Original Study Article
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Anthrax in the Russian Federation in 2023 or in other words, «the same old story»

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Abstract

Introduction. The current anthrax situation in Russia is characterized by instability. In 2023, there was an increase in the number of infection outbreaks compared to the long-term average (for five years).

The aim of the study is to assess the epizootological and epidemiological situation regarding anthrax in the Russian Federation in 2023 and the reasons for its deterioration, and to analyze data from genomic epidemiological surveillance of this infection.

Materials and methods. The information of the territorial bodies of Rospotrebnadzor on the investigation of anthrax outbreaks, reference materials about anthrax stationary hazardous areas and anthrax burials were used. The phylogenetic position of the identified *Bacíllus anthracis* strains and genomes structure were determined based on whole-genome sequencing data.

Results. In 2023 anthrax outbreaks were registered in the Chuvash Republic — Chuvashia (1), the Tyva Republic (1), Tambov (1), Ryazan (1) and Voronezh (3) regions. 14 farm animals and 19 people fell ill. The infection of animals not vaccinated against anthrax, as well as vaccinated long before contact with the source of infection, occurred mainly during grazing in the territories of old (unregistered) anthrax soil foci. Human disease is caused by contact with sick animals during care, forced slaughter, cutting, transportation of carcasses and meat, cooking processing of contaminated meat and offal, and consumption of insufficiently heat-treated liver. 17 patients were diagnosed with a cutaneous form of anthrax, while 2 had an oropharyngeal form combined with a cutaneous form of the disease.

In all cases, the genome structure typical of the *B. anthracis* species has been established. The phylogenetic relationship of *B. anthracis* isolates with *B. anthracis* strains previously isolated in Russia is shown.

Conclusion. The reason for the trouble in anthrax in 2023 was a number of violations of veterinary and sanitary-epidemiological regulations against the background of the presence of soil foci of infection. Stabilization of the situation can be achieved only in full range of regulated preventive measures are constantly implementated. The results of molecular genetic typing of B. anthracis strains isolated during the epidemiologic investigation of seven anthrax outbreaks in the Russian Federation in 2023 allow us to conclude that they are of local origin and have a genome structure typical of the species. Genetic analysis of the isolated strains demonstrated the effectiveness of the developed wgSNP typing system in the epidemiologic investigation of outbreaks.

Keywords: anthrax, soil focus, outbreak, *Bacillus anthracis*, whole genome sequencing

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Local Ethics Committee of the Stavropol Research Anti-Plague Institute (protocol No. 109, May 19, 2022).

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Оригинальное исследование
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Сибирская язва в Российской Федерации в 2023 году, или «старая сказка о главном»

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Аннотация

Введение. Современная ситуация по сибирской язве (СЯ) в России характеризуется неустойчивостью. В 2023 г. отмечено увеличение числа вспышек инфекции по сравнению со средним многолетним показателем (за 5 лет).

Цель работы — оценка эпизоотолого-эпидемиологической ситуации по СЯ, сложившейся в России в 2023 г., и причин её ухудшения, анализ данных геномного эпидемиологического надзора за этой инфекцией.

Материалы и методы. Использовали информацию территориальных органов Роспотребнадзора о расследовании вспышек СЯ, справочные материалы о стационарно неблагополучных по СЯ пунктах и сибиреязвенных захоронениях. Филогенетическое положение идентифицированных штаммов *Bacíllus anthracis* и структуру геномов определяли на основе данных полногеномного секвенирования.

Результаты. В 2023 г. вспышки СЯ зарегистрированы в Чувашской Республике (1), Республике Тыва (1), Тамбовской (1), Рязанской (1) и Воронежской (3) областях. Заболело 14 сельскохозяйственных животных и 19 человек. Заражение животных, не вакцинированных против СЯ, а также привитых задолго до контакта с источником инфекции, происходило преимущественно при выпасе на территориях старых (неучтённых) почвенных очагов СЯ. Заболевание людей обусловлено контактом с больными животными при уходе, вынужденном убое, разделке, транспортировке туш и мяса, кулинарной обработке заражённого мяса и субпродуктов, употреблением в пищу недостаточно термически обработанного ливера. У 17 заболевших диагностирована кожная форма СЯ, у 2 — орофарингеальная форма в сочетании с кожной формой болезни. Во всех случаях установлена типичная для вида *B. anthracis* структура геномов. Показана филогенетическая связь изолятов со штаммами *B. anthracis*, ранее выделенными в России.

Заключение. Причиной неблагополучия по СЯ в 2023 г. стал ряд нарушений ветеринарного и санитарно-эпидемиологического нормирования на фоне наличия почвенных очагов инфекции. Стабилизация обстановки может быть достигнута только при постоянной реализации в полном объёме комплекса регламентированных профилактических мероприятий. Результаты молекулярно-генетического типирования штаммов *B. anthracis,* выделенных в ходе эпидемиологического расследования 7 вспышек СЯ на территории России в 2023 г., позволяют сделать вывод об их местном происхождении и типичной для вида структуре генома. Генетический анализ изолированных штаммов показал эффективность применения разработанной системы wgSNP-типирования при эпидемиологическом расследовании вспышек.

Ключевые слова: *сибирская язва, почвенный очаг, Bacillus anthracis, полногеномное секвенирование*

Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациентов. Протокол исследования одобрен Ставропольского государственного медицинского университета (заключение № 109 от 19.05.2022).

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

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

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Introduction

Anthrax is a particularly dangerous zoonotic disease that is practically ubiquitous in the world [1, 2]. The danger of the disease is due to the ability of its causative agent, *Bacillus anthracis*, to form highly resistant to environmental factors spores, which, once in the soil, remain viable for decades, forming persistent soil foci of infection.

