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MEASLES IN YEKATERINBURG: THE HISTORICAL PATH FROM THE PERIOD BEFORE VACCINATION TO THE STAGE OF ELIMINATION OF THE INFECTION

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Measles is still salient due to the reports of its outbreaks in many regions of the world, including in the Russian Federation.

The aim of the study is to characterize the manifestations of the epidemic process of measles in a large industrial center with different strategies of vaccination.

The research is based on statistical reports on the incidence of measles in Yekaterinburg from 1950 to 2016. The manifestations of the epidemic process were analyzed for 6 periods: the period before vaccination (1950–1961), the period of selective immunization (1962–1965), routine vaccination of children under the age of 8 years (1966–1972), prolongation of the age for measles vaccination to 14 years (1973–1986), the revaccination of children and adolescents (1987–2001) and the period of elimination of infection (2002–2016).

In the period before vaccination the mean annual incidence was 1381.7±162.9 ‰, the seasonal rise was in December-May, children prevailed in the structure of cases. The incidence was anti-persistent with the Hurst index (H) 0.472. In the period of selective vaccination, the incidence decreased to 1082.8±189.1 ‰.

During routine vaccination of children under the age of 8 years, the incidence reduced to 219.8±110.8 ‰, with the annual decline rate of –53.0%, the trend stability of the incidence is confirmed by the Hurst index (0.529). The incidence reduced in almost all age groups, seasonality was similar to the previous periods.

While the vaccinated population under the age of 14 years increased, the incidence decreased to 89.9± 39.1 ‰. However, 2 outbreaks (in 1979 and 1984) were reported in this period. The incidence of measles in this period was anti-persistent (H = 0.381).

The revaccination against measles led to a significant decrease in incidence to 5.7±1.6 ‰, offset of seasonal rises to February-May, while cases among adolescents and adults prevailed.

In the period of elimination of infection (2001–2015), the morbidity was sporadic (0.06±0.02 ‰), due to introduction of measles from other regions without spread. In 2016, there was an outbreak of infection in the city with 72 cases.

Thus, in the historical context the strategy of vaccination determined the situation with measles. However, at the stage of elimination of infection, the possibility of outbreaks of measles remains among unvaccinated children and adults, which requires rethinking of evaluation criteria of epidemiologic safety and their constant adjustment.

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GENERATION AND CHARACTERIZATION OF GENETIC REASSORTANTS BETWEEN POTENTIALLY PANDEMIC VIRUSES (A/H9N2 OR A/H5N8) AND THE A/HONG KONG/1/68/162/35 (H3N2) MASTER DONOR VIRUS

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In this work, reassortants based on potentially pandemic viruses (A/H9N2 or A/H5N8) and the A/Hong Kong/1/68/162/35 (H3N2) (A/HKca) master virus were generated and characterized. The A/HK/HK/6:2/2016 (H9N2) (RA-52) and A/UNL/HK/2:6/2017 (H5N8) (RA-54) strains were obtained by genetic reassortment of wild viruses (A/Hong Kong/1073/99 (H9N2) or A/Common tern/Uvs-Nuur Lake/26/2016 (H5N8)) and A/HKca attenuated high yield virus, which serves as a donor of internal protein gene segments [Tsybalova, 2012]. The RA-52 reassortant was obtained after 10 passages in 10–12 day-old embryonated chicken eggs (CE); the RA-54 was obtained after 7 passages. The reassortants inherited 2 surface proteins genes from wild viruses and 6 internal protein genes from the donor strain. Genetic compositions were confirmed using restriction fragment length polymorphism analysis. The antigenic identities of reassortants and wild-type strains was confirmed by haemagglutinin inhibition reaction. Full-genome sequencing showed one amino acid substitution in the PB2 protein sequence (M475I) in RA-54, in comparison with the donor strain. RA-52 had no amino acid substitutions. Both reassortants are high virus yield in CE. The RA-52 yield was 8.5 log EID_{50}/0.2 ml; the RA-54 yield was 7.75 log EID_{50}/0.2 ml. To confirm that both were cold-adapted and temperature sensitive, the reproductive capacities at different temperatures were measured at 26°C and 39°C. The RCT_{26} for RA-52 was 1.0 log EID_{50}; for RA-54, it was 2.25 EID_{50}. The RCT_{39} values were 7.0 log EID_{50}, and 7.5 log EID_{50}, respectively. These properties were retained after 5 passages (CE), which indicates their stability.

