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G.A. Eroshenko[1], N.V. Popov[1], Zh.V. Alkhova[1], A.N. Balykova[1], L.M. Kukleva[1], N.S. Chervyakova[1], N.S. Maykanov[2], A.Kh. Sarmuldina[2], V.V. Kutyrev[1]

Circulation of *Yersinia pestis* in the Volga-Ural Sandy Focus: Spatiotemporal Analysis

[1]Russian Research Anti-Plague Institute "Microbe", Saratov, Russian Federation;
[2]Ural Control Plague Station, Uralsk, Republic of Kazakhstan

Abstract. **Aim.** The present paper provides a comparative analysis of the phylogenetic relationship between *Yersinia pestis* strains isolated in the Volga-Ural sandy natural focus during the periods of 1912–1945 and 1963–2003, which were characterised by different levels of epidemic activity, in order to identify the spatiotemporal patterns in the circulation of the plague pathogen in the North Caspian region. **Materials and methods.** We studied the properties and performed whole-genome sequencing of 18 *Y. pestis* strains from the Volga-Ural sandy focus, along with 12 strains from other foci in the North Caspian and North Aral regions, isolated from 1912 to 2003. The phylogenetic analysis was performed drawing on the whole-genome SNP analysis, which was conducted on the basis of 2188 SNPs identified in the core genome using the Wombac 2.0 program. Maximum Likelihood Dendrogram (GTR model) was used for the analysis of phylogenetic relationships between strains. **Results and discussion.** All studied strains from the foci of the North Caspian region belong to the main subspecies (biovar *medievalis*) of the plague pathogen. These are highly virulent and epidemiologically dangerous strains. The whole-genome sequencing and phylogenetic analysis of 30 strains from the Volga-Ural sandy focus, as well as adjacent plague foci, reveal that the strains (biovar *medievalis*) of two phylogenetic branches – 2.MED4 and 2.MED1 – were spread across the focus under study in the early 20th century. It is confirmed that 2.MED1 strains were the etiological agents of plague outbreaks in the Volga-Ural sandy focus during this period. The study revealed the presence of parallel evolutionary lines in 2.MED1 associated with plague outbreaks in the first half of the last century. In the second half of the 20th and early 21st centuries, the modern evolutionary line of 2.MED1 became widespread in the Volga-Ural sandy focus. The strains of this line are closely grouped, which indicates their close genetic relationship. Only sporadic cases of plague were recorded during this period. Modern strains from the Volga-Ural sandy focus (1963–2003), as well as the strains previously isolated there (1912–1945), do not originate from each other. These strains represent closely related, independent evolutionary branches, extending from the common trunk of 2.MED1. Modern strains originating from those of the North Aral desert focus (1945) form a separate cluster in the dendrogram. This suggests that, following a break in the 1950s, the Volga-Ural sandy focus was re-colonised by closely related strains from the North Aral region.

Key words: *Yersinia pestis* strains, Volga-Ural sandy focus, phylogenetic analysis, circulation of plague agent.

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

Corresponding author: Galina A. Eroshenko, e-mail: rusrapi@microbe.ru.

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Г.А. Ерошенко[1], Н.В. Попов[1], Ж.В. Альхова[1], А.Н. Балыкова[1], Л.М. Куклева[1], Н.С. Червякова[1], Н.С. Майканов[2], А.Х. Сармулдина[2], В.В. Кутырев[1]

ПРОСТРАНСТВЕННО-ВРЕМЕННОЙ АНАЛИЗ ЦИРКУЛЯЦИИ *YERSINIA PESTIS* В ВОЛГО-УРАЛЬСКОМ ПЕСЧАНОМ ОЧАГЕ

[1]ФКУЗ «Российский научно-исследовательский противочумный институт «Микроб», Саратов, Российская Федерация;
[2]РГУ «Уральская противочумная станция» Комитета контроля качества безопасности товаров и услуг Министерства здравоохранения Республики Казахстан, Уральск, Республика Казахстан