In the past, anthrax of susceptible animals and humans in Russia was widespread. Since 1900, more than 70,000 outbreaks of animal and human anthrax have been recorded in Russia on the territory of more than 35,000 stationary anthrax-affected sites [3]. Thus, in the 1920s, the number of cases among animals was measured in tens of thousands (142,800 animals in 1920–1923), among humans — in thousands (9,037 for the same period), and in some years more than 10,000 human cases per year were registered (46,326 cases in 1924–1926) [4]. Thanks to the introduction of mass prophylactic immunization of farm animals and contingents at high risk of infection through occupational contact with livestock and livestock products since the 1950s, a striking reduction in the incidence of both animal and human diseases was achieved [5].

The current situation on anthrax in Russia is characterized by instability. While in 2018–2022 there were sporadic cases in animals (1-3 per year) and humans (2–5 per year), and in 2017 anthrax was not observed at all [6–8], then in 2016, the largest epizootic was registered in the Yamalo-Nenets Autonomous District with the disease in more than 2650 reindeer and 36 people with 1 fatal case [9].

In 2023, Russia again recorded a worsening of the situation on anthrax, which makes it necessary to analyze the causes that led to the unfavorable situation on this infection.

Currently, due to the development of sequencing technologies, a large amount of data on the genomic sequences of microorganisms has been accumulated. The method of whole genome sequencing is used to study the structure of genome sequences, phylogenetic analysis of isolates, and is successfully used in the process of epidemiological investigation of outbreaks of infectious diseases, including anthrax [10-13]. In this regard, the active improvement and implementation of genomic epidemiologic surveillance methods seems to be particularly relevant.

The aim of this study is to assess the epizootological and epidemiological situation of anthrax in Russia in 2023 and the reasons for its deterioration, as well as to analyze the data of genomic epidemiological surveillance of this infection.

Materials and methods

Information from the Rospotrebnadzor offices in the Chuvash Republic, Republic of Tyva, Tambov, Ryazan, and Voronezh regions on the investigation of anthrax outbreaks in the respective subjects in 2023 was used in this work. In order to conduct a retrospective analysis of the anthrax situation in these territories, we used official reference materials on stationary anthrax-affected sites [3], anthrax burial sites [14–16], as well as databases of stationary anthrax-affected sites and anthrax burial sites created in 2023, based on updated information (database "Inpatient anthrax-affected sites in the Russian Federation", certificate of state registration from 01.08.2024 No. 2024623389).

All subjects gave informed voluntary consent to participate in these studies (according to the Federal Law "On Fundamentals of Health Protection of Citizens in the Russian Federation" of 21.11.2011 No. 323-FZ, ed. of 30.12.2021). Clinical trials were approved by the local ethical committee of the Federal State Budgetary Educational Institution of Higher Professional Education "Stavropol State Medical University" of the Ministry of Health of Russia (conclusion of the local ethical committee from 19.05.2022 № 109).

All manipulations with laboratory animals were performed according to the "European Convention for the Protection of Vertebrate Animals Used for Experiments or Other Scientific Purposes" (Strasbourg, 18.03.1986 ETS No. 123).

Laboratory diagnosis of anthrax and identification of 32 cultures of *B. anthracis* isolated in 2023 from clinical material (7), animal material (17) and environmental objects (8) were performed in accordance with the methodological guidelines "Laboratory Diagnosis and Detection of Anthrax Pathogen"[1].

B. anthracis genomes were sequenced using the DNBSEQG50RS high-throughput sequencing platform (BGI). Genotyping of 1169 *B. anthracis* strains (302 strains from the collection of the Stavropol Anti-Plague Institute of Rospotrebnadzor, 867 strain genome sequences from the international GenBank database) on the basis of wg-SNPs was performed using Parsnp [17]. The obtained SNP profiles were used to construct phylogenetic reconstruction using the maximum likelihood method according to the Tamura-Nei model [18] in the Mega10 software. To visualize the phylogenetic tree, the FigTree program (Tree Figure Drawing Tool v. 1.4.3) was used [19].

The genome sequences of *B. anthracis* strains from the collection of the Stavropol Plague Control Institute of Rospotrebnadzor are presented in the "Nucleotide sequences of complete genomes of *B. anthracis* strains isolated in Russia and neighboring countries" electronic database (certificate of state registration No. 2022620144 dated 18.01.2022), in the electronic database of the Stavropol Plague Control Institute of Rospotrebnadzor 'Full genome sequences of *B. anthra-*

[1] Methodological guidelines MG 4.2.2413-08 "Laboratory diagnosis and detection of anthrax pathogen" (approved by the Head of Rospotrebnadzor on 29.07.2008).

cis strains' (reg. No. B.ab-R-1–B.ab-R-302) and can be provided on request (stavnipchi@mail.ru).

Results

From March to September 2023, 7 outbreaks of anthrax were registered in Russia in 5 subjects of 3 federal districts of the Russian Federation: Volga, Siberian and Central (**Table**).

The first outbreak of anthrax in Russia in 2023 was recorded in March in the Chuvash Republic. In the private farm of a resident of the village of Starye Aktashevo, Tsivilsky District, a bull calf was slaughtered without anyone notifying veterinary specialists, after which 1 of 4 participants, the most active in the slaughter, became ill. In the process of culinary processing of by-products from cattle the spouse of the owner of the sick steer became infected.

It was found that the animal was not registered and was not vaccinated against anthrax. During the epidemiologic investigation, the owner of the cattle sold meat and skin of the steer to unknown persons on the highway, which were timely seized and destroyed during the epidemiologic investigation of the outbreak.

