4. ZOONOTIC AND PARASITIC INFECTIONS: CLINICAL, EPIDEMIOLOGICAL AND LABORATORY ASPECTS

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PCR ANALYSIS IN THE REAL TIME REGIMEN AS A LONG-TERM METHOD FOR LABORATORY DIAGNOSIS OF RICKETTSIOSIS

E.E. Alieva[1], E.I. Bondarenko[2], M.T. Gafarova[3], K.D. Malyi[3], E.A. Verbenets[4]

[1]N.I. Pirogov Saki Military Clinical Sanatorium, Saki, Republic of Crimea, Russia; [2]Vector-Best, Novosibirsk, Russia; [3]Medical Academy named after S.I. Georgievsky (Academic Unit) of V.I. Vernadsky Crimean Federal University, Simferopol, Republic of Crimea, Russia; [4]Sevastopol Centre for Disease Prevention and Control of AIDS, Sevastopol, Republic of Crimea, Russia

The actuality of studying the natural foci of rickettsiosis and the expediency of researches for the revealing of rickettsia DNA in ticks of the Crimea are caused by peculiarities of the region that are favorable for the circulation and preservation of pathogens in the nature.

The purpose of the study was to define the contamination of ticks by rickettsia and determine their species belonging.

Tasks: the organization of ticks collection and carrying out of their specific identification; carrying out of the laboratory researches of ticks — PCR — analysis in the real time regimen (PCR-RV).

Materials and methods — epidemiological and literary data on the study of rickettsiosis in the Crimea; parasitological methods (collection of ticks for the standard flag and dragging, manual collection from animals), specific identification of the ticks, laboratory methods (revealing of rickettsia DNA by PCR-RV using reagents set "RealBest DNA Rickettsia species" ("Vector-Best", Novosibirsk).

1342 specimens of ticks are collected from August to October 2016 and analyzed in total. Specific composition is presented by: *Haemaphysalis punctata* — 65.3%, *Rhipicephalus sanguineus* — 21.8%, *Hyalomma marginatum* — 9.5% and *Dermacentor marginatus* — 3.4%.

Using the PCR test "RealBest DNA Rickettsia species" in 470 from 1342 nucleic acid samples isolated from individual ticks suspensions, DNA marker of rickettsia revealed, a site of the citrate synthase gene *(gltA)*, was detected. 114 positive samples of rickettsia DNA were selected for additional amplicons production and sequencing of their sequences by 3–4 genes (*gltA, ompA, ompB* and *sca4*). The received results of the sequencing were compared with the nucleotide sequences of the rickettsia DNA presented in the GeneBank database. The species of rickettsia was established for 3 to 4 genes.

Analysis of nucleotide sequences indicated about circulation in four analyzed species of ticks collected in the Crimea, in six species of rickettsia, five from them are pathogenic for humans: *R. conori, R. massiliae, R. aeschlimannii, R. mongolotimonae, R. slovaca*.

The PCR test "RealBest DNA Rickettsia species" allows detecting in the extracted nucleic acid samples the DNA-marker of rickettsia circulating on the peninsula and can be considered a long-term method for laboratory diagnosis of rickettsiosis.

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LESIONS OF THE GASTROINTESTINAL TRACT IN SCHOOLCHILDREN INVASED BY LAMBLIA

R.S. Arakelyan[1], E.I. Okunskaya[2], N.A. Sergeeva[3], S.F. Karpenko[1], Kh.M. Galimzyanov[1], O.V. Konnova[1]

[1]Astrakhan State Medical University, Astrakhan, Russia; [2]Children's City Polyclinic No. 3, Astrakhan, Russia; [3]Children's City Polyclinic No. 5, Astrakhan, Russia

In recent years, more and more often among residents of the Russian Federation, especially among children, cases of parasitic infestations have been recorded, among which a special place is occupied by lambliasis, which often occurs under the mask of the lesion of gastrointestinal tract and is not always recognized in time.

The purpose of the study was to analyze lesions of the gastrointestinal tract in schoolchildren invaded by lamblia.

Under supervision there were 55 children of school age of whom 60% were children with gastrointestinal lesions. The diagnosis was confirmed by a coprological examination of feces for lamblia cysts.

