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Natural Mega-Focus of *Yersinia pestis* Main Subspecies, Antique Biovar, Phylogenetic Line 4.ANT in Gorny Altai

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Objective of this study was to investigate the areal of *Yersinia pestis* strains of antique biovar, main subspecies, phylogenetic line 4.ANT, as well as to establish the borders and spatial structure of mega-focus in the territory of Gorny Altai. **Materials and methods.** Complex comprehensive analysis of the properties in *Yersinia pestis* strains of the main subspecies, circulating in Gorny Altai has been conducted. 33 out of them, isolated predominantly in 2012–2017 have been sequenced. Whole genome SNP analysis and search of marker SNPs was performed using Wombac 2.0 software package. Tree diagram was built applying Maximum Likelihood algorithm, using PhyML 3.1 software on the basis of HKY85 model. **Results and conclusions.** Based on the results of whole genome SNPs analysis of 33 endemic strains and creation of the tree diagram of *Y. pestis* strains, the presence of natural mega-focus of *Y. pestis* belonging to the main subspecies, antique biovar, phylogenetic line 4.ANT has been substantiated. Epizootic manifestations on multiple local areas, characterized by persistent autonomous nature of plague manifestations, are registered on an annual basis. Within the boundaries of the areal of *Yersinia pestis* main subspecies, antique biovar, phylogenetic line 4.ANT, existence of joint natural foci of *Yersinia pestis* belonging to non-main subspecies *ssp. altaica* and *ulegeica* is established. Location of natural foci of the main and non-main subspecies of *Yersinia pestis* in different altitudinal belts of the Altai Mountains Range on the whole provides for observed multi-host and multi-vector feature of epizootic manifestations. For the first time ever, the data on the areal of the main subspecies of plague microbe are used for setting the boundaries of its natural focus.

Key words: Gorny Altai, mega-focus of the main subspecies of *Yersinia pestis*, joint natural foci of the main and non-main subspecies of *Yersinia pestis*, multi-host and multi-vector nature.

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Based on modern concepts of the mechanisms of enzootic zoonotic infections, the major specific component of their natural foci is the pathogen population [12]. From this perspective, the genetic characteristics of the main and non-main subspecies of the plague microbe can be used as the main criterion for determining the boundaries of their areals and typing of the focal territories [10, 18]. Nowadays, the development of a multilevel hierarchical molecular typing system for determination of attribution of *Y. pestis* strains to a particular subspecies and biovar, allowed to define the main patterns of their spatial distribution throughout Russia and other CIS countries [2, 3, 6, 11, 20]. In particular, as it is demonstrated, on this territories prevail the strains of the medieval biovar of the main subspecies *Y. pestis ssp. pestis* [14, 16]. On the contrary, it is only on the territory of Gorny Altai where the strains of main subspecies of

the antique biovar of phylogenetic line 4.ANT circulate [8, 17]. The enzootic for plague territory of Gorny Altai covers high-mountain and mountain chains located within the boundaries of the Altai and Tuva Republics, as well as the Mongolian People's Republic [15]. The 4.ANT strains are an ancient line of the antique biovar, which has diverged long time ago from the common evolution branch of *Y. pestis* [22]. In other foci of the world, these strains are not found. The strains of the non-main subspecies, including the Altai, Ulegey, Gissar, and Caucasian circulate mainly in the mountainous landscapes of Altai, Tien Shan, Caucasus and Transcaucasia [9, 15]. The objective of this work is to investigate the areal of the antique biovar of the main subspecies of *Y. pestis ssp. pestis* of the phylogenetic line 4.ANT and to establish the borders and spatial structure of its mega-focus on the territory of Gorny Altai.

Materials and methods

Y. pestis strains, culture, DNA isolation.

The strains of *Y. pestis* used in this study were obtained from the state collection of pathogenic bacteria of the Russian Research Anti-Plague Institute "Microbe". The strains were cultivated on LB agar and in LB broth (pH 7.2) at 28°C for 24–48 hours. Analysis of cultural and morphological, and differential and diagnostic characteristics was performed in accordance with standard methods of laboratory diagnostics of plague. DNA of the strains was isolated with The PureLink™ Genomic DNA Mini Kit Invitrogen kit manufactured by Thermo Fisher Scientific.

