Wild type 1 poliovirus, the causative agent of poliomyelitis outbreak in Tajikistan where vaccine coverage dramatically decreased, was imported into Russia and was isolated from poliomyelitis cases and healthy migrants’ from Tajikistan. We isolated WPV1 from three children who arrived in Russia from Tajikistan. The percentage of migrants’ children who were seronegative to three types of poliovirus was 30 times higher than it was among resident Russian children. We isolated twice as many polioviruses from healthy migrants’ children as from patients with acute flaccid paralysis in Russia.

The transmission of pathogenic revertant type 2 poliovirus from the unvaccinated paralytic patient to four healthy contacts in a hospital illustrated the emergence of VDPV with increased transmissibility. The vaccine-derived poliovirus of type 3 which displayed 1.1% nucleotide substitutions in the genomic region VP1 was isolated from a patient with VAPP who received two doses of oral polio vaccine (OPV). Another VAPP patient excreted polioviruses of types 1 and 2 after vaccination with OPV. A month later he stopped to excrete poliovirus of type 2, but he continued to excrete poliovirus of type 1 for more than 4 months. We also revealed the excretion of vaccine poliovirus of type 2 from VAPP patient till 105th day after receiving four doses of oral polio vaccine. Vaccine poliovirus of type 3 was detected in the sample of unvaccinated 11-week-old patient with VAPP. We isolated the same poliovirus from the patient’s sister who received 3 doses of inactivated polio vaccine and had high antibodies titers to polioviruses. She was the only possible source of poliovirus for the VAPP patient.

These data illustrate how poliovirus can persist in the population and confirm the possibility of limited spread of VDPV among well-immunized population. Reemergence of poliomyelitis can compromise Polio Eradication Initiative. It is indispensable to continue accurate surveillance and maintain polio free status of Russia as well as of other polio free countries.

Role of different types of enteroviruses in etiology of infection on certain territories of Russia

N.I. Romanenkov, M.A. Bichurina, N.R. Rozaeva, O.I. Kanaeva
St. Petersburg Pasteur Institute, St. Petersburg, Russia

Epidemic peaks of enterovirus infection with the prevalence of different clinical forms of infection depend on different etiological factors. Outbreaks of hand, foot and mouth disease registered in the North-West of Russia in 2011–2012 were connected with Coxackievirus A16 not detected previously in the region. The identification of two genetic variants closely related to strains isolated in France in 2010 and in Japan in 2011 suggested that Coxackievirus A16 implicated in these outbreaks had been brought to the North-West of Russia by two importation events. Echovirus 30 lineage which largely circulated in Russia in 2013 and caused outbreaks of meningitis in the North-West of Russia belonged to genotype H new for the region. Viruses implicated in outbreaks were closely related to the strains of genotype H detected in China in 2010–2013. Since earlier we detected in the country only Echovirus 30 of genotype Ec2 it is likely that Echovirus 30 of genotype H was imported into Russia from South-East Asia.

In 2016 Echovirus 30 of different variants of genotype H was implicated in epidemic peaks of enterovirus meningitis in Saratov and Kostroma regions. But a year later in Saratov region another type of enterovirus provoked a peak of enterovirus meningitis. It was Echovirus 18 which differed from viruses of the same type occasionally circulating in the North-West of Russia. In Murmansk and Leningrad regions in 2016 Coxackieviruses A6 belonging to different genetic variants were the etiological factor of hand, foot and mouth disease. In Murmansk region and in the Komi Republic the cases of enterovirus infection with exanthema in 2017 were also connected mainly with Coxackievirus A6. The strains of Coxackievirus A6 identified in the North-West of Russia belonged to three sub-genotypes of pandemic genotype of Coxackievirus A6.

Thus we detected Echoviruses 30 and 18 on territories where enterovirus meningitis was the leading form. On territories where enterovirus exanthema dominated the etiological factor of infection was Coxackievirus A6. Our studies proved that surveillance of enterovirus infection aimed at acquiring new information about circulation of enteroviruses among population on different territories in different years is indispensable for prevention of propagation of enterovirus infection and for limitation of circulation of enteroviruses including the imported new serotypes/genotypes by means of using virological and molecular methods.