Цель работы – сравнительный анализ филогенетического родства штаммов *Yersinia pestis*, выделенных в периоды 1912–1945 и 1963–2003 гг. с различной эпидемической активностью в Волго-Уральском песчаном природном очаге, для выявления пространственно-временных закономерностей циркуляции возбудителя чумы в регионах Северного Прикаспия. **Материалы и методы.** Проведено исследование свойств и полногеномное секвенирование 18 штаммов *Y. pestis* из Волго-Уральского песчаного очага и 12 штаммов из других очагов Северного Прикаспия и Северного Приаралья, выделенных с 1912 по 2003 год. Филогенетический анализ выполнен по данным полногеномного SNP-анализа на основе 2188 выявленных SNPs. Поиск SNPs в коровом геноме проведен с помощью программы Wombac 2.0. Для анализа филогенетических связей штаммов использована дендрограмма Maximum Likelihood, модель GTR. **Результаты и обсуждение.** Все исследованные штаммы из очагов Северного Прикаспия относятся к средневековому биовару основного подвида возбудителя чумы. Это высоковирулентные и эпидемически опасные штаммы. По данным полногеномного секвенирования и филогенетического анализа 30 штаммов из Волго-Уральского песчаного и сопредельных очагов чумы установлено, что в начале XX в. на территории очага были распространены штаммы двух филогенетических ветвей средневекового биовара – 2.MED4 и

2.MED1. Доказано, что штаммы 2.MED1 являлись этиологическими агентами вспышек чумы в Волго-Уральском песчаном очаге в этот период. Выявлено наличие параллельных линий эволюции в ветви 2.MED1, связанных со вспышками чумы в первой половине прошлого века. Во второй половине XX и начале XXI вв. в Волго-Уральском песчаном очаге получила распространение современная линия эволюции ветви 2.MED1, штаммы которой тесно сгруппированы, что свидетельствует о близком генетическом родстве этих штаммов. В этот период зарегистрированы лишь спорадические случаи заболевания людей чумой. Современные штаммы из Волго-Уральского песчаного очага (1963–2003 гг.) и ранее выделявшиеся штаммы (1912–1945 гг.) не ведут происхождения друг от друга, а представляют близкородственные, но независимые ветви эволюции, отходящие от общего ствола ветви 2.MED1. Современные штаммы образуют отдельный кластер дендрограммы, в основании которого лежат штаммы из Северо-Приаральского пустынного очага 1945 г. Это позволяет сделать предположение о повторном заселении территории Волго-Уральского песчаного очага после перерыва активности в 50-е годы прошлого столетия близкородственными штаммами из Северного Приаралья.

Ключевые слова: штаммы *Yersinia pestis*, Волго-Уральский песчаный очаг, филогенетический анализ, циркуляция возбудителя чумы.

Корреспондирующий автор: Ерошенко Галина Александровна, e-mail: rusrapi@microbe.ru.

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Introduction

The Russian Federation encompasses 11 natural foci of plague, which are located in the Caspian region, Siberia and the Caucasus [1, 2]. The total plague-enzootic area on the territory of the Russian Federation comes to 222,377 km^2. The North Caspian region includes the transboundary Volga-Ural sandy natural focus occupying sandy landscapes in the southern part of the Volga-Ural interfluve [3]. Administratively, the above-mentioned focus is located in the Astrakhan Region of the Russian Federation, as well as in the Ural and Atyrau regions of the Republic of Kazakhstan. This focus, encompassing a total area of 61,000 km^2 (including 8,625 km^2 on the territory of Russia), constitutes a multi-host and multi-vector system. Main plague hosts are small gerbils: midday gerbil (*Meriones meridianus*) and tamarisk gerbil (*M. tamariscinus*), accounting for 93 % of the total cultures that have been isolated from rodents since 1926. The plague vectors are parasites specific to gerbils (*Nosopsyllus laeviceps* and *Xenopsylla conformis*), accounting for 74.7 % of the total cultures that have been isolated from fleas since 1926. In the last century, the Volga-Ural sandy focus was one of the most epizootically and epidemically active natural plague foci of the Russian Federation and other countries of the Commonwealth of Independent States (CIS). The focus has been regularly monitored since 1926, with plague epizootics being first recorded in that area in 1922. From 1922 to 2007 (85 years), epizootics were recorded for 65 years (epizootic index of 0.76). Major deterioration of the epizootic situation occurred in 1937–1941, 1945–1946, 1951–1952, 1962–1963, 1966, 1971–1972, 1979–1980 and 1989–2002. The periods when infected animals were registered in different parts of the focus varied from 1 to 13 years. In separate landscape areas of the focus, intervals between infected animals being registered reached from 2–6 to 10–38 years. In 1989–2007, epizootic manifestations were recorded mainly in the northern and central parts of the focus. From 2008 to 2019, no infected animals were found on the territory of the focus. Long intervals between registered epizootic manifestations of plague on the territory of the Volga-Ural sandy focus are referred to as inter-epizootic periods [4, 5]. The mechanism involved in the subsequent reactivation of the focus remains unclear [6–8]. Significantly, long intervals between registered epizootic manifestations of plague are also characteristic of other plain natural foci of the North Caspian and North Aral regions. In particular, the cessation of epizootics that took place in 2002–2019 also occurred on the plague-enzootic territory of the Ural-Emba interfluve, which is adjacent to the Volga-Ural sandy focus, as well as in other landscape regions in the northern part of the desert zone of the Russian Federation and the Republic of Kazakhstan [9, 10]. In 1899–2019, 38 epidemiological years and 117 outbreaks were recorded in the Volga-Ural sandy focus, with most of these outbreaks being associated with livestock breeders working in sandy areas. The largest outbreaks were noted in 1905 (Beketay; 659 cases, 621 deaths); 1911 (Kulken; 148 cases, 148 deaths), 1923 (Kunbergen; 80 cases, 76 deaths) and 1937–1938 (Volga-Akhtuba floodplain, 61 cases) [11]. The total number of infected people amounted to 2,450 in 214 populated localities. The epidemic outbreaks were facilitated by the socio-economic conditions of the local population, migration when changing pastures, living in dugouts, an abundance of insects in dwellings and hunting. Bubonic forms of the disease predominated, although pneumonic forms of plague were often recorded.

