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С. Ж. Асфендияров атындағы Қазақ ұлттық медицина университеті

# Х А Б А Р Л А Р Ы

## ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК  
РЕСПУБЛИКИ КАЗАХСТАН  
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## NEWS

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**RETROSPECTIVE ANALYSIS OF ANTHRAX OUTBREAKS IN THE TURKESTAN REGION**

**Abstract.** The territory of the Republic of Kazakhstan is unfavorable for anthrax, officially registered since 1935. Millions of tenge are spent annually on a complex of anti-epidemic measures aimed at eliminating the outbreak, including the introduction of quarantine in the village, the establishment of medical supervision of contact persons, the implementation of preventive measures against people and animals, the organization of disinfection.

The article presents retrospective data on anthrax outbreaks, the results of a study of 32 strains isolated in the Turkestan region in the period from 1961 to 2011, using the MLVA - 31. Expansion of economic, trade, and tourism ties increases the possibility of the introduction and spread of the anthrax pathogen on the territory of the republic. Purpose of the study retrospective analysis of the properties of anthrax strains in the Turkestan region from 1961 to 2011, a description and assessment of the epidemiological situation in the region during the period under study and at the present time, the causes of infection in humans and animals, anti-epidemic and anti-epizootic measures. Study of the genetic properties of anthrax strains isolated during this period to establish a possible connection between the identified genotypes of *Bacillus anthracis* with the geographical origin and to improve the epidemiological monitoring of anthrax.

During the period from 1961 to 2011, retrospective studies on anthrax in the Turkestan district showed that at present, as before, there is a very high risk of infection of animals, and, as a result, people with the causative agent of anthrax in the territory of the region.

Anthrax strains circulating in the territory of the Turkestan region are variable in genotype but, as a rule, they have either one or a similar genotype if isolated during a single outbreak.

**Key words:** risk of infection, anthrax strains, multilocus analysis, epizootic index.

**Introduction.** Anthrax is a constant serious problem for the public health system and agriculture of all countries. The territory of Kazakhstan is unfavorable for anthrax, officially registered since 1935. The relative indicator of the incidence of anthrax in Kazakhstan in 1999-2020 is from 0.01 to 0.24 per 100 thousand population [1].

The Turkestan region is located on the territory of Kazakhstan with the maximum risk of infection with the causative agent of anthrax, the epizootic index is 0.41 [2]. Almost every year, cases of anthrax disease in humans and farm animals are recorded.

The article presents data on the study of 32 strains from the Turkestan region using multilocus analysis (MLVA-31). The expansion of economic, trade, and tourist ties, increases the possibility of the introduction and spread of the pathogen of anthrax on the territory of the republic. Moreover, the causative agent of anthrax can be used as a biological weapon.

In this regard, to characterize the properties of anthrax pathogen strains circulating on the territory of Kazakhstan, to differentiate them from atypical and closely related microorganisms, it is necessary to create electronic databases with molecular portraits of anthrax strains, to improve the scheme of indication and identification of culture studies to establish the connection of the identified genotypes of the anthrax pathogen, with the geographical origin, virulence, and source.

**Materials and Methods.** Epidemiological, genetic, and microbiological [3,4]. The study included DNA from 32 strains of anthrax. DNA isolation was performed using a QIA amp DNA Mini Kit manufactured by QIAGEN (USA). For molecular typing of the DNA of *B. anthracis* strains, we used multilocus tandem variable repeat number analysis (MLVA), which includes 31 loci, as described previously [5,6].

**Results.** The first cases of human and animal diseases were registered in the Sairam district, in the villages of Mankent, Sairam, and Karabulak, when several heads of farm animals were infected with anthrax, in 1955, cases of human diseases were registered in Turkestan. The last three cases of human anthrax were registered in the Turkestan region in September 2019 in the Tulkubassky district, Aryssky rural district, Maktaly village [1].

