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EXPERIMENTAL WORKS

Intraspecies Classification of Rhamnose-Positive *Yersinia pestis* Strains from Natural Plague Foci of Mongolia

M. E. Platonov[a], V. V. Evseeva[a], D. V. Efremenko[b], M. V. Afanas'ev[c], D. B. Verzhutski[c], I. V. Kuznetsova[b], M. Yu. Shestopalov[c], S. V. Dentovskaya[a], A. N. Kulichenko[b], S. V. Balakhonov[c], and A. P. Anisimov[a]

[a] *State Research Center for Applied Microbiology and Biotechnology, Obolensk, 142279 Russia*
[b] *Stavropol Research Antiplague Institute, ul. Sovetskaya 13/15, Stavropol, 355035 Russia*
[c] *Antiplague Research Institute for Siberia and the Far East, ul. Trilissera 78, Irkutsk, 664047 Russia*
e-mail: platonov@obolensk.org

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Abstract—Comparative analysis of rhamnose-positive *Y. pestis* strains, which are traditionally recognized as the biovar altaica, was performed with the use of molecular typing. The strains were isolated from the natural plague foci of the Changay mountainous system and the Sailugem natural plague focus in Mongolia. The Russian part of the latter is known as the Altai Mountains focus (focus 36). All isolates were compared with the other rhamnose-positive vole strains from the natural plague foci located in the grassland of Xilingol (focus L, China), the plateau of Qinghai-Tibet (focus M, China), the Gissarian Ridge (focus 34, Tadjikistan and Uzbekistan), and the Talassian Ridge (focus 40, Kyrgyzstan). The strains studied formed a single cluster with two branches, of which the first branch included the talassica (0.PE4?), qinghaiensis (0.PE4ab), and xilingolensis (0.PE4cd) phylogenetic groups, while the second included the hissarica (0.PE9) and altaica (0.PE1) phylogenetic groups. The results of molecular typing suggest that several strains from the Qinghai group of the Mongolian foci belong to the qinghaiensis and xilingolensis biovars but not to the altaica biovar.

Keywords: VNTR, MLVA, CRISPR, genotyping, *lcrV*, *aspA*, *Yersinia pestis*
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INTRODUCTION

According to G. Morelli et al. [14], the divergence of *Yersinia pseudotuberculosis* and *Y. pestis* occurred 2600–28000 years ago. The formation of the new species and its consequent intraspecies variability resulted in a significant number of *Y. pestis* intraspecies phylogenetic groups (Fig. 1), which differed in both their ranges of sensitive mammals and virulence [1, 7, 10, 14, 15, 17]. *Y. pestis* is known to persist in natural plague foci of populations of more than 200 mammal species (predominantly rodents) and more than 240 flea species. Also, less than half is regularly involved in the epizootic process [3, 5, 7]. Long-term interaction of a pathogen and corresponding populations of carrier and vector animals, which are specific for each plague focus, can launch pathogen microevolution, which, in turn, is important for developing genotypes to be fitted into a specific ecological niche.

Previously, using MLVA25 typing (Multilocus VNTR Analysis; VNTR stands for "Variable Number of Tandem Repeats"), we studied 41 rhamnose-positive biovars of xilingolensis (focus L, China) and qinghaiensis (focus M, China), as well as 14 biovars of altaica, hissarica, and ulegeica (focus 36, Russia; focus 34, Tadjikistan and Uzbekistan; foci of Mongolia; respectively). This resulted in the phylogenetic tree of the *Y. pestis* including a single cluster consisted of these intraspecies groups, in which each biovar forms a separate branch (clonal cluster) [12]. In more recent studies, 235 *Y. pestis* strains, which were isolated from natural plague foci located in the countries of the former Soviet Union and Mongolia, were investigated

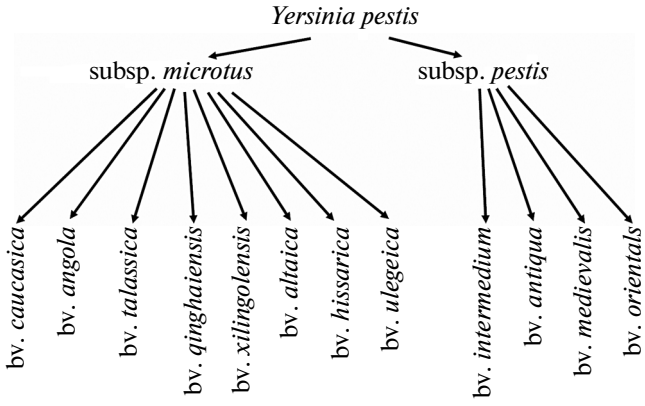


Fig. 1. Intraspecies taxonomy of *Y. pestis* [15].