Presently, the risk of plague infection is increasing due to the closer contacts of the population with natural foci (agricultural work, oil and gas extraction, an increase in the migratory activity of the population, hunting, *etc.*). The long-term environmental and epidemiological forecast for 2019–2020 indicates a high probability that the natural plain foci in the northern and eastern Caspian regions will become more active [12, 13]. In this connection, a number of measures need to be implemented in order to ensure the epidemiological welfare on the

territory of the Volga-Ural sandy focus. Firstly, the epidemiological surveillance of plague should be strengthened significantly. Secondly, it is necessary to decipher the mechanisms involved in activating the natural foci of this highly dangerous infection on the plague-enzootic transboundary territory of the Russian Federation and the Republic of Kazakhstan [14–16].

As it was previously shown using traditional microbiological methods and confirmed by molecular genetic analysis, as well as the whole-genome sequencing, *Y. pestis* strains from the natural foci of the Caspian region belong to the medieval biovar fo the main subspecies, i.e. they are highly virulent and epidemiologically significant. The *Y. pestis* strains of medieval biovar (2.MED line according to the genetic nomenclature) constitute one of the youngest evolutionary lines, which includes the following branches: 2.MED0 (strains from the Central Caucasian high-mountain focus of the Russian Federation), 2.MED1 (most foci of the Russian Federation and other CIS countries, Iran, China), 2.MED2 and 2.MED3 (foci of China) [17–19].

Over a long period of monitoring the Volga-Ural sandy focus, a large number of *Y. pestis* strains were isolated from patients, hosts and vectors (1912–2003). This collection, whose chronology reflects the history of the focus, belongs to the State collection of pathogenic bacteria housed by the Russian Research Anti-Plague Institute "Microbe". Modern technologies of molecular genetic analysis and whole-genome sequencing provide a unique opportunity to study the evolution of *Y. pestis* in the Volga-Ural sandy focus over a period of about 100 years, as well as to analyse epizootological and epidemiological data. Earlier, we published a similar work on the phylogenetic analysis of the *Yersinia pestis* strains (biovar medievalis) isolated in the North-West Caspian steppe focus [20].

Aim: The present paper provides a comparative analysis of the phylogenetic relationship between *Yersinia pestis* strains isolated in the Volga-Ural sandy focus during the periods of 1912–1945 and 1963–2003, which are characterised by different epidemic activity, as well as identifies the spatiotemporal patterns in the circulation of plague pathogens in the North Caspian region.

Materials and methods

Y. pestis strains, culture conditions, biochemical analysis. The strains of *Y. pestis* used in this work were obtained from the State collection of pathogenic bacteria at the premises of the Russian Research Anti-Plague Institute "Microbe". The strains were cultured in agar and Hottinger broth at 28 °C for 24–48 hours. The capacity of *Y. pestis* strains for the fermentation of sugars and glycerol, as well as for the reduction of nitrates was determined using standard laboratory diagnostic methods [21].

Whole-genome sequencing, identification of SNPs, dendrogram construction. The DNA of *Y. pestis* strains was isolated using a PureLink Genomic DNA Mini Kit (Invitrogen, USA). The whole-genome sequencing of *Y. pestis* strains was performed in the Ion PGM system (Life technologies), with the Ion Torrent Suite software package 3.4.2 and Newbler gsAssembler 2.6 being used for data processing. SNPs were identified by aligning the contigs of strains against the CO92 genome using Wombac 2.0 followed by the removal of 28 SNP homoplasies [22]. A model of nucleotide substitutions was selected using two programs: jMODELTEST 2.1.7 and MEGA X. Considering the AIC and BIC criteria, the GTR model was selected. Phylogenetic analysis was performed through the Maximum Likelihood method using the following programs: Mesquite 3.6 and PhyML-3.1 (500 bootstrap replicas).

