Aprobation of the Mobile Laboratories for Monitoring and Diagnostics at Epizootological Inspection of Natural Plague Foci in Siberia

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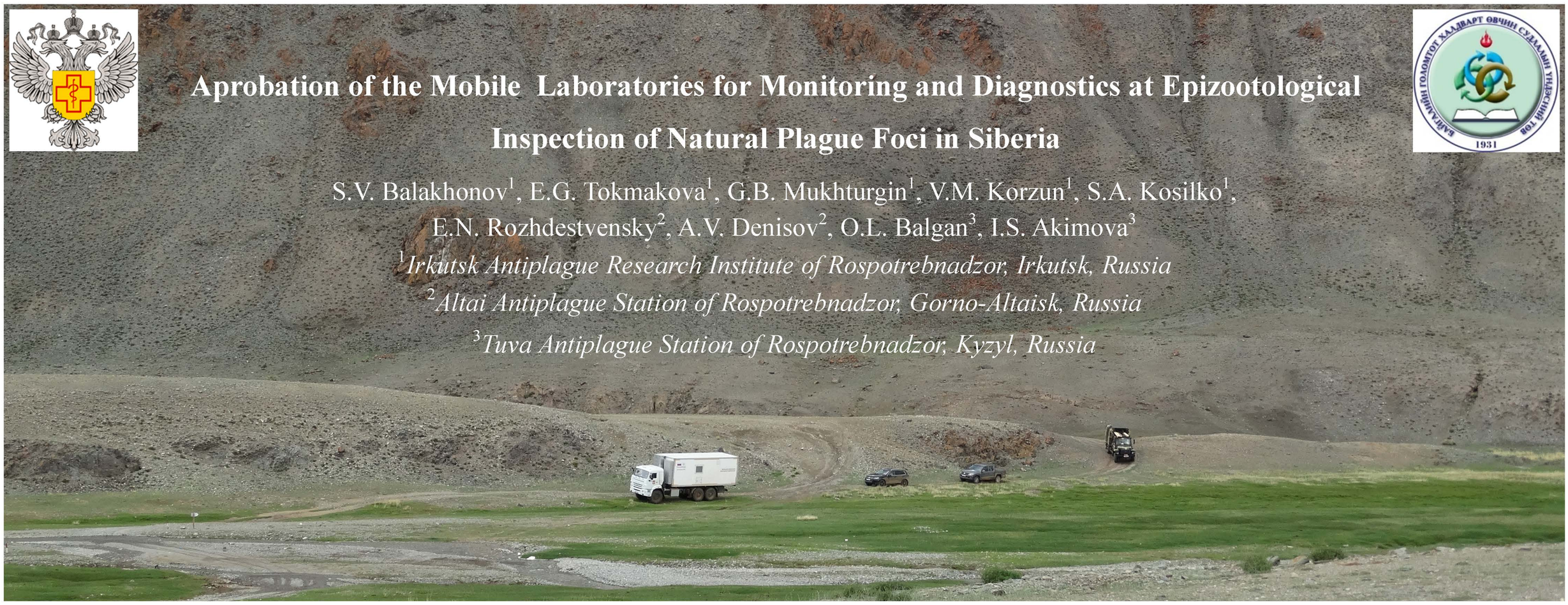

Optimization of epizootological monitoring using mobile laboratory complexes for operative detection of epizootic manifestations at the removed enzootic sites and timely response to the preconditions for epidemic complication occurrences among local population is one of the directions to improve epidemiological surveillance in the natural plague foci.

In December 2017 a mobile laboratory for monitoring and diagnostics (MLMD) on the basis of "KamAz" car appeared at Altai Antiplague Station (APS). In 2018 the Tuva Antiplague Station obtained the same complex. The mobile laboratory is intended for examination of a biological material for presence of the bacterial and virus causative agents in field conditions by molecular-genetic, serological and bacteriological methods.

The aim: Analysis of MLMD operational experience at epizootic inspection of Tuva and the Mongolian part of transboundary Sailugem plague foci.

