics of the functional activity of the phagocytic cells of animals vaccinated against anthrax. *Veterinarian*. 2020; 5: 33–39. DOI: 10.33632/1998-698X.2020-5-33-39. (in Russian)

2. Patel V. I., Booth J. L., Dozmorov M., Brown B. R., Metcalf J. P. Anthrax edema and lethal toxins differentially target human lung and blood phagocytes. *Toxins*. 2020; 12 (7):464. DOI: 10.3390/toxins12070464.

3. Andryukov B. G., Karpenko A. A., Lyapun I. N. Learning from nature: Bacterial spores as a target for current technologies in medicine (review). *Sovremennye tehnologii v medicine* [*Modern Technologies in Medicine*]. 2020; 12 (3): 105–123. DOI: 10.17691/stm2020.12.3.13.

4. Driks A. The *Bacillus anthracis* spore. *Mol. Aspects Med.* 2009; 30 (6): 368–373. DOI: 10.1016/j.mam.2009.08.001.

5. Driks A., Setlow P. Morphogenesis and Properties of the Bacterial Spore. *In: Prokaryotic Development*. Ed. by Y. V. Brun, L. J. Shimkets, Washington: ASM Press; 2000; 191–218. DOI: 10.1128/9781555818166.ch9.

6. Chada V. G., Sanstad E. A., Wang R., Driks A. Morphogenesis of *Bacillus* spore surfaces. *J. Bacteriol.* 2003; 185 (21): 6255–6261. DOI: 10.1128/jb.185.21.6255-6261.2003.

7. Driks A. The dynamic spore. *Proc. Natl. Acad. Sci. USA*. 2003; 100 (6): 3007–3009. DOI: 10.1073/pnas.0730807100.

8. Plomp M., Leighton T., Wheeler K. E., Malkin A. J. The high-resolution architecture and structural dynamics of *Bacillus* spores. *Biophys. J.* 2005; 88 (1): 603–608. DOI: 10.1529/biophysj.104.049312.

9. Plomp M., Leighton T. J., Wheeler K. E., Malkin A. J. Architecture and high-resolution structure of *Bacillus thuringiensis* and *Bacillus cereus* spore coat surfaces. *Langmuir.* 2005; 21 (17): 7892–7898. DOI: 10.1021/la050412r.

10. Westphal A. J., Price P. B., Leighton T. J., Wheeler K. E. Kinetics of size changes of individual *Bacillus thuringiensis* spores in response to changes in relative humidity. *Proc. Natl. Acad. Sci. USA*. 2003; 100 (6): 3461–3466. DOI: 10.1073/pnas.232710999.

11. Henriques A. O., Moran C. P. Structure, assembly and function of the spore surface layers. *Annu. Rev. Microbiol*. 2007; 61: 555–588. DOI: 10.1146/annurev.micro.61.080706.093224.

12. Laaberki M. H., Dworkin J. Role of spore coat proteins in the resistance of *Bacillus subtilis* spores to *Caenorhabditis elegans* predation. *J. Bacteriol*. 2008; 190 (18): 6197–6203. DOI: 10.1128/JB.00623-08.

13. Setlow P. Spores of *Bacillus subtilis*: Their resistance to and killing by radiation, heat and chemicals. *J. Appl. Microbiol*. 2006; 101 (3): 514–525. DOI: 10.1111/j.1365-2672.2005.02736.x.

14. Nicholson W. L. Using thermal inactivation kinetics to calculate the probability of extreme spore longevity: implications for paleomicrobiology and lithopanspermia. *Orig. Life Evol. Biosph*. 2003; 33 (6): 621–631. DOI: 10.1023/a:1025789032195.

15. Vreeland R. H., Rosenzweig W. D., Powers D. W. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature*. 2000; 407 (6806): 897–900. DOI: 10.1038/35038060.

16. Fox A., Stewart G. C., Waller L. N., Fox K. F., Harley W. M., Price R. L. Carbohydrates and glycoproteins of *Bacillus anthracis* and related bacilli: targets for biodetection. *J. Microbiol. Methods*. 2003; 54 (2): 143–152. DOI: 10.1016/s0167-7012(03)00095-2.

17. Tournier J. N., Ulrich R. G., Quesnel-Hellmann A., Mohamadzadeh M., Stiles B. G. Anthrax, toxins and vaccines: a 125-year journey targeting *Bacillus anthracis*. *Expert Rev. Anti Infect. Ther*. 2009; 7 (2): 219–236. DOI: 10.1586/14787210.7.2.219.

18. Fisher N., Hanna P. Characterization of *Bacillus anthracis* germinant receptors *in vitro*. *J. Bacteriol*. 2005; 187 (23): 8055–8062. DOI: 10.1128/JB.187.23.8055-8062.2005.

19. Minett F. C., Dhanda M. R. Multiplication of *B. anthracis* and *Cl. chauvoei* in soil and water. *Indian J. Vet. Sci. Anim. Husb.* 1941; 11: 308–321.