According to the results of ultrasound investigation, all children showed lesions of the gastrointestinal tract, manifested in the form of reactive changes in the pancreas — 2.1%, reactive changes in the liver — 15.2%, signs of biliary dyskinesia — 18.2%, combined liver and pancreatic lesions — 15.2%, combined liver and pancreas damage, and signs of biliary dyskinesia — 18.2%, liver damage and signs of biliary dyskinesia — 12.1%, as well as pancreatic lesions and signs of dyskinesia of bile ducts — 9.1%. In most cases — 75.8% of children received the drug Makmiror at the rate of 15–30 mg per 1 kg of body weight for 7 days. Albendazole was received by 24.2% of children at 12 mg/kg body weight.

Lambliasis was registered most often in children of primary school age, which may indicate an incomplete knowledge of the rules of personal hygiene. The main causes of the disease were non-compliance with personal hygiene and contact with domestic animals, more often with cat. The main complaints of children were abdominal pain, nausea, decreased appetite, loosening of the stool and allergic reactions to the skin.

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CLINICAL-EPIDEMIOLOGICAL AND LABORATORY-INSTRUMENTAL ASPECTS OF NON-ERYTHEMATOUS FORM IN PATIENTS WITH TICK-BORNE BORRELIOSIS

T.M. Bergelson[1], A.A. Nafeev[1], A.V. Polushkin[1], A.A. Nafeeva[2]

[1]Ulyanovsk State University, Ulyanovsk, Russia; [2]I.M. Sechenov First Moscow State Medical University, Moscow, Moscow, Russia

Tick-borne borreliosis remains like the most common natural focal disease with a transmissive mechanism of the pathogen. Despite the introduction of advanced technologies of early diagnosis and modern methods of treatment doctors are faced with such a problem as medical examination and diagnosis of patients with tick-borne borreliosis.

The purpose of the work is to develop universal recommendations for clinicians for the management of patients with tick-borne borreliosis.

Tasks of the study was to compare the distinctive features of epidemiological anamnesis, clinical manifestations, indicators of laboratory and instrumental diagnostics and criteria of dispensary registration of patients with tick-borne borreliosis of erythema and non-erythema form.

We have analyzed about 34 patients from the maps of municipal institutions in Ulyanovsk. Forms of borreliosis were divided evenly into erythemic and non-erythemic forms in 17 patients (50%)

In the first 7 days 9 (26%) patients addressed, on 8–14 days — 5 (15%), on 15–30 days — 4 (12%) and 16 (47%) arrived at a later date. The complaint in 100% was the presence of itching and in 50% of erythema, which was accompanied by subjective sensations (burning sensation or compaction — 17 (50%), increase — 9 (26%) patients. In 3 (8%) patients complications with the defeat of the musculoskeletal system (rheumatoid arthritis) were revealed. Serological diagnosis (ELISA) was performed in 17 (50%) patients. Antibodies were found in 14 (41%), IgM levels ranged from 0.470 to 0.633. Terms of appearance were different (15–43 days). In 3 (9%) people the level was below normal. The remaining half of the patients were not examined for various reasons. Clinical and electrocardiographic manifestations of dysfunction of the circulatory system were noted in non-erythematous form (50%).

Serological diagnosis of tick-borne borreliosis by ELISA, due to the late appearance of antibodies in the early stages of little informative, which necessitates the introduction of modern rapid methods. Patients with non-erythematous form of tick-borne borreliosis require more attention and detailed laboratory and instrumental diagnosis, as there is a risk of complications from vital organs and systems.

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DETECTION OF GENETIC MARKERS OF TICK-BORNE RICKETTSIOSIS WITH THE PCR

E.I. Bondarenko[1], M.T. Gafarova[2], L.D. Shchuchinova[3], L.V. Shchuchinov[3], A.N. Shvalov[4], V.A. Lubova[5], G.N. Leonova[5]

[1]"Vector-Best", Novosibirsk, Russia; [2]Vernadsky Crimean Federal University, Simferopol, Republic of Crimea, Russia; [3]Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing in the Altai Republic, Gorno-Altaisk, Russia; [4]State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russia; [5]Somov Institute of Epidemiology and Microbiology, Vladivostok, Russia