Whole genome sequencing, SNPs search, phylogenetic analysis, dendrogram building.

Generation of whole genome nucleotide sequences of *Y. pestis* strains was performed using the Ion PGM system (Ion Torrent). Data processing and assembling of *de novo* genomes were performed using Ion Torrent Suit software package 4.2 and Newbler Assembler 2.6. The obtained sequences of the lines were compiled into genomes, the coverage of which for the genome of the reference strain CO92 (GenBank accession number NC_003143.1) was about 98.84 % with 60-fold reading. The average length of the assembled genome was 4.64 Mb.

Whole genome SNP analysis and search for marker SNPs were performed using the Wombac 2.0 software. The dendrogram with application Maximum Likelihood algorithm, was built using PhyML 3.1 software on the basis of HKY85 model. Dendrograms were visualized via the FigTree 1.4.3. software. Bootstrap analysis was performed with application of 500 random samples.

Results and discussion

In the geographical borders of Gorny Altai, plague enzootic is caused by circulation of *Yersinia pestis* three subspecies: the major subspecies – *ssp. pestis* of antique biovar (the Republics of Tuva and Altai, the Mongolian People's Republic), Altai subspecies – *ssp. altaica* (Altai Republic) and Ulegey subspecies – *ssp. ulegeica* (the Mongolian People's Republic). In the Altai Republic, the circula-

tion of *Y. pestis* strains of the main subspecies of the antique biovar was first registered in 2012 [1]. The strains of the antique biovar are highly virulent, and contact with them has led to three cases of human plague infection in the Kosh-Agach district of the Altai Republic in 2014–2016. A comprehensive molecular genetic analysis of the *Y. pestis* strains isolated on the territory of the Altai and Tuva Republics, as well as in the adjacent areas of the Mongolian People's Republic (Bayan-Ulekey aimag) was performed aimed at molecular examination of these epidemiological complications cases and identification of the source of infection; it was as well aimed at studying the phylogeny of antique biovar strains in Altai. Based on the obtained data, it was established that the strains of the main subspecies from the territories of the Republics of Tuva and Altai belong to the antique biovar of the phylogenetic line 4.ANT [8, 17]. A whole genome sequencing was performed on eight strains of *Y. pestis* of the main subspecies of the antique biovar from the territory of the Republic of Tuva, isolated in 1971–2015, and 22 strains of the main subspecies of the antique biovar from the territory of the Altai Republic, isolated in 2012–2017, and one strain of *Y. pestis* I-3244 from Bayan-Ulekey aimag in Mongolia, isolated in 1988. The analysis also used the sequences of three sequenced genomes of *Y. pestis* strains of the Altai subspecies from the Gorny Altai high-mountain focus, *Y. pestis* MGJZ12 strain of 4.ANT line from the Bayan-Ulekey aimag in Mongolia (NCBI GenBank), and another seven strains from the NCBI database GenBank, representing the main lines of the evolution of the plague pathogen (Table 1).

A Maximum Likelihood tree diagram was built using the PHYML 3.1 program and the HKY85 model, based on a whole genome analysis of 42 strains and 1055 identified SNPs (Fig. 1).

It was concluded that at the base of the 4.ANT line locates a KM932 strain, isolated from the fleas of the long-tailed ground squirrel in 1987 in the territory of the Republic of Tuva. This strain is a precursor of the 30 investigated strains of the 4.ANT line. This strain branch includes the MGJZ12 strain from the Bayan-Ulekey aimag in Mongolia (2002, NCBI GenBank) and a separate cluster that includes modern strains from

Table 1

Sequenced strains of *Y. pestis* from the territories of the Republics of Tuva, Altai and Mongolian People's Republic