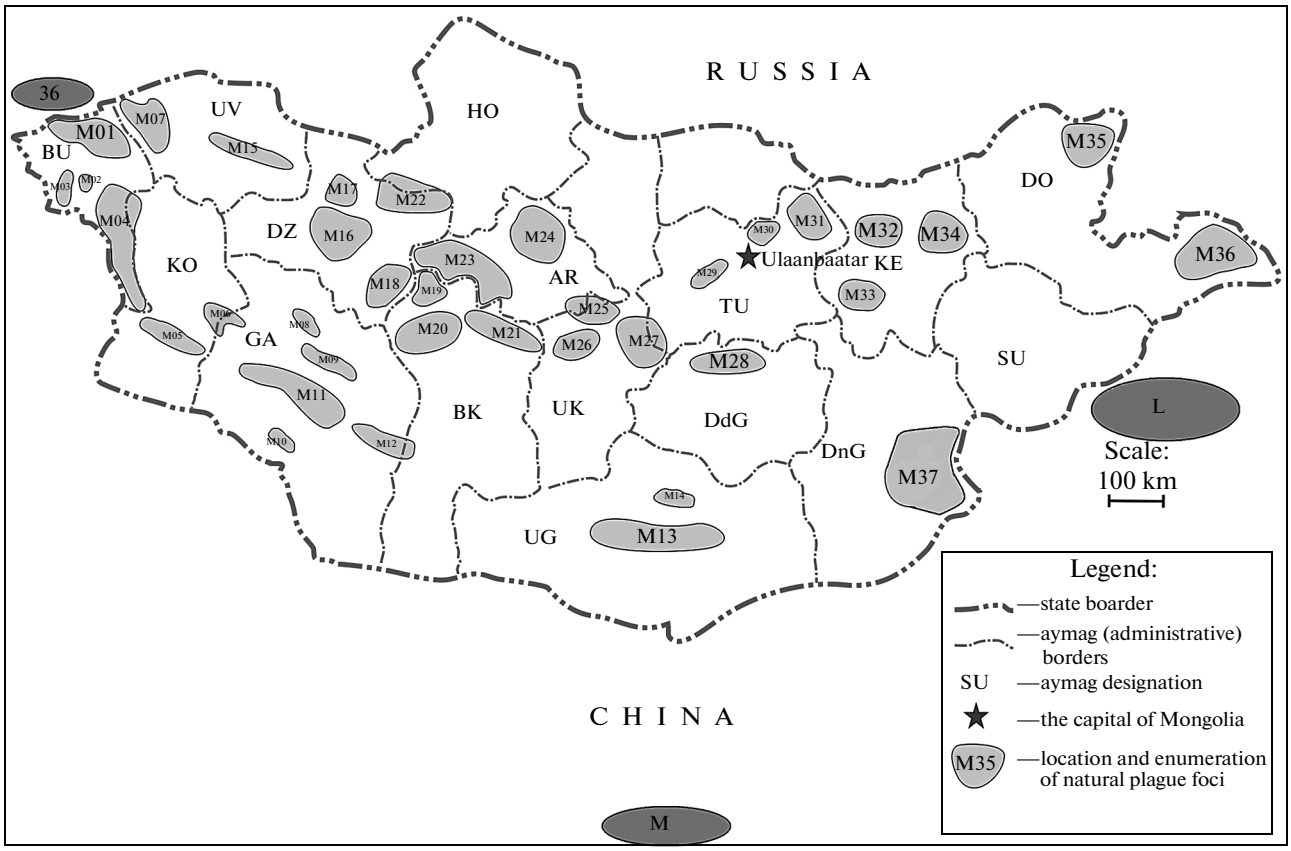


Fig. 2. Natural plague foci in Mongolia. Natural foci of Mongolian Altai Mountains: M01—Sailugem, M02—Bukhen-Ulsk, M03—Tsengel-Khairkhan, M04—KhuKh-Serkh-Munkh-Khairakan, M05—Ulan-Sunduy, M06—Sutay, M07—Kharkhira-Turgen. Natural foci of Gobi Altai Mountains: M08—Khasagd, M09—Taishir, M10—Azh-Bogdin, M11—Burkhan-Buday, M12—Gichgen, M13—*Ochotona* Gurvan-Saikhan, M14— *Meriones* Gurvan-Saikhan. Natural foci of western Changay: M15—Khan-Khukhey, M16—Yaru-Bogdyn, M17—Songin-Tudev. Natural foci of southern Changay: M18—Buyant-Otgon, M19—Shara-Usyn, M20—Zag-Baidrag, M21—Tuingol. Natural foci of northern Changay: M22—Bulnay, M23—Terkhin, M24—Khanuin-Gol. Natural foci of southeastern Changay: M25—Barum-Orkhon, M26—Khankhegsh, M27—Bayan-Under, M28—Baga-Gaderyn-Ulin. Natural foci of Khentey: M29—Nalgar-Ulin, M30—Bogd-Ul, M31—Mungun-Mort, M32—Khurakh-Gol, M33—Khurkhin, M34—Zun-Khentey. Eastern and southeastern natural foci: M35–Khoit-Kerul, M36–Tamsag-Bulag, M37–Dzamyn-Ud. *Alticola* foci of Russia and China: 36, L, and M. Aymags: AR—Arkhangei, BK—Bayan-Khongor, BU—Bayan-Ulgii, DdG—Middle Gobi, DnG—Eastern Gobi, DO—Eastern, DZ—Zavkhan, GA—Gobi-Altai, KE—Khentii, KO—Kobdos, TU—Central, UG—Southern Gobi, UK—Ubur-Khangay, UV—Ubsa-Nur.

with the use of DFR-typing [13]. It was found that some of them, which are persistent in Mongolia and classed as bv. altaica in their phenotypic characters, had the DFR type 14 [4], which is the main one for the biovars qinghaiensis and xilingolensis [13]. This fact allowed it to be suggested that they do not belong to the biovar altaica [4].