The clinical diagnosis of cutaneous anthrax in the diseased was confirmed by specialists of the Reference Center for monitoring of the anthrax pathogen (hereinafter referred to as the Reference Center), operating on the basis of the Stavropol Plague Control Institute of Rospotrebnadzor, and the Center for Hygiene and Epidemiology in the Chuvash Republic (Chuvashia) by polymerase chain reaction (PCR), based on the detection of DNA of the anthrax pathogen in skin samples, as well as positive results of additional immunological methods: detection of specific anti-anthrax antibodies by indirect fluorescent antibody method, allergy test with anthrax allergen *in vitro* using flow cytometry. *B. anthracis* culture was isolated from the source of infection — bovine meat.

The second outbreak of anthrax infection was recorded in June in Barun-Khemchiksky district of the Republic of Tyva with the disease in 2 unvaccinated horses, one of which was subjected to forced slaughter and the other fell ill. A total of 5 people fell ill; 2 of the ill persons who carried out unauthorized forced slaughter of a sick horse at a private shepherd's camp near the village of Bizhiktig-Khaya. The skin manifestations of the infection in them were preceded by the formation of inflammatory foci in the oropharynx. On the basis of clinical data, positive PCR detection of *B. anthracis* DNA both in swabs from the pharynx and in samples of skin affects, the patients were diagnosed with the oropharyngeal form of anthrax combined with the cutaneous form of the disease. Thanks to timely diagnosis and immediate start of intensive therapy of the prognostically unfavorable oropharyngeal form of the infection, fatal outcomes were avoided.

Due to the shipment of contaminated meat for sale to a butcher shop in Ak-Dovurak, 3 people became ill with cutaneous anthrax, which was diagnosed on the basis of positive PCR results: the meat transporter, the son of the shop clerk and a female customer who purchased ground beef from the shop. It was found that the meat of cattle, from which the minced beef was made, was contaminated during storage in the refrigerator in the neighborhood with infected horse meat delivered from the outbreak in the village of Bizhiktig-Khaya, during laboratory examination of which *B. anthracis* culture was isolated in the Tuva anti-plague station of Rospotrebnadzor.

An epizootic outbreak with disease in one head of cattle was detected in June in Bondarsky district of Tambov region – in a pasture located 3 km from the village of Shacha Molokanskaya, Mitropolsky rural council. This is the only outbreak of anthrax among livestock in 2023 that did not result in epidemic complications. No samples were sent to the Reference Center for research.

In June, 6 cattle fell in LLC Lenin's Way, Zakharovsky district, Ryazan region, grazing in the summer pasture in the village of Staroye Zimino. As a result of direct contact with sick animals, a cattleman became ill with anthrax. According to the veterinary service of the region, scheduled specific immunization of cattle was carried out in October-November 2022. However, the employees of LLC Lenin's Way, including the sick person, were not vaccinated against anthrax.

The diagnosis of anthrax in 2 cattle was established by the Ryazan Regional Veterinary Laboratory based on the results of bacteriological examination of pathological material samples. In the course of epidemiologic investigation Rospotrebnadzor specialists isolated cultures of anthrax microbe from soil and water samples taken in the places of animal deaths. The clinical diagnosis of cutaneous infection in a patient was confirmed by the detection of *B. anthracis* DNA in a scrape from a skin affect scab.

In 2023, special attention was focused on the situation on anthrax in Voronezh region, where in August–September, 3 outbreaks of infection with 11 people falling ill were recorded in 3 districts of the region.

The first outbreak was detected in August in Paninsky district, where the owner of a private subsidiary farm in Krasnye Kholmy village fell ill after forced slaughter of 1 head of cattle without notification of veterinarians. The animal had not been vaccinated against anthrax. The owner sold the meat to unknown persons, and as a result of search activities it was found that the resellers sold the meat to an entrepreneur of one of the markets in Voronezh, where it was promptly seized almost in its entirety, and remnants of infected meat were found in Semiluki and Novaya Olshanka villages. During material examination by specialists of the Center for Hygiene and Epidemiology in Voronezh region and Stavropol Anti-Plague Institute of Rospotreb-

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ORIGINAL RESEARCHES

Epizootological and epidemiological situation on anthrax in the Russian Federation in 2023

No. in oder	Federal District of the RF	Subject of the RF	Municipal district, locality	Number and type of sick livestock	Number of sick people	Previous outbreak
1	Privolzhsky	Chuvash Republic — Chuvashia	Tsivilsky district, v. Staroe Aktashevo	1 cattle	2	1930
2	Siberian	Tyva Republic	Barun-Khemchiksky district, v. Bizhiktig-Khaya	2 horses	5	2021
3	Central	Tambov region	Bondarsky district, v. Shacha Molokanskaya	1 cattle	-	1959
4	Central	Ryazan region	Zakharovsky district, v. Staroe Zimino	6 cattle	1	1944
5	Central	Voronezh region	Paninsky district, v. Krasnye Holmyi	1 cattle	1	1958
6	Central	Voronezh region	Bogucharsky district, v. Lebedinka	2 cattle	9	1952
7	Central	Voronezh region	Novousmansky district, farm Krylovsky	1 cattle	1	1948
Total	3	7	7	12 cattle, 2 horses)	19	1930–2021

nadzor, *B. anthracis* culture was isolated from samples of cattle skin and meat, DNA of the pathogen was found in samples of pathological skin effects of the patient.

In September, a large outbreak with the disease of 2 cattle and 9 people was registered in Bogucharsky district. Infection of 5 people occurred in the process of forced slaughter of 1 head of cattle, carried out in a peasant (private) farm in Lebedinka village without veterinary inspection, subsequent cutting of the carcass and meat. The second animal fell and was buried on the territory of the farm.

It was found that the cows in the farm were not fully vaccinated, and the diseased animals were not immunized in a planned manner. The employees of the farm were not immunized against anthrax either. During the investigation, it was determined that the owner of the farm transported part of the meat to his own cafe, and sold the rest in an unauthorized place of trade to the owner of another cafe and random persons. As a result, 4 more people became ill from contact with the purchased meat.