Results

Features of strains from the Volga-Ural sandy focus. We studied a total of 18 *Y. pestis* strains isolated in the Volga-Ural sandy focus during the 1912–2003 period. In addition, a total of 12 strains from other foci of the North Caspian and North Aral regions were taken for comparison, which included two strains from the North-West Caspian steppe focus, one strain from the Volga-Ural steppe focus, two strains from the Trans-Ural steppe focus, one strain from the Ustyurt desert focus, three strains from the North Aral desert focus, one strain from the Caspian sandy focus, as well as one strain from the Central Caucasian high-mountain focus and one strain from the Zangezur-Karabakh mesofocus of the Transcaucasian high-mountain focus (Table). Strains from the Volga-Ural sandy focus were isolated at different time periods and from different sources. Eleven strains obtained in the first half of the 20th century from (1912–1945) were isolated from humans (7 strains), camels (1 strain), house mice (1 strain), yellow ground squirrels (1 strain) and midday gerbils (1 strain).

In the second half of the 20th century (1963–2002), seven of the other studied *Y. pestis* strains were isolated in the Volga-Ural sandy focus from hosts and vectors: tamarisk gerbils (*M. tamaricinus,* 4 strains), midday gerbils (*M. meridianus,* 1 strain) and their fleas (1 strain), as well as from little ground squirrels (*C. pygmaeus*, 1 strain). Twelve strains taken for comparison from other foci of the North Caspian and North Aral regions were isolated in 1923–1992 from humans (6 strains), little ground squirrels (*C. pygmaeus*, 3 strains), great gerbils (*Rhombomis opinus*, 2 strains) and fleas (*Citellophilus tesquorum*, 1 strain) (Table). In this study, we performed a comprehensive analysis of the properties exhibited by these strains. We studied phenotypic and genetic properties; performed whole-genome sequencing of 30 strains (including 18 strains from the Volga-Ural sandy focus), as well as their phylogenetic analysis.

All strains from the Volga-Ural sandy focus exhibited cultural and morphological properties typical of *Y. pestis* and were uniform in terms of biochemical characteristics. They did not ferment rhamnose and melibiose, which suggests they belonged to the main subspecies of the plague pathogen. In addition, they uti-

Origin and characteristics of the studied *Y. pestis* strains from the State collection of pathogenic bacteria at the premises of the Russian Research Anti-Plague Institute "Microbe"

