Saint Petersburg Pasteur Institute

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Currently, it is one of the largest institutes of epidemiology and microbiology in Russia, especially in the northwestern region of Russia.
The Institute has a modern material base and a quality scientific staff that are united in five departments:

- Department of microbiology;
- Department of virology;
- Department of epidemiology;
- Department of immunology;
- Department of new technologies.

At the Institute work:

- Scientific and Methodological Center for the Surveillance of Pathogens of Infectious and Parasitic Diseases of II-IV Pathogenicity Groups in the North-West Federal District of Russia;
- North-West District Center for AIDS Prevention and Control;
- Regional Center for Epidemiological Surveillance of Poliomyelitis;
- Regional Center for Epidemiological Surveillance of Measles and Rubella.

The Institute has two reference centers of the Russian Federation:

- Federal Center for Surveillance of Typhoid Fever;
- Federal Center for Surveillance of Yersinioses.

Collaboration with WHO:

- WHO Global Polio Laboratory Network (WHO Polio Laboratory);
- WHO Subnational Measles and Rubella Laboratory (European Measles Laboratory Network).

Contents

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Genetic characteristics of *Escherichia coli* strains, O26, O55 and O111 serological groups

Acute enteric infections with signs of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) that are caused by shiga toxin-producing *Escherichia coli* (STEC) are common throughout the world and are registered as sporadic cases or outbreaks. Of *E. coli* strains, pathogenic strains synthesizing verotoxins (shiga-like toxins) are of particular importance. *E. coli* O157:H7 is the serotype that is most often associated with disease outbreaks and severe clinical outcomes. As of today, it is believed to be the most common STEC serotype in the world. At the same time, serotypes other than O157, namely O26, O55, O103, O111, and O145, are also known for their pathogenic potential and together they make the so called five serogroups of non-O157 STEC pathogenic for humans.

Materials and methods. 76 strains of *E. coli* isolated from children were examined including O26 (53 strains), O111 (17 strains) and O55 (7 strains). Serotype characteristics (O- and H-antigens) and virulence factor genes, i.e. of STX production *(stx1/stx1a, stx1c, stx1d; stx2/stx2a, stx2b, stx2c, stx2d, stx2e, stx2f, stx2g)*, α-hemolysin *(hlyA)*, enterohemolysin *(ehxA)*, the adhesion factor intimin *(eae)* and pilus conjugation formation factor *(bfp)* as well as transcriptional regulator of enteroaggregative *E. coli* (EAEC) *aggR* were examined using PCR.

Results. The findings of strain examination are summarized in the Tabl. 1. According to serotyping, the examined strains belong to six serovars: O26:H11 (53 strains), O55:H6 (5 strains), O55:H7 (1 strain), O55:H21 (1 strain), O111:H2 (12 strains), and O111:H8 (5 strains).

According to our data, the STEC group included 19 strains of *E. coli* O26:H11, five strains of *E. coli* O111:H8 and one strain of *E. coli* O55:H7. They had *stx1/stx1a, eae*, and *ehxA* genes. 34 strains of *E. coli* O26:H11, five strains of *E. coli* O55:H6 and 12 strains of *E. coli* O111:H2 *had eae, bfp,* and *hlyA* virulence genes and according to this factor they were classified as enteropathogenic *E. coli* (EPEC). One strain of *E. coli* O55:H21 had neither STEC nor EPEC virulence genes but it had EAEC-typical genes.

Conclusions. The population of *E. coli* strains, serological groups O26, O111 and O55, isolated from children is inhomogeneous in terms of serotypes and the presence of genes coding EPEC, STEC and EAEC pathogenicity factors. The obtained data show we need further research into the intraspecific biodiversity of *E. coli*, identification of pathogenicity factors or their genetic determinants along with serotyping for the final identification of *E. coli* as causative agents of acute enteric infections.

Phylogenetic structure of *S.* Typhi population isolated in Russia in 2005–2018

The global *S.* Typhi population is rather young, highly clonal and originated from a common ancestor existed so recently that multiple mutations have not yet accumulated. To evaluate the phylogenetic relatedness of the high clonal pathogen population, the genotyping method, based on the detection in the compared genomes of the whole range of core single-nucleotide variations (SNV) located both in coding and non-coding genome regions, is commonly used. The principle of SNV-typing of *S.* Typhi isolates is currently widely used to evaluate the pathogen population structure and to determine the relation between single/group of isolates.

Whole genome sequencing of *S.* Typhi was performed on MiSeq (Illumina, USA) with MiSeq Reagent Kit v3 600 cycles. Genomic DNA was isolated by the DNeasy Blood & Tissue Kit (Qiagen). Genome libraries were prepared using MiSeq Nextera XT (Illumina, USA). Genome assembly and analysis was performed using CLC Genomics Workbench 8.0 (QIAGEN, Germany).

To reconstruct the global phylogenetic tree, we analyzed a set of 1683 *S.* Typhi isolates, which included both Russian isolates and isolates sequenced in previous studies. Thus, the set of strains under our investigation was characterized by the wide isolation time period (from 1905 to 2013) and broad geographical origin (63 countries, 6 continents: Asia, Africa, North and South America, Europe, Australia and Oceania).

The detection of orthological SNV was performed using the previously developed algorithm of data analysis

Table 1. Molecular-genetic characteristics of *E. coli* O26, O55, and O111 isolated from children with the diarrheal syndrome in St. Petersburg

E. coli serotype	Number of strains	Virulence genes						
		stx1	*stx2*	*eae*	*bfp*	*aggR*	*hlyA*	*ehxA*
O26:H11	19	*stx1a*	–	+	–	–	–	+
O26:H11	34	–	–	+	+	–	+	–
O55:H6	5	–	–	+	+	–	+	–
O55:H7	1	*stx1a*	–	+	–	–	–	–
O55:H21	1	–	–	–	–	+	–	–
O111:H2	12	–	–	+	+	–	+	–
O111:H8	5	*stx1a*	–	+	–	–	–	+

(Kuleshov K.V. et al., Infect. Genet. Evol., 2016). The nucleotide sequence of *S.* Typhi CT18 strain (NCBI acc. AL513382) was used as a reference genome for reads mapping. The resulting matrix of orthologous SNV was used for phylogenetic reconstruction in RAxML software, the model GTR+I was used as a model of nucleotide substitutions. Bootstrap analysis was carried out with the number of repetitions 1000. The phylogenetic tree visualization was carried out in the program Figtree v1.3.1. Additionally, Russian *S.* Typhi isolates were analyzed by Genotyphi program (https://github.com/katholt/genotyphi) according to the author's instructions.

In the global phylogenetic tree Russian *S.* Typhi isolates were clustered into several phylogenetic groups. The most of them (82.6%) belonged to haplotype H58 (Fig. 1).

The phylogenetic line of H58 was heterogeneous: *S.* Typhi isolates were clustered into three phylogenetic groups (designated as G1, G2 and G3), and five isolates had individual genotypes (designated as S1-S5). 60.0% isolates of H58 belonged to the group G3 and had identical resistance phenotype (fluoroquinolone low-level resistance) and resistance mechanism (single nucleotide substitution in *gyrA* Asp87Asn).

The phylogenetic group G1 (11.2%) included *S.* Typhi with fluoroquinolone high-level resistance mediated by three single nucleotide substitutions: *gyrA* (Ser83Phe+Asp87Asn) + *parC* Ser80Ile. The *S.* Typhi isolates of the phylogenetic group G2 (7.8%) had the identical resistance mechanism (fluoroquinolone low-level resistance due to *gyrA* Asp87Asn), but two isolates also had additional multidrug resistance associated with the plasmid IncHI1B(R27). Five *S.* Typhi H58 isolates with individual genotypes (S1-S5) were susceptible to antibiotics or had fluoroquinolone low-level resistance due to *gyrA* Ser83Tyr — the single nucleotide substitution, which was not found in other phylogenetic groups.

The described phylogenetic groups of H58 haplotype included *S.* Typhi strains isolated in different regions of Russian during all years under study. Epidemiological data was agreed with phylogenetic analysis as well as with antimicrobial resistance phenotypes. The strains isolated in the same outbreak were clustered together in one phylogenetic group. The isolates from the same outbreak or small group cases (Saint Petersburg, 2006; Kaliningrad, 2012; Moscow, 2013; Irkutsk 2016) had identical antimicrobial resistance phenotypes due to identical single nucleotide substitutions.

In *S.* Typhi of non-H58 haplotype, almost all isolates, were susceptible to antibiotics except one isolate with fluoroquinolone low-level resistance due to the single nucleotide substitution in *gyrA* Ser83Phe, detected in other phylogenetic groups only in combinations with other single nucleotide substitution. Some strains were clustered into phylogenetic groups (designated as G4-G6), others had individual genotypes (S6 and S7).

Additional analysis of sequenced *S.* Typhi genomes by Genotyphi showed that Russian *S.* Typhi population was represented by the isolates of all four primary clusters, but mainly — by the cluster 4 (83.7%) (Tabl. 2). Within cluster 4, the majority of strains (82.6%) belonged to subclade 4.3.1. According to Wong et al. (2016) progentor *S.* Typhi isolates of this subclade are originated from the countries in Southeast and South Asia. It should be noted that all isolates of the subclade 4.3.1. belonged to the phylogenetic lineage of H58 haplotype defined by the global phylogeny. At the same time, Russian isolates of the subclade 4.3.1. were further clustered in two genetic clusters. The cluster 4.3.1.1 (68.5%) mainly included the isolates with fluoroquinolone low-level resistance due to *gyrA* Asp87Asn, and the group 4.3.1.2 (14.1%) — the isolates with fluoroquinolone low-level resistance due to non-common nucleotide substitution in *gyrA* Ser83Tyr, and the isolates with high-level resistance

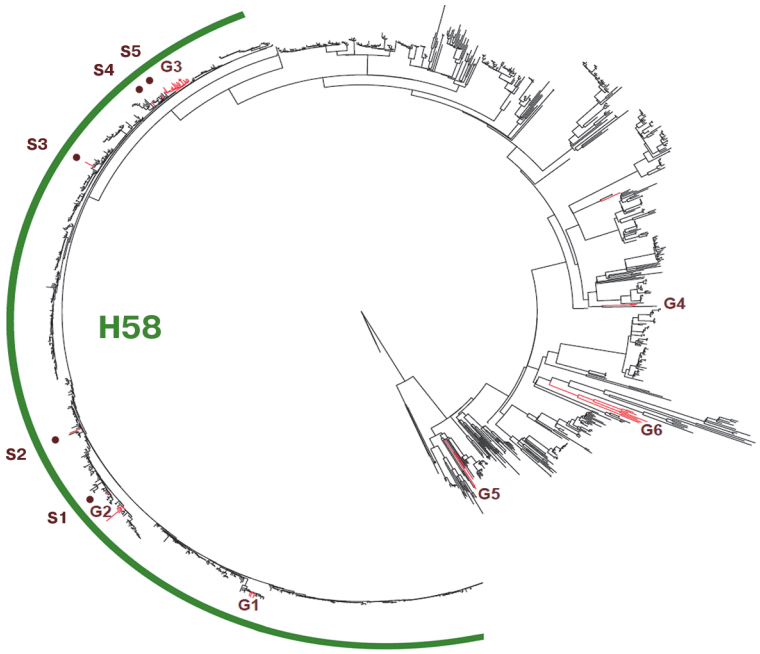

Figure 1. The global phylogenetic tree constructed on the basis of the identified ortological SNV in 1683 *S.* Typhi genomes. The tree was reconstructed by the maximum likelihood method implemented in the RAxML. The phylogenetic lineage related to haplotype H58 is highlighted in green. The tree branches with Russian *S.* Typhi isolates are marked in red. If several Russian *S.* Typhi isolates were clustered together, they were designated as a phylogroup "G", if individually — as "S"

due to three single nucleotide substitutions: *gyrA* (Ser-83Phe+Asp87Asn) and *parC* (Ser80Ile). Furthermore, within cluster 4, one antimicrobial susceptible *S.* Typhi strain, isolated in the Voronezh in 2015, belonged to subclade 4.1.1 and was probably of African origin.

Primary cluster 1, subclade 1.2.1 included eight antimicrobial susceptible *S.* Typhi isolates, also belonged to the same phylogenetic group (G6). It is interesting to note that some susceptible *S.* Typhi from our collection, isolated in Kyrgyzstan and Kazakhstan in 2010 and 2012, also belonged to this subclade. According to Wong et al. (2016) the *S.* Typhi isolates of subclade 1.2.1 originate from countries in Southeast Asia.

The primary clusters 2 and 3 and their subclades in our study were presented by single *S.* Typhi isolates full susceptible to antibiotics or with fluoroquinolone low-level resistance due to *gyrA* Ser83Phe (not detected as single substitution in other clusters).

Conclusions. More than 80.0% of *S.* Typhi isolates imported to the Russian Federation in 2005–2018 belonged

Table 2. Characteristics of *S.* Typhi strains isolated in Russia in 2005–2018 by the resistance mechanisms and phylogenetic analysis (n = 92)

Phylogroups		Resistance genotypes	Number of strains	Place and year of isolation	Geographic origin of isolates in reference set (microreact.org/project/styphi)
Wong et al., 2016	Global phylogeny				
1.2.1	nonH58_G6	WT	8	St. Petersburg 2009 and 2011; Leningrad region 2009; Moscow 2011; Tomsk 2015; Kyrgyzstan 2010; Kazakhstan 2012	Southeast Asia (100%) — Vietnam
2.0.2	nonH58	*gyrA* (Ser83Phe)	1	St. Petersburg 2017	North America (50%) — Mexico North Africa (50%) — Algeria, Tunisia
2.3.2	nonH58_G5	WT	2	Kemerovo 2012	West Africa (33%) — Nigeria, Mali South America (27%) — Argentina Southeast Asia (20%) — Vietnam, Thailand North America (13%) — Mexico West Asia (7%) — Turkey
	nonH58_S7	WT	1	Ulyanovsk 2010	
3.0.1	nonH58_G4	WT	2	St. Petersburg 2010 and 2011	North Africa (50%) — Morocco South Asia (50%) — Pakistan
3.0.2	nonH58_S6	*gyrA* (Ser83Phe)	1	St. Petersburg 2012	South Asia (100%) — India
4.1.1	nonH58	WT	1	Voronezh 2015	Southern Africa (78%) — Malawi South Africa (11%) West Africa (6%) — Mauritania Central Africa (6%) — Cameroon
4.3.1.1	H58_G3	*gyrA* (Asp87Asn)	54	St. Petersburg 2006 (outbreak), 2007, 2010–2012, 2014 and 2017; Moscow 2011 and 2013 (outbreak); Kaliningrad 2011 and 2012; Khabarovsk 2012; Voronezh 2014; Irkutsk 2015	Southeast Asia (50%) — Vietnam, Laos, Cambodia South Asia (26%) — India, Bangladesh, Pakistan, Nepal, Sri Lanka, Afghanistan East Africa (10%) — Tanzania, Kenia Southern Africa (9%) — Malawi
	H58_G2	*gyrA* (Asp87Asn)	5	St. Petersburg 2008; Khanty-Mansiysk 2009; Jewish Autonomous region 2011; Irkutsk 2017	
		gyrA (Asp87Asn) + p ncHI1B(R27)	2	St. Petersburg 2013 and 2015	
	H58_S2	WT	1	Irkutsk 2012	
	H58_S4	WT	1	St. Petersburg 2011	
4.3.1.2	H58_G1	*gyrA* (Ser83Phe+Asp87Asn) + *parC* (Ser80Ile)	9	Kaliningrad 2011 and 2012; Smolensk 2011; Kirov 2015; Khanty-Mansiysk 2016; Voronezh 2017; Krasnoyarsk 2017; St. Petersburg 2018	
		gyrA (Ser83Phe+Asp87Asn) + *parC* (Ser80Ile) + p IncI	1	Arkhangelsk 2015	
	H58_S1	*gyrA* (Ser83Tyr) + p IncHI1B(R27)	1	St. Petersburg 2006	
	H58_S3	*gyrA* (Ser83Tyr)	1	Arkhangelsk 2011	
	H58_S5	*gyrA* (Ser83Tyr)	1	St. Petersburg 2011	

WT — wild type, susceptible to fluoroquinolones and other antibiotics.

to successful international Asian clone — "subclade 4.3.1" or haplotype H58 and with high probability originated from the countries of Southeast and South Asia. In Russia, this dominant phylogenetic group mainly included the isolates with the same resistance phenotype and mechanisms: about 60.0% had fluoroquinolone low-level resistance due to the single nucleotide substitution in *gyrA* Asp87Asn. All *S.* Typhi isolates with fluoroquinolone high-level resistance (due tree single nucleotide substitutions in *gyrA* and *parC*) and multidrug resistant *S.* Typhi isolates also belonged to subclade 4.3.1. The isolates of this subclade caused the typhoid fever cases in different years in all regions of the Russian Federation. According the epidemiological data in many cases the patients were infected travelling to India (the tourists and Indian students of Russian universities). Only single *S.* Typhi isolates belonged to subclades other then 4.3.1 and differentiated by full antimicrobial susceptibility or the mutations non common for Russian *S.* Typhi population.