In the process of material examination, cultures of the pathogen were isolated from samples of clinical material, meat and cattle hides. Positive PCR results were also obtained in the study of semi-finished meat products from the cafe of the owner of the farm, intended for sale and catering of employees of his farm.

The diagnosis of cutaneous anthrax in patients was also confirmed by the detection of *B. anthracis* DNA in skin samples, specific antibodies and positive results of allergy test.

The third focus of infection was recorded in Novousman district. One farm worker in the Krylovskoye state farm became ill after contact with the carcass of fallen cattle. The clinical diagnosis was confirmed by PCR and immunological methods in the Reference Center.

A total of 32 cultures of the anthrax pathogen were isolated during the outbreaks. Final identification performed at the Reference Center using basic and additional bacteriological tests showed that all isolates were typical virulent cultures of *B. anthracis* with high sensitivity to antibacterial drugs used to treat anthrax in humans (penicillins, carbapenems, tetracyclines, fluoroquinolones, aminoglycosides, rifampicin).

As a result of molecular genetic typing, the cultures isolated in the Chuvash Republic, Ryazan and Voronezh regions were determined to belong to the main genetic clade A, canSNP-group A.Br.008/009 (A.Br.008/011) of the widespread trans-Eurasian subclade, and the isolate isolated in the Republic of Tyva — to canSNP-group B.Br.001/002 of the main genetic clade B.

According to full genome sequencing data, all strains were found to have a typical genome structure and contain a set of virulence genes characteristic of the *B. anthracis* species.

Phylogenetic analysis based on wg-SNP typing of more than 1100 *B. anthracis* strains from the collection of the Stavropol Anti-Plague Institute of Rospotrebnadzor and genomes from the international GenBank database showed that the strain isolated in the Chuvash Republic belongs to the lineage A.Br.118 (STI) (subcluster 2 of the A.Br.125 cluster) and has the closest affinity with *B. anthracis* strains previously isolated during outbreaks of anthrax in the Volgograd region (Bykovsky district, 1985) and Saratov region (Balashovsky district, 2015) (**Fig. 1**).

Cultures from the Paninsky district of Voronezh region and Zakharovsky district of Ryazan region belong to the A.Br.117 (Tsiankovskii) lineage, A.Br.215 cluster, and the strain isolated in the Anninsky district of Voronezh region in 1982 is the closest to them (**Fig. 2**).

The strains isolated in Bogucharsk district of Voronezh region also belong to the A.Br.117 (Tsiankovskii) lineage and cluster with strains isolated in Stavropol Krai (Petrovsky district — 1959, Novoselitsky District — 2001), Volgograd Region (Oktyabrsky District, 2014), Kaluga Region (Kozelsky District, 1989), Republic of Ingushetia (Malgobeksky District, 1968), Ryazan Region (1981), and Republic of Kalmykia (Gorodovikovsky District, 2002) (Fig. 2).

The culture isolated in the Barun-Khemchik District of the Republic of Tyva belongs to the B.Br.013 Asia cluster of the B.Br.012 lineage and has a close phylogenetic relationship with strains isolated in the same region of the republic in 2018 and 2021 (subcluster 1 in **Fig. 3**).

Discussion

By definition, stationary anthrax-affected sites have on their territory soil centers of anthrax, which are anthrax burial sites [20]. However, the number of stationary anthrax-affected sites, let alone outbreaks, is dozens of times greater than the number of registered anthrax burial sites. In the Republic of Tyva, while more than 300 outbreaks have been registered in 154 stationary anthrax-affected sites since 1910, there is information about only 11 burial sites of corpses/ash remains of anthrax-infected animals (of which 7 are carcass incineration sites in 2018–2023). In the Chuvash Republic, where more than 3,600 foci of infection in 1,231 stationary anthrax-affected sites have occurred since 1901, 397 anthrax burial sites were registered as of 2013 [16]; however, in accordance with the resolution of the Cabinet of Ministers of the Chuvash Republic, 345 burial sites were eliminated in 2015, including in the village of Staroye Aktashevo. In the Voronezh region, where 780 stationary anthrax-affected sites have been recorded since 1902, 81 anthrax burial sites were registered [14], but, according to the order of the Voronezh Region Veterinary Department, they were removed from the regional register in 2011. And in the Ryazan region,