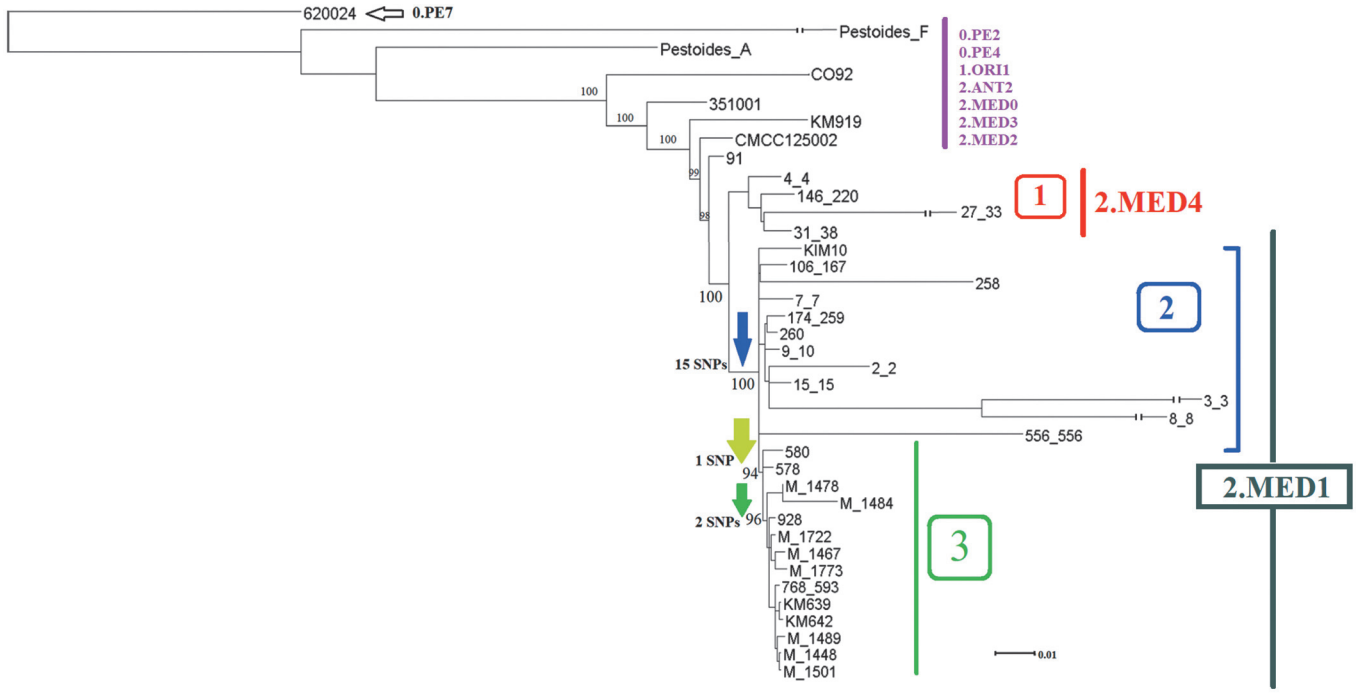
Strain No., name and No. of the focus	Isolation source	Location	Phylogenetic branch
2 Volga-Ural sandy focus (16)	human, 1912	Western Kazakhstan	2.MED1
3 Volga-Ural sandy focus (16)	*Mus musculus*, 1917	Western Kazakhstan	2.MED1
4 Volga-Ural sandy focus (16)	camel, 1917	Western Kazakhstan	2.MED4
7 Volga-Ural sandy focus (16)	human (corpse), 1922	Western Kazakhstan	2.MED1
8 Volga-Ural sandy focus (16)	human (corpse), 1922–1923	Western Kazakhstan	2.MED1
15 Volga-Ural sandy focus (16)	human (corpse), pneumonic form, 1923	Ural province	2.MED1
31 Volga-Ural sandy focus (16)	yellow ground squirrel, *Citellus fulvus*, 1924	Ural province	2.MED4
260 Volga-Ural sandy focus (16)	human, 1924	No data	2.MED1
106 Volga-Ural sandy focus (16)	midday gerbil *Pallasiomys meridianus*, 1928	Ural province	2.MED1
174 Volga-Ural sandy focus (16)	human, 1932	Kazakhstan	2.MED1
556 Volga-Ural sandy focus (16)	human (corpse), 1945	Guryev Region	2.MED1
768 Volga-Ural sandy focus (16)	tamarisk gerbils *Meriones tamaricinus*, 1963	Astrakhan Region	2.MED1
M-1722 Volga-Ural sandy focus (16)	8 tamarisk gerbils *Meriones tamariscinus*, 1977	Guryev Region	2.MED1
КМ639 Volga-Ural sandy focus (16)	tamarisk gerbils *Meriones tamariscinus,* 1980	Ural province	2.MED1
КМ642 Volga-Ural sandy focus (16)	7 tamarisk gerbils *Meriones tamariscinus*, 1980	Guryev Region	2.MED1
M-1478 Volga-Ural sandy focus (16)	3 little ground squirrels *Citellus pygmaeus*, 1992	Aygyr	2.MED1
M-1501 Volga-Ural sandy focus (16)	3 midday gerbils *Meriones meridianus* 1992	city of Sary	2.MED1
M-1773 Volga-Ural sandy focus (16)	from fleas of midday gerbil *Meriones meridianus*, 2002	Astrakhan Region	2.MED1
146 Transcaucasian high-mountain focus (Zangezur-Karabakh mesofocus, 09)	human (corpse), pneumonic form, 1931	Azerbaijan SSR, Nagorno-Karabakh	2.MED4
27 North-West Caspian focus (14)	little ground squirrel *Citellus pygmaeus,* 1924	Rostov Region	2.MED4
9 North-West Caspian steppe focus (14)	human (corpse) Demetrius, 1923	Rostov Region	2.MED1
M-1484 Volga-Ural steppe focus (16)	5 little ground squirrels *Citellus pygmaeus*, 1992	Boltay	2.MED1
M-1448 Trans-Ural steppe focus (17)	little ground squirrel *Citellus pygmaeus* 1990	Chapaev anti-plague division, Esensay	2.MED1
M-1489 Trans-Ural steppe focus (17)	great gerbil *Rhombomis opimus*, 1992	Kois	2.MED1
M-1467 Ustyurt desert focus (19)	great gerbil *Rhombomys opimus,* 1990	Guryev Region	2.MED1
578 North Aral desert focus (21)	human, bubonic form, 1945	Kazakhstan, Kyzylorda Region	2.MED1
580 North Aral desert focus (21)	human, primary pneumonic form, 1945	Kazakhstan, Kyzylorda Region	2.MED1
928 North Aral desert focus (21)	human, bubonic form, 1955	Kazakh SSR	2.MED1
258 Caspian sandy focus (43)	human, bubonic form, 1930	Astrakhan	2.MED1
КМ 919 Central Caucasian high-mountain focus (1)	fleas *Citellophilus tesquorum* from Caucasian Mountain ground squirrel *Citellus musicus*, 1986	Kabardino-Balkar ASSR, city of Kyzy-Kol	2.MED1