Based on the results of this study, the Russian *S.* Typhi Reference Center Database was created.

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LABORATORY OF MEDICAL BACTERIOLOGY

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Prevalence of infection with diphtheria-causing agents in migrant workers from Central Asia and residents of St. Petersburg and infection susceptibility

Relevance of the study. Large-scale migration that is typical of metropolitan cities including St. Petersburg calls for comprehensive research into the infection prevalence among migrant workers (MWs) who enter the country with their working visa. MWs arriving in St. Petersburg mostly come from Uzbekistan and Tajikistan. According to official data, there are no known cases of diphtheria in these countries. WHO studies have shown, however, that more than half of Tajikistan population is not protected against diphtheria. Speaking about Uzbekistan, there are no data at all. Therefore, the infection may be carried from the republics of Central Asia, especially those bordering to diphtheria-endemic countries. This is why we need data on the prevalence of infection with the diphtheria-causing agent and protection of MWs and residents of St. Petersburg against the infection.

Materials and methods. The sample material was collected from 370 migrants. The control group consisted of 320 adult residents of St. Petersburg. Throat and nasopharyngeal swabs were examined for the presence of *Corynebacterium diphtheriae*, and blood tests for the presence of antitoxic anti-diphtheria antibodies were made. *C. diphtheriae* strains were identified using biochemical tests and mass spectrometry. Diphtheria toxin was detected using genetic and phenotyping methods. Protection against diphtheria was examined by identifying the level of blood serum antitoxic antibodies through enzyme immunoassay. All the data obtained were processed using appropriate methods of mathematical statistics.

Results. In MWs *C. diphtheriae* strains were isolated in 1.6% of cases, whereas in the control group of the residents of St. Petersburg no strains were isolated. According to the epidemiology department, during the same period *C. diphtheriae* strains from the local residents of St. Petersburg were isolated in 0.02% of cases, i.e. 80 times less often. 83% of *C. diphtheriae* strains were obtained from Uzbekistan MWs, and 17% — from MWs coming from Tajikistan. Among the residents of St. Petersburg, the biological variant gravis is found in 25% of cases, whereas in migrants the percentage is 83% of cases ($p < 0.001$). The majority of those migrants come from the Samarkand Region. In the phenotyping Elek's test no isolated strains had toxigenic properties. In the polymerase chain reaction 17% of strains were positive (having the silent toxigenic gene).

The results of the study of the specific antitoxic immunity to diphtheria showed that 95% of permanent residents of St. Petersburg were protected from diphtheria, whereas among the MWs the figure was only 66% ($p < 0.001$). The average level of total antitoxic antibodies in MWs is 0.56 IU/ml, and in permanent residents of St. Petersburg it is 0.82 IU/ml. Mean avidity index of antitoxic anti-diphtheria antibodies in MWs is 38%, and in permanent residents of St. Petersburg — 56% ($p < 0.01$), whereas the protective avidity index is 30%. For MWs from both countries it is true that one half of the persons (50%) have the protective level of antibodies, and the other half (50%) only has minimal (7%) or moderate level of protection (43%) against diphtheria. No age-related differences were found in the antibody levels.

Conclusion. Such factors as high circulation rate of *C. diphtheriae* strains among MWs, the spread of gravis biochemical variant, the presence of strains with a silent gene, insufficient MW protection against diphtheria, overcrowded conditions at the places of temporary residence, social and economic difficulties in the country of temporary residence, inadequate availability and affordability of medical services are evidence of infavourable diphtheria situation among MWs and require certain measures to prevent epidemiological instability in St. Petersburg.

Serum monitoring of the pertussis infection

1. Both in Russia and in a number of other countries, the advent of vaccination of infants against pertussis in the 1950s resulted in a drop in the disease incidence. The up-to-date vaccination schedule includes primary vaccination of children in their first year of life consisting of three doses and subsequent revaccination one year after the completion of the primary series. Predominantly whole cell vaccines are used for vaccination and revaccination; cell-free vaccines are less common. Incidence rate in large cities remains high; the disease is mostly registered in school-aged children. Study objective was to assess the duration of protection against pertussis in children who received the complete vaccination course by looking into serological signs of a recent pertussis infection.

Materials and methods. Blood serum samples of 395 children from St. Petersburg aged from 3 through 13 years who completed the entire vaccination course (4 doses) were examined for pertussis toxin antibodies (IgG, IgA). IgG titre of 40 IU/ml and higher was considered a sign that the person had pertussis infection in the last 12 months; IgG titre of 100 IU/ml and higher together with any IgA value or IgG level 40–100 IU/ml with IgA level of 12 IU/ml and higher were considered a sign that the person had pertussis infection in the last 6 months.

Results. Serological signs of past infection of the last 12 months were found in 10.6% of children, the proportion of seropositive children starting to increase from the age of 7 ($r^2 = 0.812$). In this group, serological signs of pertussis infection of the last 6 months were found in 4.3% of children, the proportion of seropositive children starting to increase from the age of 10 ($r^2 = 0.786$).

Conclusions. The obtained results show that postvaccinal immunity to pertussis becomes weaker by school age and children become susceptible to the infection. This proves that revaccination is necessary at the age of 6–7, before starting the school.

2. Traditionally, pertussis is considered to be a childhood infection. For example, in 2006–2015, annually 15 to 33 cases of pertussis were recorded in St. Petersburg among adults (which corresponds to 0.34–0.84 per 100,000) and

349 to 1394 cases in children up to 14 years (64.96–241.9 per 100,000). However, studies from a number of European countries as well as Japan and the USA showed that the real incidence in adults is dozens or even hundreds of times more than stated in the official statistics. Adults (parents, other relatives, medical personnel) as well as older siblings can become a source of pertussis infection for infants. The objective of this study is to understand the real incidence of pertussis among the adult population of St. Petersburg using as a marker of disease/latent immunization the increased level of antibodies to pertussis toxin.

Materials and methods. We examined 538 adults who came to the medical centre to have laboratory tests done due to chronic non-pulmonary diseases. The age ranged from 18 to 82 (mean age was 41.2 years); there were 333 females and 205 males. Test method: EIA to detect antibodies to pertussis toxin (IgG, IgA).

Results. IgG antibodies to pertussis toxin were found in 87 persons (16.2% of all examined), in 27 of those (5.1% of all examined) serological markers of recent pertussis infection were found. The proportion of seropositive persons was maximum in the groups of 18–29 and 30–39-year-olds (21.4 and 19.9%, respectively), with the subsequent decrease to 5.7% in the group aged 50–59; among the examined 60+ persons the proportion of seropositive probands was, again, higher and amounted to 13.9%. The highest percentage of examined persons with markers of recent infection was also found in the group of 18–29-year-olds (6.4%).

Conclusion. The obtained data demonstrate active involvement of adults in the pertussis epidemic process in St. Petersburg. The risk group includes adults aged up to 40. It is disturbing that the proportion of seropositive probands aged 60+ in on the increase, since this age group faces an increased risk of complicated and more severe disease course.

Microbiological monitoring of pseudotuberculosis using genotyping methods

CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins) has evolved as a specific adaptive system to defend prokaryotes against foreign genetic material. Unique spacer consequences of CRISPR loci contain DNA fragments derived mostly from previous exposure to bacteriophages and plasmids, which reflects evolution of a bacterial cell. Therefore, spacers of CRISPR loci are a meaningful molecular marker that can be used in phylogenetic studies and intraspecies typing for the purposes of microbiological monitoring of circulating strains. High resolution of CRISPR-typing due to marked intraspecies polymorphism of spacer consequences was proven during the studies conducted on *Y. pestis*.

The objective of this study was to develop a bioinformatic algorithm of identification and analysis of *Y. pseudotuberculosis* CRISPR/Cas systems using Genbank databases.

Materials and methods. To identify CRISPR/Cas systems, whole genome sequences of *Y. pseudotuberculosis* strains that can be found in the NCBI database were used. BioEdit editor, CRISPROne and CRISPRDetect online apps were employed. We used the CRISPR Target online app and BLASTn search algorithm for screening of phages and plasmids according to spacer sites. Primers for the detection of CRISPR loci were chosen using the Primer-BLAST online app.

Results. *Y. pseudotuberculosis* CRISPR/Cas system includes one to three loci YP1, YP2 and YP3 and one set of *cas* genes (class 1 CRISPR/Cas system, type IF) containing the *cas1* gene, which is universal for all system types, and type-specific *cas3f, cas8f, cas5f, cas7f*, and *cas6f* genes. The leader sequence where the CRISPR locus transcription initiates can be situated before or after the locus. CRISPR loci are separated with a certain interval. The unique sequence of repetitions consists of 28 nucleotides. The number of repetitions and spacers in different loci differs. The YP1 locus is rather conservative. The most variable is the YP3 CRISPR locus. It is assumed that the CRISPR/Cas system of *Y. pseudotuberculosis* consisting of two or three loci at a certain interval, works mostly in relation to this locus adding new spacer sequences after interaction with foreign mobile genetic elements.

Information obtained through bioinformatic software-based method made it possible to develop a set of primers for PCR detection of *Y. pseudotuberculosis*-specific CRISPR loci. Currently, primers to YP1 and YP3 loci are used to study 34 strains of *Y. pseudotuberculosis*, O:1 and O:3 serotypes, isolated in several regions of Russia from various sources. It has been shown that 30 strains have two CRISPR loci, YP1 and YP3, 3 strains have the single YP1 locus, and 1 strain has the YP3 locus. Sequencing of YP1 and YP3 CRISPR loci (up to 1000 nucleotide sequences in size) was carried out on 5 strains. Variability of locus lengths and spacer sequences was found. YP3 loci of three strains have identical spacer sequences. This can be explained by the fact that the strains were isolated in the same geographical region. Two other strains have individual YP1 and YP3 loci. Locus sequences were deposited in the GenBank database under the numbers KX592595, KX592596, KX592597, KX592598, and KX592599. Bioinformatic search for phages and plasmids using spacer sequences gave credible results for *Y. similis* str. 228 and *Y. pseudotuberculosis* str. ATCC6904; bacteriophages of *Salmonella* and *Shigella*; plasmids of *E. coli* O157:H7 (pSS17, pEC4115), *Salmonella enterica* (pCFSAN), and *Proteus vulgaris* (Rts1). Further search and analysis of *Y. pseudotuberculosis* CRISPR/Cas systems will be carried out.

Conclusions. Bioinformatic searching algorithm for CRISPR/Cas systems in *Y. pseudotuberculosis* strains that are kept in the GenBank database was developed. It was shown that CRISPR-typing can be used for the monitoring of circulating strains.

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LABORATORY OF ZOOANTHROPONOSES

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The main line of laboratory activity in 2017–2018 was the improvement of epidemiological surveillance methods and control of relevant infections based on molecular-genetic and immunological approach. The following problems were to be addressed within this line of activity.

Plasmid analysis optimization for quick typing of *Coxiella burnetii* strains and isolates

Coxiella burnetii strains and isolates may carry one of the four plasmids with different genetic composition. Some authors believe that the plasmid type can be associated with the level of virulence of an isolate due to production of specific gene products that take part in the interaction with the host cell. Several models of plasmid typing for *C. burnetii* have been proposed previously. The methods, however, need some optimization to enable the work with nonculturable isolates with modest accumulation of the pathogen's bacterial mass.

We analyzed the nucleotide sequence of QpH1, QpRS, and QpDV plasmids, and selected oligonucleotide primers involved in the amplification of type-specific products. Specificity was checked using the strains with the plasmid type identified previously using the whole genome analysis.

Nine archival strains and three nonculturable isolates of *C. burnetii*, which showed no pathogen accumulation and were isolated in the European part of Russia, were examined. The study showed that the majority of *C. burnetii* archival strains isolated within Russia had the QpH1 plasmid type (Tabl. 3). The QpRS type was found in strains isolated in Central Asian countries and in Russian strains allegedly imported from this region with animal-derived raw materials (wool).

It was found that they all had the QpH1 plasmid type, so we tend to believe that this type is predominant in natural and anthropourgic foci of the European part of Russia.

Comparative analysis of whole genome sequences of *Coxiella burnetii* strains circulating in Russia

Coxiella burnetii is an extremely dangerous pathogen. Therefore, there is an urgent need to search for and study differential diagnostically significant genetic markers of phenotypic properties that are important in terms of epidemiology and are associated, among others, with factors of host specificity and geographical factors. Thus far, there has been no information on the whole genome structure of the *C. burnetii* strains circulating in Russia. Therefore, the analysis of genetic features of these strains will help to fill in the gaps in those highly meaningful characteristics of *C. burnetii* population.

To analyze the whole genomes, next generation sequencing (NGS) of the four strains of *C. burnetii* isolated in Russia from two types of hosts (human and arthropods) was carried out. Sequencing was carried out using the Illumina platform (USA), MiSeq technology, with the strategy of paired-end reads and barcode analysis.

Whole genome analysis of the Russian strains of *C. burnetii* that was conducted within this group and by comparing it with the whole genome sequences of *C. burnetii* strains available in the NCBI database confirmed the concept of a closed *C. burnetii* species pan-genome characterized by low intraspecific genetic diversity, including the population circulating in Russia. Nevertheless, the analysis of the variable part of the genome and the composition of unique genes revealed the deletion of some genes in the group of Russian strains, which was not found in other strains of the world collection, which suggests they could have a unique genomotype.

Analysis of possible mechanisms of patho-adaptation revealed pronounced clustering in the group of Russian strains by host type, whereas within the clusters genome differences were minor. When comparing the number

Table 3. Plasmid type of *C. burnetii* strains and isolates

Name	Year of isolation	Place of isolation	Source	Plasmid type
Ixodes — 2 — Luga	1962	Luga district of Leningrad Region	*Ixodes persulcatus*	QpH1
Ixodes — 6 — Luga	1962	Luga district of Leningrad Region	*Ixodes persulcatus*	QpH1
Cimex — 1 — Luga	1959	Luga district of Leningrad Region	*Cimex lecturalius*	QpH1
Yellow-necked mouse — Luga	1959	Luga district of Leningrad Region	*Apodemus flavicolis*	QpH1
Vologda — 2	1987	Vologda	Blood of a patient with Q fever	QpH1
Cheredov	1955	Kazakhstan	Blood of a patient with Q fever	QpRS
Leningrad — 4	1957	Leningrad	Blood of a patient with Q fever	QpRS
Ufa — 1	1960	Ufa	Blood of a patient with Q fever	QpH1
Irkutsk — Dermacentor	1969	Irkutsk Region	*Dermacentor nuttalli*	QpH1
Sorex — 2008	2008	St. Petersburg	*Sorex araneus*	QpH1
Apodemus — 2008	2008	St. Petersburg	*Apodemus agrarius*	QpH1
AM — 2010	2010	St. Petersburg	Blood of a patient with Q fever	QpH1

of deleted open reading frames for genomes of strains isolated from arthropods and from humans, it was found that the genome of strains from arthropods was significantly more reduced, despite the assertion made by a number of authors that the genome reduction of strains from arthropods is insignificant.

Thus, the obtained results enable us to describe unique genome features of the strains isolated in Russia and add new information to our understanding of *Coxiella* host specificity, encouraging further research into its mechanisms.

Infection of ticks and migratory birds with *Coxiella burnetii*

Working under the research grant of the Paris Pasteur Institute ACIP A-08-2010 included the study of infection of birds with *C. burnetii* in order to assess the risk of *C. burnetii* spread in different regions of Europe by migratory birds and Ixodidae feeding on them.

Our work involved examination of biological material (blood, blood serum and feces) from 1970 migratory birds belonging to 53 species and collected within the territory of Russia (the Baltic sea region), and Bulgaria (the regions of the Atanasovsko Lake and the city of Sofia) as well as 888 ticks removed from birds and vegetation at their stopover sites. *C. burnetii* were detected using polymerase chain reaction (PCR). Positive results were confirmed by Sanger sequencing of the 16srRNA gene target fragments. Antibodies to *C. burnetii* in the blood serum of birds were detected by the complement-fixation test.

The findings showed that 19% of migratory birds caught in the Baltic region carried *Ixodes ricinus* (L) ticks. The nymphs of these ticks removed from Erithacus rubecula as well as ticks of different species collected from plants at stopover sites of migratory birds contained *C. burnetii*. *C. burnetii* was also detected in the blood and feces of some of the birds captured at different sites, and the blood serum contained antibodies to this pathogen. The results of bird blood PCR showed 1.2% infection rate with *C. burnetii*.