Almost every year, from 1935 to 2020, outbreaks of anthrax among humans and farm animals are recorded in the territory of the Turkestan region, with the release of virulent strains of *B. anthracis*. To study the cultural and morphological properties and their genotyping in MLVA-31, the DNA of 32 anthrax strains isolated from 1961 to 2011 was taken for research (Table 1,2,3).

The study of the cultural and morphological properties of the following *B. anthracis* strains № 5-9, 11-18, 20,41, 50-59, 62, 96-98, and 130-132 showed that the strains were taken in the studies isolated in the Turkestan region from 1962 to 2011 are belong to the order Eubacteriales, the family Bacillaceae, the genus and subgenus *Bacillus*, the species *B. anthracis*. All collection strains of the anthrax pathogen have typical cultural-morphological, biochemical, virulent properties. In a polymerase chain reaction using Pag R1-R5 primers and CAP A, CAP B primers that detect pag and cap genes responsible for toxin synthesis and capsules, which are virulence factors in *B. anthracis*, were found in all studied strains. Primers Pag R1 and Pag R4 formed 300-bp amplified fragments, primers Pag R2, R3 and R5-900-bp amplified fragments [7].

MLVA profile for 32 *B. anthracis* strains was obtained using multilocus tandem variable repeat number analysis (MLVA - 31) [3]. Of the 31 analyzed loci, the variability was established in the following loci: BAMS30, pXO2, pXO1, CG3, BAMS22, vrrA, BAMS03, BAMS15, Bavnr23, BAMS28, BAMS23, BAMS13, BAMS05 и vrrC1.

Cluster analysis by the method of unweighted pairwise mean (UPGMA) made it possible to identify two lines in the strains isolated in the territory of the Turkestan region: A. Br. 008/009 (30 strains) and A. Br. Vollum (1 strain). The analyzed strains were grouped into 21 genotypes.

Thirteen strains isolated from 1961 to 1963 were clustered into 6 genotypes (Table 1). There is no data on the aforementioned anthrax outbreaks in the archival documents, which makes it difficult to compare the results of genotyping of the strains and their relationship to specific outbreaks. Nevertheless, the difference in genotypes at two or more variable loci suggests that each genotype is a separate outbreak. The phylogenetic analysis shows that genotype 13 combined 6 strains isolated in 1961-1963 in Tulkuba and Kazygurt districts. Isolation of the same genotypes in different regions indicates a connection between outbreaks, and repeated outbreaks in 1962-1963 indicate the safety of pathogens and the low efficiency of the sanitary and preventive measures taken. Analysis of the genotypes of the strains isolated in the village of Kereit in 1961 indicates the introduction of three independent genotypes or three independent outbreaks with different strains (Figure 1). Isolation of genotype No. 9 in the strain isolated in 1962 and the strain isolated in 2001 is characteristic of anthrax due to the persistence of spores. It is important to emphasize that the 20th genotype identified in strain 11 can be tentatively assigned to the A.Br. Vollum. The high genetic diversity of strains isolated in the 60s indicates a more complex epizootic situation, and the isolation of the same genotypes in different periods indicates the endemicity of the disease.