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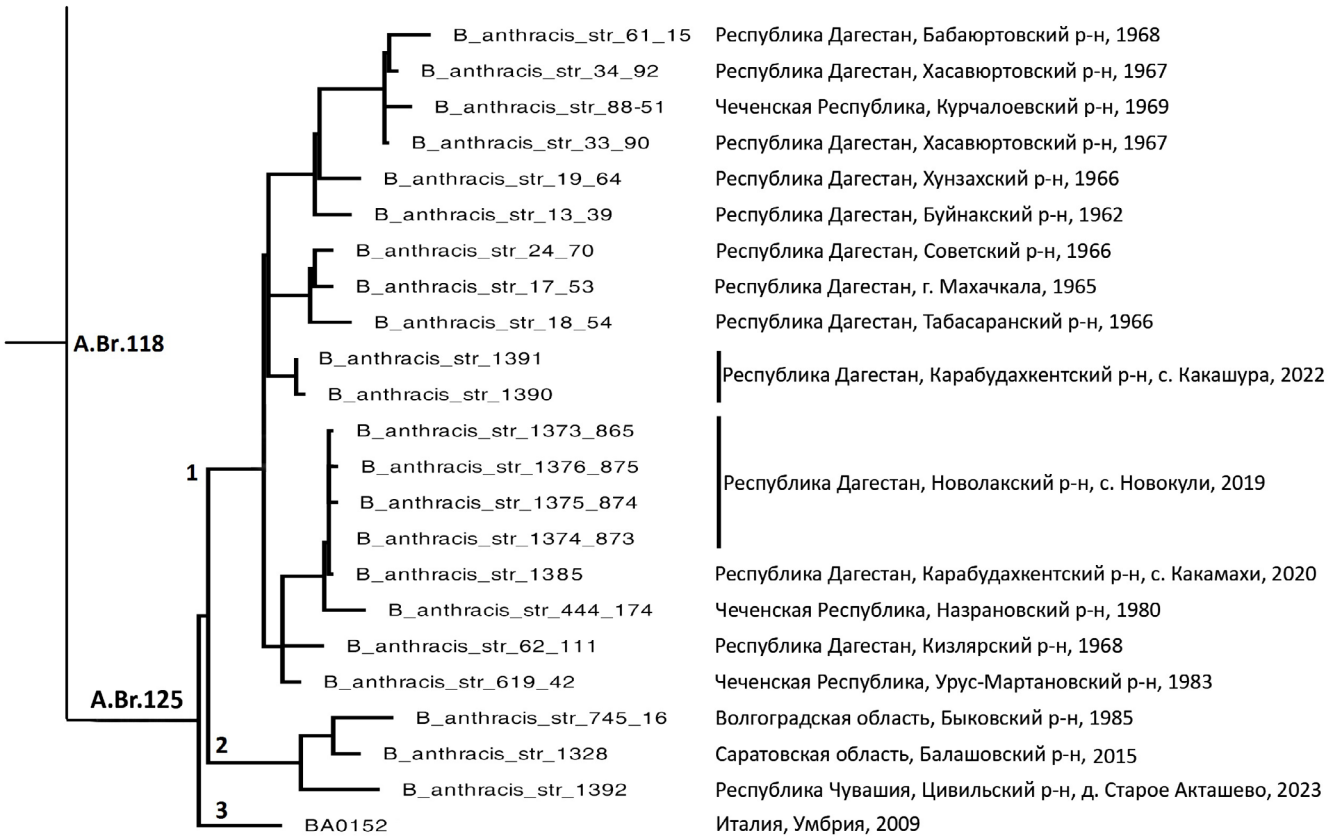

Fig. 1. Phylogenetic position of *B. anthracis* strains isolated in the Chuvash Republic — Chuvashia (2) and the Dagestan Republic (1). Fragment of dendrogram reconstructed on the basis of wg-SNP-analysis by method Maximum Likelihood according to Tamura–Nei model.

in which, since 1901, more than 1900 outbreaks have been noted on the territory of more than 900 stationary anthrax-affected sites, and in the Tambov region, where almost 700 stationary anthrax-affected sites are known to be active more than 1600 times since 1929, not a single anthrax burial site has been accounted for [14]. Thus, on the territory of the constituent entities of the Russian Federation, where outbreaks of anthrax in 2023 have been noted, there is a huge number of unattended animal burial sites that are soil foci of anthrax — unknown, due to the historical absence of their registration or incomplete registration by veterinary services in violation of the veterinary regulatory framework on the need to document and supervise anthrax[2], removed from registration.

Epidemiological investigation of the outbreak and retrospective analysis of the anthrax situation on the territory of the subjects where outbreaks were detected in 2023, conducted in accordance with the regional updated databases of stationary anthrax-affected sites, anthrax, Cadastre information [3], lists of livestock burial grounds [14–16], showed the following.

In the Tsivilsky district of the Chuvash Republic in 1901–1979, 155 cases of anthrax were reported in 72 stationary anthrax-affected sites. The village of Staroye Aktashevo is a stationary anthrax-affected site with manifestations of activity in 1929 and 1930. At a distance of 1 km from the village there is an anthrax burial site organized in 1930. It was found that cattle grazing near the anthrax burial site was impossible due to the presence of persistent snow cover and lack of vegetation in March 2023; it is possible that the animals were infected when consuming fodder prepared earlier on the territory of the anthrax burial site.

Since 1929, anthrax has been reported more than 40 times in the Barun-Khemchik District of the Tyva Republic, but no anthrax has been recorded. The previous outbreak on the territory of this district took place in 2021 in the village of Bizhiktig-Khaya [21]. Previously, the village had not been reported to be infected, but the investigation showed that cattle grazing

[2] Instruction "On measures against anthrax" (approved by the Ministry of Agriculture of the USSR on 28.02.1953); Instruction "On measures against anthrax" (approved by the Main Department of the Ministry of Agriculture of the USSR on 05.06.1981, as amended on 12.11.1982); Sanitary Rules (SP 3.1. 089-96) and Veterinary Rules (VP 13.3.1320-96) "Prevention and control of contagious diseases common to humans and animals", section 6 "Anthrax" (approved by the State Committee for Sanitary and Epidemiological Surveillance of the Russian Federation on 31.05.1996 No. 11, Ministry of Agriculture and Food of the Russian Federation on 18.06.1996 No. 23).

