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HETEROGENEITY OF POPULATIONS OF THE FLEA *CITELLOPHILUS TESQUORUM ELBRUSENSIS* DETECTED ON THE BASIS OF ANALYSIS OF PROTEOMIC PROFILES BY THE METHOD OF MALDI-TOF MS

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The aim of the work was to analyze proteomic profiles of imago *C. t. elbrusensis*, the basic vector of the causative agent of plague in territory of the Central-Caucasian high-mountain natural focus of plague.

Proteomic profiles of 49 specimens of imago *C. t. elbrusensis* collected in populations of East and North Prielbrusye in June-August, 2017 were analyzed in the course of this work. All parasites were previously characterized by the following signs: sex, state of gastrointestinal tract, generative state of females. Each sample was studied individually by homogenization and extraction of proteins in 80% TFA. Spectra were collected and analyzed on MALDI-TOF mass spectrometer Microflex LT (Bruker, Germany) by using pre-established programs Flex Control V 3.3.5 and Flex Analysis v 3. (Bruker, Germany). The additional analysis of signal frequency and statistical processing were carried out using programs Microbe MS (Lash P., 2016).

The MSP analysis of the dendrogram constructed on the basis of super-spectra (generalized spectra of each sample) on the basis of differences in their protein composition showed clearly that *C. t. elbrusensis* was clustering into two basic geographical groups: a group of East Prielbrusye and a group of North Prielbrusye. At the same time the analysis of proteomic profiles of fleas of each of these groups revealed heterogeneity of protein composition of samples collected from points, most remote from each other in the region of 2–12 000 Da. It makes possible to differentiate some local proteotypes in populations of *C. t. elbrusensis* of basic geographical groups, each of which is characterized by certain frequency of both ribosomal and individual proteins denoting sufficiently long isolation of the given local populations of parasites — vectors of the causative agent of plague, owing to disconnection of settlements of their hosts — mountain sousliks in the conditions of mountain landscape of Prielbrusye.

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THE GRANULOCYTES PHAGOCYTIC CAPACITY TO *YERSINIA PESTIS* IN BLOOD SAMPLES OF ANTI-PLAGUE VACCINATED PEOPLE ACCORDING TO FLOW CYTOMETRIC ANALYSIS DATA

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Phagocytosis is the basis of cellular immunity in bacterial infections, but there is currently no information on the phagocytic activity of human blood granulocytes for killed *Yersinia pestis* cells grown at 28°C and the effect of anti-plague vaccination on this indicator. In this work a problem of obtaining such information was solved for the first time with the help of flow-cytometric technology, which allows to evaluate objectively the indices of the phagocytic reaction in whole blood samples without allocation of phagocytes and serum from it. Heat-killed *Y. pestis* (EV NIIEG), *Escherichia coli* (25922ATS) and *Staphylococcus aureus* (209P) cells, stained with FITC, were used in the experiments. Individual phagocytic reaction indicators were determined in blood samples of people, living in the territory of the Caspian sandy natural plague focus (130 persons), before and one month after anti-plague vaccination with respect to three types of bacteria after 15 min of incubation *in vitro* by the method of Hasui M. et al. (1989), modified by us according torecommendations of White-Owen C. et al. (1992). The results were taken into account on the CyAn ADP™ Dako Cytomation flow cytometer using the Summit v.4.3 Built 2445 software. Against the background of high phagocytic indices for *E. coli* and *S. aureus*, respectively 97.3±0.24 and 98.5±0.13%, in relation to *Y. pestis* were recorded the reduced phagocytic activity of granulocytes 55.6±2.1% in blood samples before vaccination.The phagocytic numbers measured in the FITC fluorescence intensity units for blood granulocytes that absorbed *Y. pestis* cells were on average (Mean) twice lower than for *E. coli* and *S. aureus* at significantly higher coefficients of variation on this parameter (CV = 169±3.7%) in comparison with CV for *E. coli* (68.8±1.6%) and *S. aureus* (66.1±0.9%). A month after the anti-plague vaccination, the blood granulocyte phagocytic activity to *Y. pestis* increased to 82.4±2.8 ($p < 0.001$), indicating that a new cellular test for anti-plague immunity evaluation in humans may be developed on the basis of the rapid whole blood granulocyte phagocytic activity to *Y. pestis* cells determination *in vitro*.