20. Hugh-Jones M., Blackburn J. The ecology of *Bacillus anthracis*. *Mol. Aspects Med*. 2009; 30 (6): 356–367. DOI: 10.1016/j.mam.2009.08.003.

21. Girault G., Parisot N., Peyretaillade E., Peyret P., Derzelle S. Draft genomes of three strains representative of the *Bacillus anthracis* diversity found in France. *Genome Announc*. 2014; 2 (4):e00736-14. DOI: 10.1128/genomeA.00736-14.

22. Brahmbhatt T. N., Janes B. K., Stibitz E. S., Darnell S. C., Sanz P., Rasmussen S. B., O'Brien A. D. *Bacillus anthracis* exosporium protein BclA affects spore germination, interaction with extracellular matrix proteins, and hydrophobici-

ty. *Infect. Immun*. 2007; 75 (11): 5233–5239. DOI: 10.1128/IAI.00660-07.

23. Antwerpen M., Ilin D., Georgieva E., Meyer H., Savov E., Frangoulidis D. MLVA and SNP analysis identified a unique genetic cluster in Bulgarian *Bacillus anthracis* strains. *Eur. J. Clin. Microbiol. Infect. Dis*. 2011; 30 (7): 923–930. DOI: 10.1007/s10096-011-1177-2.

24. Aikembayev A. M., Lukhnova L., Temiraliyeva G., Meka-Mechenko T., Pazylov Y., Zakaryan S., et al. Historical distribution and molecular diversity of *Bacillus anthracis*, Kazakhstan. *Emerg. Infect. Dis*. 2010; 16 (5): 789–796. DOI: 10.3201/eid1605.091427.

25. Jensen G. B., Hansen B. M., Eilenberg J., Mahillon J. The hidden lifestyles of *Bacillus cereus* and relatives. *Environ. Microbiol*. 2003; 5 (8): 631–640. DOI: 10.1046/j.1462-2920.2003.00461.x.

26. Saile E., Koehler T. M. *Bacillus anthracis* multiplication, persistence, and genetic exchange in the rhizosphere of grass plants. *Appl. Environ. Microbiol*. 2006; 72 (5): 3168–3174. DOI: 10.1128/AEM.72.5.3168-3174.2006.

27. Dey R., Hoffman P. S., Glomski I. J. Germination and amplification of anthrax spores by soil-dwelling amoebas. *Appl. Environ Microbiol*. 2012; 78 (22): 8075–8081. DOI: 10.1128/AEM.02034-12.

28. Shishkova N. A., Marinin L. I., Mokrievich A. N. Interaction between earthworms and soil-inhabiting anthrax microbe spores. *Problemy Osobo Opasnykh Infektsii* [*Problems of Particularly Dangerous Infections*]. 2012; 1 (111): 66–69. DOI: 10.21055/0370-1069-2012-1(111)-66-69. (in Russian)

29. Schuch R., Fischetti V. A. The secret life of the anthrax agent *Bacillus anthracis*: bacteriophage-mediated ecological adaptations. *PloS ONE*. 2009; 4 (8):e6532. DOI: 10.1371/journal.pone.0006532.

30. Kiel J. L., Parker J. E., Holwitt E. A., McCreary R. P., Andrews C. J., De Los Santos A., et al. Geographical distribution of genotypic and phenotypic markers among *Bacillus anthracis* isolates and related species by historical movement and horizontal transfer. *Folia Microbiol*. 2008; 53 (6): 472–478. DOI: 10.1007/s12223-008-0074-2.

31. Eremenko E. I., Ryazanova A. G., Buravtseva N. P. The current situation with anthrax in Russia and the world. Main trends and features. *Problemy Osobo Opasnykh Infectcii* [*Problems of Particularly Dangerous Infections*]. 2017; 1: 65–71. DOI: 10.21055/0370-1069-2017-1-65-71. (in Russian)

32. Klee S. R., Ozel M., Appel B., Boesch C., Ellerbrok H., Jacob D., et al. Characterization of *Bacillus anthracis*-like bacteria isolated from wild great apes from Cote d'Ivoire and Cameroon. *J. Bacteriol*. 2006; 188 (15): 5333–5344. DOI: 10.1128/JB.00303-06.

33. Leendertz F. H., Lankester F., Guislain P., Néel C., Drori O., Dupain J., et al. Anthrax in Western and Central African great apes. *Am. J. Primatol*. 2006; 68 (9): 928–933. DOI: 10.1002/ajp.20298.

34. Okinaka R., Pearson T., Keim P. Anthrax, but not *Bacillus anthracis*? *PLoS Pathog*. 2006; 2 (11):e122. DOI: 10.1371/journal.ppat.0020122.

35. Brézillon C., Haustant M., Dupke S., Corre J. P., Lander A., Franz T., et al. Capsules, toxins and AtxA as virulence factors of emerging *Bacillus cereus* biovar *anthracis*. *PLoS Negl. Trop. Dis*. 2015; 9 (4):e0003455. DOI: 10.1371/journal.pntd.0003455.