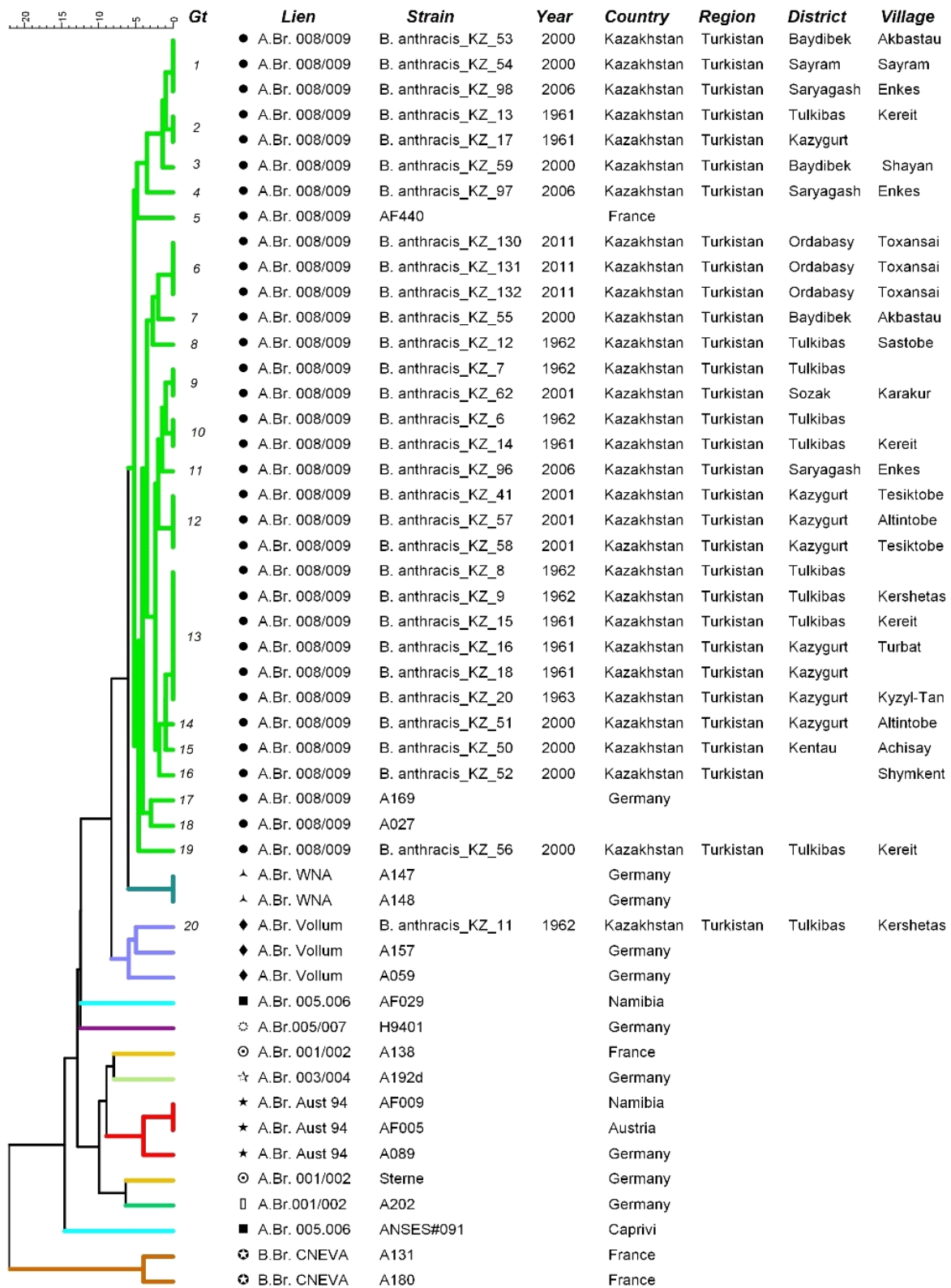


Figure 1 – Phylogenetic analysis of anthrax strains isolated in the Turkestan region in the period from 1961 to 2011.

Table 1-Genotypes of anthrax strains isolated during anthrax outbreaks in 1961-1963 in the Turkestan region