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ANTI-PLAGUE VACCINATION STIMULATES THE NEUTROPHIL EXTRACELLULAR TRAPS FORMATION TO INCREASE THE YERSINIA PESTIS KILLING EFFICIENCY *IN VIVO*

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Neutrophil extracellular traps (NETs) formation is a recently described anti-microbial mechanism of neutrophils which involves the release of chromatin decorated with granular proteins in order to bind extracellularly and kill microorganisms. However, the role of NETs in anti-plague immunity is unknown. Our aim was to show that NETs participate in *Yersinia pestis* killing and significantly increase the bacterial clearance *in vivo*, when post-vaccination anti-plague immunity in mice is created. BALB/c mice were immunized subcutaneously by protective dose of live *Y. pestis EV* NIIEG cells (2.5×10^4) and results were recorded on the 21st day after vaccination. Contributionof NETs to bacterial killing was determined by intraperitoneal (i.p.) inoculation of 150 U/mouse micrococcal nuclease (MCN) or EDTA-inactivated MCN to vaccinated and control mice 10 min before i.p. challenge of 10^8 live *Y. pestis EV* cells, grown 48 h at 28°C. After 4 h, animals were killed and the collected peritoneal lavage (PL) were seeded on polylysine pretreated coverslides, where the percentage of NET-forming neutrophils (NFN) were determined by fluorescence microscopy using DNA staining with propidium iodide. Colony-forming units (CFU) in PL were evaluated using Hottinger agar after 72 h ofbacterial grown at 28°C. Phagocytic capacity of neutrophils to i.p. injected FITC-labeled *Y. pestis* cells was measured in PL samples byflow cytometry. Vaccinationstimulated NETs formationin response to live *Y. pestis* cells (from control NFN values 8.3±0.9 to 41.5±2.3%, $p < 0.001$ forn = 6) and this accompanied the increased bacterial killing, reflected in 10-fold decreasing of CFUin PLof vaccinated animals, against the background of the absence

of significant differences to 4 h in cell phagocytic capacity *in vivo*. MCN treatment decreased the NFN and increased the CFU values in vaccinated mice reaching control values, and this effect was reversed when MCN was inactivated. These results highlight the contribution of NETs is as an important cellular defense mechanism in anti-plague immunity.

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HLA GENE POLYMORPHISM IN PERSONS VACCINATED AGAINST PLAGUE

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The live-attenuated vaccine based on the *Yersinia pestis* strain EV NIIEG is still in use in the Russian Federation for the protection of people living in territories endemic for plague and provides a high degree of efficacy, but fluctuations in individual values of adaptive immunity in response to vaccination necessitate the establishment of genes that control the variability of the immune response. Human Leukocyte Antigen (HLA) genes play a decisive role in this process. In this study the distribution of HLA genes in people, vaccinated EV NIIEG live vaccine and living in the Caspian sand plague focus (Kalmykia and from Astrakhan), was investigated for their connection of HLA genes with indicators of immunity factors. The study involved 120 people. HLA gene typing was performed by multiplex PCR. Production of cytokines was determined by enzyme immunoassay. Statistical processing of the results was performed using the program "Statistica" 10.0. We determined that HLA-DRB1 alleles were more often in both regions *04(20–21%), *03(18%), *07(15–16%) and *01(10–15%). No significant difference was found, as well as in the reaction of cytokines in the inhabitants of both regions. The difference in the distribution of variants of the gene DRB1 and DQA1 was found in residents of the Lagan district of Kalmykia — the predominance of allele group DRB1*04 (40%) compared to DRB1*03(10%). The dynamics of cytokine production also varied by region of residence. 1 month after the vaccination, the levels of TNFα and IL-10 production increased in the residents of the Lagan district, and the inhabitants of the Black Soil district showed their decrease. The difference in cytokine production among residents of the Lagan district may be related to the special distribution of haplotypes of HLA.