36. Kolonin G. V. On the role of birds in epizootology of anthrax. T*he Russian Journal of Ornithology*. 2017; 26 (1397): 327–329. eLIBRARY ID: 27664072. (in Russian)

37. Turner W. C., Kausrud K. L., Krishnappa Y. S., Cromsigt J. P., Ganz H. H., Mapaure I., et al. Fatal attraction: vegetation responses to nutrient inputs attract herbivores to infectious anthrax carcass sites. *Proc. Biol. Sci*. 2014; 281 (1795):20141785. DOI: 10.1098/rspb.2014.1785.

38. Dragon D. C., Bader D. E., Mitchell J., Woollen N. Natural dissemination of *Bacillus anthracis* spores in northern Canada. *Appl. Environ Microbiol*. 2005; 71 (3): 1610–1615. DOI: 10.1128/AEM.71.3.1610-1615.2005.

39. Shishkova N. A., Tyurin E. A., Marinin L. I., Dyatlov I. A., Mokrievich A. N. Modern state of the anthrax problem. *Bacteriology*. 2017; 2 (3): 33–40. DOI: 10.20953/2500-1027-2017-3-33-40. (in Russian)

40. Popova A. Yu., Ezhlova E. B., Demina Yu. V., Kulichenko A. N., Ryazanova A. G., Buravtseva N. P., et al. Ways to improve epidemiological surveillance and control of anthrax in the Russian Federation. *Problemy Osobo Opasnykh Infectcii* [*Problems of Particularly Dangerous Infections*]. 2017; 1: 84–88. DOI: 10.21055/0370-1069-2017-1-84-88. (in Russian)

41. Belchikhina A. V., Shibaev M. A., Klinovitskaya I. M., Karaulov A. K. The state of animal waste rendering and disposing system in the subjects of the Russian Federation. *Veterinary Science Today*. 2019; 4: 54–60. DOI: 10.29326/2304-196X-2019-4-31-54-60. (in Russian)

42. Simonova E. G., Kartavaya S. A., Loktionova M. N., Ladnyi V. I. Epidemiological hazard of anthrax animal burials: Theoretical and methodological aspects. *Medicine in Kuzbass*. 2013; 12 (2): 26–31. eLIBRARY ID: 20371381. (in Russian)

43. Dugarzhapova Z. F., Rodzikovsky A. V., Chesnokova M. V. Epidemiological surveillance for anthrax using GIS-technologies at the territory of large industrial project constructions. *The Far Eastern Journal of Infectious Pathology*. 2010; 17 (17): 216–219. eLIBRARY ID: 18379625. (in Russian)

44. Simonova E. G., Galkin V. V., Loktionova M. N., Ladnyi V. I. Anthrax cattle burial grounds in Russia and their biosafety. *Zhurnal mikrobiologii, èpidemiologii i immunobiologii* [*Journal of Microbiology, Epidemiology and Immunobiology*]. 2010; 4: 23–26. eLIBRARY ID: 17949167. (in Russian)

45. Ivanova S. V., Melnikova L. A., Rodionov A. P., Makaev K. N., Safina G. M., Murtazina G. K., et al. Analysis of the epizootic situation and improvement of the scheme for the specific prevention of anthrax. *Int. J. Res. Pharm. Sci*. 2020; 11 (1): 949–952. DOI: 10.26452/ijrps.v11i1.1919.

Received on 12.02.2021
Approved for publication on 16.03.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Alexander. P. Rodionov, Junior Researcher, Laboratory for Collection of Strains of Microorganisms, FSBSI "FCTRBS-ARRVI", Kazan, Russia.

Родионов Александр Павлович, младший научный сотрудник лаборатории коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», г. Казань, Россия.

Elena A. Artemeva, Candidate of Science (Veterinary Medicine), Head of Laboratory for Collection of Strains of Microorganisms, FSBSI "FCTRBS-ARRVI", Kazan, Russia.

Lilia A. Melnikova, Candidate of Science (Veterinary Medicine), Associate Professor, Leading Researcher, Laboratory for Collection of Strains of Microorganisms, FSBSI "FCTRBS-ARRVI", Kazan, Russia.

Maxim A. Kosarev, Candidate of Science (Biology), Head of Department of Bacteriology, FSBSI "FCTRBS-ARRVI", Kazan, Russia.

Svetlana V. Ivanova, Candidate of Science (Biology), Leading Researcher, Laboratory of Viral Anthropozoonoses, FSBSI "FCTRBS-ARRVI", Kazan, Russia.

Артемьева Елена Александровна, кандидат ветеринарных наук, заведующий лабораторией коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», г. Казань, Россия.

Мельникова Лилия Арсентьевна, кандидат ветеринарных наук, доцент, ведущий научный сотрудник лаборатории коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», г. Казань, Россия.

Косарев Максим Аркадьевич, кандидат биологических наук, заведующий отделением бактериологии ФГБНУ «ФЦТРБ-ВНИВИ», г. Казань, Россия.

Иванова Светлана Викторовна, кандидат биологических наук, ведущий научный сотрудник лаборатории вирусных антропозоонозов ФГБНУ «ФЦТРБ-ВНИВИ», г. Казань, Россия.