<i>Y. pestis</i> strain №	Location, source, year of isolation, phylogenetic line
The Altai Republic, 4.ANT	
I-1454	The corps of the long-tailed ground squirrel <i>Spermophilus undulatus</i> , 2012
517	Patient, 2014
1530	Altai marmot <i>Marmota baibacina</i> , 2014
337	Remains of the table of birds of prey, 2015
338	Altai marmot <i>M. baibacina</i> , 2015
349	Fleas <i>Oropsylla silantievi</i> of Altai marmot <i>M. baibacina</i> , 2015
157	Patient, 2016
162	Patient, 2016
367	Altai marmot <i>M. baibacina</i> , 2016
368	Altai marmot <i>M. baibacina</i> , 2016
299	Altai marmot <i>M. baibacina</i> , 2016
45	Remains of the table of birds of prey (marmot), 2016
44	Remains of the table of birds of prey (marmot), 2016
46	Remains of the table of birds of prey (marmot), 2016
25	Remains of the table of birds of prey (marmot), 2016
37	Marmot bones, 2016
133	Remains of the table of birds of prey (marmot), 2016
142	Remains of the table of birds of prey (marmot), 2016
145	Remains of the table of birds of prey (marmot), 2016
848	Long-tailed ground squirrel <i>S. undulatus</i> , 2017
174	Remains of the table of birds of prey (marmot), 2017
175	Remains of the table of birds of prey (marmot), 2017
18	Remains of the table of birds of prey (marmot), 2017
257	Steppe polecat <i>Mustela eversmanni</i> , 2017
The Republic of Tuva, 4.ANT	
KM932	Fleas <i>Citellophylus tesquorum</i> , 1987
M-1944	Tarbagan marmot <i>M. sibirica</i> , 2012
633	Fleas <i>Frontopsylla elatoides</i> , 2013
863	Long-tailed ground squirrel <i>S. undulatus</i> , 2013
272-276	Long-tailed ground squirrel <i>S. undulatus</i> , 2014
2437	Flea <i>Neopsylla mana</i> , 2014
1636	Fleas <i>C. tesquorum</i> , 2015
209	Long-tailed ground squirrel <i>S. undulatus</i> , 2015
The Republic of Altai, 0.PE4	
I-2998	Pallas's pika <i>Ochotona pallasi</i> , 1982
B1313	Fleas <i>Paradoxopsyllus scorodumovi</i> of Pallas's pika <i>O. pallasi</i> , 2014
2751-55	Pallas's pika <i>O. pallasi</i> , 2012
The Mongolian People's Republic (Bayan-Ulekey aimag)	
I-3244	Daurian pika <i>Ochotona daurica</i> , 1988
NCBI GenBank	
Pestoides A	0.PE4
B42003004	Altai marmot <i>M. baibacina</i> , China 2003, 0.ANT
Antiqua	Patient, Congo, 1965, 1.ANT
Nepal516	Patient, Nepal, 1967, 2.ANT
MGJZ29	Fleas <i>O. silantievi</i> , Mongolia, 1998, 3.ANT
MGJZ12	Altai marmot <i>M. sibirica</i> , Bayan-Ulekey aimag, Mongolia, 2002, 4.ANT
KIM10	Patient, Iran / Kurdistan, 1968, 2.MED
CO92	Patient, USA, 1992, 1.ORI

the territory of the Republics of Tuva (2012–2015) and Altai (2012–2017). The subcluster of modern Tuvian strains is accurately divided into two groups: the first includes three strains of 2012–2013 (4 unique SNPs), and the second – four Tuva strains isolated in 2014–2015 (2 unique SNPs) (Table 2). The entire subcluster of modern Tuvian strains has four marker SNPs. The second subcluster is also divided into two groups. The first group includes modern strains from the Altai Republic (2 unique SNPs), and the second group includes one strain I-3244 from Mongolia, 1988 (4 unique SNPs) (Table 2).