In Mongolia, there are 37 natural plague foci, which are combined into eight groups and extend from the Tansagbulag mesofocus in the northeast to the Sailugem and Kharkhir-Turgem foci in the northwest of the country (Fig. 2). Until recently, strains of three biovars were known to persist in these foci. The strains of subsp. *pestis* bv. antiqua can be isolated basically from Tarbagan marmot (*Marmota sibirica*), long-tailed ground squirrel (*Spermophilus undulates*), Daurian pika (*Ochotona dauurica*), and their fleas. The strains of subsp. *microtus* bv. altaica (leucine-dependant) and bv. ulegeica (trega-lose-negative) can be isolated in the northwest of the country (Sailugem and Bukhen-Ulsk foci) and southern Gobi Desert, basically from Mongolian pika (*Ochotona pallasi pricei*), flat-headed vole (*Alticola strelzovi*), and their fleas. In addition, strains of bv. altaica (leucine-independent) were found in Brandt's vole (*Lasiopodomys brandit*) populations in the mesofoci located in the southeast of the group of the natural foci belonging to the Changay mountainous system (foci Terkhinsky, Shara-Usynsky, and Yaru-Bogdynsky) [1]. As has been shown recently, the strains of subsp. *microtus* bv. xilingolensis persist in the Central and Bayan-Khongorsk aymags of Mongolia [17].

In this study, the results of MLVA25-typing of the rhamnose-positive strains of the plague pathogen isolated predominantly from natural foci in Mongolia and the Altai Mountains focus in Russia are represented. In addition, we tried to support our results on

MLVA25-clusterization based on CRISPR-typing (Clustered Regularly Interspaced Short Palindromic Repeats) [9], as well as the results of *Y. pestis lcrV* and *aspA* gene sequencing.