The results allow for the conclusion that the sites of migratory birds' stopover in the Baltic region (Russia) and in the area of Atanasovsko Lake and Sofia (Bulgaria) are natural foci of *C. burnetii* infection. It is confirmed that migratory birds are a natural reservoir of this pathogen and can play an important role as hosts transporting ixodid ticks infected with *C. burnetii*. It can be assumed that in places of mass stopovers during long-distance migration migratory birds can be exposed to or exchange the pathogen. Moreover, these processes can take place both during migrations from Central and Western Europe (the vast number of migratory birds, 43.4%), and during migration from India (6%) and Africa (39%) with stops in Bulgaria and later in northwest Russia. However, to confirm such assumptions further molecular genetic studies of the pathogen from these regions is needed.

Molecular diagnostics of Q fever in Russia

Antibodies are formed in the system rather late (10 days after the infection), therefore molecular diagnosis of Q fever in the blood of febrile patients is a relevant diagnostic method. The amplification of a gene fragment encoding superoxide dismutase was used to study 86 whole blood samples obtained from patients with suspected Q fever. The main infection manifestations were: influenza-like illness, community-acquired pneumonia or prolonged low-grade fever (over 1 month). A standard PCR was performed with subsequent result verification by Sanger sequencing. At the same time, an enzyme immunoassay was performed to detect antibodies to *Coxiella burnetii* in blood serum obtained from the same patients.

Antibodies to the pathogen were not detected in the studied samples. Using standard PCR, DNA of the pathogen was detected in three samples (3.49%). The analysis of amplified DNA fragments showed 100% homology of the nucleotide sequence with strains of *C. burnetii*, which confirms the specificity of the analysis. Positive PCR results were observed in two cases of influenza-like illness and in one case of chronic low-grade fever. Detection of *C. burnetii* by molecular genetic methods allows the diagnosis of Q fever in the early stages of the disease even if there is no immunologic response, which is an important aspect of using the method in clinical laboratory diagnosis.

Prevalence of tick-borne infections in the Northwestern region of Russia

We continued our work in the international joint project with the Norwegian Institute of Public Health Tick-borne diseases in the Barents Sea region and at the western coast of Norway aimed at studying the spread of tick-borne pathogens for the purposes of surveillance of tick-borne diseases and for defining the boundaries of occurrence of pathogens causing tick-borne infections.

Infection of ticks with agents causing tick-borne encephalitis, ixodid tick-borne borreliosis, Q fever, and human granulocytic anaplasmosis was studied. Ticks were collected from the vegetation in St. Petersburg city, Leningrad, Pskov, Arkhangelsk Regions and the Republic of Karelia in spring-summer 2015–2016 by flag dragging.

In endemic areas of 5 regions of Russia 1499 ticks were collected and analyzed by RT-PCR. It was found that in the Northwestern region the average infection of ticks with tick-borne encephalitis virus, *Borrelia burgdorferi* s.l. and *C. burnetii* was 2.6%, 15.4% and 9.2% respectively. Genotyping of *B. burgdorferi* s.l. revealed an equal ratio of genome types *B. afzelii* (50%) and *B. garinii* (50%) in the studied samples. The data obtained indicate that there are natural foci of tick-borne diseases in the studied areas.

During the period from 1996 to 2016, together with the Federal state state-financed health care institution Federal Centre for Hygiene and Epidemiology of Rospotrebnadzor and the Department of Parasitology of the Centre for Hygiene and Epidemiology in St. Petersburg, an analysis of the ecological and epidemiological situation of tick-borne encephalitis in St. Petersburg, the northernmost metropolis of Russia with persistent foci of tick-borne encephalitis, was carried out.

- It was found that carriers of the tick-borne encephalitis virus in St. Petersburg were Ixodes persulcatus (85%) and Ixodes ricinus (15%) ticks.
- The number of patients seeking medical advice after tick bites in St. Petersburg is growing; the incidence tends to decrease, but at a slower rate than in Russia as a whole.
- The main risk group for tick-borne encephalitis includes children aged 3–14 years, in which regard St. Petersburg is starkly different from other regions of Russia. Therefore, it is recommended that preventive measures be focused on this age group.

- The level of immunization of the population of St. Petersburg against tick-borne encephalitis is gradually growing, but still remains low. However, the lack of patients with tick-borne encephalitis in professionally threatened groups of the population is evidence of the efficacy of prevention carried out in these groups.

Human impact such as cutting down and burning of primary forests in the peripheral parts of St. Petersburg and in Leningrad Region resulted in the increasing number of ticks and growing spread of viral tick-borne encephalitis and ixodid tick-borne borreliosis among the population of these regions. We have used molecular genetic methods to detect the tick-borne encephalitis virus, *B. burgdorferi* sensu lato and *Rickettsia* sp. in ticks removed with people.

Infection rate with the tick-borne encephalitis virus was 1%. Borrelia DNA was detected in 9.8% of samples; sequencing of the ospC gene showed that all samples contained *Borrelia afzelii* DNA. No rickettsiae were detected in congested ticks. The prevalence of pathogens was compared to the data of domestic authors and authors from western countries. The data obtained can serve as a basis for improving the scheme of anti-epidemic measures in the surveyed regions. The results of the work show that further monitoring of tick infections in the north of European Russia is required in order to ensure the protection of public health from these infections which are moving farther to the north.

Study of infection of migrant workers in St. Petersburg with agents causing zoogenous infection

In order to determine the rate of their infection with agents causing zoogenous infection (brucellosis, Q fever, leptospirosis), blood serum samples from 142 migrant workers (MWs) from Uzbekistan and Tajikistan were studied, 85% males and 15% females. The age structure of migrants from Uzbekistan and Tajikistan was almost the same: 97% of the examined migrants were under 50 years of age. The comparison group consisted of 320 apparently healthy residents of St. Petersburg. The gender and age composition of this group was close to the study group of migrants.

Testing MW serum samples for zoogenous infections revealed antibodies to all three pathogens (Tabl. 4).

Significant differences revealed in *Brucella* and *Coxiella* infection rate of MWs compared to permanent residents (PRs) of St. Petersburg allow us to assume with high probability that the infection occurred outside the metropolis. This assumption is indirectly confirmed by the high prevalence of brucellosis and *Coxiella burnetii* infection in Uzbekistan and Tajikistan due to their social and economic structure and the transfer of livestock from collective to individual farms. This results in decreased efficacy of preventive anti-epizootic and anti-epidemic measures, and these infections become epidemic. In contrast to brucellosis and *Coxiella burnetii* infection, infection of MWs with *Leptospira* probably occurred in St. Petersburg. This is confirmed by virtually identical Leptospira infection rates in MWs and PRs of St. Petersburg. In addition, in MW samples antibodies to Leptospira serogroups Icterogemorrhagiae and Canicola were found that are typical of the leptospirosis etiology in St. Petersburg.

The possibility of MWs infection with *Leptospira* in St. Petersburg is largely due to the nature of their work. Since they usually provide low-skilled labour at construction sites or markets (e.g., as loaders) or in communal services (e.g., as street cleaners or garbage collectors), and the living conditions may be poor, it is possible that they contact with synanthropic rodents, which, as is known, are the source of infection for more than 30% of patients in a city.

Human, as a rule, is a biological (ecological) deadlock for agents causing zooanthroponoses, therefore, unlike anthroponoses; migrants with the history of infection with agent causing leptospirosis, Q fever or brucellosis do not pose a real danger to PRs of St. Petersburg. However, these pathogens in some cases persist in the human body for a long time. Chronic course typical of brucellosis and *Coxiella burnetii* infection and pathological changes persistent for a long time after the (in some cases undiagnosed) disease significantly reduce the quality of life of MWs, worsen their performance and increase the load on health facilities of St. Petersburg.

Study of biological diversity of *Leptospira* spp. pathogens

Working under the research grant of the Paris Pasteur Institute PTR No. 30-17 Global diversity, genomic epidemiology and pathogen evolution of *Leptospira* spp. (GlobE Evolution), we prepared 50 original strains of *Leptospira* spp. from the collection of the Laboratory of Zooanthroponotic Infections of the St. Petersburg Pasteur Institute. The strains represent 5 serological groups (Icterohaemorrhagiae, Canicola, Grippotyphosa, Pomona, and Tarassovi). Typing of strains was conducted using the MAT and standard agglutinating sera. High-quality genomic DNA was isolated from 30 strains.

As part of this project, the identification of the *Leptospira* spp. isolate was carried out at the serovar level by MALDI-TOF mass spectrometry.

Leptospira spp. strain was isolated from the lungs of the person who died of leptospirosis. Using the microscopic agglutination test (MAT), we were able to classify the isolate as serogroup Icterohaemorrhagiae. Mass spectra were obtained from samples of ethanol-formic acid extracts of the studied strain cells and 10 reference strains of *Leptospira* spp. used in MAT on the Microflex mass-spectrometric analyzer (Bruker Daltonik). Isolate identification was carried

Table 4. Results of examination of migrants working in St. Petersburg and permanent residents of St. Petersburg for the presence of antibodies to zoogenous infections

Country of the principle place of residence	Antibodies found to the following pathogens			
	Total investigated	*Coxiella burnetii*	*Brucella*	*Leptospira*
Uzbekistan	113	11 (9.7%)	2 (1.8%)	3 (2.7%)
Tajikistan	29	3 (10.3%)	4 (13.8%)	1 (3.4%)
Russia — St. Petersburg	320	2 (0.6%)	1 (0.3%)	10 (3.1%)

out by comparing the mass spectrum of the sample with the previously obtained data of the main spectra profiles (MSPs) of reference strains. The created MSP project database was added to the main taxonomic library of reference spectra of the Biotyper 3.1 software (Bruker Daltonik) we use. The result of the matching process was evaluated by the correlation coefficient of spectral peaks of the isolate and the reference spectra. We proposed to consider coefficient values > 2.1 as possible identification up to serovar, coefficient values < 2.1 — as no proper serovar identification.

The highest correlation coefficient of studied sample spectrum (2.152) was recorded with the spectrum of the reference *L. interrogans* strain, serovar copenhageni. There was also a slightly lower estimated correlation of the isolate with the *L. interrogans* strain, serovar icterohaemorrhagiae (2.123) and non-pathogenic *L. biflexa* strain, serovar patoc (2.149). The values of coefficients with other reference spectra of serovars were lower than 2.1. Analysis of the spectra of the isolate and serovars copenhageni, icterohaemorrhagiae, and patoc showed that they have a large number of identical peaks, including possibly ribosomal proteins, which is evidence of their close evolutionary relationship. The difference in spectra was observed in the range of molecular masses m/z = 3000–3500 Da, where all strains had clusters of peaks typical of linear polysaccharides. Molecular masses of the highest points of such clusters were: for the patoc serovar m/z = 3301 Da, and for the isolate, serovars copenhageni and icterohaemorrhagiae m/z = 3209 Da. The spectrum of the serovar icterohaemorrhagiae had a peak at m/z = 2958 Da which was absent in the isolate and serovar copenhageni.

Thus, the obtained results indicate that the isolate belongs to the serogroup Icterohaemorrhagiae, serovar copenhageni, and the proposed method can be used to determine the serovariant of isolated strains of *Leptospira*.

Assessment of Q fever prevalence in the Republic of Guinea for subsequent development of effective prevention measures

This work was carried out within the framework of international cooperation with the Republic of Guinea and included a serological examination of blood serum samples of relatively healthy residents of the Republic of Guinea (596 samples) for the presence of IgG antibodies to the causative agent of Q fever. It was found that the proportion of Q fever-seropositive persons among the relatively healthy residents of the Republic of Guinea was 5.4%. Biological material (250 pools of ticks collected from cattle) was examined for the presence of DNA of *Coxiella burnetii*, the causative agent of Q fever, using a species-specific test system developed at the Federal state-financed scientific institution St. Petersburg Research Pasteur Institute. Genetic markers of *Coxiella burnetii* infection were found in 3% of the examined material. The obtained data indicate that there are foci of Q fever in Kindia Prefecture and blood-sucking arthropods are involved in the circulation of the pathogen as vectors.

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Department of Virology

LABORATORY OF ETIOLOGY AND VIRAL INFECTIONS CONTROL

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Monitoring polio and enteroviral infections in Russia

The Subnational Poliomyelitis Laboratory of the Pasteur Institute of St. Petersburg, part of the WHO Polio Laboratory Network, is responsible for the virological surveillance of acute flaccid paralysis (AFP) and paralytic poliomyelitis associated with vaccination (PPAV) in children under 15 years of age in 14 administrative territories of Russia. Administrative territories send stool samples of children with AFP or PPAV and their contacts (children living in the immediate vicinity), as well as stool samples from children of migrant families, patients with enteroviral infections, and samples of sewage to examine. The diagnostic procedure is carried out in accordance with the WHO protocol.

Virological surveillance of cases of acute flaccid paralysis and paralytic poliomyelitis associated with vaccination remains the "gold standard" of the global polio eradication program. At the same time, the role of complementary surveillance (environmental monitoring and surveillance of at-risk population groups) is becoming increasingly important during the post-certification period of global polio eradication.

During the surveillance of poliomyelitis and acute flaccid paralysis in recent years (2016–2018), 343 stool samples were examined, 3 strains of poliovirus type 1 and 4 strains of poliovirus type 3. All strains of poliovirus were vaccinal according to the results of typical intra differentiation. In addition 7 strains of nonpolio enteroviruses (Echovirus 6, Enterovirus 71, Coxsackievirus A4, Coxsackievirus B1–6) were isolated.

Among 167 cases of acute flaccid paralysis two cases were classified as paralytic poliomyelitis associated with vaccination. From 1998 to 2017, 132 cases of acute flaccid paralysis were registered in Russia, 109 cases from 1998 to 2008 and 23 cases from 2009 to 2017. These data can be seen on Fig. 2. Until 2008 most cases were recorded in children who had received poliomyelitis vaccine, then cases of paralytic poliomyelitis associated with vaccination were reported in unvaccinated children living near newly vaccinated children.

The following is a description of two cases of paralytic poliomyelitis associated with vaccination that occurred in the North-West region of Russia in 2016 and 2017.

A case of VAPP (vaccine-associated paralytic polio) in the child T.: Acute flaccid paralysis was recorded in a 1 year 9 months old child after the first dose of poliomyelitis vaccine. Immunization was carried out not with inactivated (as per National vaccination schedule) but with oral poliomyelitis vaccine. When choosing the vaccine health professionals looked at the child's age, not at real immunization status. Acute flaccid paralysis developed 12 days after the OPV vaccination. Type 1 and 3 polioviruses were isolated from stool samples. Blood serum samples taken late after the beginning of paralysis were found to contain poliovirus antibodies in the following titres: PV1 — 1:256, PV3 — 1:32. The examination at day 60 of the disease revealed residual paralysis. Final diagnosis was VAPP in a recipient of the oral poliomyelitis vaccine.

A case of VAPP in the child K.: Acute flaccid paralysis was recorded in a 10-week-old child not vaccinated against poliomyelitis. The infant spent more than a month in the intensive care unit on assisted ventilation. Type 3 poliovirus was isolated from stool samples taken on days 3 and 5 of the disease. The blood serum sample taken on day 2 of the disease was found to contain antibodies to type 1, 2, and 3 polioviruses in the titre 1:32 (probably maternal), the serum sample taken on day 20 of the disease showed the 4-fold increase of antibodies only to type 3 poliovirus (1:128). 7 exposed persons were examined. Type 3 poliovirus was also isolated from the 19-month-old sister of the patient, who received three doses of inactivated polio vaccine (before 10 months of age). The girl was found to have antibodies to type 3 poliovirus in the titre > 1:512, titres of antibodies to type 1 and 2 polioviruses were 1:32. It seems that the sister was the source of poliovirus for the patient K., the source of poliovirus for her could not be identified. The examination of patient K. at day 60 revealed residual paralysis. Final diagnosis was paralytic polio associated with the vaccine in an exposed person.

Since the risk of importing wild polioviruses into polio-free countries exists up to the global polio eradica-

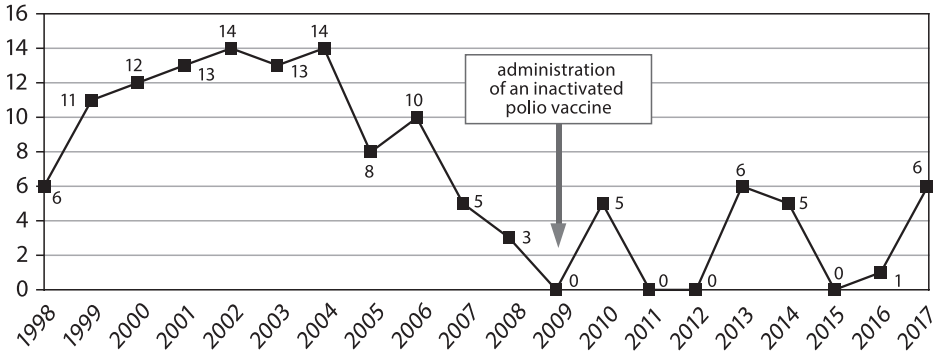

Figure 2. Cases of paralytic poliomyelitis associated with vaccination in Russia in 1998–2017

tion, the Poliomyelitis Laboratory of the Pasteur Institute of St. Petersburg has carried out enhanced virological surveillance of at-risk population, including children of migrant families from Central Asian countries, Ukraine and some other countries newly arrived in St. Petersburg.