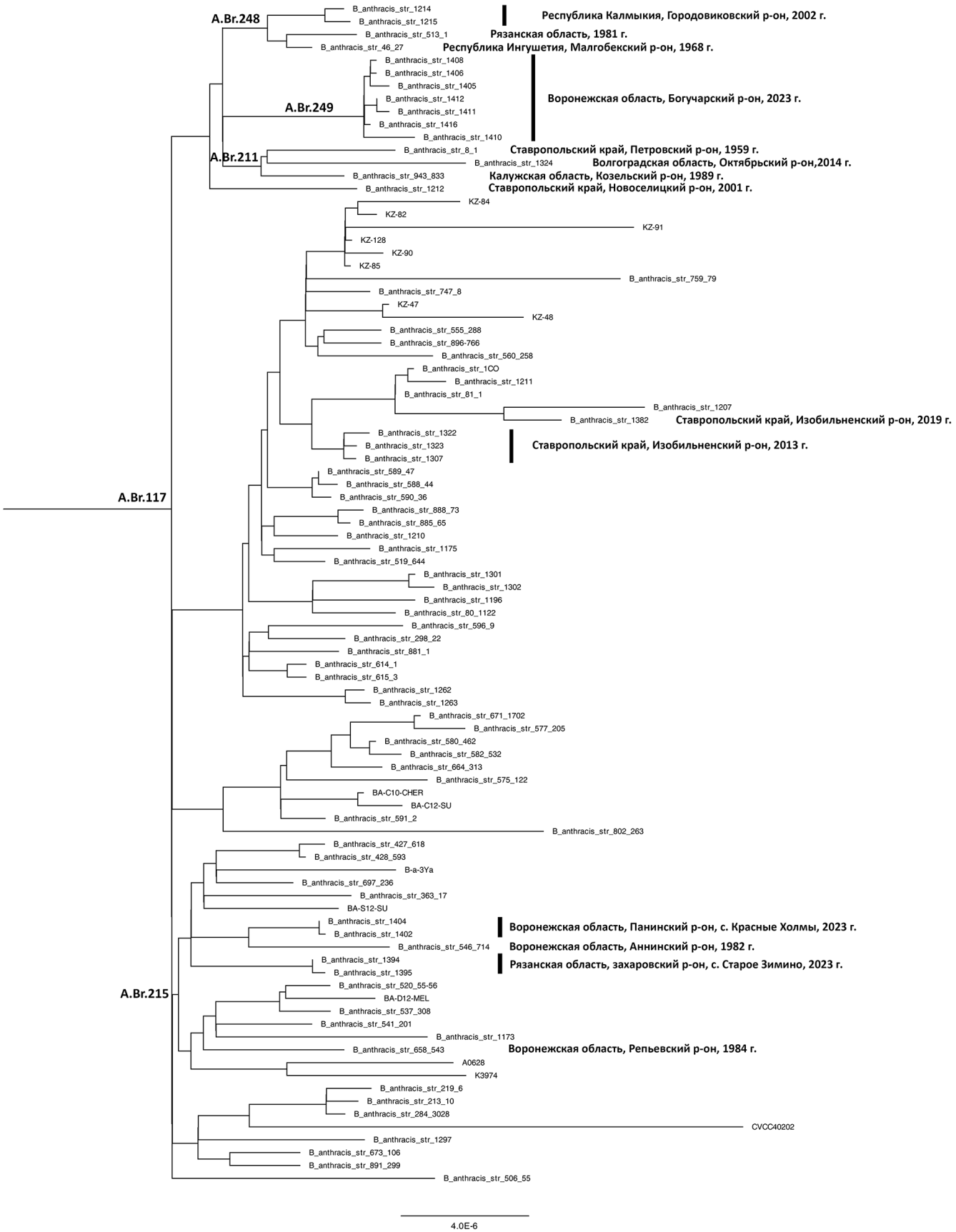

Fig. 2. Results of phylogenetic analysis of B. anthracis strains isolated in the Voronezh and Ryazan regions, Stavropol Territory. Fragment of dendrogram reconstructed on the basis of wg-SNP-analysis by method Maximum Likelihood according to Tamura–Nei model.

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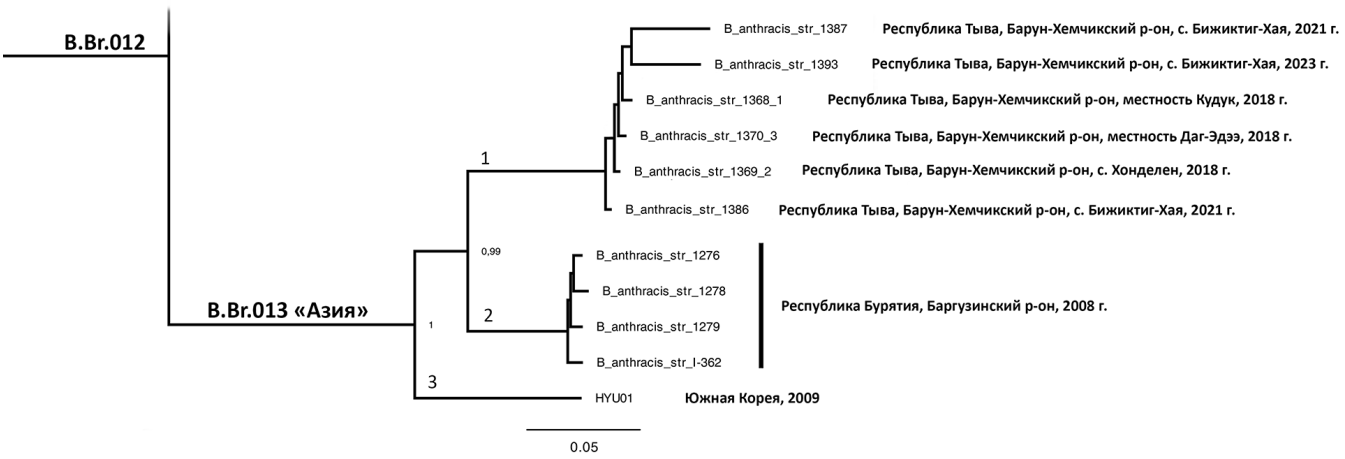

Рис. 3. Филогенетическое положение штаммов *B. anthracis*, выделенных в Республике Тыва (1) и Республике Бурятия (2).

Фрагмент дендрограммы, реконструированной на основе wg-SNP-анализа методом максимального правдоподобия в соответствии с моделью Tamura– Nei.

Fig. 3. Phylogenetic position of B. anthracis strains isolated in the Tyva Republic (1) and the Buryatia Republic (2).