Strain cipher	Information about anthrax strains: district, locality, date of isolation, object of isolation	Genotype(Gt)
Almaty_6	Tulkubassky, collective farm of the XX Party Congress, Kara-Uygur tract. Date of isolation: 15.07.1962, isolated from the spleen of small cattle (sheep).	10
Almaty_7	Tulkubasskiy, sub-farm of the lead plant. Date of isolation: 07/17/1962, isolated from the contents of the human carbuncle.	9
Almaty_8	Tulkubassky, collective farm of the XX party congress. Date of isolation: 18.07.1962, isolated from the skin of small cattle (sheep).	13
Almaty_9	tulkubassky, Antonovka village. Date of isolation: 30.07.1962, isolated from the skin of small cattle (sheep).	13
Almaty_11	Tulkubassky, Antonovka village. Date of isolation: 30.07.1962, isolated from the spleen of small cattle (sheep).	20
Almaty_12	Tulkubassky, Sastobe village, St. Abaya 5. Date of separation: 31.07.1962, isolated from the skin of small cattle (sheep).	8
Almaty_13	Tulkubassky, Kereit collective farm. Date of isolation: 16.07.1961, isolated from the contents of a human carbuncle.	2
Almaty_14	Tulkubassky, Kereit collective farm. Date of isolation: 18.07.1961, isolated from sheep skin.	10
Almaty_15	Tulkubassky, Kereit collective farm. Date of isolation: 13.07.1961, isolated from the contents of a human carbuncle.	13
Almaty_16	Kazygurt district, Turbat village. Date of isolation: 31.07.1961, isolated from sheep skin.	13
Almaty_17	Kazygurt district, S. Communism. Allocation date: 7.08.1961, isolated from the skin of small cattle (sheep).	2
Almaty_18	Kazygurt district. Date of isolation: 7.08.1961, isolated from sheep skin.	13
Almaty_20	Kazygurt district, the Kyzyl-Tan village. Date of isolation: 06.07.1963, isolated from the contents of a human carbuncle;	13

In 2000, 33 cases of anthrax were registered in the Turkestan region [8]. 31 people had a cutaneous form of anthrax, which ended in recovery, two cases of the disease ended in death.

According to official data alone, 17 heads of farm animals died from anthrax in the Turkestan region in 2000, 33 cases of the disease were registered among people. The total number of contacts in anthrax foci was 273 people, in 2000 17 strains of *B. anthracis* were isolated. Diseases of people and farm animals were registered in Tulkubassky, Kazygurt, Baidibek, Tolebiysky, Ordabasinsky districts, and Kentau city (Achisay village).

In the Tulkubassky district (the village of Kereit) in two foci from 03.07.2000 to 25.07.2000, 12 people who directly participated in the slaughter and processing of two forcedly slaughtered cows with anthrax from private possessions fell ill with anthrax.

In the Kazygurt region, during the period from 22.07.2000 to 26.07.2000 in the village of Zhana-Bazar, during the forced slaughter of a cow from an individual farm, three people who were directly involved in the slaughter of an animal fell ill with a skin form of anthrax. Anthrax carbuncles were on the hands and fingers. The diseases of people ended in recovery. From a septic form of anthrax in the Kazygurt region in the village of Altyn-Tobe, a 47-year-old man died, who on August 31, 2000 slaughtered the fell calf.

In the Tolebiysky district, in the Kazygurt village, a man has a case of a cutaneous form of anthrax.

In the area of Baidybek from 26.07.2000 to 06.08.2000. in 2 centers of anthrax (the villages of Turakty and Akbastau), 10 people directly participated in the cutting of the following forcedly slaughtered farm animals: six heads of cattle, one horse, four heads of small cattle.

In the vicinity of Kentau (Achisai village), three people (skin form) with localization of anthrax carbuncles on the fingers, hands, and forearm fell ill with anthrax in one outbreak on 16-17-07.2000. All of them participated in the slaughter of a cow from private ownership that was of necessity to be slaughtered without a veterinary examination.

In the Ordabasyn district, on 11.09.2000, a 70-year-old woman fell ill with anthrax (skin form) in the village of Kok-Bulak. In the Shymkent city on 16.09.2000, a cleaning woman of the meat pavilion located in the market died of anthrax in septic form. The anthrax carbuncles were located on the cheeks, the patient was self-medicated for a long time.

The improvement of the epizootic and epidemic situation was noted in 2001, 10 cases of anthrax were registered in the region. In 2001, 6 people fell ill with anthrax in two foci in the Kazygurt district. In the first foci in the village of Tesik-Tobe (Zhanabazar rural district), five cases of anthrax were registered in people, in the second – one case was detected.



