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### GENETIC DETERMINANTS CHARACTERISTIC FOR *YERSINIA PSEUDOTUBERCULOSIS* STRAINS ISOLATED FROM PATIENTS WITH FAR-EAST SCARLETT-LIKE FEVER

S.A. Ermolaeva<sup>1</sup>, N.F. Timchenko<sup>2</sup>, E.A. Psareva<sup>2</sup>, R.R. Adgamov<sup>1</sup>

<sup>1</sup>Gamaleya State Research Center of Epidemiology and Microbiology, Moscow, Russia; <sup>2</sup>Somov Research Institute of Epidemiology and Microbiology, Vladivostok, Russia

The illness caused by *Yersinia pseudotuberculosis*, which was firstly described in Russia (Vladivostok, 1959) and named Far East Scarlett-like Fever (FESLF), is manifested via fever, rash and injury of liver and joints. Our study was aimed to reveal phylogenetic relationships of the FESLF isolates with the *Y. pseudotuberculosis* population. Totally, 64 *Y. pseudotuberculosis* strains including 37 isolates from 6 FESLF outbreaks and 19 sporadic cases were used. A previously described MLST scheme was used to characterize clonal diversity. MLST analysis was extended by sequencing virulence genes *inv*, *yadA*, *yopE*, *cnf*. We found three MLST types among FESLF isolates: ST2 (n = 33), ST26 (n = 5), and ST32 (n = 3; specific for serotype O3). All but 1 vegetable isolate belonged to ST2, which was also found in 9 (60%) of 15 rodent isolates. ST2 prevailed among isolates from all sources. The ST2/ST26/ST32 sequence types formed a cluster at the eBURST scheme with ST2 and ST32 belonged to separating subclusters descended from ST26. Combining MLST with virulence gene sequence typing gave rise to 6 MVLST types. The concatenated sequences of 10 MVLST genes were used to build a maximum likelihood tree that divided into 2 subclades. One subclade united MVLSTs found in FESLF isolates and MVLST6, which was found in rodent isolates only. The second subclade united MVLSTs found in rodent and vegetable isolates. The analysis of virulence gene diversity revealed predominance of nonsynonymous substitutions among virulence genes, whereas basic parameters of nucleotide diversity were similar in virulence and house-keeping genes. Notably, unique *yopE* and *inv* alleles and a deletion of 946 bp in the *cnf* gene encoding cytotoxic necrotizing toxin were found in all FESLF isolates independently on MLST type. The deletion in *cnf* resulted in a loss of the Rho-binding domain and toxin inactivation. The plasmid pYV was found in all strains. Additional plasmid pVM82 was found in all but 4 ST2 strains but not in other genotypes. The fact that full FESLF symptomatology is caused by several distinct genotypes supports the view that specific virulence traits are characteristic of FESLF-associated strains and suggests that the dominance of the ST2 genotype could be caused by its epidemiologic advantages rather than its pathogenic traits. This suggestion was supported by evolutionary analysis that rejected the hypothesis of equality of evolutionary rates for ST2 and other genotypes ( $p < 0.05$ ).

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### DYNAMICS OF MORBIDITY OF THE WEST NILE FEVER IN THE ASTRAKHAN REGION

S.F. Karpenko<sup>1</sup>, Kh.M. Galimzyanov<sup>1</sup>, B.I. Kantemirova<sup>1</sup>, R.S. Arakelyan<sup>1</sup>, A.R. Kurbangaliev<sup>2</sup>, G.L. Shendo<sup>2</sup>

<sup>1</sup>Astrakhan State Medical University, Astrakhan, Russia; <sup>2</sup>Center for Hygiene and Epidemiology in the Astrakhan Region, Russia

The variety and wide prevalence of arbovirus infections, the possibility of adverse outcomes determine the relevance of their study. In the territory of the Astrakhan re-

gion the epidemic focus of West Nile Fever is registered. The purpose of this study was to analyze the dynamics of the morbidity of the West Nile Fever in the Astrakhan region from 2014 to 2017. The analysis of "Data on infectious and parasitic diseases" (Form 1) in the Astrakhan region was carried out.

