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

Yu.A. Panferova[1], O.A. Freylikhman[1], N.K. Tokarevich[1], A.A. Nafeev[2], E.I. Sibaeva[2]

[1]St. Petersburg Pasteur Institute, St. Petersburg, Russia;
[2]Center for Hygiene and Epidemiology in the Ulyanovsk Region, Ulyanovsk, Russia

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

M. Picardeau

Institut Pasteur, Paris, France

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.