It should be emphasized that the I-3244 strain from Mongolia, 1988 is phylogenetically more “young” not only in comparison with the precursor strain K932 from the Tuvan mountain heartland of 1987, but also comparing to the MGJZ12 strain of 2002 again from Bayan-Ulekey aimag. All the strains 4.ANT, isolated in 2012–2017. And it is notable that all 4.ANT strains isolated in 2012–2017 in the Altai Republic had the same type of SNPs, including strains 517 (2014), 157 (2016) and 162 (2016), obtained from infected people. Two strains were an exception – 145 (marmot, 2016) and 257 (steppe polecat, 2017), each having one marker SNP.

The technique of multi-locus analysis of the variable number of tandem repeats – MLVA for the 15 selected VNTR loci with the highest resolution relative to these strains M06, M18 (Y3336), M22, M25 (Y0920), M27, M28, M34 (ms62), M43, Y3701, Y3515, N1606, N2117, N2577, N4268 (Y4892), ms09 [19, 21, 23] was used, aimed at further differentiation of 22 strains of *Y. pestis* of the main subspecies isolated in the Altai Republic in 2012–2017. As it was consequently concluded, the *Y. pestis* 517 strain, isolated during an epidemic complication from a patient in 2014, has an identical VNTR profile with strain 1530, obtained from marmot organs – the assumed source of infection. Moreover, it was found that two clinical strains of *Y. pestis* 157 and 162, isolated in 2016, and strains 367 and 368, obtained from marmots, hunted in the same year, in the Irbistu tract, where was located the encampment of livestock farmers and occurred the case of human infection with plague, have identical VNTR profiles, which proves their clonal origin. Therefore, the

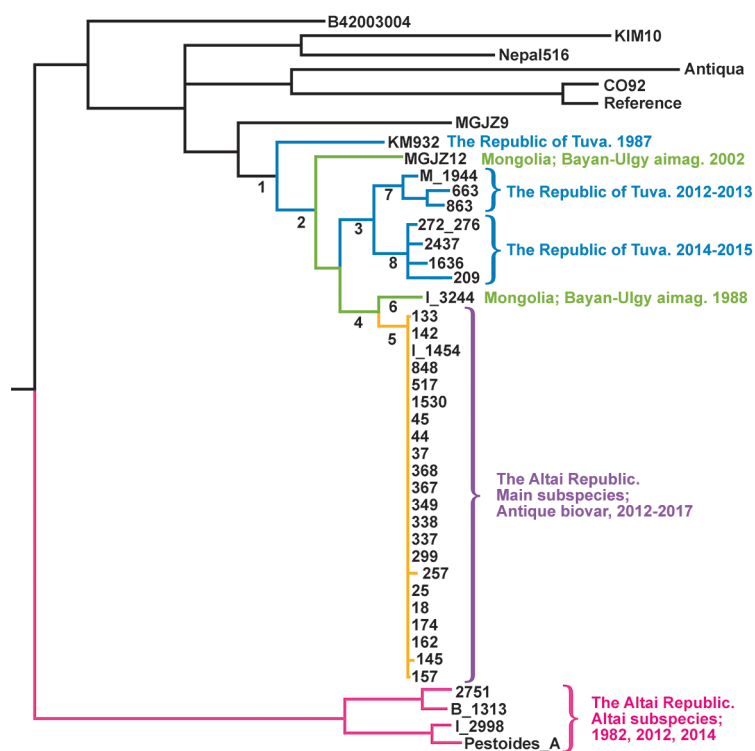


Fig. 1. Comparative phylogenetic analysis of *Y. pestis* strains of the main subspecies, antique biovar, 4.ANT branch, isolated in the territory of the Republics of Altai (2012–2017) and Tuva (1987–2015). Tree diagram is built applying Maximum Likelihood algorithm, using PhyML 3.1 software based on the data from whole genome SNP analysis of the strain, covering 1055 SNPs

identity of the VNTR profiles of *Y. pestis* 517 and 1530 strains (2014) and *Y. pestis* strains 157, 162, 367 and 368 (2016) confirms the conclusion of the epidemiological inspection report that the plague infection of people is caused by hunt for infected marmot.