The results show that the reassortants are antigenically identical to wild-type strains and that they inherited high yields, cold adaptation, and temp. sensitivity from the master strain. They can be used for development of both inactivated and live influenza pandemic vaccines. Both reassortants were deposited in the State Collection of Viruses under the numbers 2884 (RA-52) and 2885 (RA-54). Nucleotide sequences have been deposited in GISAID under numbers EPI_ISL_321099 (RA-52) and EPI_ISL_321098 (RA-54).

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FURTHER DEVELOPMENT OF "IgY-TECHNOLOGY": AN ELISA SYSTEM BASED ON SPECIFIC ANTIBODIES FROM EGG YOLKS AS A SURROGATE VARIANT OF THE NEUTRALIZATION TEST

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Neutralization test (NT) remains the "gold standard" in seroepidemiological and diagnostic studies of infections associated with poliovirus (PV). This is due to functional nature of the NT, which reflects the real immune status of the test serum. However, the NT uses cell cultures and live viruses, which requires compliance with relevant biosafety requirements. In accordance with the plans of the WHO Polio Eradication Initiative, work with PV (wild or Sabin strains) in the near future will be sharply limited (and is already limited for PV Sabin type 2) by the requirements of the containment and will be possible only in a small number of specially accredited institutions.

In this report, we present the results of the development of blocking ELISA as a surrogate variant of NT for the detection of antibodies to PV based on the use of specific IgY antibodies isolated from egg yolks of chickens immunized with PV and inactivated standard poliovirus antigen.

The results of comparison of two tests (NT and blocking ELISA) are presented when titrating 90 blood serums of children who received 2 doses of IPV following 3 doses

of OPV. according to National vaccination schedule. Mean values (arithmetic mean) of NT titers/blocking ELISA were for PV type 1 — 128/60; type 2 — 131/20 and type 3 — 54/25. In this case, the sensitivity of the ELISA relative to the NT for PV type 1 was 98%, type 2 — 100%, type 3 — 98%. Correlation coefficient *r* for PV type 1 — 0.67, type 2 — 0.61, type 3 — 0.76, which corresponds to the definitions "mean correlation" (for types 1 and 2) and "high correlation" (for type 3). Thus, the presented results demonstrate the further development of "IgY-technology" in combination with certain serological technique — surrogate version of NT (blocking ELISA) for use in large-scale seroepidemiological studies of poliomyelitis. Blocking ELISA does not require the use of live PV, which allows it to be used in laboratories of different levels in PV containment conditions. The duration of the test (24 hours) is its additional advantage compared to the NT (5–7 days), which opens the prospect of its use for rapid diagnosis of poliovirus infection.

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REALIZATION OF POLIO ERADICATION PROGRAM IN THE RUSSIAN FEDERATION: CURRENT STATUS AND CHALLENGES OF THE PERIOD AFTER CERTIFICATION OF THE EUROPEAN REGION, 2003–2017

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In 2002 Russia, as part of WHO European Region, was certified as "polio-free country" and has successfully maintained this status for more than 15 years. This is guaranteed by a high (97–99%) polio vaccine coverage and effective epidemiological surveillance of acute flaccid paralysis (AFP). Nevertheless, Russia faced a number of challenges, expected at the final stage of polio eradication: importation of wild poliovirus (WPV) from endemic regions, vaccine-derived PV (VDPV), poliomyelitis cases associated with using of oral poliovirus vaccine (OPV).