Molecular-genetic characteristics of the Coxackie A10 enterovirus that was circulating in the constituent entities of the Russian Far East

E.Yu. Sapega, L.V. Butakova, O.E. Trotsenko
Khabarovsk Research Institute of Epidemiology and Microbiology, Khabarovsk, Russia

The 2017 epidemic season of enterovirus infection (EVI) was conditioned by circulation of Coxackie A10 (CA10) in several constituent entities of the Far Eastern Federal District (FEFD) — the Khabarovsk, Primorsky Territories, Republic Sakha (Yakutia), Jewish Autonomous Region (JAR), Amursk and Magadan Territories. During the previous years the CA10 was identified in individual cases and only in 2016 it was the cause of outbreaks in the Amursk city (the Khabarovsk Territory).

A total number of 90 strains of CA10 were sequenced. A following phylogenetic analysis with the aid of BEAST program software and reference sequences obtained from the GenBank database was executed. A model of molecular clock was used to perform the evolutionary analysis.

Two genetic lines of CA10 (A and B) were circulating in the observed constituent entities of the FEFD. The line A included enteroviruses (EV) isolated in the Republic Sakha (Yakutia) and Khabarovsk Region during 2016 as well as those that circulated in different regions of Russia in 2009–2013 and Europe in 2003–2010. The presented genetic variant was the source of the outbreaks in the Amursk city (the Khabarovsk Territory) registered in 2016. Divergence of the characteristics between Far Eastern and other Russian EV strains most likely took place in 2011 (CI: 2009–2012). The genetic line B was presented by CA10 isolated in the FEFD in 2016–2017. The B-line strains isolated in the FEFD were divided into two clusters. First cluster was presented by the strains that circulated in the Khabarovsk, Primorsk, Magadan Territories and JAR in 2017 as well as those isolated in China in 2015. The most recent common ancestor (MRCA) for EV of the first cluster existed in 2013 (CI: 2012–2015). The second cluster included strains from the Republic Sakha (Yakutia) and Amur region isolated in 2016. However, this CA10 variant did not circulate in the constituent entities of the FEFD in 2017.
During the last two years of observation the molecular-genetic research allowed to reveal circulation of the two Far Eastern CA10 genetic lines of different origins and identify the time to their MRCA.

3.40

DISTRIBUTION OF ROTAVIRUS G-, P-, I-, AND E-GENOTYPES IN NIZHNY NOVGOROD, RUSSIA

T.A. Sashina1, O.V. Morozova1,2, N.V. Epifanova1, T.A. Migunova1, N.A. Polyakov2, N.A. Novikova1,2

1 FGUP SPbNIIVS FMBA Russia, St. Petersburg, Russia
2 Lobachovsky State University, Nizhny Novgorod, Russia

Rotavirus infection is an important health problem all over the world. In Russia, under the conditions of the beginning of vaccination against this infection, knowledge about its pathogen is limited by the characteristic with the binary classification (G[P]-genotypes), based on the properties of the VP4 and VP7 genes encoding the rotavirus outer capsid proteins. Information about the other gene segments genotypes, as well as unusual and reassortant strains is not sufficient. The aim of this study was to determine the I (VP6) and E (NSP4) genotypes of rotaviruses detected in Nizhny Novgorod using the multiplex PCR method.

We used 55 rotavirus-positive fecal samples from children hospitalized with acute intestinal infection from January to May 2018. RNA of rotaviruses was extracted using “RIBO-prep” reagent kit (AmpliSens, Russia). RT-PCR was carried out with reagents manufactured by “Sileks” (Germany). G- and P-genotypes of rotaviruses were determined using previously published primers. To identify I- and E genotypes in multiplex PCR, fragments of 195 bp (I1), 273 bp (I1), 358 bp (I2) and 233 bp (E3), 305 bp (E2), 443 bp (E1), respectively, were amplified and detected by agarose gel electrophoresis.

I- and E-genotypes were determined in 51 samples (92.8%). In one sample only E-genotype (1.8%) was revealed, and in three — only I-genotype (5.4%). Mostly, the genotypes were detected in combination I1-E1 (52.7%). The set of I2-E2 was found in 30.9% of cases. In addition, the genotype I1-E2 (5.6%) was identified in three samples, I2-E1 and I3-E3 (3.6% together) were shown to be sporadic. The following combinations of G-, P-, I-, and E-genotypes were determined: G1-P[8]-I1-E1 (9.1%), G4-P[8]-I1-E1 (7.3%), G9-P[8]-I1-E1 (32.7%), G4-P[8]-I1-E2 (5.5%), G3-P[x]-I2-E2 (1.8%), G2-P[4]-I2-E2 (29.1%), G2-P[4]-I2-E1 (1.8%), G2-P[4]-I2-Ex (3.6%), G9-P[8]-I1-Ex (1.8%), Gx-P[8]-I1-E1 (5.5%), and Gx-P[x]-I3-E3 (1.8%).