MATERIALS AND METHODS

Bacterial strains. In this study, a *Y. pestis* intraspecies classification corresponding to the International Codex of Bacterial Nomenclature [11, 15–17] was applied. In total, 59 strains of *Y. pestis* (Fig. 3), representing all known biovars of the subsp. *microtus* [15], were used. The exceptions were bv. angola, bv. caucasica, and eight strains of bv. antiqua of the basic subspecies isolated in Mongolia and the focus 40 of Kyrgyzstan. Two coupled foci are thought to exist in the focus 40: bv. antiqua (marmot) and bv. talassica (vole) [5]. The genotypes of 25 strains were studied during this investigation. The other isolate data are known from our previous studies [4, 6, 9, 12] available from the complete nucleotide sequences and presented by J.M. Riehm et al. [17]. Bacteria were grown and genomic DNA was isolated as described previously [12].

Molecular typing. The primer sequences, PCR conditions, MLVA25-typing [12], CRISPR-typing [9], and *lcrV* [6] and *aspA* gene sequencing [8] were described previously. The published complete sequences of the strains *Y. pestis* Pestoides A, 91001 and *Y. pseudotuberculosis* IP31758 (Genbank/EMBL/DDBJ IDs ACNT01000001–ACNT01000037, AE017042–AE017046 and CP000720.1, CP000719.1, CP000718.1, respectively) were used for molecular typing in silico.

Nomenclature of natural plague foci. The natural plague foci of China are designated by the letters A to O [19] and the foci of the countries of the former Soviet Union are designated by numbers [5, 7]. The geographic location and description of these foci have been given in previous publications [5, 7, 19]. The locations of the foci (M01 to M37) on the administrative territories of Mongolia are shown in Fig. 2.

Data analysis. The results were deposited in the database of the Bionumerics 5.1 computer program. A Neighbor-Joining method with a category coefficient (*Y. pseudotuberculosis* IP31758 as a root species) was used to create an MLVA25 dendrogram. The SNP types are given in accordance with [10, 14] regarding linking strains [17] and/or based on the law of homologous series in hereditary variability [18].

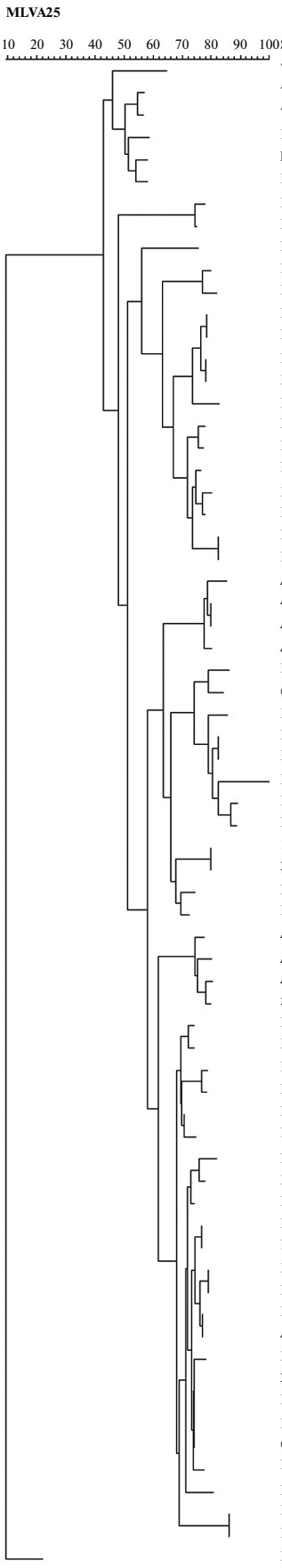
RESULTS AND DISCUSSION

Figure 3 shows results of MLVA25-analysis of 59 vole rhamnose-positive strains of *Y. pestis*. These include the biovar altaica isolated in Mongolia from the Sailugem natural plague focus (focus M01) and the group of foci belonging to the Changay mountainous system (foci M20 and M27), as well as the Altai Mountains focus located in Russia (focus 36); the biovars xilingolensis and qinghaiensis isolated in China from the focus L (the grassland of Xilingol) and the focus M (the plateau of Qinghai-Tibet), respectively; the biovar hissarica (focus 34, Tadjikistan and Uzbekistan); and the biovar talassica (focus 40, Kyrgyzstan). For comparison, eight strains of the plague pathogen of the basic subspecies were included: the biovar antiqua and those isolated from the foci M14, M21, M27, and M32 (Mongolia) and the focus 40 (Kyrgyzstan).