In 2010, Russia was involved in a large-scale outbreak of poliomyelitis in Tajikistan caused by WPV1. Newly arrived migrants from countries bordering Tajikistan as well as unvaccinated citizens of several Russian regions were affected. The outbreak was interrupted by vigorous vaccine interventions.

After certification, VDPV strains were not found through routine AFP surveillance and special studies of persons with immunodeficiency undertaken in Russia. At the same time, VDPVs2 were detected during supplementary surveillance for PV. In 2015, highly divergent (17.6% nucleotide substitutions in VP1 region) VDPV2 was isolated from the wastewater. The divergence rate of the virus is most likely indicative of its long-term (> 15 years) excretion by the immunodeficient person. In 2016, after "switch" from tOPV to bOPV, two VDPV2 were isolated from healthy unvaccinated children of different regions of Russia. The genetic association of the isolates and their origin from the PV2 Sabin was confirmed by the presence of 10 and 13 nucleotide substitutions compared to vaccine PV2, 10 of which were common. Epidemiological investigation revealed family ties and contact children in one household. This "event" in the period after the cessation of use of PV2 in oral polio vaccine required an assessment of risk of spread, large-scale organizational and vaccination activities.

The occurrence of polio cases associated with vaccine (VAPP) is particularly unacceptable at the final stage of eradication. During 2003–2017, there were 76 cases of VAPP in Russia. The introduction of IPV in the National Immunization Schedule in 2008 did not lead to a complete elimination of VAPP, among 26 cases registered in 2008–2017, VAPP in unvaccinated children prevailed.

This experience allows conclude the necessity to continue both AFP and supplementary poliovirus surveillance following the global certification of poliomyelitis eradication.

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ENVIRONMENTAL AND HUMAN SURVEILLANCE OF POLIOVIRUSES AND OTHER ENTEROVIRUSES IN MADAGASCAR. IMPACT OF THE TRIVALENT TO BIVALENT ORAL POLIO VACCINE SWITCH

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Poliomyelitis has been a major public health concern and currently, efforts are being made towards eradicating poliovirus type 2 (PV2). A global switch from trivalent oral poliovirus vaccine (tOPV) to bivalent oral poliovirus vaccine (bOPV without PV2) has been organized by the World Health Organization (WHO) to prevent epidemics of recombinant type 2 pathogenic circulating vaccine-derived polioviruses (cVDPVs). In an attempt to monitor the decline of OPV2 following the switch and the possible effect on other enteroviruses in the human population, environmental and human surveillance was conducted before, during, and after the switch in three regions of Madagascar. The developed WHO "gold standards" for detecting PV consists of isolation on cell lines and characterization by rRT-PCR assays. Other enterovirus isolates can be identified using sequencing. These methods are poorly conducive to large environmental studies, investigating multiple enteroviruses in one sample. Therefore, we developed an RT-PCR assay where we designed degenerate primers for conserved regions of the genome capable of sequencing the whole genome for all enteroviruses (A, B, C, and D). For this study, stool samples from healthy children (> 200) and sewage samples (> 400) were collected, concentrated, and inoculated on RD and L20B cells.

To date, the results from sewage and stool samples collected indicate that prior to the switch from tOPV to bOPV in April 2016, all Sabin strains were detected until July 2016. After which, Sabin 2 was no longer isolated in either sample sets analyzed. For stool samples, the majority of the enteroviruses detected were EV-B (81%) followed by EV-C (12%) and EV-A (7%). For sewage samples, EV-B (98.6%) was detected in the majority followed by EV-C (1.2%), and EV-A (0.2%). EV-D was not detected in any samples collected in this study. We were successful in detecting and characterizing, in a single sequencing run, all enteroviruses present in mixtures containing up to five different serotypes. We were able to confirm the results concerning PV obtained with the classical WHO method thus, validating the presence/absence of polioviruses and other enteroviruses using a metagenomics methodology. Additionally, this study allowed us to gain a deeper understanding of the enterovirus ecosystem and diversity and opened the way to study the genetic interactions among viruses that favor the emergence of pathogenic recombinant cVDPVs in Madagascar.