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INTERNATIONAL COLLABORATIVE PROJECT ON TICK-BORNE ENCEPHALITIS IN THE BARENTS REGION

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An international joint project on the surveillance of tick-borne encephalitis (TBE) in the Barents region was implemented in Norway and in NW Russia.

The project objective was to analyze hard ticks in endemic, non-endemic and borderline endemic areas within the Barents region, to verify the range of Ixodidae occurrence, and to define the northern limit of tick-borne encephalitis virus (TBEV) distribution.

Ticks were flagged in 2014–2015 (May-June) at several sites in Norway: from 58°N (Mandal) to 65°N (Brønnøysund), and in Russia: from 57°N (Pskov) to 64°N (Zachapino, the Arkhangelsk Oblast). TBEV was detected by real-time PCR.

Ticks collected in Russia were mostly *I. perculcatus*, while all those in Norway were *I. ricinus*. Each tick was studied individually. TBEV detected in Russian samples belonged to Siberian genotype, while in Norwegian samples it was only European genotype. TBEV prevalence in ticks collected in Russia was: 0.5% in St. Petersburg and in the Leningrad Oblast, 1.3% in the Pskov Oblast, 3.9% in the Arkhangelsk Region, 4.4% in Karelia.

In Russia fifty years ago scanty TBE cases were reported only in the south of the area under study, but now TBE is registered in most of districts, including the north of Arkhangelsk Oblast. In Norway TBE cases in humans are currently reported only in the south, however, TBEV is detected in questing ticks up to Brønnøy county. This northward shift of TBE in the northern Europe is a serious challenge to public health care.

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IS THERE A TRANSOVARIAL TRANSMISSION OF TAIGA TICK *(IXODES PERSULCATUS Sch.)* AND THE SHEEP TICK *(IXODES RICINUS (L.))* THE CAUSATIVE AGENT OF IXODID TICK-BORNE BORRELIOSIS *(BORRELIA BURGDORFERI* s.l.*)*?

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Ixodes ricinus (L.) and *Ixodes persulcatus Sch.* (Acari: Ixodidae) — the main vectors of pathogens of tick-borne borreliosis of humans. Transovarial transmission of the pathogen from the infected female to the eggs can serve as a mechanism for the vertical transmission of *Borrelia* to new generations of ticks in nature. At present, this issue has not been finally resolved, although it is generally believed that the transovarial transmission of *Borrelia* has no appreciable significance in maintaining their circulation and forming the level of infestation of adult ticks of the following generations (Korenberg et al., 2013).

Collected in May 2018 in natural biotopes of the Leningrad Region, adult females and males of taiga and sheep ticks were planted on rabbits for feeding. Of the 20 females of each species, 15 females of *I. persulcatus* and 17 females of *I. ricinus* were feed in June. In July, 15 females of *I. persulcatus* and 13 females of *I. ricinus* laid eggs. Determination of the presence of *B. burgdorferi* sensu lato complex DNA in females and samples of their clutches was carried out using the PCR method with hybridization-fluorescent detection in real time using a commercial set of AmpliSens (Interlabservis, Russia).The amplification was performed on a Quantcudio 3 thermocycler (Applied Biosystems, USA) A positive response to *B. burgdorferi* was found in 5 (38.5%) of *I. ricinus* females and 7 (46.7%) of *I. persulcatus* females. No laying eggs positive reaction did not. According to our results, transvarial transmission of *B. burgdorferi* sensu lato in *I. persulcatus* and *I. ricinus* is absent.

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FEATURES OF *BACILLUS ANTHRACIS* IDENTIFICATION BY MALDI-TOF MS

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Identification of *B. anthracis* is an important stage in laboratory diagnosis of anthrax, a dangerous infectious disease of humans and animals. The application of sensitive and rapid method of MALDI-TOF MS for this purpose is difficult owing to considerable homology of protein spectra of *B. anthracis* and closely related saprophytes of the genus *Bacillus*. It requires creation of databases of reference mass spectra of various representatives of the given genus and development of algorithms of their analysis.

The aim of the work was to develop a technical approach for reliable identification of *B. anthracis* with using MALDI-TOF MS.

We used 72 strains of saprophytes of the genus *Bacillus*, including strains belonging to the group *Bacillus cereus*, and 37 strains of the causative agent of anthrax, differing in their biological properties. To prevent spore formation and eliminate signals of spore proteins from spectra under study, cultures were reinoculated twice. Samples were prepared by lysis of 18-hour cultures and extraction of acid-soluble proteins by 80% TFA with the subsequent ultra-micro-centrifuge filtration. Collection of spectra

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