lised glycerol and didn't reduce nitrates which proves their belonging to the biovar *medievalis* of the main subspecies *Y. pestis*. The strains taken for comparison from other foci (North-West Caspian steppe focus, Volga-Ural steppe focus, Trans-Ural steppe focus, Ustyurt desert focus, Caspian sandy focus and North Aral desert focus) belonged to the medieval biovar of the main subspecies and exhibited the same set of properties.

The phylogeny of strains from the Volga-Ural sandy focus. In order to construct a phylogenetic tree of strains from the Volga-Ural sandy focus, we analysed the whole-genome sequences of all 18 strains from this focus and 10 strains from the adjacent foci of the North Caspian and North Aral regions, as well as 1 strain from the Central Caucasian high-mountain focus and 1 strain from the Zangezur-Karabakh mesofocus (Transcaucasian high-mountain focus), which we sequenced. The analysis also included strains of other phylogenetic lines from different world foci, whose sequences were taken from the NCBI GenBank database. These include the following genomes: Pestoides F (ssp. *caucasica*), Pestoides A (ssp. *altaica*), 620024 (ssp. tibetica), C092 (main subspecies, biovar *orientalis*), 351001 (main subspecies, biovar *antiqua*), 91 (main subspecies, biovar *medievalis*, 2.MED2), CMCC125002 (main subspecies, biovar *medievalis*, 2.MED3).

Drawing on the whole-genome SNP analysis of these genomes performed on the basis of 2188 found SNPs, we constructed a dendrogram showing phylogenetic relationships between strains from the Volga-Ural sandy focus (Figure). As it follows from the dendrogram, all strains from the Volga-Ural sandy focus are divided into three phylogeographic groups (No. 1, No. 2, No. 3) in accordance with the time and place of their isolation.

Two strains – 4 (1917) and 31 (1924) – were included into a separate early diverged branch of the medieval biovar strains, which is denoted as 2.MED4. In the dendrogram, 2.MED4 (Figure, No. 1) precedes a large clus-



Dendrogram of the phylogenetic relationship between *Y. pestis* strains from the Volga-Ural sandy focus constructed in accordance with the whole-genome SNP analysis. The Maximum Likelihood Dendrogram showing 38 genomes of *Y. pestis* strains was constructed on the basis of 2188 SNPs using PHYML 3.1 and the GTR model

ter of strains belonging to the biovar *medievalis* of the phylogenetic branch 2.MED1. In addition to two strains from the Volga-Ural sandy natural focus (4 and 31), 2.MED4 includes strain 27 (1924) from the North-West Caspian focus and strain 146 (1931) from the Zangezur-Karabakh high-mountain focus. Strain 146 was isolated from a human (1 case), the other three strains were obtained from a camel (1 case) and ground squirrels (2 cases), which confirms the circulation of *Y. pestis* belonging to 2.MED4 in the natural biocenosis of the Volga-Ural sandy focus. Thus, strains belonging to the early evolutionary branch of medieval biovar (2.MED4) were isolated in these foci in the early 20th century, with their subsequent disappearance from this territory.