In general, the obtained data of phylogenetic analysis demonstrate a close genetic relationship between the *Y. pestis* strains isolated in the territories of the Republics of Tuva, Altai and the Mongolian People's Republic (Bayan-Ulehey aimag) and also highlights the integrity of the focal territory of Gorny Altai, where takes course the overall evolution of strains of 4.ANT line (Fig. 2). In the course of this process, in the common evolution branch of the 4.ANT line, there is a sequent change of genotypes that are being distributed from the territories of the different Gorny Altai foci. Apparently, evolution of the 4.ANT line, alternate predominance of genotypes and fluctuation of the sizes of epizootic areas, are mediated by changes in climatic conditions and the associated changes in the natural biocenosis of plague foci. In general, it should be concluded that an active process of evolution of the 4.ANT line of the antique biovar of the plague pathogen is taking course throughout the focal territory of the mountain Altai along with extension of the territory of the circulation of strains of this line in the modern period.

The results of genetic investigations also allow us to conclude that in the orographically integral system of Gorny Altai the territory of the natural mega-focus of the *Y. pestis* main subspecies of the antique biovar of the phylogenetic 4.ANT line includes the high-mountainous areas of the mountain ranges of Sailugem, Mongolian Altai, South Chuy, North Chuy, Chikhachev, Shapshalsky, Tsagan-Shibet, western and eastern Tannu-ola, as well as the area of the Mongun-Taiga mountain site. Due to the mosaic pattern of the mountainous terrain, the epizootic manifestations of the plague are microfocal, as in the high mountain natural plague



Fig. 2. Spatial structure of the natural mega-focus of *Yersinia pestis* main subspecies, antique biovar, phylogenetic line 4.ANT in Altai Mountains. Digits indicate areas of strains' detection: 1 – main subspecies; 2 – main and *altaica* subspecies; 3 – main and *uleigea* ssp.

Table 2

List of SNPs, that are marker ones for the strains of *Yersinia pestis* of the main subspecies, antique biovar, 4.ANT branch, from the territories of the Republics of Altai, Tuva, and Mongolian People's Republic

SNP position relatively to the beginning of the genome*	Mutation	Gene, intergenic space	The strains of <i>Y. pestis</i> antique biovars of 4.ANT line, with mutation
576681	C → T	leuB	Mongolian, Tuvan, Altai (№ 1 in Fig. 1)
2046555	C → A	flgE	
2416791	C → T	YPO2148	
2463301	G → A	oppF-YPO2186a	
2809913	C → A	rbsA	
3279062	G → T	YPO2935	
3765150	T → G	eno	
4266465	C → A	YPO3799	
4645860	G → A	atpG	
394234	C → A	purA-YPO0379	Tuvan, Altai with I-3244 (№ 2 in Fig. 1)
1484392	C → T	dacC	
1996447	C → T	YPO1751a	
3270727	G → A	murQ	
3718023	A → G	YPO3332	
4151377	G → A	malG	Tuvan (№ 3 in Fig. 1)
1387707	C → T	YPO1226-YPO1228	
1938023	A → C	YPO1700	
3091698	C → A	YPO2758	
3263892	G → A	purL	Altai and I-3244 (№ 4 in Fig. 1)
2092484	T → G	yecC	
3693309	G → T	YPO3311	
4339797	G → A	wzzE	Altai (№ 5 in Fig. 1)
3495476	C → T	acrB-rpmE2	
300402	G → T	terE-tnp	I-3244 (№ 6 in Fig. 1)
2506456	T → C	araD	
3658483	G → A	rldD	
3689067	G → A	YPO3308-YPO3309	
3850025	G → A	valS	Tuvan, 1 (№ 7 in Fig. 1)
325289	T → A	qor	
1345125	G → A	YPO1194-YPO1195	
3972331	C → T	gltD	Tuvan, 2 (№ 8 in Fig. 1)
2774153	G → A	YPO2472	
3705268	C → A	cybB-YPO3322	

*The position for the genome of the reference strain *Y. pestis* CO92 (access number in NCBI Genbank No. NC_003143.1).

foci of the Tian Shan, [4]. It should be noted that in the territory of the Kosh-Agach District of the Altai Republic in 2012–2017 numerous micro foci of the main subspecies *Y. pestis* ssp. *pestis* of the antique biovar of the phylogenetic 4.ANT line were first found in the high mountains of the Sailugem, South-Chuy, North-Chuy, Chikhachev ranges. Before that period no systematic annual epizootiological investigation of high mountain areas (more than 2500 masl) was

conducted. Significant isolation of the epizootic foci revealed in 2012–2017 implicitly indicates an autonomous nature of the plague manifestation of each of these cases.