MLMD autonomy permitted to conduct researches in immediate proximity from the surveyed points with daily material delivery. The field material arriving in MLMD was distributed as follows (Fig. 1):

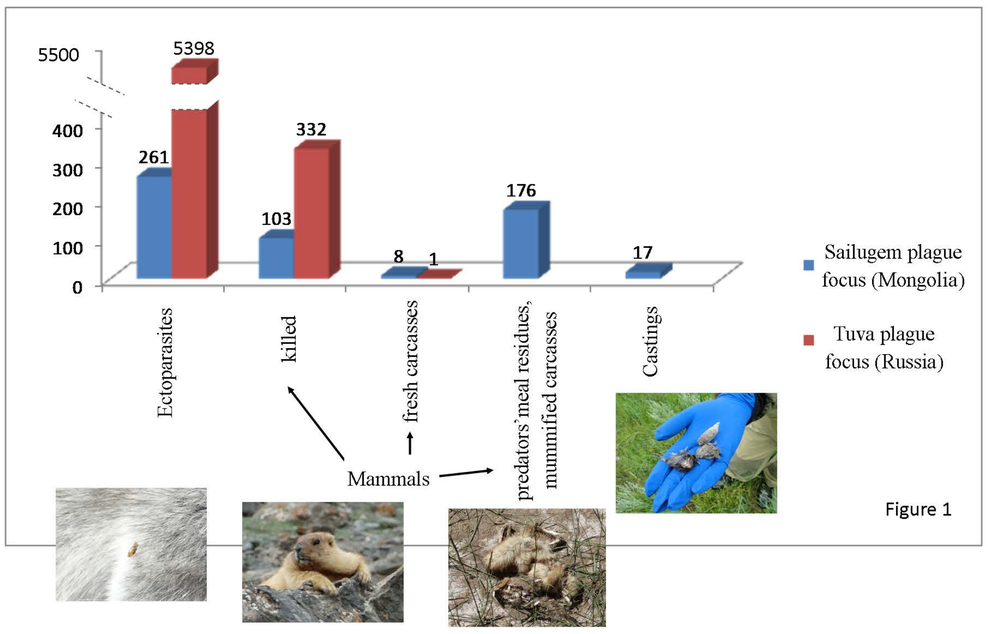

Figure 1

Grey marmots (197 individuals) in weight to 6 kg prevailed among the mammals examined in the transboundary Sailugem natural plague focus. The zoological group attached to MLMD in the Tuva focus revealed only one Mongolian marmot, all the other mammals belonged to small ones: long-tailed Siberian souslik, Mongolian and Daurian pikas, silvery and red-grey voles, house mouse. Different field material caused the peculiarities in its processing in MLMD. In the Salugem focus its sorting and registration, sampling was conducted in specially equipped yurta. Spinal cord slices were the most preferable material from the fresh meal remains as it was taken easily and in sufficient amount; when rotting was observed the marrow from tubular bones was taken. The zoologists selected some tubular bones from the dry meal residues, stale and mummified carcasses. Often incomplete residues mainly skulls were found. The marrow from lower jaw was convenient to use and besides, it perfectly remained even in the dried by sight bones. Materials except for ectoparasites were transferred to MLMD in microtest tubes in volume of 1,5 ml. Microtest tubes of two types were used: with a round bottom for sample crushing in a homogenizer and conic tubes if such processing was not required. The scheme of the material preparation and examination is resulted on Fig. 2.

In the Tuva focus the caught mammals were combed in a tent. Then they were transferred in MLMD for autopsy carried out in class II B Cabinet of Microbiological Safety (CMS). Ectoparasite identification was performed in the tent due to its big number.