Fragment of dendrogram reconstructed on the basis of wg-SNP-analysis by method Maximum Likelihood according to Tamura–Nei model.

from Bizhiktig-Khaya village and Kyzyl-Mazhalyk village had not been reported. Kyzyl-Mazhalyk, located at a distance of 6 km from the analyzed village, are grazing on a common pasture, and the village of Kyzyl-Mazhalyk. Kyzyl-Mazhalyk is a stationary anthrax-affected site with five times of activity from 1941 to 1989. Thus, infection of livestock both in 2023 and 2021 occurred during grazing on the territory of old soil outbreaks on the territory of the pasture. Prior to that, in 2018, 3 outbreaks were recorded in Barun-Khemchik District (in Khondelen village and two nearby areas – Kuduk, Dag-Edey, and Edegey tract), in which anthrax had been observed many times in the past with the last occurrences in 1950 and 1982, respectively. It was also revealed that the emergence of epizootic foci in 2018, 2021 and 2023 was preceded by heavy rainfall alternating with high ambient temperatures – this gave birth to the term "anthrax weather" [22–24]. This contributed to the release of spores of the anthrax pathogen on the surface of soil foci with subsequent drying and spreading, which facilitated the infection of livestock by aspiration and alimentary routes during grazing.

In Bondarsky district, Tambov region, more than 50 outbreaks of anthrax were recorded in 26 locations in 1933–1974. On the territory of Mitropolsky rural council, which includes the v. Shacha Molokanskaya, anthrax was registered 16 times in 1933-1959, in connection with which it is quite obvious that there are soil foci here – old burials of fallen animals, including in the pasture, where, probably, the infection of cattle occurred.

There are 71 known outbreaks of anthrax in 26 settlements of Zakharievsky District, Ryazan region, in 1904-1980, but there are no recorded cases of anthrax in the district. The village of Staroye Zimino, where the summer pasture of LLC "Lenin's Way" is located, belongs to stationary sites with outbreaks of activity in 1911 and 1944. It was noted that the emergence of the epizootic in the Ryazan region, as well as in the Republic of Tyva, was preceded by heavy precipitation and hot weather, which contributed to the activation of soil foci, resulting in infection during grazing in the territory of the old burial site with unknown localization even of immunized animals.

In Panin district, Voronezh region, 70 outbreaks of infection were recorded in 1938–1984 on the territory of at least 28 counted SNPs. Anthrax in the village of Krasnye Kholmy, the first outbreak focus in the Voronezh region 2023, was reported in 1955 and 1958. In the district, one anthrax was previously registered in the village of Chernavka. Thirty-four stationary anthrax-affected sites have been registered in Bogucharsky District, Voronezh region, in which 74 outbreaks were noted in 1948–1981. No SNPPs are registered in the district. There are no data on the registration of anthrax cases in Lebedinka village, where the second outbreak in the region took place, but outbreaks were registered in other nearby settlements of Pervomaisky rural settlement, which includes this village (Plesnovka village in 1948, Batovka village in 1952). In Novousman district of Voronezh region, where the third outbreak of infection took place, 20 SNPs with activity 42 times in 1941–1997 were recorded, and one stationary anthrax-affected site was registered earlier (Petropavlovka village). According to archival data, the registration of anthrax in 1948 on the territory where the Krylovsky state farm is currently located is not excluded. Thus, infection of animals in Voronezh region also occurred during grazing in the territories of old anthrax-infected soil foci.

A number of violations of the requirements of the legislation in the field of veterinary medicine[3], sanitary and epidemiological well-being of the population[4] and the regulatory and legal acts adopted in accordance with it contributed to the formation of the disease in 2023[5]. First of all – concealment of livestock during registration by owners of private subsidiary farms in the Chuvash Republic, Paninsky district of Voronezh region, the Republic of Tyva and the peasant farm enterprise in Bogucharsky district of Voronezh region, as a result of which farm animals were not covered by specific immunization in a planned manner, which caused the disease of animals in contact with the pathogen. The reasons for the disease of 6 cattle in Ryazan region vaccinated against anthrax are probably related to the fact that routine vaccination was carried out in the fall of 2022, i.e., long before the most dangerous spring-summer season in terms of anthrax infection, and did not ensure the preservation of proper immunity in animals; infection could have been promoted by the entry of a massive dose of the pathogen into the organism, as well as the realization of the vector-borne mechanism of transmission of the sybillivirus microbe from the diseased animal to the others through the bites of blood-sucking insects. There is no information about immunization of farm animals in the foci of Tambov region and Novousman district of Voronezh region; it is obvious that the diseased animals were also not accounted for and, accordingly, were not routinely vaccinated against anthrax.

The next violation, which caused human infection, is the forced slaughter of sick livestock without notifying the veterinary service, whereas the owners were obliged to report within 24 hours by any available means about the case of disease or death of an animal to a veterinary specialist, who determines the order of further actions on site based on the results of the inspection.

Also, the requirements of points 1098–1102 of SanPiN 3.3686-21 on routine vaccination of persons exposed to the risk of occupational infection due to their occupation, which caused the disease in workers of livestock farms in Ryazan region, Bogucharsky and Novousman districts of the Voronezh region.

A gross violation punishable under Article 236 (part 1) of the Criminal Code of the Russian Federation was the sale by owners of farm animals of knowingly contaminated meat from sick animals subjected to forced slaughter and fallen livestock, as a result of contact with which people fell ill in the Republic of Tyva and Bogucharsky district of Voronezh region. Also, a veterinarian of one of the markets in Voronezh city authorized the sale of dangerous products accepted without veterinary accompanying documents from resellers of infected meat from the Paninsky district of Voronezh region.

As a result, the described violations, first of all, of owners of private subsidiary farms and private farms, which initially led to non-coverage of livestock with routine vaccination, caused the formation of epizootological-epidemiological disadvantage for anthrax, epidemiological investigations and implementation of a set of anti-epidemic measures required considerable labor inputs from specialists of Rospotrebnadzor, Rosselkhoznadzor, Ministry of Health of Russia, as well as territorial structures of the Ministry of Internal Affairs of Russia and the Federal Security Service of Russia, which provided substantial assistance in the investigation of outbreaks and large expenditures of budget funds.