As our research has shown, West Nile Fever in the Astrakhan region is currently characterized by a low intensity of the epidemic process. 5 people in the Astrakhan region were affected by the West Nile Fever in 2014, the morbidity rate per 100 000 of the population was 0.5. The number of cases increased by 3.0 and 4.8 times respectively in 2015 and 2016. 15 people fell ill with West Nile Fever in 2015, and 24 people — in 2016. The mortality rate per 100 000 of the population was equal to 1.5 and 2.4 respectively. It should be noted that the number of people with West Nile Fever in Russian Federation as a whole increased by 1.5 and 4.9 times in 2015 and 2016, compared to 2014. The source of infection in West Nile Fever is mainly wild birds. The increase in the incidence rate in 2015 and 2016 in the Astrakhan region and in Russia may be associated with increased infection of migratory birds during their seasonal migration from the natural foci of West Nile Fever. Only one case of West Nile Fever was registered in the Astrakhan region in 2017, the mortality rate per 100 000 of the population decreased in 24 times compared to the previous year and amounted to 0.1. Children under the age of 14 years were 11.1% of all the patients with this arbovirus infection from 2014 to 2017.

Thus, the natural focus of the West Nile Fever remains in the Astrakhan region, which activity depends on both the sources of infection and its vectors influenced by the intensity of the epidemic process in endemic foci, seasonal migration of sources of infection and climatic conditions.

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### POSSIBILITIES OF NON-INVASIVE METHODS APPLICATION FOR DIAGNOSIS OF YERSINIOSIS IN CHILDREN

G.I. Kokorina<sup>1</sup>, M.D. Shestakova<sup>2</sup>

<sup>1</sup>St. Petersburg Pasteur Institute, St. Petersburg, Russia;

<sup>2</sup>St. Petersburg State Pediatric Medical University, St. Petersburg, Russia

The term "*Y. enterocolitica* and *Y. pseudotuberculosis* infections" or "yersiniosis" is applied for two infectious diseases caused by *Yersinia*. These are pseudotuberculosis and intestinal yersiniosis followed by intoxication, injuries to the gastrointestinal tract and multiple organ disorders in case of miscellaneous and multi-disorder disease types. According to course duration of the disease it is classified as an acute (lasts for one month), a protracted (no longer than 3 months) and a chronic form (longer than six months). Nowadays in the acute period and at relapse of the disease bacteriological and PCR methods are used for diagnosis. Sokolova and co-authors (2016) analysed data of the diagnosis procedure of infants with acute diarrhea treated in an infectious diseases unit and proved 3 times more of yersiniosis to be detected by the PCR technique than by using bacteriological tests. Thus, non-invasive PCR technique should be used more widely for acute form *Y. enterocolitica* and *Y. pseudotuberculosis* infections diagnosis.

During the winter rise in the incidence of yersiniosis we investigated the appendix tissue (n = 60) taken from a surgical unit in the acute period of the disease of the children and demonstrated 20% of positive results obtained by PCR (11 DNA of *Y. pseudotuberculosis* and 1 DNA of *Y. entero-*

*colitica*), only one *Y. pseudotuberculosis* strains (1.7%) was detected by the bacteriological method. The set of pathogenicity factors of this strain didn't prove to be usual for the strains isolated in the Russia which in most have the YPM genes (the superantigen *Y. pseudotuberculosis*-derived mitogen) and responsible for the typical pseudotuberculosis symptoms (rash, skin desquamation, red tongue). The obtained strain proved to have "high pathogenicity islands" (HPI) genes with ypm being absent. These "european" strains cause pseudotuberculosis with such symptoms as mesenteric lymphadenitis, acute appendicitis and gastrointestinal features. The traditional bacteriological technique proved to be effective both in theory and in practice.

Necessity of using both classical bacteriological and PCR methods thus proves to be important for understanding the symptoms yersiniosis.

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### ONCE UPON A TIME IN THE FAR EAST: ON THE 50<sup>TH</sup> ANNIVERSARY OF THE DISCOVERY OF "REAL PSEUDOTUBERCULOSIS" AMONG HUMANS

A.M. Korolyuk

*St. Petersburg State Pediatric Medical University, St. Petersburg, Russia*

After the first description of pseudotuberculosis and its pathogen in 1884, the infection was for a long time considered a classic zoonosis. Single cases of human disease usually resulted in death. In the mid twentieth century W. Masshoff and W. Knapp (1953, 1954) described rare sporadic cases of the appendicitis caused by infection of mesenteric lymph nodes. However, the decisive turn in the discovery of the true face of human pseudotuberculosis occurred only after the unusual events in the Far East of the USSR. It all started with the fact that in Vladivostok in the spring of 1959 an infection affected more than 300 young sailors of one military unit. 200 people with a severe form of the disease were placed in the hospital. Epidemiologists believed that the cause of this outbreak could be massive foodborne infection.