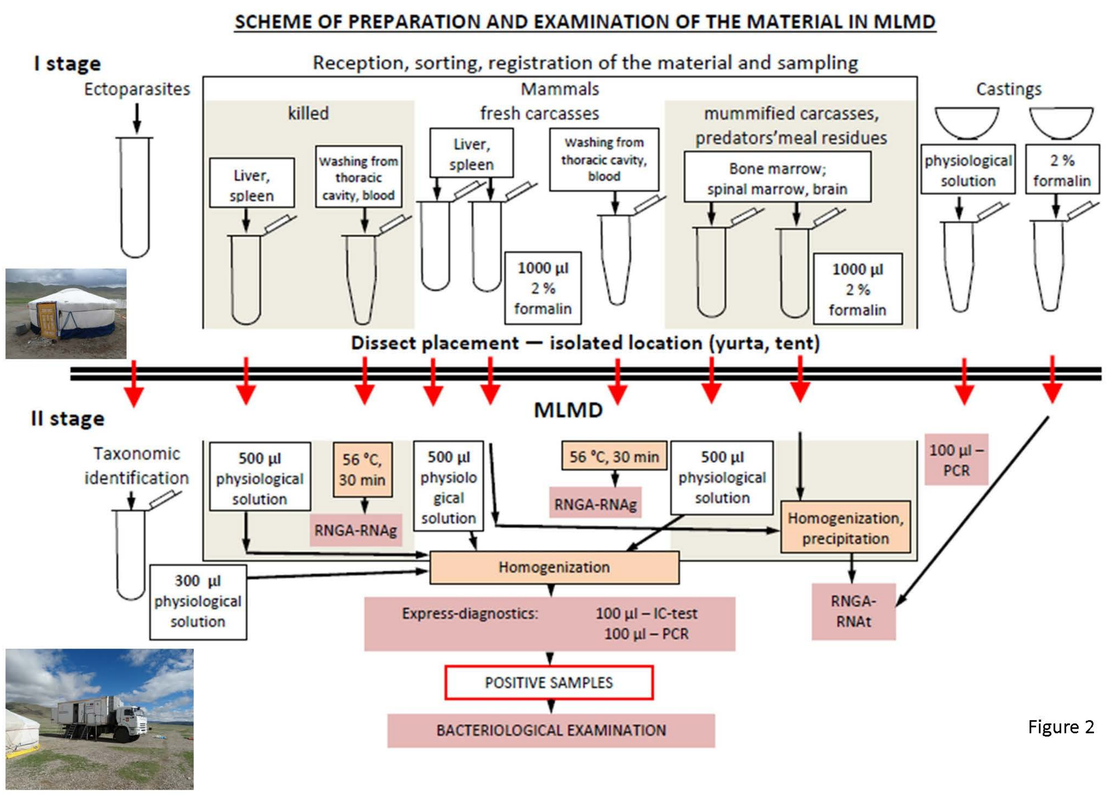

Figure 2

The selected samples were treated in class II B CMS (Fig. 3a). Samples with addition of formalin and physiological solution were crushed in homogenizer TissueLyser LT (QIAGEN, Germany) within 2 min and precipitated by centrifugation some seconds at 3000 rpm. All samples were tested in real-time PCR using Rotor Gene Q device (QIAGEN, Germany). DNA for PCR was isolated in class II B CMS with "RIBO-prep" set (Central Research Institute of Epidemiology) by the manufacturer's instruction (Fig. 3b). Preparation of reaction mixtures

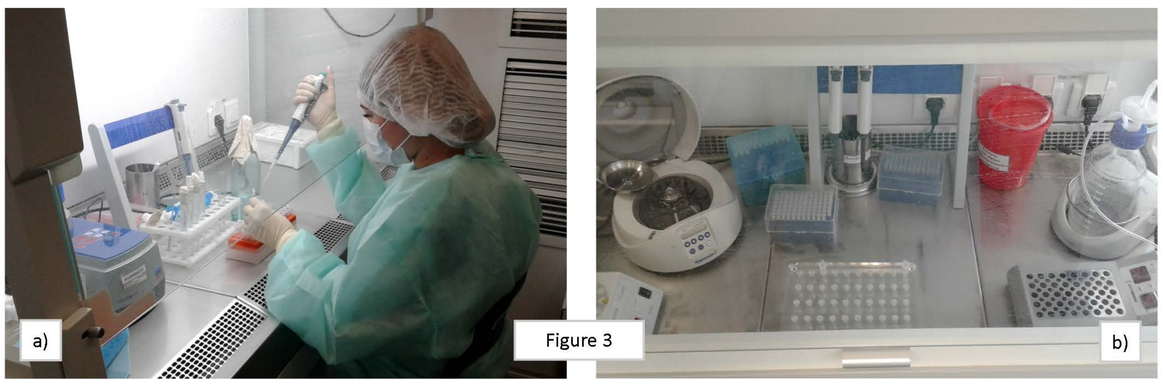

Figure 3

and filling of the amplifier RotorGene cells was performed in PCR-cabinet. After each stage of the manipulations the protective clothing was changed. Serological reactions were performed by micromethod with erythrocytic diagnosticum: plague immunoglobulin and plague antigen (M. Aikimbayev Kazakh Scientific Center of Quaratine and Zoonotic Diseases) at the table between the cabinets.

The number of the samples tested for plague in each MLMD is presented in the Table. Despite qualitative and quantitative distinctions in the processed material during one month the both laboratories treated the similar number of samples. It can be accepted as a conditional optimum volume of field material testing in MLMD. In the Sailugem focus after amplification the positive samples were placed on agar plates with nutrient medium for cultivation and plague agent isolation (State Research Centre of Applied Microbiology and Biotechnology, Obolensk). After isolation of a pure culture of the plague agent, its subspecies was specified in PCR with yp2769ms06 and yp3057ms09 primer set. PCR result was considered by electrophoresis in agarose gel. Subspecies of the isolated *Yersinia pestis* strains also were determined by arabinose, rhamnose, melibiose (1 % and 0,5 %) and maltose (1 % and 0,5 %) fermentation. There were no positive reactions in PCR in the Tuva focus. Therefore, all samples were subjected to bacteriological examination; no culture was isolated.