The western part of the natural focus of the main subspecies of the antique biovar *Y. pestis* ssp. *pestis* of the 4.ANT line is located in the territory of the Kosh-Agach district of the Altai Republic and the Bayan-Ulekey aimag of the Mongolian People's Republic. At the territory of the Altai Republic, the circulation of strains of the main subspecies of the antique biovar *Y. pestis* ssp. *pestis* of the 4.ANT line is mostly registered at altitudes of 2.1–2.6 thousand masl. The gray marmot is the dominant species of rodents on this territories. Long-tailed ground squirrels, flat-headed voles, Mongolian pikas and other background species of rodents are sporadically involved in the epizootic process among gray marmots. Total of 131 cultures of the main subspecies of the antique biovar of the 4.ANT line were isolated in 2012–2017. Most of these cultures (117, 89.3 %) originated from the gray marmot and its ectoparasites – 55 from animals, 45 from fleas, 8 from lice and 9 from ticks collected from wool. Single strains were isolated from the long-tailed ground squirrel (3), its fleas (6) and lice (2); from the steppe polecat (1), from fleas of the Mongolian pika (1), from the fleas from the entrances of the holes of this animal (1). In 2016, the DNA of *Y. pestis* ssp. *pestis* was detected in the long-tailed ground squirrel (remains of the table of birds of prey) via PCR method. Infected animals were found in 36 epizootic areas located in the high mountains of the Northern Chuy (8), South Chuy (2), Sailugem (14), and Chikhachev (12) ranges (Fig. 3). The strains of the main subspecies of the antique biovar *Y. pestis* ssp. *pestis* of 4.ANT line isolated within the period 2012–2017 in the high-mountainous regions of the ranges, the North-Chuy, South-Chuy, Sailugem, Chikhachyov have no difference between each other in genetic aspect. The lack of genetic differences of the strains of the main subspecies of the plague microbe isolated in 2012–2017 in the highland regions of the mountain ranges isolated from each other, implicitly confirms the unified mechanism of formation and the relative antiquity of this part of the areal of the main subspecies of the antique biovar *Y. pestis* ssp.