After increasing the number of strains that derived from the Sailugem and Hissar foci, as well as including in the analysis four strains from the Talas focus and five strains from the Changay focus group, all located in Mongolia, the overall picture of the genetic diversity of the plague microbe persistent in this part of the Central Asian zone of the natural plague foci became more complete. The rhamnose-positive strains studied formed a single cluster, of which the first branch included the phylogenetic groups of talassica (0.PE4?), qinghaiensis (0.PE4ab), and xilingolensis (0.PE4cd) and the second branch included the hissarica (0.PE9) and altaica (0.PE1) phylogenetic groups (Fig. 3). Moreover, the strains of each biovar were constituents of autonomous clonal clusters, which had their counterparts as the corresponding CRISPR types. The exceptions were five strains (I-3088, I-3132, I-3134, 162, and 2420) that were classified as bv. altaica. Of these, three strains phenotypically corresponded to the leucine-independent ecovar bv. altaica and the other two had no data on their leucine-dependency. All these strains were isolated from the *L. brandi* animals, which are also the principal plague carriers in the focus L (bv. xilingolensis) in China [19]. The strain I-3088 was isolated in 1983 in the Uburhangay aymag (focus M27). The strains 162 and 2420 were isolated in the Bayan-Khongor aymag (focus M20) in 1956, and the strains I-3132 and I-3134 were isolated in the same focus in 1984.

Three isolates, I-3088, I-3132, and I-3134, according to MLVA25-typing and regarding our previous DFR-typing [4], belonged to bv. xilingolensis, while their plasmid spectrum represented by plas-

Fig. 3. N-J dendrogram of MLVA25-types of *Y. pestis* strains: biovars of altaica, antiqua, hissarica, qinghaiensis, talassica, ulegeica, and xilingolensis. Strain and F designate a strain name and a natural focus, respectively; ssp stands for a subspecies (P, *pestis*, and M, *microtus*); bv designates a biovar: an—antiqua, al—altaica, h—hissarica, q—qinghaiensis, t—talassica, u—ulegeica, x—xilingolensis; al>x (al>q) means that a strain was initially referred to bv. altaica; however, later results on genotyping indicate to bv. xilingolensis (qinghaiensis); Ypa, Ypb, and Ypc designate a CRISPR-type [9]; LcrV stands for the V-antigen type [6] (ND—not determined); pCad means no pCad plasmid; and SNP means that SNIP-types are represented according to [10, 14] with regard to linking strains [17] and/or based on the law of homologous series in hereditary variability [18].