There are seven species of pathogenic rickettsia belong to the spotted fever group were reported to be circulating in Russian: *R. conorii, R. sibirica, R. heilongjiangensis, R. slovaca, R. aeschlimannii, R. helvetica* and *R. raoultii*. But only first three of them were reported to cause the confirmed diseases in our country. The regions that are endemic for *R. sibirica* and *R. heilongjiangensis* are: coast of Primorsky Krai, the Amur River, coast of lake Baikal, the Reserve Krasnoyarsk Stolby, Altai, Khakassia. Crimean peninsula was reported to be endemic by *R. conorii*. All of these regions are the popular places for tourism. Real-time PCR test system "RealBest DNA Rickettsia species" (AO "Vector-Best", Novosibirsk) was developed to detect the DNA markers of the pathogenic rickettsia in clinical specimens. It also can be used for detection of rickettsia in ticks without defining the species. Using the developed test system, more than 7000 tick from 10 regions of Russia were tested. The percentage of rickettsia-infected ticks varied from 7 to 92% in dependence of region and tick's species. After the sequencing of DNA of positive samples in regions of genes *gltA, ompA, ompB* and *sca4* it was determined that there are 11 rickettsia species are circulating with 7 of them that are pathogenic: *R. sibirica, R. heilongjiangensis, R. conorii, R. slovaca, R. aeschlimannii, R. massilae* and *R. mongolotimonae*.

Also using the developed test system the DNA-markers of *R. sibirica* and *R. heilongjiangensis* were determined in the clinical samples (blood samples, urine, swabs of skin eschar, eschar biopsy) derived from patients that were hospitalized in the Far East, Western and Eastern Siberia with the diagnosis "tick-borne rickettsiosis". With the goal of the ability of determination of these two pathogens, the PCR test system "RealBest DNA Rickettsia sibirica/Rickettsia heilongjiangensis" was developed additionally. Using this test system it was found, that the frequency of presence of *R. heilongjiangensis* in tick varies from 0.7 for *I. persulcatus* to 29% for *H. oncinna*. The occurrence of the *R. sibirica* varies from 0.6 for *D. silvarum* up to 17% в *D. nuttalli*. It was proven that both of the developed PCR test systems can be successfully used for determination of circulating pathogenic rickettsia in natural foci, detection of their DNA markers in ticks for the diagnosis of the tick bitten people, as well as for analysis of clinical samples in the laboratory diagnostics tick-borne rickettsiosis.

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DATABASE OF LEPTOSPIRA PROTEIN SPECTRA FOR MASS-SPECTROMETRY IDENTIFICATION

N.V. Breneva[1], A.P. Samsonova[2], E.M. Petrov[2], Zh.A. Konovalova[1], A.S. Ostyak[1], S.V. Balakhonov[1]

[1]Irkutsk Antiplague Research Institute of Rospotrebnadzor, Irkutsk, Russia; [2]Gamaleya State Research Centre for Epidemiology and Microbiology, Moscow, Russia

Leptospirosis is found all over the world both in humans and in many species of agricultural, domestic and wild animals. The disease caused by individual serovars of the pathogen is characterized by a severe clinic and high mortality. *Leptospira* grow very slowly and only on special nutrient media. Together with the difficult pathogen isolation there is also the problem of its identification. According to the modern genosystematics several molecular biology methods were proposed to determine the *Leptospira* species. Mass-spectrometry direct profiling of proteins is easy to set up and widely used to diagnose most bacterial infections, while the available databases of *Leptospira* spectra are absent.

The aim of this study was the development of a protein spectra database for identification of the *Leptospira* species.

Our database contains information about 28 *Leptospira* reference strains of 28 serovars including eight most common species *L. interrogans*, *L. borgpetersenii, L. kirschneri, L. santarosai, L. noguchii, L. inadai, L. weilii, L. biflexa*, as well as the protein spectra of these strains in the format for the software "MALDI Biotyper 3.0". According to the serological classification the presented strains belong to 21 serogroups: *Icterohaemorrhagiae, Grippotyphosa, Canicola, Pomona, Tarassovi, Australis, Sejroe, Autumnalis, Bataviae, Ballum, Pyrogenes, Javanica, Hebdomadis, Louisiana, Panama, Lyme, Sarmin, Djasiman, Mini, Manhao, Sema-*