The clinical findings of the disease were very polymorphic, which made diagnosis difficult. Most patients in the early days had mainly typical symptoms of scarlet fever, some — signs of hepatitis, others — appendicitis or arthritis. A new disease was called "Far Eastern scarlet fever — FESF" before the discovery of etiology (I. Grunin, G. Somov, I. Zalmover, 1960). Similar collective outbreaks began to be recorded annually in other regions of the Far East.

However, the nature of the infection remained a mystery for several years, until in 1966 the naval bacteriologist Vladimir Znamenskij did announce that he and his colleagues found in the feces of patients *Yersinia pseudotuberculosis*. However, authoritative scientists refused to believe a little-known provincial microbiologist. On the recommendation of reviewers, the scientific journal rejected the article sent. So, in order to prove his rightness, Dr. Znamenskij in January 1967 in Leningrad infected himself with the culture of *Y. pseudotuberculosis*, taken from a patient. After 6 days, he developed a severe septic form of FESF.

Doctors of the Military Medical Academy studied it in details and successfully cured. The results fully confirmed Znamenskij's hypothesis that FESF is a previously unknown form of human pseudotuberculosis. From then a new stage in the study of pseudotuberculosis began, which significantly changed the old ideas about this infection and its pathogen.

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### MONITORING OF VACCINAL PROCESS IN HUMANS RESIDING IN THE ALTAI HIGH-MOUNTAINOUS NATURAL PLAGUE FOCUS

K.M. Korytov<sup>1</sup>, S.V. Balakhonov<sup>1</sup>, V.V. Voitkova<sup>1</sup>, V.I. Dubrovina<sup>1</sup>, A.K. Noskov<sup>1</sup>, A.I. Mishchenko<sup>2</sup>, L.V. Shchuchinov<sup>3</sup>, A.A. Makin<sup>4</sup>

<sup>1</sup>Irkutsk Antiplague Research Institute of Rospotrebnadzor, Irkutsk, Russia; <sup>2</sup>Altai Antiplague Station of Rospotrebnadzor, Gorno-Altai, Russia; <sup>3</sup>Administration of Rospotrebnadzor in Republic Altai, Gorno-Altai, Russia; <sup>4</sup>Kosh-Agach Regional Hospital, Kosh-Agach, Altai Republic, Russia

The Altai high-mountainous natural plague focus has high epidemic potential; in 2014–2016 three cases of bubonic plague are registered in local residents.

The aim of this work — complex immunological examination of the humans residing at the territory of Gorno-Altai high-mountainous natural focus, vaccinated with a live plague vaccine.

Sixty volunteers earlier non-vaccinated against plague and living in the focus took part in the experiment. Blood sampling was performed before vaccination/revaccination after 1, 3 and 6 months.

Immunological efficiency of vaccination was estimated by: lymphocyte subpopulational composition; index of CD4<sup>+</sup>/CD8<sup>+</sup> cells; IFN $\gamma$ , TNF $\alpha$ , IL-4 production; NST-test; specific antibody titer to *Yersinia pestis* F1; typing of HLA class II genes. Processing of statistical data was performed using parametrical and nonparametric criteria.

No pathological alterations in lymphocyte subpopulational composition were revealed. Decrease of T-lymphocyte was registered in 3 months after revaccination due to increase of CD3<sup>-</sup>CD19<sup>+</sup>-cells. Increase of T-helper percentage, raising immunoregulatory index value and the general tendency to functional activity increase of immunocompetent cells in the NST-test in 1 month after revaccination indicates the presence of adaptive cellular immunity.

Positive seroconversion in the overwhelming majority of the vaccinated humans taking part in the testing indicates the adequate immune reconstruction of the body and development of specific antibody in reply to plague vaccine introduction. Increase of TNF $\alpha$  and IFN $\gamma$  production, and also IFN $\gamma$ /IL-4 ratio after vaccination indicate the increase of Th1-cell activity and development of the cellular immune response in humans. At the same time, decrease of IFN $\gamma$ /IL-4 ratio and also TNF $\alpha$  associated with Th-1-cells occurs after revaccination indicating the shift to humoral immunity.

Commonly encountered HLA-DRB1 (\*03, \*04, \*07, \*08, \*11, \*13), HLA-DQB1 (\*02, \*03:01) and HLA-DQA1 (\*05:01) gene alleles are defined. Possible associations of these alleles with TNF $\alpha$  and IL-4 production level and also with relative T-helper content and CD3<sup>-</sup>CD16<sup>+</sup>-cells are revealed.

Comparative analysis permitted to detect a number of the major parameters indicating to activation of cellular and humoral immunity in humans vaccinated against plague. Further immunological monitoring of the vaccinal process is necessary.