Table

Number of samples examinated to plague in MLMD/positively responded to plague

Methods of testing	Sailugem high-mountainous plague focus	Tuva mountainous plague focus
PCR	345 / 60	363 / 0
IC-test	189 / 39	Not tested
Serological	305 / 50 Ag + 10 Ig	332 / 17 Ig
Bacteriological	60 / 47	332 / 0

After each stage completion damp cleaning with application of "DP-Altai"(Production and Commercial Company "WEST", Russia) disinfectant was performed and UV-irradiators inside MSC and in the laboratory were switched on for 30 minutes.

In MLMD involved in Sailugem focus inspection, immunochromatographic test-system for express-detection and identification of the plague agent («IC *Y. pestis* test-system», State Research Centre of Applied Microbiology and Biotechnology, Obolensk, IC-test) was actively used. 100 µl of the sample just transferred in liquid phase was placed in round receiving window of IC-test. Appearance of two parallel stained strips in rectangular window indicated the positive result. IC-tests were approved for application in bacterial soliquids and suspensions of the mammal internals (Belkova S.A. et al., 2009; Belkova S.A., Balakhonov S.V., Biketov S.F., 2011; Belkova S.A., Balakhonov S.V., 2014). Experience of its application to *Y. pestis* detection in ectoparasite suspensions was not described. We found out its suitability for express-examination of the *Oropsylla silantiewi* flea samples with I-II stages of blood digestion from a marmot – birds of prey meal residues (Fig. 4). Subsequently, specific DNA fragments of *Y. pestis* were amplified at early cycles (since the 5th) from these samples as well as from the others (total 39 samples) with positive result in IC-test and the plague agent cultures were isolated. In total 60 positive results in PCR for *Y.pestis* DNA presence were received included 50 tests confirmed by F1 detection in RNGA-RNAt. Forty seven strains of an epidemiologically significant variant *Y. pestis* subsp. *pestis* were isolated (Fig. 5).

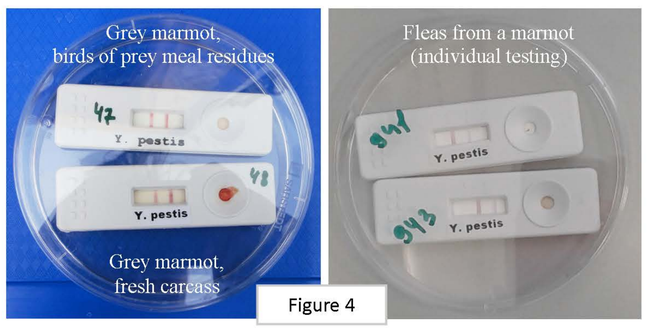

Figure 4

Conclusion. Thus, MLMD application in the Sailugem natural plague focus demonstrated high efficiency and productivity for inspection of Russian and Mongolian frontier territories and permitted to reveal epizootic plague manifestations at the area of 1544 sq. km. In Tuva focus - for the first time in 10 years MLMD application provided promotion of the full epidemiological brigade to the points removed from a stationary laboratory, material examination lost-free its properties in the course of storage and delivery and the definition of the points of *Y. pestis* preservation at total area of 155 sq.km. It is advisable to perform the field material preprocessing outside of MLMD in the advanced temporary placement (tent, yurta). Such division permits to reduce essentially the time necessary for field material sample examination due to number reduction of intermediate current disinfections. Full cycle of manipulations in MLMD is possible at small amount of field material with prevalence of small mammals. The approved order of processing and material examination was included into the project of methodical instructions of Federal level «Organization and carrying out of laboratory diagnostics of natural focal and other dangerous infectious diseases in a mobile laboratory for monitoring and diagnostics».

Comparative results of the use of bacteriological and molecular diagnostic (PCR) methods when conducting an inspection of the transboundary Sailyugem plague natural focus in 2018.

Places of *Y. pestis subsp. pestis* strains isolation

Places of *Y. pestis* DNA revealing

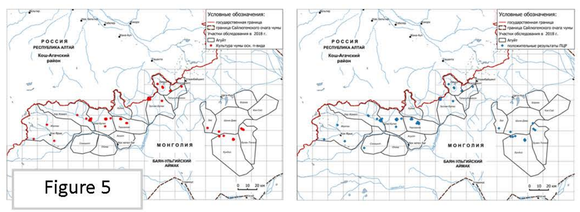

Figure 5

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