During the surveillance of children from migrant families stool samples from 375 children were examined. In unvaccinated children 2 strains of type 1 and type 3 poliovirus were isolated. According to the results of typical intra-differentiation, the poliovirus strains were vaccinal. Nonpolio enteroviruses were isolated from 37 children examined (9.9%). These are Echovirus 6, 11 and 30, Coxsackievirus A, Coxsackievirus B1–6 and other nonpolio enteroviruses.

In two years (2017–2018) 9360 samples taken from patients with enterovirus infection were studied through virological method in different areas.Vaccinal polioviruses have been isolated in a limited number of cases in children vaccinated against poliomyelitis. Nonpolio enteroviruses (953) were isolated in 10.2% of the samples, mostly Coxsackieviruses A of various serotypes (46%), Coxsackievirus B1–6 (15%), Echovirus 6 (10%), Echovirus 30 (10.4%), Enterovirus 71 (4.8%) and Echovirus (5% in 2017). Echoviruses 7, 9, 11, 13, 25 were also isolated, as well as a small percentage of other Echovirus cases.

A molecular study was carried out and the nucleotide sequences of nonpolio enterovirus isolated in the different population groups (patients with AFP and enterovirus infections, children of migrant families) were identified. Echovirus 6, Echoviruses 9, 11, 13, Echovirus 18, Echovirus 30, Coxsackievirus B2, B3, B4, B5, Coxsackievirus A2, A3, A4, A6, A9, A10, A16 and A24 were identified as well as enteroviruses 75 and 99.

In 2017, the increase in the morbidity index of enteroviral infections was reported in some regions of Russia. In Saratov 65% of cases were represented by viral meningitis caused by Echovirus 18. The etiological factor has been established by virological and molecular methods. The share of enterovirus ECHO 18 accounted for 42% of all enteroviruses isolated from patients. In the Murmansk region and in the Republic of Komi, exanthematous diseases that accounted for 95% and 60% of all cases of enterovirus infection were mostly caused by Coxsackievirus A6.

The molecular study showed that strains of Echovirus 18 isolated in Russia in recent years belonged to three groups. The strains that caused meningitis in Saratov belonged to group 3. These strains were formed separately from other Echovirus 18 strains circulating in the North-Western region of Russia. Fig. 3 represents the phylogenic tree of genomic region VP1 of Echoviruses 18.

Most of the Coxsackievirus A6 strains identified in our regions belonged to the two subgenotypes 6 and 8 of the Coxsackievirus A6 pandemic genotype that predominate in the structure of Coxsackievirus A6 in the North-Western region of Russia and across the Russian Federation. Fig. 4 represents the phylogenic tree of genomic region VP1 of Coxsackievirus A6.

The period of summer and autumn 2018 showed no increased incidence of enteroviral infection (EVI) in the administrative territories supervised by St. Petersburg resource centre as compared to the previous year. In five territorial units out of 14 diseases with the clinical signs of enteroviral meningitis were predominant. Their share in the total number of EVI was 88.9% in the Republic of Karelia, 65.4% in Arkhangelsk Region, 64.7% in Saratov Region, 48.5% in St. Petersburg, and 47.8% in Novgorod Region. In the other territories the disease primarily had clinical symptoms of a viral exanthema of the oral cavity and limbs or herpetic angina. These forms of EVI were common in the Komi Republic (61.3%), Murmansk Region (61.2%), Leningrad Region (49.4%), Kostroma Region (42.2%) and Vologda Region (35%).

In some territorial units, group diseases were registered in children. For example, in September 2018, three children aged 3 to 6 years fell ill and were admitted to hospital with the diagnosis of aseptic meningitis in one of the children institutions of St. Petersburg. Clinical material from two patients and ten exposed persons was sent to a laboratory in our ins-

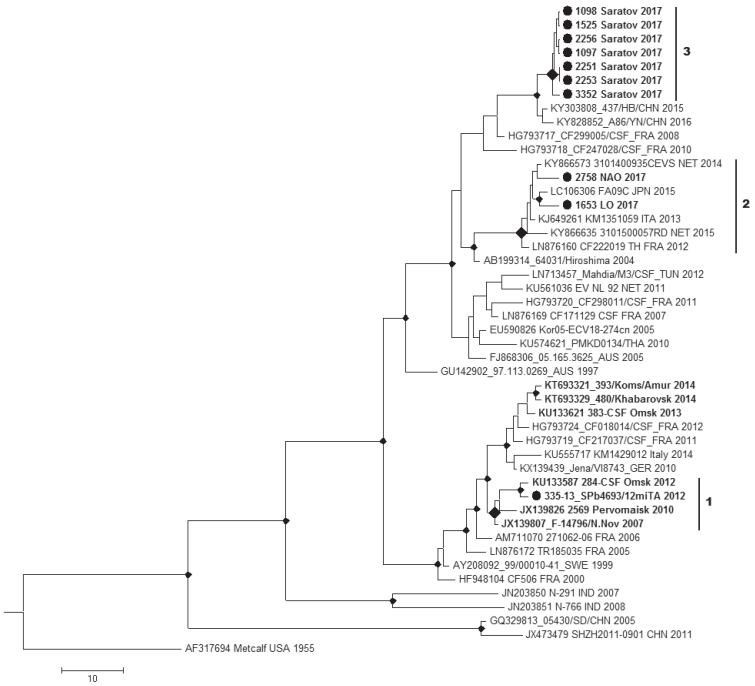

Figure 3. Phylogenetic tree of genomic region VP1 of Echovirus 18

titution for virological examination. Non-polio enteroviruses were isolated from the samples of two patients and seven exposed children. Virus identification revealed the causative agent of the disease, i.e. Coxsackie B5 enterovirus. In two child care centres of Novgorod Region group cases of EVI with the clinical picture of enteroviral exanthema of the oral cavity and limbs were recorded (three children in each child care facility). The Laboratory of Virology of the Pasteur Institute (St. Petersburg) isolated enteroviruses from the samples of six children and identified them. The causative agent of EVI turned out to be Coxsackie A6 enteroviruses.

In conducting environmental monitoring in 2017–2018 we studied 3690 samples of sewage waters. Among the strains isolated, 115 (31.0%) were identified as poliovirus, 256 (69.0%) were identified as non-polio enteroviruses. All strains isolated from the poliovirus proved to be vaccinal. The isolation frequency of non-polio enteroviruses was 6.9%. Most nonpolio strains of enterovirus belonged to Coxsackievirus B1–6 (38.0%), Coxsackievirus A (12.5%), Echovirus 30 (12.5%) and Echovirus 6 (9.0%). Others have been identified as Echoviruses serotypes 7, 9, 11, 13, 25 and other serotypes.

Serotype correlation of enteroviruses ECHO 6, ECHO 30, Coxsackievirus B1–6 and Coxsackievirus A with different serotypes isolated from samples of enterovirus infection patients, as well as sewage samples, confirmed the intense circulation of these enteroviruses in different regions.

The results of the study of stool samples of children with AFP or PPAV and their contacts, children from migrant families and patients with enterovirus infection have shown that all the territories of the North-Western region under surveillance have remained polio-free. The high coverage of polio immunization among children of all age groups should be maintained, and a good quality of epidemiological and virological surveillance of poliomyelitis and acute flaccid paralysis should be ensured.

Evidence shows that only the combination of acute flaccid paralysis, identifying population at risk, and environment surveillance can provide us with accurate data on poliovirus circulation, including wild-type strains and VDPVs in the population and in the environment. This can guarantee the polio-free status of the region under surveillance even after importations of wild polioviruses.

Improving epidemiological monitoring of measles and rubella during the elimination stage

In 2017, 17 samples of blood serum from measles patients, 45 samples of blood serum from rubella patients and 316 samples of blood serum from patients with exanthema diseases from 11 territorial units of the Northwestern Federal District (NWFD) were examined.

In St. Petersburg, a total of 3 cases of measles in adults were registered (0.02 per 1,000,000 of population). All cases of measles were confirmed serologically (EIA). One case was imported from the Chechen Republic. Molecular genetic testing was carried out in the material from the patient with the primary diagnosis of measles. He had measles virus of the D 8 Frankfurt Main Deu/17.11 genotype.

Retesting of individuals seronegative to the measles virus was continued in some indicator groups in the territorial units of the NWFD. St. Petersburg resource centre laboratory retested 221 EIA-negative blood serum samples from 5 territorial units of the NWFD. Study findings were confirmed in 100% cases in three territorial units (Komi Republic, Novgorod Region and Leningrad Region), in two more territorial units the confirmation percent ranged from 94.6% to 97%.

No cases of rubella were registered in the region in 2017.

The standard of the internal laboratory quality control of ZAO Vector Best was introduced in the laboratory. Monthly monitoring of control samples during EIA laboratory tests with test systems for measles and rubella was carried out.

Analytic overview "Measles situation in the territorial units of the NWFD and issues of diagnosis of sporadic cases" has been published.

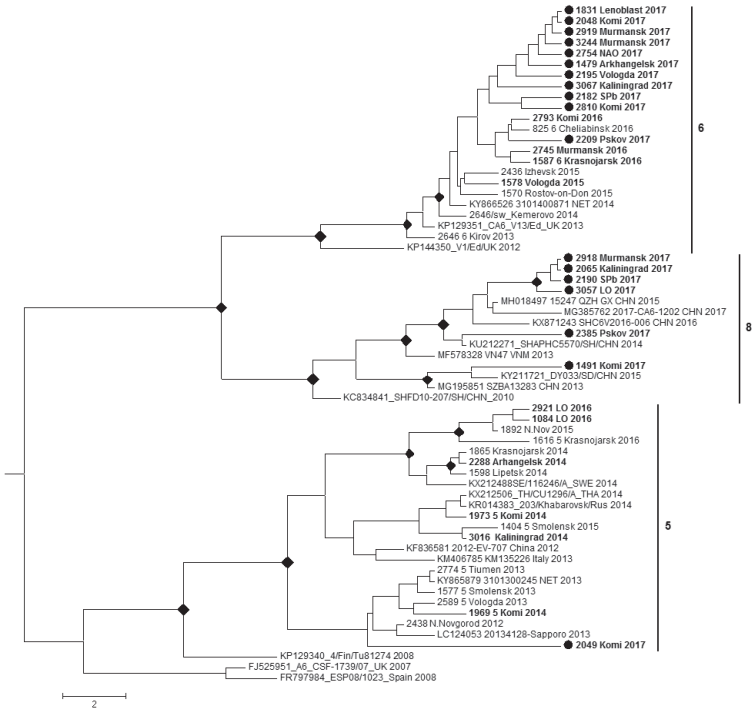

Figure 4. Phylogenetic tree of genomic region VP1 of Coxsackievirus A6

Table 5. Findings of the molecular-genetic examination of the material from measles patients in 2018 (NWFD)

Territorial unit	Date of disease onset (month)	Measles virus genotype
Kaliningrad Region	February	1 — D8 Frankfurt
St. Petersburg	March	3 — B3 Kabul
Vologda Region	March	1 — B3 Kabul
St. Petersburg	June–July	2 — B3 Dublin 1 — D8 Cambridge 1 — D8
	June–December	7 — D8 Gir Somnath
Leningrad Region	June	1 — D8 Frankfurt
Republic of Karelia	July	2 — D8 Gir Somnath

In 2018, testing of 186 samples of blood serum from patients with measles, 48 samples of blood serum from patients with rubella and 344 samples of blood serum from patients with exanthema diseases from 11 territorial units of the NWFD was carried out.

In 2018, the increase in measles incidence was evidenced, a total of 109 cases were recorded (0.8 per 100,000 of population) (Fig. 5).

Measles was recorded in 6 territorial units of the region. Most cases were registered in St. Petersburg, i.e. 54 cases (0.2 per 100,000 of population), the highest incidence was in the Republic of Karelia — 4.62 per 100,000 of population. Of all cases of measles in the region, 5 were imported from Italy, France, Turkey, and Ukraine and 25 cases were imported from other territorial units of the Russian Federation. There were 3 foci of nosocomial transmission and 3 family foci in St. Petersburg, 2 family foci in Leningrad Region and an outbreak of measles in the Republic of Karelia (29 cases). The majority of patients (64.2%) were adults and persons not earlier vaccinated (53.2%). In 18.4%, earlier vaccinated persons were affected.

Findings of the molecular-genetic examination of the material from measles patients evidence the diversity of measles virus genotypes found and multiple cases of virus import into the territorial units of the region (Tabl. 5).

For example, genotypes B3 Kabul, B3 Dublin, D8 Cambridge, and D8 Gir Somnath were found in St. Petersburg.

Of particular interest is the outbreak of measles in one of the districts of the Republic of Karelia. The first case was diagnosed with enterovirus infection, manifested with high fever and rash and was not examined in the laboratory. Blood sample of the patient was collected 26 days later, and then IgM to measles were found. This patient had visited the local polyclinic, which resulted in the measles outbreak. 71.4% of patients were adult persons, 41.4% had been revaccinated earlier. In the blood serum of these patients high titre of IgG (more than 3.0 IU/ml) and high percent of IgG avidity (more than 94.3%) was found in the early stages,

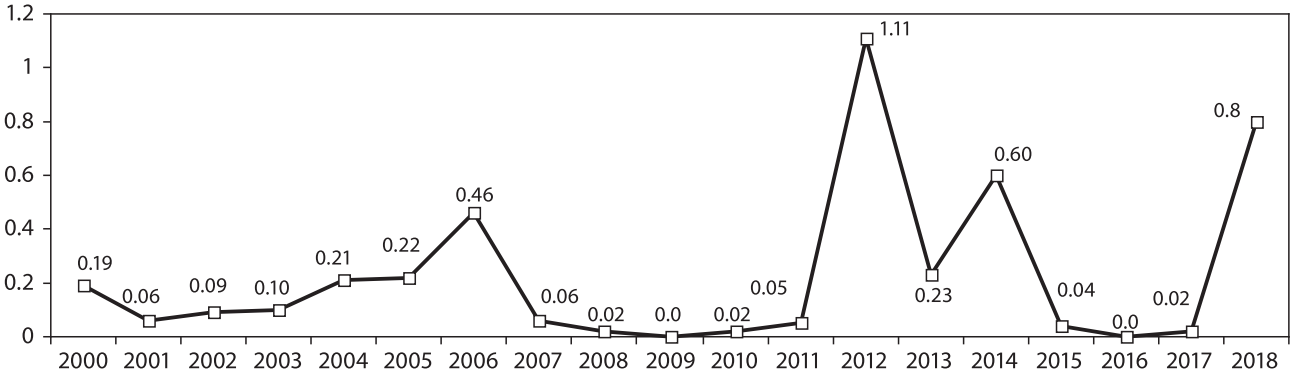

Figure 5. Measles incidence in the territorial units of the Northwestern Federal District in 2000–2018 (per 100,000 of population)

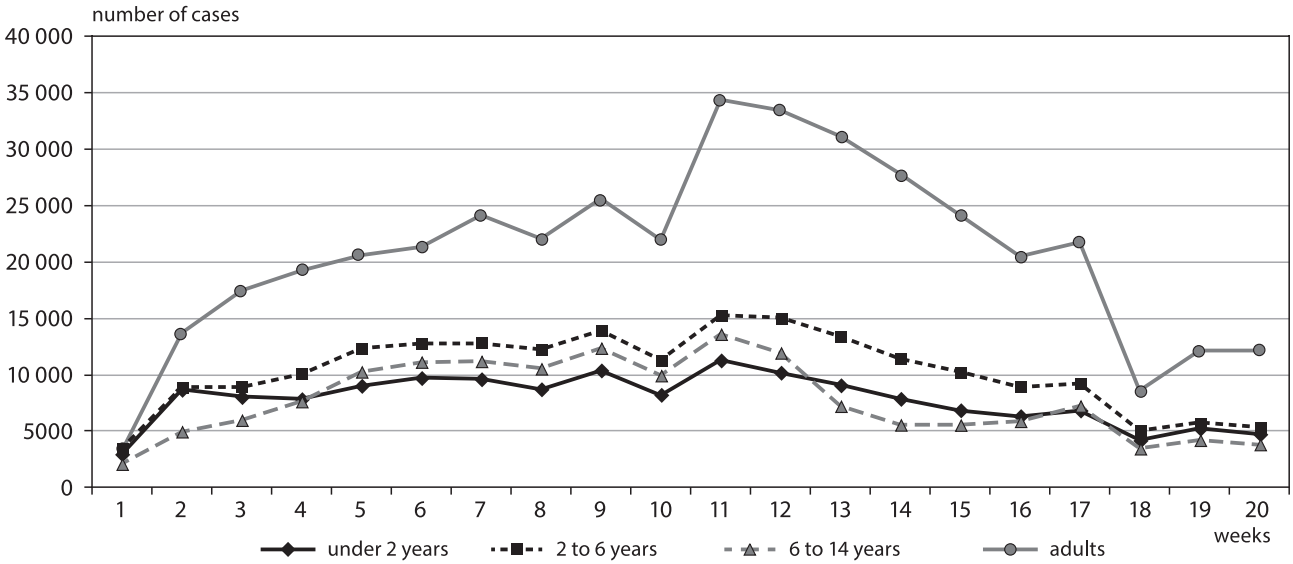

Figure 6. Recorded cases of flu and ARVI during the epidemic increase – 2018 in St. Petersburg

which confirmed that they had been vaccinated previously and showed secondary response. The interval after the re-vaccination in patients was 15 to 32 years.