In the Sairam district, the infection of a resident of the village of Kyzyl-Kyshlak occurred as a result of the slaughter of a cow with anthrax on 03.07.2001. In Shymkent, two residents bought beef and lamb meat and liver at the market on 03.07.2001. Both became infected with anthrax while cutting meat, the disease proceeded in a skin form and ended in recovery. Two more people were infected with anthrax while cutting meat bought at the market in Shymkent on 09.07.2001.

In the Sozak district, during the forced slaughter of a cow on 23.08.2001, one person fell ill. The causative agent *B. anthracis* was isolated from the contents of the patient's carbuncle on 06.09.2001.

Phylogenetic analysis using MLVA-31 clustered eight anthrax strains isolated in 2000 into seven genotypes (Table 2). The first genotype combined strain 53 isolated from the contents of the carbuncle of a person living in the village of Akbastau and strain 54 isolated from a weigher wash out. It is tempting to assume that the source of human infection and the contamination of the weigher is identical; additional epidemiological information has made it possible to refine this hypothesis.

The remaining strains represent separate genotypes and are not related to each other geographically or by a time interval, so each strain is considered as a separate outbreak. Three strains (41, 57, and 58) isolated in the Kazygurt district in 2001 have an identical genotype. Epidemiological analysis and genotyping results allow us to link strain No. 57 isolated from a patient engaged in meat resale with an outbreak that occurred in the village of Tesik-Tobe.

Table 2-Genotypes of anthrax strains isolated in 2000-2001 during anthrax outbreaks in the Turkestan region

Strain cipher	Information on the strains of anthrax: region, locality, date of isolation, source of isolation	Genotype
Almaty_50	Kentau city, Achisay village. Date of isolation: 07/20/2000, isolated from the soil from the place of slaughter of a sick cow	15
Almaty_51	Kazygurt district, Altyn-Tobe village. Date of isolation: 09/08/2000, isolated from the contents of the human carbuncle.	14
Almaty_52	Shymkent city. Date of isolation: 09/19/2000, isolated from the contents of the human carbuncle.	16
Almaty_53	Baidybek district, Akbastau village. Date of isolation: 08/20/2000, isolated from the contents of the human carbuncle.	1
Almaty_54	Sairam district, Sairam village. Separation date: 08/20/2000, isolated from the washout from the weigher.	1
Almaty_55	Baidybek district, Akbastau village. Date of isolation: 04.28.2000, isolated from the soil from the place of slaughter of cattle sick with anthrax.	7
Almaty_56	Tulkibassky district, with. Kereitis. Date of isolation: 07/27/2000, isolated from the contents of the human carbuncle.	19
Almaty_59	Baidybek district. Isolation date: 08/09/2000 Source: isolated from the contents of a human carbuncle.	3
Almaty_41	Kazygurt district, Abay village. Isolation date: 28.07.2001, ; isolated from horse meat.	12
Almaty_57	Kazygurt district, Altyntobe village. Date of isolation: 22.07.2001, isolated from grass from the place of slaughter of a cattle patient with anthrax.	12
Almaty_58	Kazygurt district, the Abay village. Date of isolation: 20.08.2000, isolated from horse meat.	12
Almaty_62	Sozaksy district. Date of isolation: 20.09.2001, isolated from the contents of the human carbuncle.	9

We typed six more strains using multilocus analysis of the number of variable tandem repeats, which includes 31 locus of anthrax strains.

Three strains were isolated in the period from 10 to 29.09.2006 in the Saryagash region, where 5 foci of anthrax were registered, in which 9 people and 5 heads of cattle fell ill. The first outbreak was registered in the Kurkule rural district, the Yenkes village. Three cases of human infection with the anthrax pathogen were recorded in the outbreak. On 10.09.2006, two residents of the village of Yenkes carried out a forced slaughter without a veterinary examination of cattle at home. Another human case of anthrax was registered in the village of Yenkes on 26.09.2006. Infection of people occurred during the slaughter of cattle. Anthrax strains were isolated in the village of Yenkes (Table 3). MLVA-31 typing of strains revealed three genotypes.