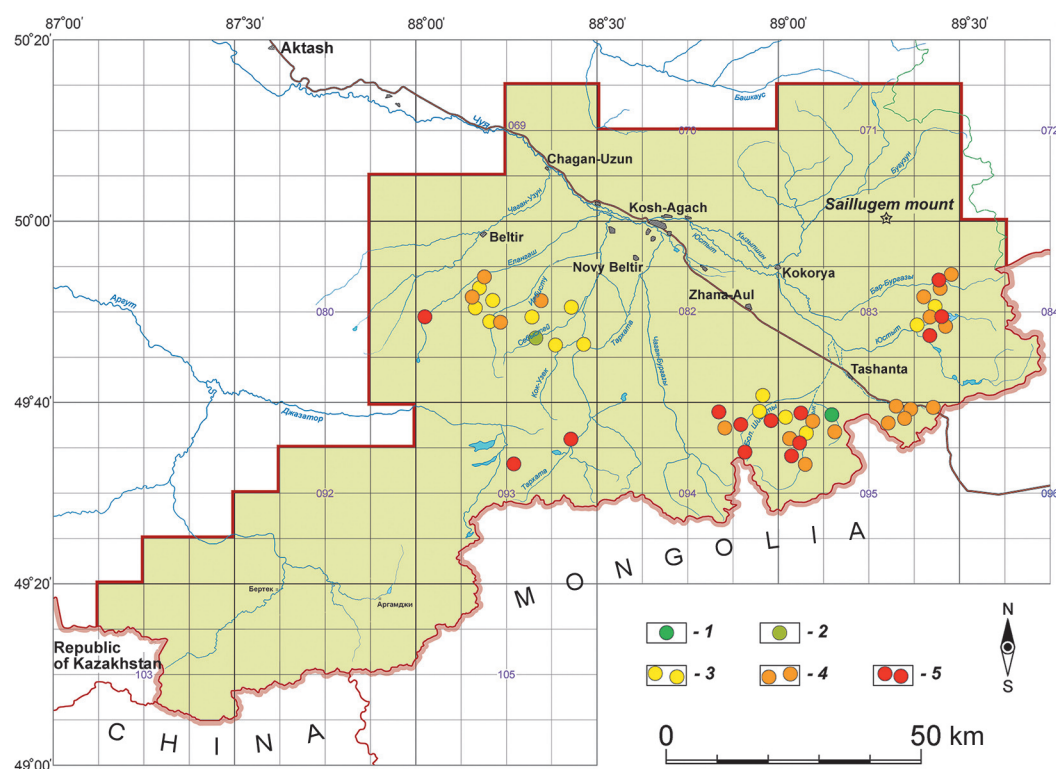


Fig. 3. Location of the regions where *Y. pestis* main subspecies, antique biovar, 4. ANT branch are detected in the territory of Kosh-Agach district of the Republic of Altai in 2012–2017:

Years of registration: 1 – 2012; 2 – 2014; 3 – 2015; 4 – 2016; 5 – 2017

pestis of 4.ANT line. At the same time, numerous registrations of the circulation of genetically homogeneous strains on the same areas within 3–5 years period clearly indicates the independence of the manifestations of plague on each of these areas.

The circulation of strains of plague microbe main subspecies has been known on the territory of the Bayan-Ulekey aimag of the Mongolian People's Republic since 1988. In particular, the *Y. pestis* I-3244 strain was detected in this location. Based on the presence of a marker replacement of single nucleotide G → A in the YPO1418 locus, this strain is referred to the 4.ANT line [7]. Previously, only one strain of the 4.ANT genetic variant isolated in Mongolia was presented in the scheme of world genetic diversity, [22]. 4.ANT genetic variant strains are isolated only on the western border of Mongolia with Russia, and, therefore, they are endemic to the Bayan-Ulekey aimag territory. During an epizootiological investigation of the territories adjacent to the southern macroslope of the Sailugem range in 2017, eight cultures of the plague pathogen of the main subspecies were detected, including seven cultures from

marmots, and one from the long-tailed ground squirrel. Specific antibodies to the plague microbe were found in 25.6 % of gray marmots, in 18.0 % of long-tailed gophers, in 6.2 % of Mongolian pika [5]. The circulation of strains of the main subspecies of the plague microbe is registered in the eastern part of the natural foci on the territory of the Tuva Republic, in mountainous and highland areas, where the dominant species of rodents is the long-tailed ground squirrel. The epizootic manifestations of plague were detected in a wide altitude range from 1500 (Despen-Beldyr tract) to 2500 masl. (Ak-Khem tract). In 1964–2016, 1691 cultures of the main subspecies of the plague microbe were isolated in this area.

It is notable that 94.5 % of all the cultures of plague agent were found in long-tailed ground squirrel and its ecoparasites. Tarbagan infections (1984 and 2012), as well as infections of other rodent species, have sporadic character in this area. The independent circulation of the main subspecies of the plague microbe was identified in 11 meso-foci. Numerous local epizootic zones were defined on these territories.

Conjugacy of natural foci of the main sub-

species of *Y. pestis* ssp. *pestis* of the antique biovar of line 4.ANT and the Altai subspecies - ssp. *altaica* on the territory of the Altai Republic. Based on the tree diagram (Fig. 1), the strains of the antique biovar of 4.ANT line and the minor Altai subspecies are phylogenetically distant from each other. They differ in virulence and epidemic significance. Efficient separation methods by the PCR method with real-time hybridization-fluorescence accounting of results, and VNTR method have been developed for differentiation of these strains [1, 13]. The analysis of the spatial distribution of the antique biovar strains of 4.ANT line and the non-main Altai subspecies in the Kosh-Agach district of the Altai Republic implicitly indicates the presence of their conjugated foci in this location.

