and their analysis were carried out using Microflex LT (Brucker) and its programs v. 3.3.64 and v. 3.3.65.

At the first stage we created 2 databases of mass spectra of reference strains: 1) saprophytes of the genus Bacillus and 2) strains of *B. anthracis*. When carrying out "blind" tests we revealed that fragments of peptide complexes over the range 2–12 000 Da in all representatives of both groups practically did not differ because of high degree of affinity. Thus, strains of closely related saprophytes were identified as *B. anthracis* and strains with high indicator SV on the contrary as saprophytes. When all spectra of cultures of both groups were pooled, identification became more correct, allowing to obtain the highest values of SV for strains of one species. The most optimum results of specific identification were obtained when identification of cultures was carried out using the program MALDI Biotyper RIC and construction of MsP-dendrogram was carried out using the program FlexAnalysis. In obtained dendrograms samples under study were clearly clustered with one of bacilli species represented in the base.

Thus, perfection of the scheme of reliable identification of *B. anthracis,* including accurate differentiation from closely related bacilli on the basis of MALDI TOF MS continues to remain urgent.

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SEARCH FOR SPECIES-SPECIFIC MARKERS FOR *BACILLUS ANTHRACIS* BY MALDI-TOF MASS SPECTROMETRY

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The use of the sensitive and rapid method of MALDI-TOF MS for identification of cultures of the causative agent of anthrax requires not only strict specificity, but also universality for all strains irrespective of their intraspecific variability of phenotypic properties.

The aim of the work was to reveal species-specific signals, common to all *B. anthracis* strains with various complexes of phenotypic properties.

We used 37 strains which included strains atypical in capsule formation, toxin production, nutritional requirements, activities of protease, lecithinase and hemolysins, ability to hydrolyze carbohydrates, as well as strains with different MLVA- and SNP-genotypes. Samples were prepared by lysis of 18-hour cultures in 80% TFA followed by ultra-micro-centrifuge filtration. The studies were carried out using Microflex LT instrument (Bruker). Collection of mass spectra and analysis of data were carried out using the programs v. 3.3.64 and v. 3.3.65. Analysis of spectra for frequency of signals was carried out using the program Microbe MS.

The occurrence of various combinations of phenotypic properties made it possible to discriminate 11 phenotypes. Individual spectra of each of these phenotypes (20 spectra of each strain) were analyzed and peak frequency was determined. For the further analysis we used peaks occurring at the frequency ≥ 95%, with their numbers in various groups varying from 2 to 32.

When comparing the peak frequency of all the 11 phenotypic groups we revealed the absence of common peaks with the frequency ≥ 95%. The distribution of signals which were identified in all the groups most often were as follows: 2601 Da — 82.2%; 4367 Da — 81.7%; 4666 Da — 76.4%; 6445 Da — 73.8%; 5206 Da — 72.8%. Earlier these peaks were not considered as specific markers of *B. anthracis*. The approach to choose markers we used when analyzing strains with a great number of phenotypic groups, including rare strains, may account for this. Markers of the system of ribosomal proteins, SASP and histone proteins, earlier described as species-specific markers, also occur in the spectra of strains from various groups, but at much lower frequency, and that may be connected with the production of various proteins or with various levels of their expression.

Thus, selection of species-specific peaks for identification of *B. anthracis* strains should be carried out taking into account the variability of their biological properties.

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ETIOLOGICAL CHARACTERISTICS OF MALARIA AND PREVALENCE OF HEMOGLOBINOPATHIES IN PATIENTS IN THE REPUBLIC OF GUINEA

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According to WHO, in 2016, malaria affected 216 million people in 91 countries, which is 5 million more than in 2015. The number of deaths from malaria in 2016 was 445 000 people. 90% of cases and 91% of deaths from malaria was from Africa.

There are more than 50 different types of hereditary hemoglobinopathies. They are most often found in regions with a tropical and subtropical climate, which correspond to geographic regions endemic for malaria.