Three more anthrax strains taken for typing were isolated in the Ordabasyn district during the anthrax outbreak in 2011, it was determined that all strains belong to genotype 14 (Table 3).

Table 3-Genotypes of anthrax strains isolated in 2001 and 2011 in the Turkestan region

Strain cipher	Information about anthrax strains: district, locality, date of isolation, source of isolation	Genotype
Almaty_96	Saryagash district, Enkes village. Date of isolation: 22.09.2006, isolated from the contents of the patient's ulcer.	11
Almaty_97	Saryagash district, Enkes village. Date of isolation: 22.09.2006, isolated from the contents of the patient's ulcer.	4
Almaty_98	Saryagashsky district, Enkes village; Date of isolation: 27.09.2006, isolated from manure from the slaughter site.	1
Almaty_130	Ordabasyn district, Toksansai, Kazhymukan street, b/n. Date of isolation: 24.06.2011, isolated from patient B., born in 1949.;	6
Almaty_131	Ordabasyn district, Toksansai, Kazhymukan street, b/n. Date of isolation: 22.06.2011, isolated from the carbuncle of the patient R., born in 1967.	6
Almaty_132	Ordabasyn district, Toksansai, Kazhymukan street, b/n. Date of isolation: 24.06.2011, isolated from the spleen of forcedly killed cattle.	6

**Discussion.** The results of phylogenetic analysis indicate that strains of 20 genotypes circulate in the territory of the Turkestan region, of which 13, 1, 6, and 12 are most represented. The identification of identical genotypes from the territory of settlements with an interval from 2 to 37 years is noted. At the same time, there are cases of isolation of two genotypes in one outbreak with a difference of 1-2 tandem repeats at hypervariable loci.

The last outbreak among people and farm animals in the Turkestan region was registered in September 2019 in the Tyulkubas district, Aryssky rural district, the village of Maktaly.

**Conclusion.** The results of a retrospective study indicate that the Turkestan region is the most unfavorable for anthrax in Kazakhstan.

Natural and climatic factors contribute to the preservation of the pathogen of anthrax in the soil. 222 SNPs, 403 epizootic and epidemic foci of anthrax, 48 soil foci were registered in the region. There are 475 cases of human diseases and 2695 cases of farm animal diseases were registered in the period from 1935 to 2019. Almost every year, anthrax outbreaks among farm animals and people occur on the territory of the Turkestan region.

The anthrax surveillance system should include monitoring the dynamics of the biological properties of the pathogen, the molecular and genetic characteristics of circulating strains, and their variability.

The results of the phylogenetic analysis of anthrax strains isolated on the territory of the Turkestan region indicate that the anthrax strains isolated in the period from 1961 to 2011 are characterized by significant variability, as well as the circulation of the same genotypes and clusters in different areas.

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## ТҮРКІСТАН ОБЛЫСЫНДАҒЫ КҮЙДІРГІНІҢ ӨРШҮІН РЕТРОСПЕКТИВТІ ТАЛДАУ

**Аннотация.** Қазақстан Республикасының аумағы 1935 жылдан бері ресми тіркелген күйдіргі бойынша қолайсыз.

Түркістан облысының аумағында жыл сайын дерлік адамдар мен ауыл шаруашылығы жануарларының күйдіргімен ауыру жағдайлары тіркеледі.

Зерттеудің мақсаты 1961-2011 жылдар аралығындағы Түркістан облысындағы күйдіргі штамдарының қасиеттерін ретроспективті талдау, зерттелетін кезеңдегі және қазіргі уақытта облыс аумағындағы эпидемиологиялық жағдайды сипаттау және бағалау, осы кезеңде бөлінген күйдіргі штамдарының генетикалық қасиеттерін зерттеу.