The results show that the polymorphism of HLA genes has an effect on the level of cytokine secretion in response to the vaccinated EV NIIEG live vaccine. Further study of genes regulating the production of immune factors, will improve the understanding of the mechanisms of the immune response after vaccination, as well as contribute to the prediction of immunogenicity and effectiveness of vaccine products developed.

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WHOOPING COUGH — AN UNDERESTIMATED "ADULT" INFECTION

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Whooping cough is traditionally considered as a childhood infection. However, studies carried out in several countries, have shown that the actual incidence among adults is 10–100-fold higher than official statistics. Adults and older siblings become sources of pertussis for infants.

The aim of this study was to determine the true incidence of whooping cough in the adult population of St. Petersburg. The objective was to estimate the circulation of pertussis causative agent among the adult population of St. Petersburg (age ≥ 18 years), using antibody level to pertussis toxin as a marker of disease/natural booster in the last 12 months.

We examined 538 adults who applied to the medical center for blood tests for diagnosis of chronic nonpulmonary diseases, aged 18 to 82 years (mean age 41.2 years), 333 women, 205 men. Method: ELISA for the detection of antibodies to pertussis toxin (IgG, IgA). The IgG value ≥ 40 IU/ml was defined for categorization of whooping cough or contact with the patient during the last 12 months; including the IgG level ≥ 40 IU/ml in combination with a positive IgA level (≥ 12 IU/ml) or IgG ≥ 100 IU/ml with any IgA value for categorization of current or recent infection.

Anti-pertussis toxin IgG were detected in 87 patients (16.2% of those examined), including 27 patients (5.1%) with serological markers of recent infection. The proportion of seropositive persons was highest in the groups of 18–29 and 30–39 years (21.4 and 19.9%, respectively), followed by a decrease to 5.7% in the 50–59 age group; in the group of 60 years and older, the proportion increased to 13.9%. The proportion of patients with serological markers of recent infection was highest in the group of 18–29 years too (6.4%).

The wide involvement of adults in the epidemic process of whooping cough in St. Petersburg was revealed, particularly in the age group 18–39 years. Attention is drawn to the increase in the proportion of seropositive patients older than 60 years due to the increasing risk of a more severe and complicated course of the disease in this age group. It is necessary to include pertussis as a cause of prolonged cough in the training cycles of the post-graduated medical education for the "adult" physicians.

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CHARACTERISTICS OF A MOBILE LABORATORY FOR MONITORING AND DIAGNOSTICS DURING EPIZOOTOLOGICAL INVESTIGATION IN THE MONGOLIAN PART OF THE TRANSBOUNDARY SAILUGEM PLAGUE FOCUS

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Spread of *Yersinia pestis* of the basic subspecies in the Russian part of the transboundary Sailugem natural plague focus and the followed epidemiological complications required the assessment of the situation in the Mongolian part of the focus. So, since 2017 joint Russian-Mongolian epizootological examinations are performed at its frontier sites. Peculiarities of the investigations in 2018 were connected with using of a mobile laboratory for monitoring and diagnostics (MLMD) on the basis of "KAMAZ" lorry that appeared in the Altai Antiplague Station in 2017.

MLMD autonomy permitted to conduct researches in immediate proximity from the examined sites with daily delivery of the material. Combing, dissection, sampling were performed in a specially equipped jurt. Two samples were taken from the whole mammal carcasses: liver and spleen pieces were placed in a plastic test tube for homoge-