In this group, 67.0% of patients had mild disease; there were no complications. In the group of persons not vaccinated earlier in 30% of patients complications were found. Molecular-genetic testing of the material revealed one genotype of influenza virus, i.e. D8 Gir Somnath.

In 2018, one case of rubella was recorded in the region, incidence rate 0.007 per 100,000 of population.

Study of influenza etiology in St. Petersburg, 2017–2018

The increase in incidence of influenza and ARVI in December 2016 — February 2017 in St. Petersburg was significantly lower than during the previous epidemic season (January–March 2016). Epidemic threshold (11,138 cases daily) was crossed in mid-December (12,356 cases daily). In March 2017, the incidence remained at 8000–9000 cases daily. Virological and serological studies showed that in 2017 the seasonal increase in incidence was mainly due to the circulation of the influenza A virus (H3N2) which was antigenically related to the reference strain A/Hong Kong/4801/2014, the circulation of the influenza B virus Victorian which was antigenically related to B/Brisbane/60/08.

In winter 2018, the epidemic threshold was crossed in February, the incidence peaked in March (on 12.03.18 there were 16,365 cases) and remained high in the first week of April (Fig. 6).

The total number of patients with influenza and ARVI in St. Petersburg from the 1st through the 20th week of 2018 was 932,684, including 820 cases of influenza confirmed in a laboratory.

Analysis of age structure in influenza and ARVI incidence showed that the highest values at week 11 were recorded in the age group of 3–6-year-olds (maximum of 678 per 100,000 of population), in the age group of 0–2-year-olds it was 547 per 100,000 of population, in 7–14-year-old children — 421 per 100,000 of population, the incidence in the group of persons aged 15 and older was low and amounted to 76 per 100,000 of population (Fig. 7).

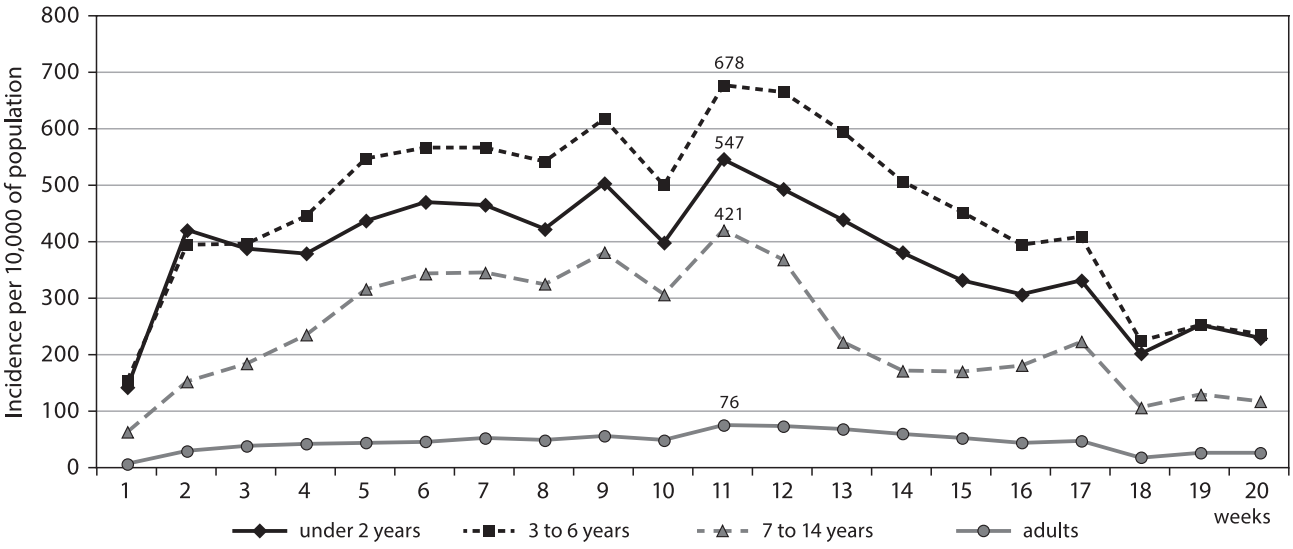

Figure 7. Influenza and ARVI incidence in different age groups during the epidemic outbreak in St. Petersburg in 2018

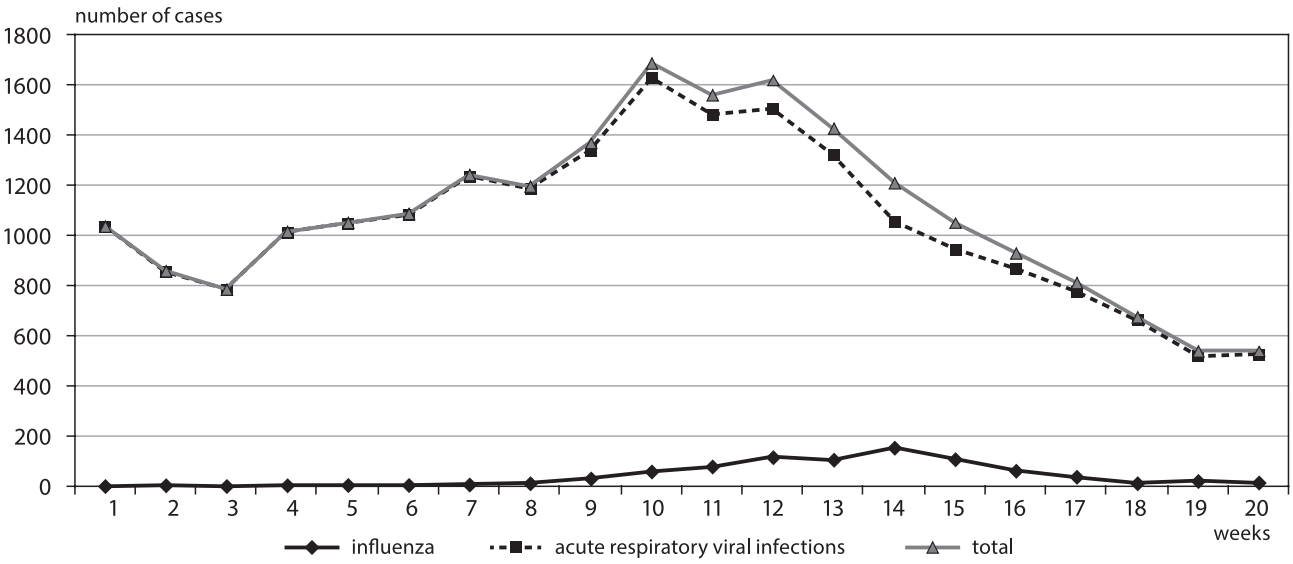

Figure 8. Number of patients with influenza and ARVI admitted to hospitals during the epidemic season 2018

All cases of influenza were moderate severe or severe and required hospital admission. A total of 20,880 patients with influenza and ARVI were admitted to city hospitals from the beginning of the year through May 2018 (Fig. 8).

Comparative analysis of laboratory data in 1152 patients aged from 16 to 91 years who were admitted to an infectious hospital in St. Petersburg with the diagnoses influenza and ARVI from January through May 2018 was carried out. From patients who were admitted before the 7th day of the disease a brushing from the nasal mucosa and oropharynx was performed for further virological and PCR examination.

According to PCR, influenza A(H3N2) viruses were predominant, they were found in 15.9%, the second most often found virus was influenza B Yamagata-lineage virus — 14.0%. Influenza viruses A(H1N1)pdm09 and B Victoria-lineage were found in approximately 5% of the patients, so were rinoviruses.

In January–May 2018, 111 nasopharyngeal swabs from patients hospitalized with the diagnoses influenza and ARVI were received at the laboratory for further virological examinations. PCR-positive for influenza samples were passaged on MDCK cell culture in accordance with the guidelines.

After two passages of fifty samples PCR-positive to influenza-viruses on MDCK cell culture, 26 strains of influenza virus were isolated and identified. Typing of influenza virus isolates with diagnostic sera and sera to epidemic strains of influenza virus showed that in January–March 2018 all three serotypes of influenza virus were circulating in St. Petersburg. In 50% of cases influenza B Yamagata-lineage viruses were detected which are antigenically related to the B/Phuket/3073/13 reference strain; 30.8% of isolates belonged to the A(H3N2) serotype and were antigenically related to the A/HK/4801/14 strain, and 19.2% of isolates were closely related to the pandemic version of the A(H1N1) pdm09 influenza virus.

Among the influenza viruses isolated and identified in a laboratory during the epidemic season 2018 no B Victorian-lineage influenza virus was isolated that dominated in the circulation in the previous years.

To two isolated A (H1N1)/SPb/24/18 and B/SPb/9/18 influenza viruses rat sera were prepared by three-fold intraperitoneal administration (5 ml each) of the fluid containing the virus with the 10-day intervals.

The serum prepared to the B/SPb/9/2018 virus had the homologous titre 320. The B/SPb/9/2018 strain interacted with antisera obtained for the versions of the B/Phuket/3073/2013 reference strain up to ½ of the homologous titre, which means this isolate belongs to the Phuket antigen and genetic group. This virus also interacted at ½ of the homologous titre with antisera to the B/Hong Kong/3417/4 and B/SPb/118/17 strains isolated in recent years.

The serum prepared to the cell variant of the A(H1N1) SPb/24/2018 virus had the homologous titre 160. This virus interacted with the antiserum prepared to the A/California/7/09 reference strain up to ½ of the homologous titre. With the antiserum to the A/Michigan/45/15 modern candidate to vaccine strains the isolated virus interacted up to ¼ of the homologous titre, which is evidence of the insignificant antigen shift of the isolate. The A(H1N1)SPb/24/2018 virus also interacted with the serum to the modern A/Israel/Q-504/15 reference strain up to ½ of the homologous titre, which is evidence of their antigen relatedness.

Therefore, the etiology of the epidemic increase of influenza incidence in St. Petersburg in January–March 2018 was deciphered: it was mainly caused by two serotypes of influenza virus strains: B Yamagata-lineage and A(H3N2). High influenza incidence predominantly of the pediatric population in 2018 is due to non-matching strains that were part of the 2017 vaccine (B Yamagata-lineage influenza virus) and those that caused the epidemic increase of influenza incidence in 2018 (B Yamagata-lineage influenza virus).

Unpredictable circulation of two lines of influenza virus B requires the inclusion of both variants into the vaccine.

Ethical aspects of preventive vaccination against infections

The study included issues of identification and analysis of the ethical component of different stages of the complex of measures associated with the organization, carrying out and control of immunologic preventive measures. The main ethical challenges associated with vaccination are the necessity to overcome the conflict of interests between an individual and the society; respect of the individual autonomy; adherence to the right of optional choice; fairness when distributing benefits and risk/damage; taking into account cultural and religious diversity; providing vulnerable groups with special conditions; non-discrimination; availability and affordability; social responsibility and solidarity.

Situations requiring the use of the above-named universal ethical principles may arise throughout the entire vaccination process: from vaccine development to its administration. Looking at the modern socially important aspect of the study, we decided to address issues of ethical and legal regulation of vaccine testing and administration, trying to achieve balance between benefits and risks when using vaccines and fairness in terms of equal access to vaccination. Ethical conclusions are grounded in original projects on studying the factors of trust and relation to vaccination in different cohorts; taking part in WHO programs for the elimination of relevant infections and direct involvement in the international dialogue on understanding and creating the recommendation resource for ethical accompaniment of vaccine prevention within the framework of WHO and UNESCO projects.

For the first time we turned to study the algorithm of ethical principles used when implementing the policy of global infection elimination/eradication using the WHO Polio eradication initiative. Comprehensive analysis including the complex of bioethical, epidemiological, virological, social and legal criteria of the infection eradication made it possible to identify the ethical components and show the meaning of complying with ethical principles for shaping the strategy of infection eradication. Modern realities set new problems associated with the validity research of the interdisciplinary analysis findings as exemplified by the WHO program of measles elimination. The fact that the deadline of the WHO strategic plan of measles elimination was postponed twice because of uncoordinated preventive measures is an unmistakable sign of how essential it is to comply with the ethical principles such as solidarity of actions and collaboration of all management and control systems. The diversity of situations related to the implementation of the WHO strategic plan in different regions of the world or related to cohorts with various cultural, social, religious, economic and psycholo-

gical status requires the commitment to ethical principles of human vulnerability, respect of personal integrity, equality, fairness, equal rights, and taking into account cultural diversity and pluralism. The analysis of administration and management systems at every individual level of implementation showed the need for commitment to ethical principles of preserving human dignity and human rights, individual and social responsibility, shared use of goods, protection of future generations and environment, biosphere and biodiversity. It was found that all the elements of scientific research both in laboratory practice and in improving vaccines when implementing the stages of elimination program could be achieved following the principles of transnational practices, technological exchange and creating access to education as well as professional training in bioethics. It is important that the global strategy of the WHO plan included as an essential component the clause devoted to "shaping public confidence in and demand for immunization" is, generally speaking, evidence of the entire WHO Strategic Plan being focused on implementing ethical principles at different stages of decision-making and acting. This commitment obliges all the participating parties at the professional, national and international levels to support and promote solidarity based on personal and social responsibility, fairness, openness and accountability to the civil society.

Another line of work was studying the ethical component in the process of establishment and counteraction to anti-vaccination movement. Based on the principle of commitment to personal responsibility for complying with moral and ethical norms, in this section we prioritize building a fully-fledged dialogue with the civil society as the only constant participant and addressed party in the opposition between vaccination champions and refusers. This possibility seems to be a real and ethically acceptable step leading to a breakthrough in the counteraction to anti-vaccination lobby. Our task in terms of ethics is to analyze vaccination-related crisis situations and ethical errors that can contribute to the shaping of the anti-vaccinal movement. It is essential to take into account, though, that the degree of opposition always depends on socio-cultural and politico-economic level of social formation. The reasons for mistrust in vaccination are multi-factorial and on the whole are spurred by raising doubts in the spiritual and legal aspect of the vaccination intervention, exaggerating/overstating when talking about vaccination being not safe. The new feature of the modern trend is that it the information flow is uncontrollable and often also completely anonymous, comes in large quantities, spreads quickly through modern means, and first of all, the Internet. We should admit, however, that the anti-vaccinal movement often builds upon the imperfections of vaccine prophylaxis. The first and main object of critics is the scientific base for vaccine development, which is not always perfect, because the scientific and social significance of any preparation is an ethical canon both at the stage of examination and administration. Continuing to talk about the importance of knowledge (scientific integrity of information), we should emphasize once again that the statement "it is unethical not to know and still recommend" refers to both parties, champions as well as opponents of vaccination. To take a non-biased stand against vaccination opponents and gain public confidence, we should constantly advance in knowledge of vaccination benefits/hazards and on that ground be able to quickly improve the drugs and vaccination policies, consequently drive home new information, increase understanding and acceptance in the society. If there is not enough evidence that existing drugs are produced at the high scientific and technological level and have the optimal benefit/risk balance, a possible solution of structures responsible for vaccination policies may be to discontinue or stop the administration of such vaccine until the risk is eliminated through targeted and prioritized drug improvement. Along with the scientific integrity of the preparation according to the benefit/risk balance, scientific integrity in the aspect of social practicality of its administration in a certain cohort and in given socio-economic and epidemic conditions has to be achieved. An example can be the need for thorough research into the infection burden in a given country before the vaccine is introduced to the market. All this is evidence not only of the need for true and impartial information, but talking about the factor under consideration, i.e. scientific approach to vaccinations, we should once again emphasize that the scientific base of vaccination must be absolute and cover the broad spectrum of goals. The complex of scientific responsibility on the side of the government includes the drug quality, study of the infection burden, the status of the community immunity, knowledge of the pathogenesis of adverse events and their treatment, examination of the social and cultural spectrum of the society, development of special programs of training of specialists, media, and various community groups. Such an approach is pivotal also in terms of transparency to the society and last but not least — gaining confidence of various strata of the civil society based on thorough examination of the efficacy of various vaccines against different chronic diseases, examination of the structure and pathogenesis of vaccination-related adverse events and complications, development and justification of treatment schedules for children with postvaccinal complications. It is the scientific basis and affordability of the whole complex of measures that play the essential role in vaccination advocacy and overcoming mistrust in vaccination and help to significantly reduce contraindications by including into the vaccination patients with chronic diseases who are most vulnerable to infection. Besides, it is necessary to create an open media landscape engaging both people responsible for vaccination and the society. This is underpinned by our solid belief that any privileged information (especially if it related to mass and global actions for wider groups of society) encourages mistrust and corruption and makes free space for the alternative information, which is used by members of the anti-vaccinal movement. Thus, important ethical principles for counteracting the anti-vaccinal lobby are identified by building a dialogue with the civil society based on solidarity, collaboration, equal rights, and fairness (individual and social).