Stabilization of the anthrax situation consists in the continuous full implementation of the main regulated prophylactic countermeasures. Since it is not possible to determine the localization of old burial sites, which serve as a permanent risk factor for complication of the situation, the priority is to ensure biological safety of known burial sites (landscaping, veterinary and sanitary control of the condition, prevention of the use of burial sites for economic needs, establishment of sanitary protection zones for anthrax burial sites) and to prevent the de-registration of anthrax burial sites. In order to prevent the formation of new soil foci of infection during the burial of livestock remains obtained by burning with the use of improvised means, which does not always allow to achieve guaranteed destruction of the pathogen, it is advisable to purchase mobile incinerators that ensure the burning of animal carcasses to a safe inorganic ash residue.

The basis for preventing anthrax in animals is to ensure their universal coverage with specific immunization in threatened areas, which can be implemented only if additional measures are taken to ensure complete cattle registration. Strict control of routine vaccination of persons at high occupational risk of infection is necessary. Important aspects of prevention include educating

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[3] Law of the Russian Federation from 14.05.1993 № 4979-1 "On veterinary medicine" (ed. from 25.12.2023).

[4] Federal Law of 30.03.1999 № 52-FZ "On sanitary-epidemiological well-being of the population" (ed. of 24.07.2023).

[5] Federal Law of 27.12.2018 No. 498-FZ "On Responsible Treatment of Animals and on Amendments to Certain Legislative Acts of the Russian Federation"; Sanitary Rules and Norms SanPiN 3.3686-21 "Sanitary and Epidemiological Requirements for the Prevention of Infectious Diseases" (approved by Resolution of the Chief State Sanitary Doctor of the Russian Federation of 28.01. 2021 No. 4); Veterinary rules for the implementation of preventive, diagnostic, treatment, restrictive and other measures, establishment and lifting of quarantine and other restrictions aimed at preventing the spread and elimination of anthrax outbreaks (approved by the order of the Ministry of Agriculture of the Russian Federation of 28.01.01. Order of the Ministry of Agriculture of Russia No. 648 of 23.09.2021); Veterinary rules for slaughtering animals (Annex No. 1 to Order of the Ministry of Agriculture of Russia No. 269 of 28.04.2022, as amended on 18.11.2022); Rules for animal registration (approved by Resolution of the Government of the Russian Federation No. 550 of 05.04.2023).

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the population about the risk factors of infection and the danger of anthrax, the inadmissibility of concealing the actual number of farm animals on the farm, which entails not including unaccounted livestock in the vaccination plan, forced slaughter of sick animals without veterinary examination, sale of raw materials and livestock products, purchase of meat products in places of unauthorized trade. The use of the algorithm of genetic analysis of isolated strains, which makes it possible to identify modifications of the genome structure and to establish the probable origin of isolates, contributes to the improvement of surveillance of anthrax and the efficiency of epidemiological investigation.

Molecular genetic monitoring as part of microbiological monitoring of infectious agents is an integral component of modern epidemiological surveillance of infectious diseases. The algorithm of wg-SNP-typing of *B. anthracis* strains developed using the data on the genetic structure of *B. anthracis* populations obtained in the course of Reference Center research was used in the investigation of anthrax outbreaks in 2023. The algorithm is intended for solving operational tasks and allows:

- identify atypical, modified, new forms of the pathogen by comparative analysis of the genome structure;
- increase the reliability of determining the origin and possible routes of spread of strains.

This approach was previously used by us in the epidemiologic investigation of anthrax outbreaks accompanied by the formation of epidemic foci, during which cultures of anthrax pathogen were isolated in the Yamalo-Nenets Autonomous District (2016), Stavropol Krai (2019), Republic of Tyva (2018, 2021), Republic of Dagestan (2019, 2020, 2022) [25–28].

Using genomic surveillance data, it was shown that the strains that caused the above-mentioned outbreaks were of local origin. Analysis of the target regions of the strains' genome, primarily pathogenicity factor genes, showed a typical genome structure for the *B. anthracis* species.

Conclusion

The analysis of the situation with anthrax in Russia in 2023 indicates that the reason for the formation of epizootic foci was the contact of unrecorded and, consequently, unvaccinated animals, vaccinated livestock with insufficiently strained level of specific immunity, with the soil of old unsupervised anthrax, as well as, probably, with fodder harvested in the territory of soil foci. A number of violations of veterinary and sanitary-epidemiological regulations, which led to contact with sick animals during care, forced slaughter, cutting and transportation of carcasses, preparation and cooking of infected meat and by-products, consumption of liver of insufficient heat treatment, caused the disease of people, both unvaccinated against anthrax of farm workers and persons not belonging to the contingent of risk of occupational contamination.

The complex of anti-epidemic measures in the course of anthrax outbreaks, carried out in the format of interdepartmental cooperation, allowed timely localization and elimination of infection foci and avoidance of even greater epidemic complications. Genetic analysis of the isolated strains indicated their local origin and absence of modifications in the genome structure, demonstrating the suitability of the developed wg-SNP typing system for epidemiologic investigation of outbreaks.

The results of molecular genetic typing of *B. anthracis* strains isolated during the epidemiologic investigation of seven anthrax outbreaks in the Russian Federation in 2023 allow us to conclude that they are of local origin and have a genome structure typical of the species. Genetic analysis of the isolated strains demonstrated the effectiveness of the developed wgSNP typing system in the epidemiologic investigation of outbreaks.

The results of the investigation of the reasons for the complication of the anthrax situation in Russia in 2023 show that the incomplete implementation of the regulated set of preventive measures can actually lead to a situation that will become a new chapter of the "old tale" about anthrax.

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