Strain	F	ssp	bv	Ypa	Ypb	Ypc	LcrV	SNP
790	40	P	an	a1-a2-a3-a4-a5-a6-a7	b1-b2-b3-b4	c1-c2-c3	D	
791	40	P	an	a1-a2-a3-a4-a5-a6-a37	b1-b2-b3-b4	c1-c2-c3	D	
793	40	P	an	a1-a2-a3-a4-a5-a6-a37	b1-b2-b3-b4	c1-c2-c3	D	
I-2976	M21	P	an	a1-a2-a3-a4-a5-a6-a7	b1-b2-b3-b4-b48	c1-c2-c3	D	0.ANT
I-3075	M14	P	an	a1-a2-a3-a4-a5-a6-a7	b1-b2-b3-b4-b48	c1-c2-c3	D	
I-2188	M14	P	an	a1-a2-a3-a4-a5-a6-a7	b1-b2-b3-b4-b48	c1-c2-c3	D	
I-3104	M32	P	an	a1-a2-a3	b1-b2	c1-c2-c3	D	
I-3081	M27	P	an	a1-a2-a3	b1-b2	c1-c2-c3	D	
MNG2972	10*	M	u	a1-a2-a3-a4-a5-a37'-a82-a85-a86	b1-b2-b3-b4-b10	c1-c3	ND	
MNG2955	10*	M	u	a1-a2-a3-a4-a5	b1-b2-b3-b4	c1-c3	ND	
MNG2959	15*	M	u	a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4	c1-c3	ND	
I-3189	M13	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10'	c1-c3	D	
I-3190	M13	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10'	c1-c3	D	
I-2487	M13	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10'	c1-c3	D	
I-2457	M13	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	D	
MNG3096	8*	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	ND	0.PE8
I-2239	M01	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	D	
I-2238	M01	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	pCad-	
I-2231	M01	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	pCad-	
I-2236	M01	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	D	
I-2226	M01	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	pCad-	
I-2836	M02	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	D	
I-2422	M02	M	u	a1-a2-a3-a4-a5-a37'-a82	b1-b2-b3-b4-b10	c1-c3	B	
A-1807	40	M	t	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	D	
A-1802	40	M	t	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	D	0.PE4?
A-1804	40	M	t	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	D	
A-1820	40	M	t	a1-a4-a6	b1-b2-b3-b4'	c1-c2-c3	D	
L1970015	L	M	x	a1-a4-a6	b1-b2-b3-b4	c1	E	
@91001	L	M	x	a1-a4-a6	b1-b2-b3-b4	c1	E	
I-3088	M27	M	al>x	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2	E	
I-3132	M20	M	al>x	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2	E	
I-3134	M20	M	al>x	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2	E	0.PE4cd
MNG3142	33*	M	x	a1-a4-a6'	b1-b2-b3-b4-b10	c1-c2	ND	
MNG3129	23*	M	x	a4-a6'	b1-b2-b3-b4-b10	c1-c2	ND	
MNG3128	23*	M	x	a1-a4-a6'	b1-b2-b3-b4-b10	c1-c2	ND	
162	M20	M	al>q	a1-a4-a6'	b1-b2-b3-b4-b10	c1-c2-c3	E	
2420	M20	M	al>q	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	pCad-	
M2000005	M	M	q	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	E	0.PE4ab
M2001006	M	M	q	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	E	
A-1728	34	M	h	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	C	
A-1249	34	M	h	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	E	
A-1725	34	M	h	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	pCad-	0.PE9
5307-Gis.	34	M	h	a1-a4-a6	b1-b2-b3-b4-b10	c1-c2-c3	pCad-	
I-2377	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	pCad-	
I-2359	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	A	
I-3455	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	A	
I-3517	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3518	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3516	36	M	al	a1-a4-a6	b1-b3-b4-b10	no spacers	D	
I-3547	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3443	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-2629	M01	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3442	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3447	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3515	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3519	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	0.PE1
I-3467	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
A-513	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3252	M01	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
2131	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3214	M01	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
I-3446	36	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
@Pestoides A	?	M	al	a1-a4-a6	b1-b3-b4-b10	c1-c2	D	
MNG2197	8a*	M	al	a1-a4-a6'	b1-b3-b4-b10	c1-c2	ND	
MNG2198	8a*	M	al	a1-a4-a6'	b1-b3-b4-b10	c1-c2	ND	
MNG3125	7*	M	al	a1-a4-a6'	b1-b3-b4-b10	c1-c2	ND	
MNG3126	7*	M	al	a1-a4-a6'	b1-b3-b4-b10	c1-c2	ND	
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mids with molecular size 8, 47, and 75–80 MD significantly differed from the typical set of the plague pathogen [2]. The other two strains (2420 and 162) had MLVA25-type relevant to bv. qinghaiensis. Moreover, all five strains, as well as the strains of the biovars xilingolensis and qinghaiensis, had the type E of their LcrV amino acid sequences, which is different from the types D and A relevant to the typical strains of the biovar altaica. The results of CRISPR-typing confirmed the belonging of the strains 162 and 2420 to the biovar qinghaiensis (Fig. 3). At the same time, the strains I-3088, I-3132, and I-3134 turned out to be recent representatives of a hypotetic CRISPR-cluster [9], which diverged from the Cc3 cluster; includes the biovars talassica, hissarica, and qinghaiensis; and was a predecessor for the CRISPR-clusters of Cc2 (bv. altaica) and Cc1 (bv. xilingolensis). As was noted, the recent representatives of this predicted cluster (including three previously described strains, from which two were isolated in the same area [17]), according to MLVA25-typing, are the closest neighbors to bv. xilingolensis. For this reason, it has been suggested that the divergence of two closely related biovars, qinghaiensis and xilingolensis, occurred in the Bayan-Khongorsk aymag area and spread to the foci L and M of China only after this event.