Thus, the new method to identify the I- and E-genotypes was tested and their distribution was determined. Various combinations of G-, P-, I-, and E-genotypes of rotaviruses have been shown. The genotype G9-P[8]-I1-E1 was predominant (32.7%). The G4-P[8]-I1-E2, G3-P[x]-I2-E2, G2-P[4]-I2-E1 strains had probably a reassortant origin.

The study was funded by RFBR (research project No. 18-34-00858).

3.41
doi: 10.15789/2220-7619-2018-4-3.41

DIAGNOSIS OF CYTOMEGALOVIRUS AND PARVOVIRUS B19 INFECTIONS IN SPECIAL GROUPS OF PATIENTS

V.M. Semenov, I.I. Dmitrachenko, V.U. Harbachou, A.V. Redenko

Vitebsk State Medical University, Vitebsk, Republic of Belarus

Some researchers described the reactivation of cytomegalovirus infection in immunocompetent patients with sepsis, burns, blood transfusions, massive surgical interventions, prolonged mechanical ventilation, use of steroids and vasopressors. In addition to herpesviruses, the reactivation of other latent viruses, in particular, parvovirus B19 (B19V), can also occur with developing immunodeficiency phenomena. With the existing concomitant pathology, these viruses significantly burdens the condition of patients.

For this reason the need for a qualitative and timely diagnosis of viral infections is increasing. PCR assay which capable of detecting even a few molecules of DNA is a progressive diagnostic method due to its high sensitivity. In this regard, quantitative detection of viral DNA can serve as a reliable criteria for significant activity of the pathogen, proving its etiological role in the development of a clinical syndromes.

The aim of the study was to create a test systems for quantitative DNA detection of CMV and B19V with hybridization-fluorescent detection of amplification products in the “real time” mode. It will help to establish the frequency of reactivation of latent viral DNA in critical condition and subsequently determine its effect on the course of the pathological process.

As a result of the studies for the first time in the Republic of Belarus a test systems for the qualitative and quantitative detection of CMV and B19V DNA by the real-time PCR method was created and registered by the Ministry of Health. The main characteristics of the developed test systems showed high values of analytical sensitivity (≥ 2 copies per run of 500 ME/ml), analytical and diagnostic specificity (100%), linear range (≥ 8 logarithms).

The created test systems, in addition to its use as a diagnostic tool, also can be used as a prognostic marker of infection, as a therapeutic marker for monitoring the success of antiviral therapy as well as for assessing the contagious nature of biological fluids. Thus, during the conducted studies using the test system, reactivation of CMV was detected in 28.6% (6 of 21) of patients in a critical condition with a viral load of 10 to 111 copies/ml. Also, a strong correlation between reactivation of CMV and established diagnosis of sepsis was found (r = 0.73). Reactivation of B19V was not detected in any of the 15 patients, which is inconsistent with the existing literature data and requires further researches.

3.42

IMPROVEMENT OF TECHNOLOGY OF PRODUCTION OF HERPETIC VACCINE, CULTURAL, INACTIVE

G.S. Shitikova, E.P. Turova

FGUP SPhNIIVS FMBA Russia, St. Petersburg, Russia

To improve methods of production and control of the vaccine, with the aim of developing a new innovative form of herpetic vaccine.

Vaccine strains of herpes simplex virus (HSV) type I (strain “US”) and type II (strain “VN”) are used as a seed material for preparing of herpetic vaccine. The monoculture cell culture (CC) of the primary fibroblasts of chick embryos (FECI) and the diploid cells of the human lung embryo (FLEC) were used for preparing of vaccine. Harvest virus of HSV-I and II types are collected in semi-finished products, which after freezing and thawing are inactivated with formalin. In a comparative plan, the semi-finished products accumulated on different cell substrates are monitored, in accordance with the production schedule and the current regulatory documents. Semi-finished products are controlled for infectious activity, safety, toxicity, and absence of extraneous contamination. Control of specific activity is carried out in experiments on white rats.