

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.

5.14

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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 NIEG 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 NIEG 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 TNF α 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 NIEG 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.

5.15

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WHOOPIING 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 \geq 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 \geq 40 IU/ml was defined for categorization of whooping cough or contact with the patient during the last 12 months; including the IgG level \geq 40 IU/ml in combination with a positive IgA level (\geq 12 IU/ml) or IgG \geq 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 old (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.

5.16

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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 yurt. Two samples were taken from the whole mammal carcasses: liver and spleen pieces were placed in a plastic test tube for homoge-

nizing, chest cavity lavage — in a usual microtest tube 1.5 ml. The same samples and one more probe of parenchymatous bodies in a test tube were taken from fresh carcasses for homogenizing with addition 2%-formalin in 1000 µl volume for detection of *Yersinia pestis* capsule antigen (F1). Spinal or bone marrow from birds-of-prey food debris, mummified carcasses, bones were taken in two test tubes for homogenizing (one tube with formalin).

In MLMD ectoparasite taxonomic identification was performed. After the necessary sample preparation the agent express-diagnostics was conducted in all received probes with the subsequent bacteriological examination only the positive samples. 100-µl liquid phase samples from agonizing, dead and birds' pecked animals, ectoparasites found out on them, bone remains were used for ICH-tests (FBUN GNTS PMB, Obolensk). All samples were examined by real-time PCR on a Rotor Gene Q instrument (Qiagen, Germany) and RNGA-RNAt. Specific fragments of *Y. pestis* DNA were amplified from all 39 ICH-positive samples at early cycles (since the 5th) and *Y. pestis* cultures were isolated. In total 60 positive responses were received in PCR including 50 replies that were confirmed by F1 detection in RNGA-RNAt, 47 *Y. pestis* subsp. *pestis* cultures were isolated.

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CLONING OF THE YERSINIA PESTIS TRANSALDOLASE GENE

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One of the antigenic complexes of the plague pathogen is fraction V (FV) (Bozhko et al., 2006). Investigation of the components of FV using two-dimensional protein electrophoresis, Western blott and mass-spectrometric analysis allowed determining that the composition of FV includes transaldolase, which has immunochemical activity with the monoclonal antibodies against FV (Arsenieva et al., 2017; Trukhachev et al., 2017). Transaldolase is an enzyme of the pentose phosphate cycle. The characteristics of the *Y. pestis* transaldolase and the *Y. pseudotuberculosis* transaldolase are similar. Perhaps, transaldolase of pathogenic *Yersinia* is the "moonlighting protein" (González-Rodríguez et al., 2012, He Y. et al., 2015).

The aim of the study was the cloning of the transaldolase gene of *Y. pestis* (*talB*) into a high-copy vector. After amplification of DNA with talF- and talR-primers, the 1059-bp PCR fragment including the *talB* gene of *Y. pestis* was cloned into the corresponding sites of pGEM-T using the set of reagents pGEM[®]-T Easy Vector Systems (Promega), resulting in pGEM-T-tal. Transformation of *E. coli* strains was performed using standard protocols (Sambrook J., 2001). The recombinant clones was selected on LB agar containing 100 µg/ml ampicillin, 0.5 mM IPTG and 80 µg/ml X-Gal. The few selected recombinant clones were detected insert of about 1000 nucleotide pairs in the plasmid vector with help the method extraction plasmid DNA (Kado et al., 1981). Analysis of the recombinant DNA of these strains using talF-, talR- and pT7-primers in PCR showed that there was an embedding of a fragment containing a *talB* under the control of the T7 promoter. The resulting recombinant *E. coli* pGEM-T-tal strain which carried the plasmid containing the *Y. pestis talB* gene reacted with the horse hyper immune antiplague serum and serum from rabbits

immunized against FV in the gel precipitation reaction. The control strain containing only the vector plasmid pGEM-T didn't react with the serum. The *Y. pestis EV* strain served as a positive control.

Thus, recombinant strain of *E. coli* pGEM-T-tal containing gene of immunologically active *Y. pestis* transaldolase in plasmid was obtained. A tool for further study of immunogenic and protective properties of one of the components of FV *Y. pestis* was created.

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THE PRESENT EPIDEMIOLOGICAL CHARACTERISTICS OF YERSINIOSIS IN THE RUSSIAN FEDERATION

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The objective of this study was to carry out a retrospective analysis of pseudotuberculosis and intestinal yersiniosis surveillance data in the Russian Federation during the period 2013–2015.

Federal statistical observation data and federal subjects of Russia data were analyzed.

Presently the intestinal yersiniosis prevails in the etiological structure of yersiniosis, with proportion of 60%. A statistically significant decreasing trend in incidence is observed, the annual average incidence of pseudotuberculosis and intestinal yersiniosis per 100 000 population is 0.82±0.05 and 1.90±0.11 correspondingly.

The intensity of the epidemic process of yersiniosis varies greatly across different regions of the country. The registration of pseudotuberculosis is noted in approximately 49% of the entities of the Russian Federation, intestinal yersiniosis is registered more evenly — in 77% of the entities. The maximum incidence of yersiniosis is noted in a number of the entities of the North-West Federal District, the Siberian Federal District, the Far Eastern Federal District, where morbidity rates exceeded the federal average rate by 2–15 times.

The proportion of outbreak morbidity of pseudotuberculosis decreases, sporadic cases prevail. For the time period 2013–2015 10 outbreaks with a total of 110 diseased persons were reported. The incidence of intestinal yersiniosis is sporadic.

In the age structure of patients with pseudotuberculosis children predominate (65%) mainly in the age group 3–6 years (32%). In 2015 incidence in this age group was 5.2 per 100 000 persons, this is 17 times higher compared with adults and 2 times higher compared with children in the age group 1–2, 7–14 years. The ratio of children and adults with intestinal yersiniosis is practically 1:1 — 45 and 55%. The maximum incidence is noted among children in the age group 1–2, 3–6 and 7–14 years (3–32%) — 3.5, 3.4, 2.9 per 100 000 persons respectively. The incidence among adults was lowest (0.8 per 100 000 persons).

During this epidemiological study it was shown what the pathogens are principally transmitted to humans through fresh vegetables (11–61%) and fruits (3–32%). Thus meat and meat products, milk and dairy products are often not investigated as the possible sources of intestinal yersiniosis infection. The diagnosis is confirmed mainly by the serological methods — 49–91% of cases, by the PCR — only 1–15%.