The strains bv. altaica described by J.M. Riehm et al. [17] were isolated from the foci 36 and M01 and differed from the rest of this biovar strains by a mutation in the a6 spacer (a6' allele) of the Ypa CRISPR-locus. This resulted in their autonomous position in the MLVA25-dendrogram as a member of the clonal cluster (Fig. 3).

In this study, increasing the number of the bv. ulegeica strains from 3 [12] to 11, as well as including four bv. ulegeica strains in the dendrogram based on the previous study [17], did not change the position of this autonomous phylogenetic group relative to the other branches of the MLVA25-dendrogram. The reliability of the clonal clusterization obtained with the use of MLVA25-typing is confirmed by the fact that the bv. ulegeica strains form a single group according to the CRISPR-typing results (Fig. 3). Association of certain MLVA25-types with the sites of their isolation is evident within the clonal clusters. The first group is formed by the strains derived from the foci of Mongolian Altai (M01 and M02). The second group is formed by the strains persistent in the Gobi Desert (focus M13). The strains described by Riehm and et al. [17] were isolated in the areas located between Mongolian Altai and the southern part of the Gobi Desert and remained autonomous within their clonal cluster (Fig. 3).

All the strains studied, which constituted a group joining the biovars talassica, qinghaiensis, xilingolensis, hissarica, and altaica (0.PE4, 0.PE9, and 0.PE1 in Fig. 3), have valine (CTG) in the aspartase AspA amino acid sequence at the position 363 in the same way as in the case of the *Y. pestis* predecessor *Y. pseudotuberculosis*. In contrast, this amino acid residue is substituted for serine (GTG → TTG) in the strains of the basic subspecies (0.ANT in Fig. 3) and leucine (GTG → TTG) in all bv. ulegeica strains (0.PE8 in Fig. 3).

Polymorphism of the V-antigen amino acid sequence (Fig. 3) is characteristic of only a some of the *Y. pestis* strains belonging to the nonbasic subspecies. The values of the specific fraction of the isolates with the V-antigen types, which are different from that relevant to the basic subspecies type D (phylogenetically closest to the most ancient V-antigen of the predecessor, *Y. pseudotuberculosis* [6]), vary among the clonal clusters. This fact suggests a continuing microevolution of *Y. pestis*.

Intraspecies identification of the Pestoides A strain is a case of applied use of MLVA25-typing for molecular epidemiology in this study. The results of molecular typing in silico (Fig. 3) are unambiguous and indicate that the isolation site of Pestoides A is located in the Russian (focus 36) or Mongolian (focus M01) part of the Sailugem natural plague focus.

In conclusion, analysis of the natural *Y. pestis* isolates confirmed the association of the MLVA25- and CRISPR-types with certain natural foci. In addition, a possible approach to more precise intraspecies taxonomy of isolates and collection strains of *Y. pestis* was demonstrated.

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REFERENCES

1. Ad'yasuren, Z., Tserennorov, D., Otgonbaatar, D., Balakhonov, S.V., Innokentyeva, T.I., Agiimaa, Sh., et al., Clinical-epidemiological features of plague in Mongolia, *Probl. Osobo Opasn. Infekts.*, 2010, vol. 103, pp. 30–33.
2. Balakhonov, S.V., Tséndzhav, S., and Erdénébat, A., New plasmidovars of *Yersinia pestis* isolated in Mongolia, *Mol. Genet. Microbiol. Virusol.*, 1991, vol. 11, pp. 27–29.
3. Goncharov, A.I., Tokhov, Yu.M., Plotnikova, E.P., and Artyushina, Yu.S., *List of Species and Subspecies of Fleas Infected by Yersinia pestis in Nature*, Stavropol: RIO IDNK Publ., 2013.
4. Platonov, M.E., Evseeva, V.V., Efremenko, D.V., Kuznetsova, I.V., Chirkova, E.V., Dentovskaya, S.V., et al., DFR-typing of *Yersinia pestis* strains from the CIS natural foci, *Probl. Osobo Opasn. Infekts.*, 2011, vol. 108, pp. 42–45.