The natural foci of the non-main Altai subspecies of the plague microbe (ssp. *Altaica*) is located in the spurs of the North-Chuy, South-Chuy, Kuray, Sailugem ranges at altitudes of 2.0–1.7 thousand masl.

Temporary circulation of strains of the Altai subspecies of plague microbe is detected in mountain landscapes, where the Mongolian pika is the dominant species among small mammals; and the long-tailed ground squirrel is the subdominant species. Usually, during periods of deep depressions in the population of Mongolian pikas, the isolation of the strains of Altai subspecies was interrupted. 2419 cultures of the minor subspecies *Y. pestis* ssp. *Altaica* were isolated in 1961–2017 from the field material. Wherein, 2037 plague microbe cultures of the Altai subspecies were isolated from the Mongolian pikas and their ectoparasites, which is 84.2 % of the total number. Involvement of other species of rodents and lagomorphs in the epizootic process, caused by the minor subspecies of *Y. pestis* ssp. of the plague microbe *altaica* is sporadic. In particular, in 1961–2017 the number of isolated strains *Y. pestis* ssp. *altaica* was: from the gray marmot and its fleas *Oropsylla silantievi* – 7 (0.3 %); from the long-tailed ground squirrel and its ectoparasites – 75 (3.1); from the Daurian pikas – 171 (7.1), from the flat-headed vole – 97 (4.0). Infected animals were detected on the territories of 43 epizootic zones, characterized by persistent nature of epizootic manifestations.

The conjugated natural foci of the main subspecies of the antique *Y. pestis* biovar 4.ANT line and the non-main subspecies can be potentially located on the southern slopes of the Sailugem range and in the spurs of the Mongolian Altai range (Bayan-Ulehey aimag, Mongolian People's Republic). The strains of the Ulehey subspecies are only found in Mongolia, where they circulate in the territory of several aimags, including the Bayan-Ulgii aimag. The strain *Y. pestis*. I-2422 was detected here in 1974. This strain refers to ssp. *ulegeica*, as proved by the biochemical features of this strain, whole genome sequencing and the presence of marker SNPs. As in the Altai Republic, the strains of the main subspecies of the antique *Y. pestis* biovar of 4.ANT line circulate mainly in mountainous and highland landscapes (up to 2500 masl), where the dominant species of rodents is the gray marmot. Strains of the minor subspecies *Y. pestis* ssp. *ulegeica* circulate in areas located at altitudes of less than 2100–1500 masl, where the most widespread species among small mammals are the Mongolian pika and the long-tailed ground squirrel. The conjugate natural foci of strains of the main subspecies of the ancient biovar *Y. pestis* of 4.ANT line and minor subspecies (Altai *Y. pestis* ssp. *altaica* and Ulgei *Y. pestis* ssp. *ulegeica*) in different altitudinal zones of Gorny Altai mountain chains demonstrate generally apparent multi-host and multi-vector epizootic manifestations.

In conclusion, it should be noted that in Gorny Altai area, within the areal border of the main subspecies of the antique biovar *Y. pestis* of 4.ANT line, epizootic manifestations are confined to the highland and mountainous landscapes of the Altai and Tuva republics and the Bayan-Ulehey aimag of the Mongolian People's Republic. Moreover, the data of phylogenetic analysis confirms the close genetic relationship of the *Y. pestis* strains isolated in the investigated focal area of Gorny Altai, where the coevolution of the 4.ANT line strains has been taking course for a long time. All the above mentioned generally confirms the common mechanism of formation and the relative antiquity of the areal of the main subspecies of the antique biovar *Y. pestis* ssp. *pestis* 4.ANT line, including in the territories of the Altai Republic.

Conflict of interest. The authors confirm the absence of a conflict of financial/non-financial interests associated with writing an article.

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