The aim of our study was to determine the etiological structure of malaria and to assess the prevalence and variants of hemoglobinopathies in patients with malaria in the territory of the subrefecture Fria of the Republic of Guinea.

The study included 300 cases of malaria aged 0 to 70 years, from the hospital "RUSAL FRIGUIA" in town Fria from May to December 2017. Malaria was determine by a rapid test for the differentiated determination of antigen *P. falciparum* and pan-malarial antigen, with verification and validation of parasitemia by the method of thick drop and smear. The species belonging to the plasmodium was confirmed by the PCR method followed by sequencing. The type of hemoglobin was determined by method of electrophoresis.

The average age of patients was 15.8 years (from 1 month to 65 years), men — 53%. In 99% cases causative agent was *P. falciparum*, with parasitemia from 16 to 20 000 tr/μL. Hemoglobinopathy revealed in 20% of patients, first of all, sickle-cell anemia (85%). Lethal outcome was registered in 11 patients at the age from 2 to 14 years.

High parasitemia was associated with a more severe course of the disease. In patients with concomitant hemoglobinopathy revealed a less severe clinical course of malaria, characterized by relatively small parasitemia.

100% dominance of *P. falciparum* in patients with malaria in this region defines clinical vigilance regarding the severity of the course and the prognosis of the disease. Identifying concomitant hemoglobinopathies allow us to predict a favorable prognosis of malaria.

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PECULIARITIES OF MASS SPECTROMETRIC ANALYSIS OF BRUCELLA S- AND L-FORMS

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The causative agent of brucellosis, like many bacteria, is able to transform from S- and R- forms into L-form under the influence of various factors changing its biological

properties. Sometimes it is not diagnosed by the available certified diagnostic preparations and test systems. In this regard, it is urgent to develop an effective method for identifying the pathogen using MALDI-TOF MS.

The aim is to study peculiarities of protein profiles of *Brucella* S- and L-forms using mass spectrometric analysis.

The following *Brucella* strains of S- and L-forms were used in this study: *B. abortus* 544, *B. melitensis* 16 M, *B. suis* 1330, *B. abortus* I-206 of S- and L-forms, L-form of *Brucella* I-6, and L-form of *Brucella* I-7 from the collection of microorganisms of Irkutsk Antiplague Research Institute. Cultures were grown on Albimi agar at 37°C for 48 hours. Extraction was carried out with 70% formic acid followed by the addition of acetonitrile according to the "Instruction for Sample Preparation and Subsequent Mass Spectrometric Analysis of Pathogens of 1–3 Risk Groups" (Irkutsk, 2011). The spectra were collected using MicroFLEX mass spectrometer (Bruker Daltonics, Germany).

In the absence of *Brucella* spp. protein profiles in the database, identification of the pathogen did not provide reliable results. Therefore, during the first stage of the work the protein profiles of the following reference strains were added to the database: *B. abortus* 544, *B. melitensis* 16 M, and *B. suis* 1330. Thereafter the mass spectrometric study of the other representatives of these three species allowed achieving the reliable identification to the species level except L-forms of *B. abortus* I-206. After the introduction of *B. abortus* I-206 in L-form into the database, it became possible to identify L-forms of this species, in particular L-forms of *Brucella* I-6 and *Brucella* I-7.

Based on the results, it can be assumed that L- and S-forms of the same species differ significantly in protein profiles. Thus, we can recommend mass spectrometry with matrix-activated laser desorption/ionization for the accelerated identification of *Brucella*. For effective application of the method, it is necessary to create a representative electronic database of mass spectra of collection *Brucella* strains in both S- and L-forms.