As a conclusion we could reason that to score points in the opposition of the vaccination and anti-vaccination movement in the modern society, one should comply with the ethical principles of the professional duty which will fill the information vacuum creating mistrust through open dialogue within the society. Still, it is important to note that this is not as much about fighting between the parties, as about making vaccination opponents mindfully turn into its champions.

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LABORATORY OF EXPERIMENTAL VIROLOGY

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In 2017–2018, the research in the Laboratory of Experimental Virology was carried out along the following main lines of research:

1. Description of B19 parvovirus isolates circulating in the territorial units of the Northwestern Federal District of the Russian Federation;
2. Study of the prevalence of parvoviral infection markers in risk groups;
3. Study of community immunity to measles in the population of the Republic of Guinea;
4. Study of antiviral activity of camphor preparations.

1. Characteristics of the isolates of Parvovirus B19 circulating in Russian Federation

The aim of our work was genotyping and molecular genetic characterization of PVB19, prevalent in the North-West of Russia.

Materials and methods

In the study blood samples negative for measles and rubella IgM-antibodies received by the St. Petersburg Regional Center for Surveillance for Measles and Rubella in the North-West Federal District of the Russian Federation (NWFD) from 821 patients with maculopapular rash in 2009–2017 were examined.

Detection of IgM antibodies to PV B19 was performed by means of ELISA using diagnostic kits (EUROIMMUN, Germany). Quantitative determination of the PVB19 DNA in the samples were performed by means of PCR. Overlapping pairs of primers of the locus (NS1-VP1u) of the following structure were used for genotyping: PVB191F: CAATTGTCACAGACACCAGTA; PVB192F: CCCGCGCTCTAGTACGCCCA; PVB191R: ACTTAGCCAGTTGGCTATACCT; PVB192R: TTGCGGGGGCCCAGCTTGTA. The sequencing reaction was performed in 3 readings on forward and reverse primers.

Results

IgM-antibodies to PVB19 were detected in 139 (16.9%) samples; PVB19 DNA was detected in 59 (42.4%) seropositive patients, in 54 samples the viral load exceeded 104 copies/ml.

To assess the heterogeneity of the isolates, 14 samples were taken from patients from geographically remote regions of the NWFD, of different ages, regardless of gender, with a high viral load (10^6–10^7 copies/ml), including 1 isolate imported from the Moscow Region.

Phylogenetic analysis showed that all isolates belong to genotype 1A. In this case, the nucleotide sequences can be divided into two subgroups: 13 isolates (92.8%) belong to subgroup 1A2, one isolate to subgroup 1A1 (Fig. 9).

Comparison of the nucleotide sequences of different strains showed a high level of identity. By pairwise comparison, the nucleotide identity of the isolates in subgroup 1A2 varied from 98.3 to 100%.

When compared with the reference isolate NC_000883 (Genbank), from 1 to 10 mutational substitutions were found in the nucleotide sequences. In the surveyed region of the NS1 gene, amino acid substitutions of F554L in isolates MF481196, MG711455, MF408298, JX644429 and F554S in isolate MF405142 were detected in comparison with the reference isolate NC_000883. In the VP1 gene of the studied isolates, form 1 to 5 amino acid substitutions were detected: E14K, Q21H, V30L, S98N, D107N, Q124P.

Discussion

Thus, isolates that are widely represented throughout the world, as well as isolates characteristic of the North-West of the Eurasian continent, were identified. All these cases were detected in 2010–2011 among our patients and in the years 2005–2011 in the works of other research groups, which indicates the continuing viral circulation. However, some level of clustering of viruses by year may indicate repeated virus imports or a renewable reservoir of the pathogen. The isolates identified in 2014–2017 demonstrated unique nucleotide and amino acid substitutions not previously represented in the international Genbank database, including the only isolate obtained in 2014 and belonging to the B19 1A1 group imported from the Moscow Region.

The implementation of screening of patients with a fever/rash for B19 can provide significant information on the prevalence of parvovirus infection. The systematic application of molecular phylogenetics in the parvovirus B19 isolates analysis will help to understand the epidemiology of the infectious process, to identify the characteristics of the spread of the virus and its genovariants.

2. Study of markers of paraboveric infection distribution in risk groups

2.1. Impact of coinfection of PVB19 on the course and prognosis of malaria caused by *Plasmodium falciparum*

Parvovirus infection complicates the course of diseases accompanied by immunodeficiencies (oncological, hematological, etc.). According to several authors, infection with PVB19 can aggravate the course of malaria.

Malaria is a vector-borne parasitic disease caused by the protozoa of the genus *Plasmodium*, that is widespread in the countries of Equatorial Africa, Southeast Asia, Oceania, Central and South America. The problem of malaria is especially urgent for the Sub-Saharan Africa. This territory accounts for 90% of cases and 92% of deaths from malaria in the world. According to WHO, in the Republic of Guinea (GR) in 2015, 811,000 cases of laboratory-confirmed malaria were detected. The disease proceeds with cyclic fever, febrile paroxysms, hepatorenal and anemic syndrome. Severe complications of malaria leading to death can be cerebral edema, cerebral (malarial) coma, splenic and kidney failure, disseminated intravascular coagulation syndrome

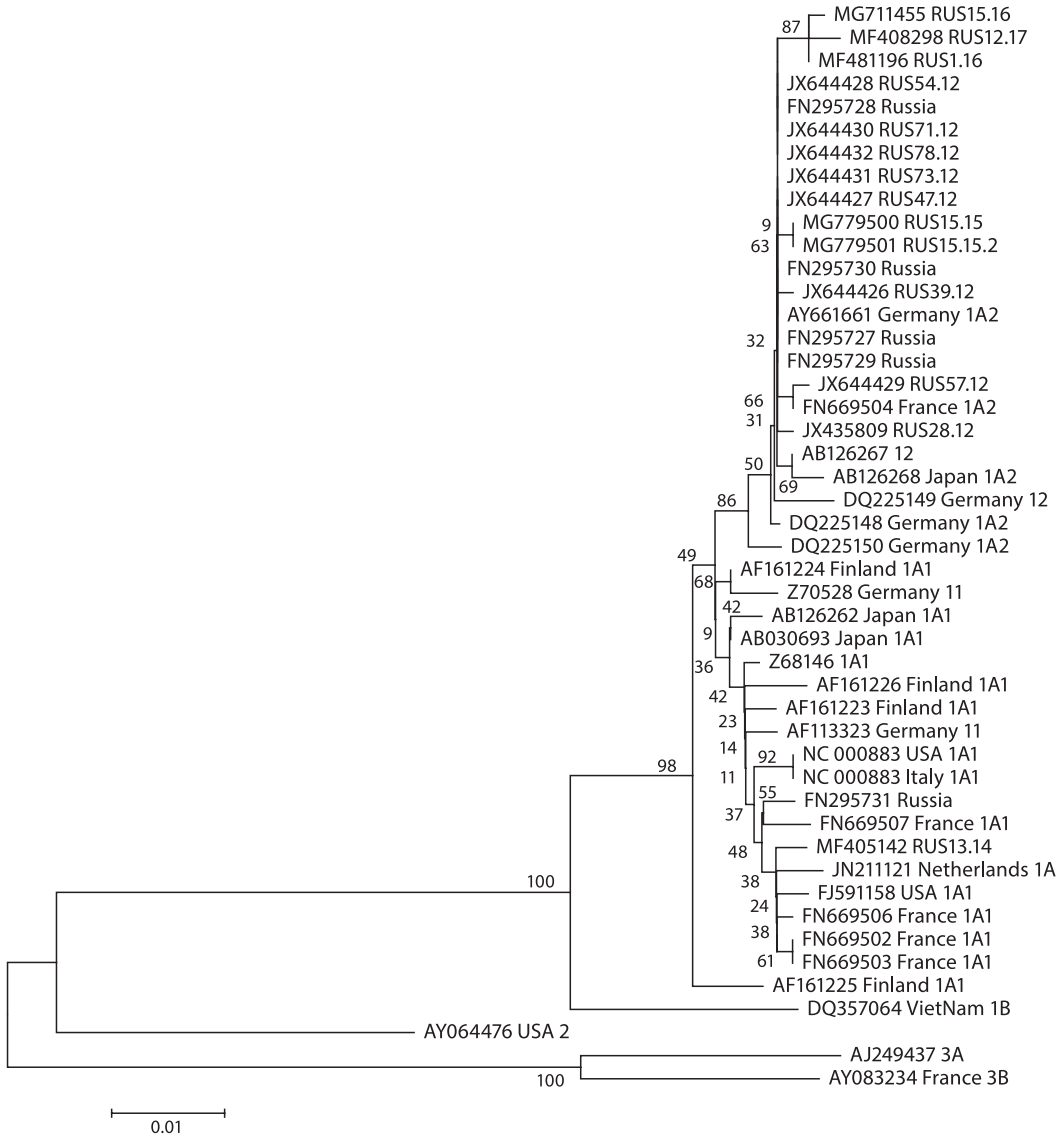

Figure 9. Dendrogram of phylogenetic relationships between the studied PVB19 isolates extracted from patients in the North-West Federal District compared with the reference sequences presented in GenBank

(DIC-syndrome), acute massive hemolysis, hemoglobinuria, hemorrhagic syndrome.

The objective of the present study was to evaluate the effect of parvovirus B19 infection on the clinical course of malaria and the outcome of the underlying disease.

Materials and methods

During the period 2016–2018 blood plasma samples of 316 patients from the hospital of the Friya Prefecture of the Republic of Guinea (GR) with confirmed diagnosis of malaria were examined for the presence of PVB19 DNA. DNA PVB19 was detected by PCR using the sets of reagents Ampliprime "RIBO-prep" and "AmpliSens® Parvovirus B19-FL" (Central Institution of Epidemiology, Rospotrebnadzor, Russia) in accordance with the manufacturer's instruction.

Statistical treatment of the results was carried out using the analysis of frequency distributions and conjugacy tables using the GraphPadInStat 3 software. The significance of the differences and the confidence interval were determined by the Student's t-test. Differences were considered significant at $p < 0.01$.

Results

The clinical course of malaria in 316 examined patients was divided into group of either mild or complicated. A mild course of malaria was detected in 177 (56.01±2.79%) patients. This form of the disease was characterized by fever with temperature not exceeding 39°C, general weakness and moderate anemia (hemoglobin concentration higher than 70 g/l). 139 patients (43.99±2.79%) manifested the complicated course of malaria that was accompanied by a rise of the temperature up to 40°C, nausea, vomiting, severe anemia (hemoglobin concentration lower than 70 g/l), high levels of transaminases, creatinine, a decrease in the total protein in the blood. In 8 (2.53±0.88%) cases, the severe course of the disease led to a fatal outcome.

Plasma samples of patients with mild and complicated forms of malaria were tested for the presence of PVB19 DNA. The results are shown in Tabl. 6.

In total, PVB19 DNA was detected in blood plasma in 55 of 316 patients (17.41±2.13%). In groups of both PVB19-positive and negative patients, cases of mild and complicated as well as fatal outcomes were detected. However, in the group

Table 6. The effect of parvovirus B19 infecting on the course of malaria in patients of Fria Prefecture of the Republic of Guinea

Course of malaria	Number of patients	Presence of PV19 DNA	
		Positive (abs./%)	Negative (abs./%)
Mild	177/56.01±2.79%	15/27.27±2.75%	162/62.07±3.0%
Complicated	139/43.99±2.79%	40/72.73±2.75%	99/37.93±3.0%
Lethal outcome	8/2.53±0.88%	6/10.91±4.40%	2/0.77±0.54%
Total	316/100%	17.41±2,13%	261/82.6±2.13%

with co-infection of PVB19 and *P. falciparum*, complications and mortality rates were significantly higher. Indeed, complications were observed in 40 of 55 (72.73±2.75%) patients, and in 6 of 55 cases (10.91±4.40%) the disease resulted in death. In the group of patients with malaria without PVI, complications occurred in 99 of 261 patients (37.9±3.0%); of those 2 (0.77±0.54%) died. Thus, the probability of developing a complicated course of malaria with a co-infection is significantly higher than in the absence of PVI ($p < 0.0001$; RR = 1.917; 95% CI: 1.532 to 2.399).

To study the age structure of patients with malaria, they were distributed into seven age groups: 0–5, 6–10, 11–15, 16–25, 26–45 and 46–65 years old (Tabl. 7). It was found that the most numerous group in the structure of malaria patients is represented by children under 5 (median 3) years (89, or 28.25±2.53%). The lowest number of malaria cases falls into the age group of 46–65 (median 57) years — 17 cases, or 5.4±0.71%. It is noteworthy that the DNA of PVB19 in the blood plasma of patients of this group has not been detected.

In other age groups of patients with malaria, the presence of PVB19 DNA in the blood plasma is characterized by the following rates: among children under 5 years (median 3 years) it was detected in 17.98±4.07% of patients; in the group of 6–10 years (median 8 years), the detection rate increased to 28.85±6.28, and reached a maximum of 34.29±8.02% in the group of 11–15 years (median 13 years). These results indicate a wide prevalence of parvovirus infection among children and adolescents and correspond to the data available in the literature. In older patients, this value was reduced to 15.38±5.0% in the group of 16–25 years (median 20 years), to 5.71±3.22% in the group of 26–45 years (median 36.5 years) and zero in the age group of 46–65 years (median 57 years). The severity of PVB19-associated malaria was analyzed in different age groups. The results are summarized in Tabl. 8.

As can be seen from the results presented, the maximum number of cases of complicated malaria with PVI (n = 15) was observed in patients under 5 years old, where it accounts for 93.75±6.05% of cases comparing to 27.27±7.04% among all PVB19-positive patients. The likelihood of developing a complicated course of malaria with a co-infection in this age group is significantly higher than in the absence of PVI ($p = 0.0001$; RR = 2.44; 95% CI: 1.780–3.357). It is important to note that 6 out of 8 deaths occur in this group, that is significantly higher than in the absence of infection with PV B19 ($p = 0.0003$, RR = 13.688, 95% CI: 3.034–61.740). With age increase, the incidence of complicated course of PVI-combined malaria decreases, although the trend persists in individuals under 26 years of age. Thus, among patients of 6–10 years of age, complicated malaria was recorded in 18.18±5.75% of PV B19-infected persons; in the group of 11–15 years — in 14.55±5.14% of cases; in persons from 16 to 25 years in 9.09±4.07% of cases. However, the differences in the course of malaria between patients infected and not infected with parvovirus B19 are not statistically reliable in all these age groups.

Discussion

It is known that in seronegative individuals with primary infection parvovirus in the acute phase can cause a failure of erythrocytes formation for up to 5–7 days, which leads to a significant decrease in hemoglobin. Erythrocytes are also the main target of the malarial plasmodium, which multiplies within and destroys them thus causing anemia of varying severity. According to several authors, infection with PVI occurs after decrease in cellular immunity caused by *P. falciparum*. There is evidence that in severe malaria-endemic regions severe forms of anemia are the main cause of child mortality. Thus, severe anemia is considered the cause of childhood death in malaria in 17–54% of cases.

Our results correlate with the data of other researchers who studied the PVI-associated malaria in children in malaria-endemic regions: among children under 5 years, the absolute majority of cases of PVI was accompanied by a complicated course of malaria.