Жұмыста эпидемиологиялық, генетикалық, микробиологиялық әдістер қолданылды.

Мақалада күйдіргінің өршуі бойынша ретроспективті деректер, 1961-2011 жылдар аралығында Түркістан облысында бөлінген 32 штаммды МЛВА - 31 пайдалана отырып зерттеу нәтижелері ұсынылған. Экономикалық, сауда және туристік байланыстарды кеңейту республика аумағында күйдіргі қоздырғышының әкеліну және таралу мүмкіндігін арттырады. Талданған 31 локустың ішінде өзгергіштік мына локустарда орналасқан: BUMS 30, pXO2, pXO1, CG3, BAMS 22, vtrA, BEAMS 03, BAMS15, Bavntr23, BAMS28, BAMS23, BAMS13, BAMS05 және vtrC1. Өлшенбеген жұптық орта (UPGMA) әдісімен кластерлік талдау Түркістан облысының аумағында бөлінген штаммдарда екі жолға сәйкестендіруге мүмкіндік берді: A. Bg. 008/009 (30 штамм) және A. Bg. Volume (1 штамм). Талданған штаммдар 21 генотипке топтастырылды.

Түркістан облысының аумағында бөлінген күйдіргі штаммдарының филогенетикалық талдау нәтижелері әртүрлі аудандарда штаммдардың бірдей генотиптер мен кластерлердің айналымымен сипатталатынын көрсетеді. Түркістан облысының аумағында айналымдағы күйдіргі штаммдары генотип бойынша вариабельді, бірақ, әдетте, егер бір өршу кезінде бөлінсе, олар бір немесе ұқсас генотипке ие болады.

**Түйінді сөздер:** жұқтыру қаупі, күйдіргі, мультилокусты талдау, эпизоотиялық индекс.

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## РЕТРОСПЕКТИВНЫЙ АНАЛИЗ ВСПЫШЕК СИБИРСКОЙ ЯЗВЫ В ТУРКЕСТАНСКОЙ ОБЛАСТИ

**Аннотация.** Территория Республики Казахстан является неблагополучной по сибирской язве, официально регистрируемой с 1935 года. На территории Туркестанской области почти ежегодно регистрируют случаи заболевания людей и сельскохозяйственных животных сибирской язвой.

Целью исследования является ретроспективный анализ свойств штаммов сибирской язвы в Туркестанской области в период с 1961 по 2011 годы, описание и оценка эпидемиологической ситуации на территории области в изучаемый период и в настоящее время, изучение генетических свойств штаммов сибирской язвы, выделенных в этот период. В работе были использованы эпидемиологические, генетические, микробиологические методы.

В статье представлены ретроспективные данные по вспышкам сибирской язвы, результатам изучения 32 штаммов, выделенных в Туркестанской области в период с 1961 по 2011 годы, с использованием мультилокусного анализа (MLVA – 31). Из 31 анализируемого локуса вариабельность установлена в локусах: BAMS30, pXO2, pXO1, CG3, BAMS22, vtrA, BAMS03, BAMS15, Bavntr23, BAMS28, BAMS23, BAMS13, BAMS05 и vtrC1. Кластерный анализ методом невзвешенного попарного среднего (UPGMA) позволил идентифицировать две линии в штаммах, выделенных на территории Туркестанской области: A.Bg. 008/009 (30 штаммов) и A.Bg. Vollum (1 штамм). Анализируемые штаммы сгруппировались в 21 генотип.

Результаты филогенетического анализа штаммов сибирской язвы, выделенных на территории Туркестанской области свидетельствуют о том, что штаммы характеризуются циркуляцией одних и тех же генотипов и кластеров в разных районах. Штаммы сибирской язвы, циркулирующие на территории Туркестанской области вариабельны по генотипу, но, как правило, имеют или один или сходный генотип, если выделены во время одной вспышки.

**Ключевые слова:** риск заражения, сибирская язва, мультилокусный анализ, эпизоотический индекс.

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