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COXIELLA BURNETII PREVALENCE IN TICKS IN THE ULYANOVSK REGION

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Q fever is on record in the Ulyanovsk Region, and 3 cases were reported in 2013–2017. (average annual incidence rate over the period is only 0.08 per 100 000), but in fact the spread of Q fever is much higher judging by the results of seroprevalence survey in some districts where the antibodies to *Coxiella burnetii* were detected in 3.7% of healthy population. T*he role of ticks* in the direct transmission of *C. burnetii* to humans is small, however, being important participants of the pathogen circulation in natural and mixed foci of the infection they pose a real threat to animals, including agricultural, that contribute much to Q fever outbreaks in humans. Hence, monitoring of *C. burnetii* in ticks is essential for Q fever prevention.

The study objective was to assess the *C. burnetii* prevalence in ticks and to conduct subsequent genetic analysis of PCR products.

709 adult ticks (*Ixodes ricinus, Dermacentor marginatus*, *D. pictus*, *D. reticulatus*) were flagged from vegetation in forest and forest-meadow sites in some districts of the Ulyanovsk Region, and examined individually using standard PCR with the genus-specific primers flanking the 16S ribosomal RNA gene site. For PCR-positive results the amplicons were sequenced.

Genetic markers of *C. burnetii* DNA were detected in 5 ticks (*I. ricinus, D. marginatus, D. reticulatus)* from the Cilninsky, Ulyanovsky, Melekessky, Kuzovatovsky and Novospassky districts. The homology of the nucleotide sequence of the 16S rRNA gene of four PCR products was 99% as compared to the reference Nine Mile strain, while for one of them it was only 95%, that justifies the need to further study the heterogeneity of the microorganisms of the genus *Coxiella*. One *D. marginatus* (Novospassky district) was possibly infected with a less-investigated coxiella-like microorganism.

The existence of natural foci of Q fever was confirmed in 5 districts of the Ulyanovsk region. The genetic heterogeneity of *C. burnetii* circulating in the region was shown for the first time. The advisability of further study on the heterogeneity of microorganisms of the genus *Coxiella* is argued.

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WHOLE GENOME-BASED PHYLOGENETIC DIVERSITY AND GENOMIC EPIDEMIOLOGY OF LEPTOSPIRA

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Leptospirosis is an emerging zoonotic disease caused by pathogenic *Leptospira* strains. Each year, there is an estimated 1 million severe cases of leptospirosis and nearly 60 000 deaths worldwide. The genus *Leptospira* is highly heterogeneous and currently consists of 23 species and more than 300 serovars that can be isolated from diverse ecological niches and animal reservoirs. According to their phylogeny, *Leptospira* species are distributed in 3 groups: the pathogens, the intermediate species, which cause a milder disease, and the saprophytes, which do not cause disease in human nor animals.

Different serological and molecular typing methods have been used to study the epidemiology of *Leptospira*, but they are performed by few reference laboratories and usually designed for the most commonly found pathogens. Since the first complete *Leptospira* genome sequence was published in 2003, it is now possible to sequence bacterial genomes in a few hours at reduced cost. Whole-genome sequencing (WGS) has emerged today as an ultimate tool for both the identification of relevant genetic variations linked to virulence and for bacterial strain typing.

In this study, the taxonomic status of all species of the genus *Leptospira,* as well as 81 strains isolated from the natural environment across a wide geographic range, was evaluated by comparative genomics. Our results reveal that the genus *Leptospira* now contains 65 named species, including species from a new sub-lineage. Our findings show that the genus has a large and open pan-genome which further confirms the complexity of this genus. The availability of whole-genome sequences of *Leptospira* also allowed us to develop a core genome MLST (cgMLST) scheme targeting the entire genus of *Leptospira*. Our cgMLST represents a standardized, accurate, highly discriminatory, and reproducible method for differentiation among *Leptospira* isolates, allowing for comparison of and sharing typing results among laboratories worldwide.

This study will advance many aspects of the leptospirosis field including epidemiology, diagnostics, and basic knowledge including species diversity, evolution, ecology, and virulence.