Having in mind that according to studies, in African countries 50% to 90% of the population by age 6 have IgG antibodies to PVB19 and, based on the results obtained,

Table 7. Age distribution of malaria patients with combined parvoviral infection

Age group, years	Number of patients (abs./%)	PVB19-positive (abs./%)
0–5	89/28.25±2.53	16/17.98±4.07%
6–10	52/16.51±2.09	15/28.85±6.28%
11–15	35/11.11±1.77	12/34.29±8.02%
16–25	52/16.51±2.09	8/15.38±5.00%
26–45	70/22.22±1.16	4/5.71±3.22%
46–65	17/5.40±0.71	0/0.0%
Total	315/100%	55/17.46±2.14%

Table 8. Mild and complicated course of malaria associated with parvovirus B19 infection in different age groups

Age, years	Mild disease, abs./%	Complicated disease, abs./%
0–5	1/1.82±1.80%	15/27.27±7.04%
6–10	5/9.09±3.88%	10/18.18±5.75%
11–15	4/7.27±3.50%	8/14.55±5.14%
16–25	3/5.45±3.06%	5/9.09±4.07%
26–45	2/3.64±2.52%	2/3.64±2.57%
46–65	0/0.0%	0/0.0%
Total (n = 55)	15/27.27±6.01%	40/72.73±11.50%

it can be assumed that the probability of complicated course of PVI-associated malaria depends on the age of the patient. The older the patient, the higher the probability of having immunity to parvovirus, and, therefore, the less influence the associated infection demonstrates.

In contrast, the primary parvovirus infection, which normally occurs in early childhood, is acute and can aggravate the course of malaria, especially when combined with other unfavorable conditions (iron deficiency, malnutrition, helminthic infections, co-infections, etc.). Thus, infection with PVB19 becomes a critical factor, which can provoke a severe life-threatening anemia, and also cause other complications.

In general, the results of this study indicate a high medico-social significance of parvovirus infection for countries endemic for malaria.

2.2. Parvovirus B19 incidence, specific antibody response, and delayed hematopoietic recovery after allogeneic hematopoietic stem cell transplantation

Parvovirus B19 (PVB19) is a well known small DNA virus from Erythrovirus genus which is in scope of pediatricians for decades being associated with erythropoiesis disturbances, arthropathies, myocarditis and other disabling clinical conditions (Asano, Yoshikawa, 1993). PVB19 shows an affinity for the group P antigen of red blood cells, with lesser amounts in blood plasma (Lee et al., 2011). The major hematotoxic effect of the virus is believed to occur at the pronormoblast stage, thus causing arrest of erythroid differentiation. PVB19 was occasionally found in aplastic anemias and pure red cell aplasia (Urban et al., 2011). In this respect, most studies concerned resistant anemia cases in the patients subjected to renal transplantation where the PVB19 was not a rare finding (Egbuna et al., 2006).

Over past years, many cases of severe myocarditis and hepatitis were shown to be associated with parvovirus infection, as based on positive PVB19 antigen or DNA findings in affected tissues. Meanwhile, a latent persistence of PVB19 was quite common in skin, synovial tissues, myocardium and bone marrow (Corcioli et al., 2008). The viral DNA was detectable in peripheral blood from 5% of healthy donors (Gama et al., 2017). The authors suggest only small risk for the donor-recipient viral transmission upon hematopoietic stem cell transplantation (HSCT).

Following allogeneic HSCT, a regular activation of herpesviruses and other latent pathogens is observed, due to acute myelosuppression and cellular immune deficiency (Chukhlovin, Pankratova, 2017). Clinical significance of the PVB19 in immunocompromised patients is not yet properly evaluated. E.g., a prolonged study of the PVB19 viral load has been performed in 53 patients after allo-HSCT using quantitative PCR (Rahiala et al., 2013). Specific viral DNA was detectable in blood serum from 30% of the HSCT recipients, either before, or after HSCT, at maximal viral load observed 2 months post-transplant. However, the patients with detectable PVB19 did not show specific clinical symptoms that could be ascribed to parvovirus infection.

The issue of optimal PVB19 diagnostics in heavily treated hematological patients is not yet clear, since, according to Lundqvist et al. (1999), the results of serological tests (serum IgM and IgG antibodies) did not correlate with detection of viral DNA in blood serum or bone marrow from the HSCT recipients.

Hence, the aim of our work was to compare the PVB19 DNA levels prior to allogeneic HSCT, and at 1–2 months post-transplant, as well as search for correlations with specific antibody levels, and rates of hematopoietic recovery within 60 days after allo-HSCT. Our preliminary data point to a prognostic significance of parvovirus DNA detection and increased antibody levels as possible predictors for delayed engraftment and febrile neutropenia.

Patients and methods

A total of 54 pediatric and adolescent patients were involved into the study at the median age of 7.2 (0.6 to 19 years old), who had a malignant disease of hematopoiesis or inherited disorders as initial diagnosis who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT). Fifty-one patient of this group were observed for at least 2 months (60 days) after HSCT. Selection of the patients for allo-HSCT, choice of conditioning regimens, prophylaxis of acute graft-versus-host disease was performed according to current EBMT recommendations. Most part of this group was represented by the patients with acute myeloblastic leukemia (AML, n = 16; 30%), acute lymphoblastic leukemia (ALL, n = 14; 26%); severe anemias (SAA) of different origin (13%; n = 7). 33% of the patients were in first remission after previous treatment.

Bone marrow was used as a source of hematopoietic stem cells in 83% of cases (45 of 54 patients), infusion of peripheral stem cells was applied in the rest of cases. Allo-HSCT from unrelated donors was performed in 45% of cases (24 of 54); grafting from matched related donors or haploidentical transplant was carried out in, resp., 20% (11/54) and 35% (19/54) patients. Reduced-intensity conditioning was used in nearly all cases (51 of 54 patients). Development of acute GvHD within early period after allo-HSCT was observed in 25 patients, of them, 9 exhibited grade 3–4 GvHD.

Regular examination of the patients before and after HSCT was carried out according to a standard local clinical protocol. He study was approved by the Local Institutional Board at the St. Petersburg State I. Pavlov Medical University. The laboratory studies included routine blood counts and serum biochemistry, urinalysis etc. Quantitative determination of the PVB19 DNA as well as herpesviruses (CMV, EBV, HSV) and polyomaviruses (BK, JC) in blood plasma was performed before conditioning therapy which preceded allo-HSCT, as well as on day+30 (D+30) and day+60 (D+60) post-transplant. DNA extraction from the samples and quantitative evaluation of PVB19 DNA in the samples were performed by means of PCR with specific fluorescent probes using "Ampliprime" amplification kits and "Amplisens® Parvovirus B19-FL" (Moscow, Russia). Moreover, quantitative determination of IgM and IgG antibodies to PV B19 was performed by means of ELISA at 0, +30 and +60 days post-transplant using "Anti-Parvovirus B19 ELISA IgM" и "Anti-Parvovirus B19 ELISA IgG" kits (EUROIMMUN, Germany). The diagnostic kits were used according to instruction. Statistical evaluation of the data was performed with a Winstat software package.

Results

Transplant-related changes of PVB19 DNA levels and anti-PVB19 antibodies

The pre-transplant contents of PVB19 DNA and IgG antibodies to the virus showed a broad-range scatter (Tabl. 9). PVB19 was found in only 31.5% of this group. Meanwhile,

68% of the patients exhibited increased levels of IgG-anti-PVB19 antibodies (> 10 IU/ml), thus reflecting high prevalence of adaptive immune response. However, mean pre-transplant contents of PVB19 DNA did not correlate either with age of the patients, or with clinical disease status, physical state, or activation of other latent viruses.

Prevalence and mean levels of PVB19 DNA as well as concentrations of anti-PVB19 antibodies did not show any significant changes at 30 or 60 days after HSCT. However, actual scatter of these data proved to be rather sufficient, thus suggesting some correlations between these laboratory indexes and clinical signs in the individual patients. In particular, positive (non-zero) viral loads have been registered in 28% before allo-HSCT, 29% and 30.4% on day+30 and day+60 after allo-HSCT, i.e., ca 70% of the patients showed negative results for PVB19 over the early period after allo-HSCT.

Interaction between the PVB19 presence and specific antibody response after Allo-HSCT

Detection of PVB19 DNA, both before and after allo-HSCT was not accompanied by IgM antibody detection at any observation point, thus suggesting absence of acute infectious process caused by parvovirus infection.

Meanwhile, a significant positive correlation was revealed between the overall PVB19 viral load and serum levels of IgG antiviral antibodies ($r = 0.351$; $p = 8 \times 10^{-6}$, 153 assays in 54 clinical cases). In particular, a significant correlation was shown between initial viral load and anti-PVB19 levels at all three terms of the study being, however, maximal at the day+60 after allo-HSCT.

As seen from Fig. 10, a significant correlation exists between pre-transplant PVB19 load and expressed antibody response detected 60 days after allo-HSCT, i.e., the non-zero viral loads were associated with higher contents of specific antibodies, thus suggesting an association between the PVB19 persistence and production of virus-specific antibodies (Fig. 10).

A half-life time for endogenous IgG antibodies in humans is about 1 to 4 weeks (Kontermann, 2009). These findings suggest an opportunity of specific antibody production at early terms after imtensive cytostatic treatment, due to potential activity of surviving memory cells, e.g., plasmocytes which able to function for months and even years after their maturation.

Parvovirus B19 activation and hematopoiesis recovery

In our clinical series, altered engraftment was, generally, more common at increased IgG PVB19 antibody levels when determined 60 days after HSCT ($r = 0.315$; $p = 0.034$; $n = 46$).

Specifically, significant correlations were shown between initial parvovirus DNA detection, and delayed reconstitution of erythrocytes and platelets in peripheral blood (respectively, $r = -0.281$; $p = 0.02$; $r = -0.303$, $p = 0.01$). Moreover, a marked correlation was shown by the day+60 between decreased neutrophils and platelet counts, and increased anti-PVD19 antibody levels (Fig. 11–13). This association may suggest a relation between continued parvovirus persistence and slower hematopoiesis recovery at 30 to 60 days post-transplant.

In general, altered engraftment was also registered in cases with higher IgG antibodies against parvovirus 60 days after HSCT ($r = 0.315$; $p = 0.034$; $n = 46$).

Parvovirus detection and febrile neutropenia

Positivity for PVB19 DNA by the day +30 after allo-HSCT was, in all cases (14/14), associated with febrile neutropenia (FN), as compared to 68% in patients with nondetectable PVB19 (23/34; $p = 0.015$, see Tabl. 10). Hence, active parvovirus infection could be a sufficient factor of common febrile reactions observed in early posttransplant neutropenia, thus supporting pathogenic significance of this infection, at least, in a subgroup of immunocompromised

Table 9. Mean and median values for serum IgG PVB19 antibodies and specific viral DNA before and at different terms after allo-HSCT

Parameters studied	IgG-antibodies to PVB19 (ME)		
Conditions de l'étude	Pre-HSCT	Day+30	Day+60
Number of cases	54	51	49
Mean values	21.7	24.8	22.8
SE	3.7	3.4	3.6
SD	26.9	24.1	25.4
Median	10.7	18.8	12.5
Minimum	0.0	1.6	1.5
Maximum	100.0	96.3	110.0
	Parvovirus B19 DNA, copy number × 10^3/mL		
Study terms	Pre-transplant	Day+30	Day+60
Number of cases	54	51	49
Mean values	27.2	32.9	25.7
SE	6.7	9.7	6.8
SD	49.5	68.8	47.3
Median	0	0	0
Minimum	0	0	0
Maximum	178.3	280.9	222.2
	Correlations between the pre-transplant viral load and IgG anti-PVB19-antibodies at different terms after HSCT		
Study terms	Pre-HSCT (Day 0)	Day+30	Day+60
R quotient	0.367	0.274	0.464
P significance level	0.003	0.02	0.0004

patiens. Development of these febrile responses could be either virus-induced, or combined with secondary bacterial infections caused by slow recovery of cellular immunity.

Conclusions

1. Presence of parvovirus B19 in peripheral blood of children before allogeneic hematopoietic stem cell transplantation is followed by increased PV-specific antibodies of IgG class in blood serum at all terms after allo-HSCT.
2. Increased IgG levels of antibodies in blood of the patients after allo-HSCT is associated with relative neutropenia and thrombocytopenia at first 2 months after allo-HSCT.
3. Detection of parvovirus DNA at initial terms (before HSCT) and and 30–60 days later may be followed by development of early febrile neutropenias and slower recovery of erythrocyte and platelet counts in peripheral blood.

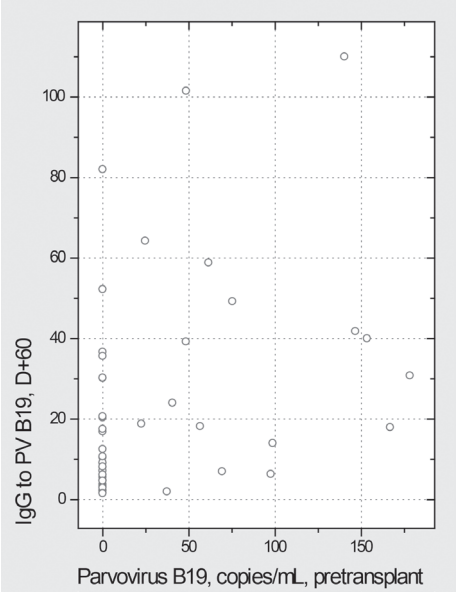

Figure 10. Association between initial PVB19 viral load (day 0), and IgG antibodies to PVB19 by day 60 (r = 0.464; p = 0.0004). Absciss, parvovirus concentration (copies per mL); ordinate, amounts of specific IgG antibodies, expressed as IU/ml, at 2 months after HSCT

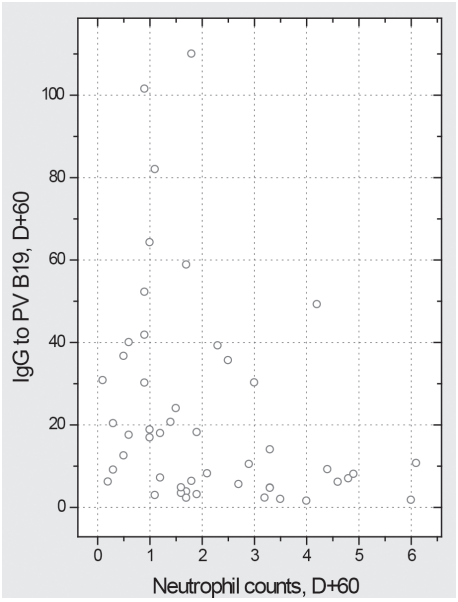

Figure 12. Negative correlation between the blood neutrophil counts, and amounts of PVB19-specific antibodies, expressed as IU/ml, at the day+60 post-HSCT (r = –0.422; p = 0.002)

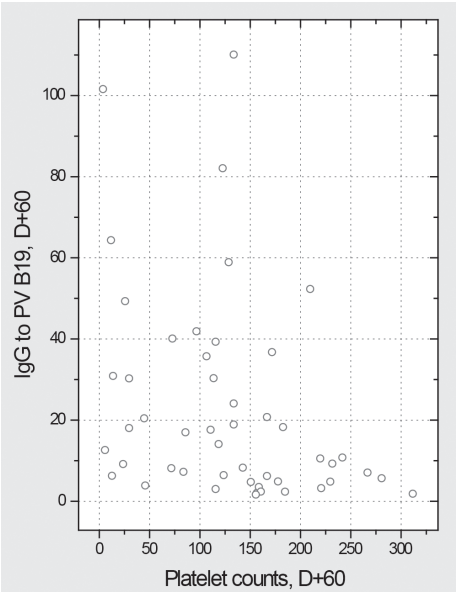

Figure 11. Negative correlation between blood platelet counts (10^9 per mL), and IgG anti-PV B19 antibodies, expressed as IU/ml, at the day+60 post-HSCT (p = 0.001; r = –0.422)

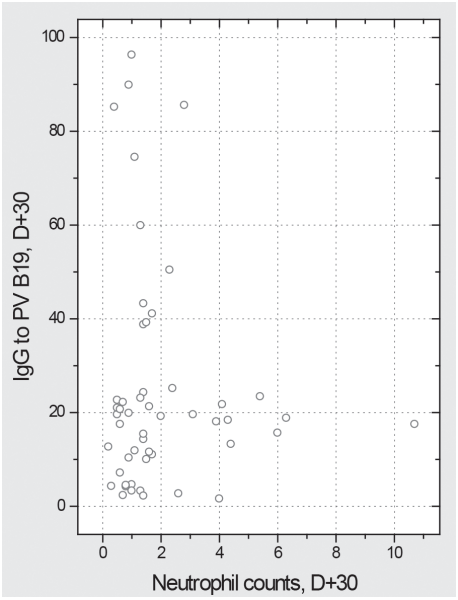

Figure 13. Negative correlation between blood neutrophil contents, and amounts of PVB19-specific antibodies, expressed as IU/ml, at the day+30 post-Allo-HSCT (r = –0.380; p = 0.003)

3. Study of the immunity level to measles in individual groups of the Republic of Guinea population

Despite all the progress made in measles control, epidemic outbreaks of measles are still being registered worldwide. In 2010–2016, outbreaks of measles were recorded including in the USA and a number of European countries with high vaccination coverage. Major outbreaks of measles were also registered in Africa, i.e. in the Democratic Republic of Congo (over 121,000 cases), in the south of Africa (176,000 cases), in Nigeria (ca. 30,000 cases), and some other African countries.