The remaining sequenced strains from the Volga-Ural sandy focus, obtained in the first half of the 20th century (1912–1945), make up another group of strains that belongs to 2.MED1 (Figure, No. 2). The 2.MED1 population of this period in the Volga-Ural sandy focus includes a number of separate evolutionary lines represented in the dendrogram by single strains and one cluster, which consists of 7 strains isolated in 1912–1932, mainly in Western Kazakhstan. This cluster, in turn, includes two subclusters, one of which consists of two strains, 174 (1932) and 260 (1924); whereas the other subcluster includes strains 2 (1912) and 15 (1923), as well as a far branch consisting of two strains 3 (1917) and 8 (1922–1923). Strain 9 (1923) from the North-West Caspian steppe focus is also included in the same cluster. In addition to the cluster made up of seven strains, the phylogenetic group No. 2 comprises strains forming separate evolutionary lines: 7 (1922, human), 106 (1928, midday gerbil), 556 (1945, human), as well as a single strain 258 (1930, human) from the Caspian sandy focus. Thus, of nine 2.MED1 strains isolated in 1912–1945 in the Volga-Ural sandy natural focus, 7 strains were obtained from humans. Clearly, this cluster is represented by epidemic strains – aetiological agents of plague outbreaks in this focus.

In general, 2.MED1 strains isolated in the Volga-Ural sandy focus in the early 20th century (1912–1945) are characterised by the presence of separate parallel evolutionary lines having long branches, which indicates their rapid evolution, possibly associated with the occupation of new territories and/or changes in climatic conditions. Many of these strains were obtained from humans, proving that outbreaks that occurred in this area in the early 20th century were caused by the *Y. pestis* of the 2.MED1 phylogenetic branch (biovar *medievalis*), which circulated in the natural biocenosis of the Volga-Ural sandy focus. Strains belonging to the phylogenetic lines of 2.MED1 (1912–1945), subsequently disappeared from this territory.

The phylogeny, differing from the strains of the early 20th century, is represented by *Y. pestis* strains isolated in the Volga-Ural sandy focus in the second half of the 20th and early 21st centuries, which form a separate phylogenetic group within 2.MED1 (Figure, No. 3). The strains in question were obtained from hosts and their fleas in 1963–2003. The entire cluster is preceded by strains from the North Aral desert focus – *Y. pestis* 578 (1945) and 580 (1945). The group of modern strains from the Volga-Ural sandy natural focus is divided into three subclusters, the first of which, includes strain M-1478 (1992) from the Volga-Ural sandy natural focus and strain M-1484 (1992) from the Volga-Ural steppe

focus. The strains of this phylogenetic group are further divided into two subclusters, which are preceded by *Y. pestis* strain 928 (1955) from the North Aral focus. One of them (strains isolated in 1976–2003) includes a separately located strain M-1722 (1977) from the Volga-Ural sandy focus, as well as two related strains: M-1773 (2002) from the Volga-Ural sandy focus and M-1467 (1990) from the Ustyurt desert focus (19). The other large subcluster includes six strains, mainly from the Volga-Ural sandy focus (1963–1992). This subcluster contains a separately located strain 768 (1963). Two strains KM639 (1980) and KM642 (1980) also form a separate group in the subcluster. One more group consists of two strains: M-1501 (1992) from the Volga-Ural sandy focus and M-1448 (1990) from the Trans-Ural steppe focus. In the dendrogram, these two strains are preceded by strain M-1489 (1992) from the Trans-Ural steppe focus (17).

In general, the modern strains from the Volga-Ural sandy focus isolated from hosts and vectors in 1963–2003 exhibit a close clustering of strains, which indicates close genetic affinity of these strains and the constancy of their living conditions that are not associated with climate changes and/or the occupation of new territories. Evidently, they are well adapted to the current biocenosis of the Volga-Ural sandy focus. These strains are not associated with plague outbreaks, which distinguishes them from strains isolated at the beginning of the 20th century in the Volga-Ural sandy focus. In general, the strains of these two phylogenetic groups do not originate from each other; they represent closely related, independent branches of evolution, extending from the common trunk of 2.MED1.