5. *Natural Plague Foci in the Caucasus, Caspian Sea Region, Central Asia, and Siberia*, Onishchenko, G.G. and Kutyrev, V.V., Eds., Moscow: Meditsina, 2004.
6. Anisimov, A.P., Dentovskaya, S.V., Panfertsev, E.A., Svetoch, T.E., Kopylov, P.Kh., Segelke, B.W., et al., Amino acid and structural variability of *Yersinia pestis* LcrV protein, *Infect. Genet. Evol.*, 2010, vol. 10, no. 1, pp. 137–145.
7. Anisimov, A.P., Lindler, L.E., and Pier, G.B., Intraspecific diversity of *Yersinia pestis*, *Clin. Microbiol. Rev.*, 2004, vol. 17, no. 2, pp. 434–464.
8. Bearden, S.W., Sexton, C., Pare, J., Fowler, J.M., Arvidson, C.G., Yerman, L., et al., Attenuated enzootic (pestoides) isolates of *Yersinia pestis* express active aspartase, *Microbiology*, 2009, vol. 155, no. 1, pp. 198–209.
9. Cui, Y., Li, Y., Gorgé, O., Platonov, M.E., Yan, Y., Guo, Z., et al., Insight into microevolution of *Yersinia pestis* by clustered regularly interspaced short palindromic repeats, *PLoS One*, 2008, vol. 3, no. 7, p. e2652.
10. Cui, Y., Yu, C., Yan, Y., Li, D., Li, Y., Jombart, T., et al., Historical variations in mutation rate in an epidemic pathogen, *Yersinia pestis*, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, vol. 110, no. 2, pp. 577–582.
11. Kiefer, D., Dalantai, G., Damdindorj, T., Riehm, J.M., Tomaso, H., Zöller, L., et al., Phenotypical characterization of Mongolian *Yersinia pestis* strains, *Vector Borne Zoonotic. Dis.*, 2012, vol. 12, no. 2, pp. 183–188.
12. Li, Y., Cui, Y., Hauck, Y., Platonov, M.E., Dai, E., Song, Y., et al., Genotyping and phylogenetic analysis of *Yersinia pestis* by MLVA: insights into the worldwide expansion of Central Asia plague foci, *PLoS One*, 2009, vol. 4, no. 6, p. e6000.
13. Li, Y., Dai, E., Cui, Y., Li, M., Zhang, Y., Wu, M., et al., Different region analysis for genotyping *Yersinia pestis* isolates from China, *PLoS One*, 2008, vol. 3, no. 5, p. e2166.
14. Morelli, G., Song, Y., Mazzoni, C.J., Eppinger, M., Roumagnac, P., Wagner, D.M., et al., *Yersinia pestis* genome sequencing identifies patterns of global phylogenetic diversity, *Nat. Genet.*, 2010, vol. 42, no. 12, pp. 1140–1143.
15. Platonov, M.E., Evseeva, V.V., Dentovskaya, S.V., and Anisimov, A.P., Molecular typing of *Yersinia pestis*, *Mol. Genet. Microbiol. Virusol.*, 2013, vol. 28, no. 2, pp. 41–51.
16. Platonov, M.E., Evseeva, V.V., Svetoch, T.E., Efremenko, D.V., Kuznetsova, I.V., Dentovskaya, S.V., et al., Phylogeography of *Yersinia pestis* vole strains isolated from natural foci of the Caucasus and South Caucasus, *Mol. Genet. Microbiol. Virusol.*, 2012, vol. 27, no. 3, pp. 108–111.
17. Riehm, J.M., Vergnaud, G., Kiefer, D., Damdindorj, T., Dashdava, O., Khurelsukh, T., et al., *Yersinia pestis* lineages in Mongolia, *PLoS One*, 2012, vol. 7, no. 2, p. e30624.
18. Vavilov, N.I., The law of homologous series in variation, *J. Genet.*, 1922, vol. 12, no. 1, pp. 47–89.
19. Zhou, D., Han, Y., Song, Y., Huang, P., and Yang, R., Comparative and evolutionary genomics of *Yersinia pestis*, *Microb. Infect.*, 2004, vol. 6, no. 13, pp. 1226–1234.

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