Discussion

The *Y. pestis* strains of the main subspecies (biovar *medievalis*) are widespread in the natural foci of the Russian Federation, other CIS countries, Iran and China. These are highly virulent and epidemiologically dangerous strains. In the late 18th – early 20th centuries, numerous plague outbreaks of unknown aetiology were recorded in the Caucasus and the Caspian region. The study results presented in this article along with the data that we have published previously [12], prove that at least since the beginning of the 20th century (1912), outbreaks in the North Caspian region have been caused by the strains of a biovar *medievalis*. The strains belonging to 2.MED4, which were isolated in the early 20th century in the foci of the Caspian region (Volga-Ural sandy focus, North-West Caspian steppe focus) and the Transcaucasian high-mountain focus, were detected for the first time. This branch which diverged earlier from the common evolutionary trunk might be associated with the plague outbreaks that occurred there in the late 18th – early 20th centuries. A younger phylogenetic branch 2.MED1 diverged from the common evolutionary trunk of the medieval biovar later than 2.MED4. However, 2.MED1 strains are currently widespread in the various foci (mountain, high-mountain, low-mountain, steppe and desert) of the Russian Federation and other CIS countries in the Caucasus, in Caspian region and Central Asia. The reasons for biovar *medievalis* being adapted to different geographical landscapes and, especially, to foci characterised by a highly arid climate (where other strains of the plague pathogen are not found) are not known. It is also unknown when the medieval biovar occupied these territories. Paleogenetic studies, which have become possible with the development of high-resolution whole-genome sequencing, can provide the answer to this question. It is known that *Y. pestis* strains (biovar *antiqua*) of the 1.ANT branch were isolated on the territory of the 16th century Tatarstan. These strains – not biovar medievalis strains, as previously thought – supposedly caused the second plague pandemic [17]. We assume that the biovar *medievalis* became widespread later – in the 18th century, at the end of the second plague pandemic. The data obtained in this study confirm that 2.MED1 strains underwent intensive evolution in the early 20th century on the territory of the North Caspian region, with the development of independent parallel evolutionary branches of 2.MED1, whose strains occupied new territories or adapted to a changing habitat. In the early 20th century, these strains served as aetiological agents for a large number of high-mortality plague outbreaks in the foci of the Caspian region. Mass high-mortality outbreaks resulted from a wide distribution of these strains in nature; a high number of hosts in the foci of the Caspian region, as well as the proximity of a large, at that time, population to rodent colonies, the abundance of fleas and a low social level. In the years following the 1940s, such strains were no longer isolated. The evolution of 2.MED1 continued with the formation of the modern branch, which includes strains isolated in the second half of the 20th century. For at least 10 years (1950s), there was a temporary break between strain isolations of the first and second halves of the 20th century in the Volga-Ural sandy focus. During this period, the strains were not isolated; no epizootic manifestations, as well as no outbreaks and sporadic cases of plague in humans, were observed. The last point mentioned indirectly confirms the “healing” of the focus as a result of *Y. pestis* strains of various phylogenetic groups being eliminated under the influence of climatic and anthropogenic factors. At the same time, strains of the modern phylogenetic group have been isolated here since 1962. According to the phylogenetic analysis, they are closely related to strains that were previously isolated there in the first half of the 20th century; however, they do not originate directly from them. The dendrogram shows that modern strains from the Volga-Ural sandy focus are preceded by strains from the North Aral desert focus isolated in 1945. This may indicate that, following a break associated with unfavourable climatic conditions, the territory of Volga-Ural sandy focus was once again populated by closely related strains of 2.MED1, which originated from the northern subzone of the Aral deserts. Prior to that, strains belonging to one of the indepen-

dent evolutionary branches of 2.MED1 (presumably from the Volga-Ural sandy focus) could be introduced into the North Aral desert focus in the first half of the 20th century. A plague epidemic was recorded there in 1945, followed by epidemic outbreaks in 1947, 1955, 1966 and 1967. Thus, it can be assumed that, following a break in the 1950s, the territory of the Volga-Ural sandy focus was re-colonised by closely related *Y. pestis* strains, which 'returned' from the North Aral desert region. However, in order to confirm the phylogenetic relationships of strains from the North Caspian and North Aral regions, it is necessary to study a larger number of strains from the North Aral desert focus. From this point of view, it is the elimination of the plague pathogen that is one of the possible reasons for no infected animals being currently found in the enzootic territory of the northern part of the desert zone of the Russian Federation and the Republic of Kazakhstan. In this regard, the conditions for another plague expansion on the territory of the Volga-Ural sandy focus will be entirely determined by the epizootic activity of adjacent natural plague foci in the North Caspian and North Aral regions.

In terms of virulence, there is no difference between the strains of the biovar *medievalis* of the beginning and the second half of the 20th century. Differences in the incidence rate of plague are associated with the following points: difference in the epizootic activity; a wide distribution of *Y. pestis* strains in the foci of the Caspian region at the beginning of the 20th century; a greater population density in the foci; as well as lower living standards in the early 20th century.

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Authors:

Eroshenko G.A., Popov N.V., Alkhova Zh.V., Balykova A.N., Kukleva L.M., Chervyakova N.S., Kutyrev V.V. Russian Research Anti-Plague Institute "Microbe". 46, Universitetskaya St., Saratov, 410005, Russian Federation. E-mail: rusrapi@microbe.ru.

Maykanov N.S., Sarmuldina A.Kh. Ural Control Plague Station. 36/1, Chapaeva St., Uralsk, Republic of Kazakhstan. E-mail: pchum@mail.ru.