Due to the increased incidence, the new Strategic Plan was adopted worldwide with other deadlines for measles elimination in the certain WHO regions. Measles elimination in the Western Pacific region was planned to be achieved by 2012; in the European and East-Mediterranean regions — by 2015; in the African region — by 2020. In the Southeast Asia, the goal was to reduce measles mortality by 2015. At the present moment, the date set for the implementation of the Global Measles and Rubella Plan has been postponed once again.

The population of the Republic of Guinea is more than 12 million people, including ca. 2 million children under 5. Measles immunization is carried out once at the age of 9 months. According to the WHO, in the period from 1980 to 2001 the incidence of measles in Guinea was high, in some years up to 15,000–18,000 new cases annually (Fig. 14). In 2002, the number of measles cases was 3.4 times lower than in 2001 and 8.4 times lower than in 1999. Later only singular measles cases were recorded. In 2014–2015, however, the increased measles incidence rate was registered in the Republic of Guinea: according to the WHO, in the 11 months of 2016 the number of cases suspected for measles was 616, whereas the number of cases confirmed in a laboratory was 290. The incidence was 2.68 per 100,000 of population. It is evident that such an increase in incidence is due to the measles vaccination coverage that dropped from 90–99% in 2010–2013 to 60–62% in 2014–2015 because of the Ebola epidemic in these years.

To reduce the risk of major measles epidemics on the African continent, the WHO recommends carrying out campaigns of additional measles immunization after the Ebola virus spread has stopped. It is evident that an increase in the efficacy of such campaigns is due to the identification of epidemically significant age groups of population in need of additional vaccination.

Table 10. Differences in PVB19 levels for the patients with/without febrile neutropenia (p = 0.015)

	Febrile neutropenia	Free of febrile neutropenia
Number of patients	37	11
Average number of copies PVB19	42.37	0
Mean average deviation	77.01	0
Average error mean	12.7	0

The objective of this study was to assess the measles immunity level in different age groups of the population of the Republic of Guinea.

Materials and methods

Examined: 257 samples of blood serum from children and adults who stayed for inpatient treatment in the hospital of Kindia (Republic of Guinea). Clinical samples were received in 2015–2016.

Blood serum samples were tested for measles IgG antibodies using the Anti-Measles Virus ELISA test system by Euroimmun Medizinische Labordiagnostika AG (Germany) in accordance with its directions for use.

Statistical data processing was carried out using parametric statistics with the Student t-test to assess the differences between the events. Differences were considered significant at $p < 0.05$.

Results et discussion

Testing for IgG to measles of 210 blood serum samples of measles patients of known age who stayed for inpatient treatment in Kindia hospital in 2015–2016 showed that 36 (17.1%) persons were seronegative. The age of another 25 patients seronegative to measles was not known. The total number of seronegative persons was 61, corresponding to 23.7% of all examined persons.

Breakdown of patients' serum samples by age and titre of measles IgG antibodies is shown in Tabl. 11.

In the age group of persons under 17 there were seven patients not having measles IgG. In 16 patients of this age

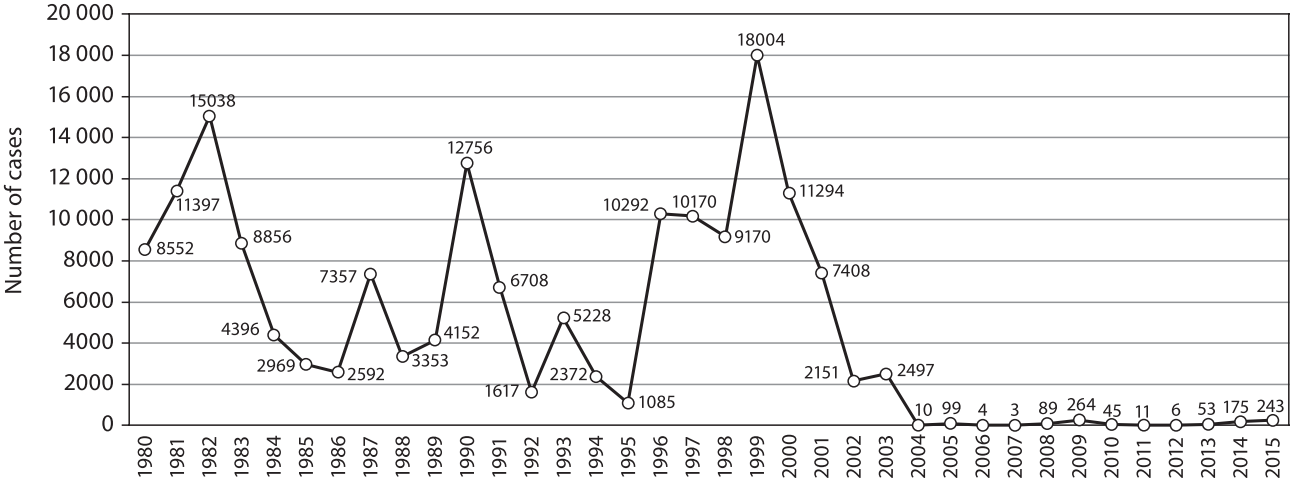

Figure 14. Number of cases of measles in the Republic of Guinea in 1980–2015 (WHO/IVB, 2016)

Table 11. Breakdown of patients' serum samples by age and titre of measles IgG antibodies

Optical density, IU/L	Age (years)					
	Under 17	18–22	23–40	41+	Total	
< 200.0	7	14	14	1	36	17.1%
201.0–1000.0	16	20	35	32	103	82.9%
1001.0–3000.0	3	4	14	13	34	
> 3000.0	5	6	8	18	37	
Total	31	44	71	64	210	100%

group IgG titres were not high but rather ranged from 201 to 1000 IU/L. These were likely post-vaccinal antibodies. In 8 children and adolescents high antibody titres (more than 1001 IU/L) were found which is evidence of a recent measles disease. High measles IgG titres were found, among others, in two children aged 2 years 7 months (1600 and 3200 IU/L) also meaning that they had had measles recently. These children had obviously not been vaccinated.

High titres of IgG (1001–3000+ IU/L) were recorded in 10 of 44 examined persons aged 18–22 (22.7±6.4%). At the same time, in the group of persons 23 years old and older, these titres were found in a significantly higher number of patients, i.e. in 53 persons (39.2±6.2%) ($p < 0.05$). These antibody titres are obviously a sign of earlier measles.

In the age group of 18–22-year-olds 14 of 44 examined persons were seronegative to measles (31.8%); in patients aged 23–40, 14 of 71 examined persons were seronegative to measles (19.7%). On the whole, in the age group aged 18–40 the share of seronegative patients was 24.3±5.1% which is significantly higher ($p < 0.05$) than among patients over 41 where only 1 of 64 examined persons was found to be seronegative to measles (1.6±3.6%).

Taking into account that major outbreaks of measles in the Republic of Guinea were recorded in 1981–1982 and in 1996–2000 with the incidence of 15,000–18,000 per 100,000 of population, it is fair to assume that the revealed considerable predominance of persons seropositive to measles among the examined patients aged 23 and over of the Kindia hospital is due to measles those persons had during epidemic outbreaks of 1980–1990s. Considering the fact that in 2015–2017 a new increase in measles incidence was recorded in the Republic of Guinea, one can assume that 37 of 210 examined persons with measles IgG titres above 3000 IU/L had measles recently. Importantly, 76% of persons aged under 22 had no or low titres of measles antibodies which can be evidence of the disturbed children vaccination schedule.

Conclusion

Many years of international experience in global measles control convincingly demonstrate the epidemiologic efficacy of measles vaccine prophylaxis if high vaccine coverage (95% and more) of cohorts subject to vaccination is achieved and maintained.

For example, in the Russian Federation the implementation of the program for measles elimination by 2010, developed in accordance with the WHO resolution, contributed to considerable reduction of measles incidence, strengthening of the national system of measles immunization, improvement of epidemiological surveillance of measles and other exanthema diseases. More than 95% of children in Russia have received two doses of live measles vaccine.

The study of measles immunity level in the Republic of Guinea showed that measles IgG were found in the majority of children and adults whose samples were examined in a laboratory. Every age group was found to include seronegative persons, however, as well as persons with low level of anti-measles antibodies. On the whole, we found 23.7% of examined patients of Kindia hospital to be measles seronegative. In 18–40-year-old patients the proportion of seronegative persons was 28.5±5.1 of all examined. This cohort is susceptible to infection with the measles virus and may contribute to maintaining and activization of the epidemic process in the case of measles epidemic outbreaks in the Republic of Guinea.

The World Health Organization states that even short-term disruptions of the vaccination schedule result in the growing number of individuals susceptible to the infection and increased risk of disease outbreaks that can otherwise be prevented through vaccination. To reduce the risk of major measles outbreaks in the regions free from Ebola virus the WHO recommends massive measles vaccination campaigns.

4. Assessment of anti-influenza activity of novel derivatives of camphor

In 2017–2018 we focused our study on the assessment of anti-influenza activity of novel derivatives of camphor. Previously, we identified derivatives of camphor as effective inhibitors of influenza virus replication in cell culture. In the course of current study, we have deciphered the mechanism of activity of the lead compound, camphecene (2-(E)-((1R,4R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene-aminoethanol) and identified its target (Stage 1). In an attempt to optimize the pharmacological properties of camphecene, a chemical library was constructed based on the scaffold of camphecene. The modifications included introduction of mono-and bicyclic heterocyclic moieties in place of the terminal hydroxyl group of camphecene (Stage 2).

1. Stage 1

1.1. Materials and methods

1.1.1. Viruses and cells

The A/Puerto Rico/8/34 (H1N1) influenza virus was obtained from the collection of viruses of Smorodintsev Research Institute of Influenza. In preparation for experimentation, the virus was propagated in the allantoic cavities of 9–11 day old chicken embryos for 48 hours at 36°C. The infectious titer of the virus was determined in MDCK cells (ATCC # CCL-34) using 96-well plates.

1.1.2. Animals

Female BALB/c mice, 6–8 weeks old, were obtained from the Russian Academy of Medicine's Rappolovo animal breeding facility (Rappolovo, Russia), and they were qua-

rantined for one week prior to experimental manipulation. Mice were fed standard rodent feed and were provided unlimited access to water. Animal experiments were conducted in accordance with the principles of proper laboratory animal care (Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington DC, 1996) and were approved by the Institutional Ethical Committee.

1.1.3. Virus resistance assay

Camphecene in serial concentrations (500–1.5 μM) was incubated with MDCK cells for 1 h at 36°C. The cell culture was then inoculated with influenza virus (moi 0.01) for 1 hour. Unbound virus was washed off with MEM and a fresh portion of camphecene was added. The plates were then incubated for 24 h at 36°C in the presence of 5% CO_2. The virus titer in the culture medium was determined in a separate passage after 48 hours of cultivation. To detect the virus, the medium (100 μL) was placed into the wells of a round-bottom plate, and an equal amount of a 1% suspension of chicken erythrocytes in saline was added. The reaction was evaluated after 60 min of incubation at room temperature. Each concentration of the compounds was tested in triplicate. Virus titers were plotted against the logarithm of concentration, and the IC_{50} values for each virus were calculated using GraphPad Prism software.

1.1.4. *In vitro* selection and analysis of resistant mutants

In order to study the development of resistance to camphecene, the A/Puerto Rico/8/34 (H1N1) (PR8) influenza virus was serially passaged in MDCK cells in the presence of increasing concentrations of the compound resulting in CF+ virus. Cells were infected with the virus and incubated for 3–5 days at 36°C with 5% CO_2 until a cytopathic effect was observed. The culture supernatants were centrifuged, and aliquots were used for sequential selection. Two initial passages were performed in the presence of 100 μM camphecene followed by four passages at 200 μM. The control virus (CF–) was serially passaged the same number of times in MDCK cells in the absence of the antiviral agent.

After the passaging procedure, we had three viruses: the initial PR8 that was not passaged; CF–; and CF (two wells each). Culture media from two parallel wells of these viruses were mixed and viruses were plaque-purified in MDCK cells. Three clones of each virus were propagated in chicken embryos in the absence or presence of 200 μM camphecene. Viral RNA was extracted using an RNAzol® kit (Gibco BRL). After reverse transcription, cDNA was amplified using four pairs of primers.

The cycling conditions were as follows: (i) denaturation (3 min at 95°C), (ii) 20 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 45 s and (iii) 30 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 60 s with final elongation at 72°C for 7 min. PCR products were sequenced using an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, USA) using the Big-Dye Terminator Cycle Sequencing Kit and ABI PRISM 3100-Avant Data Collection software provided by manufacturer. All sequences were compared with the amino acid sequence of A/Puerto Rico/8/34 virus (access code P03452).

1.1.5. Virus yield reduction assay

Camphecene stock solution (5 mM) was prepared in MEM with 1 μg/ml trypsin, serially diluted (1500 — 2 μM), and incubated with MDCK cells for 1 h at 36°C. Each concentration of camphecene was tested in triplicate. Cell cultures were then infected with A/Puerto Rico/8/34 (H1N1) influenza virus (moi 0.01) for 24 h at 36°C in the presence of 5% CO_2. Titers of virus in supernatants were determined by hemagglutination test after cultivating of the virus progeny in MDCK cells for 48 h at 36°C in the presence of 5% CO_2. The 50% inhibiting concentrations (IC_{50}) and the selectivity index (SI, the ratio of CC_{50} to IC_{50}) were calculated from the data obtained.

1.1.6. Transmission electron microscopy (TEM)

MDCK cells in 6-well plates were incubated with A/Puerto Rico/8/34 (H1N1) influenza virus, m.o.i. 100, at 4°C for 1 hour. Unbound virions were removed by washing the cells twice with cold MEM, the medium containing 100 μM camphecene and 1 μg/ml trypsin was added and cells were incubated for 1.5 hours at 36°C in 5% CO_2. In wells with control virus, MEM with trypsin (without camphecene) was added. After incubation, cells were collected from the wells, transferred into tubes, and centrifuged at 2000g for 15 min. Cell pellets were fixed with 1.5% glutaraldehyde in PBS overnight, followed by post-fixation with 1.5% OsO_4 for 1 h and uranyl acetate for 45 min at room temperature. They were then dehydrated in graded acetone and embedded in Epon/Araldit resin (Serva Feinbiochemica, Heidelberg, Germany). Thin sections (90 nm) were stained with lead citrate and examined in a JEM-100S electron microscope (JEOL, Tokyo, Japan) at 2000–50,000 magnification. The numbers of intact virions seen attached to inner endosomal membranes and the number of virions seen fusing their membranes with endosome membranes were counted. In each experimental group, 20 visual fields were analyzed containing 82 virions in the control specimen and 74 virions in camphecene-treated specimen.

1.1.7. *In vivo* experiments

In order to compare the virulence of the camphecene-sensitive and the camphecene-resistant viruses, mice (10 per group) were infected with three doses of either control or camphecene-resistant virus. Viruses were administered intranasally (50 μl volume). Each group was monitored daily for body weight and death for two weeks post inoculation. Additionally, two groups (n = 10 mice in each) were infected with 5×10^5 pfu of each virus. On days 3 and 6 post infection, five mice in each group were euthanized, their lungs harvested and homogenized in ten volumes of saline, and viral activities were measured in homogenates by titration in MDCK cells.

1.1.8. Statistical treatment of results

Viral titers were plotted against the logarithm of concentration, and the IC_{50} values for each virus were calculated using GraphPad Prism software. Survival rates were analyzed using the log rank Mantel–Cox test in Statistica 8.0 software. Viral titers in lung tissue, as well as animals' weight, were compared by one-way ANOVA. The numbers of virions counted using TEM were compared using the Student's t-test. P values of < 0.05 were considered statistically significant.

1.1.9. Molecular modeling

The interaction of camphecene with wild-type and mutant (camphecene-resistant) hemagglutinins was performed by molecular modeling as well as calculation of HA stability. The HA2 subunit was chosen as the key biological target. Two crystal structures were downloaded from the Protein Data Bank (Rose at al., 2017) (PDB codes: 1RU7 (Gamblin at al., 2004). Two polypeptide chains (two monomers of the HA trimer) were considered in the calculation, and the third one was excluded from analysis. Hydrogen atoms were added to amino acid residues and optimized at pH 5.0±0.2. All molecules of water were accounted for. The geometrical parameters of the protein

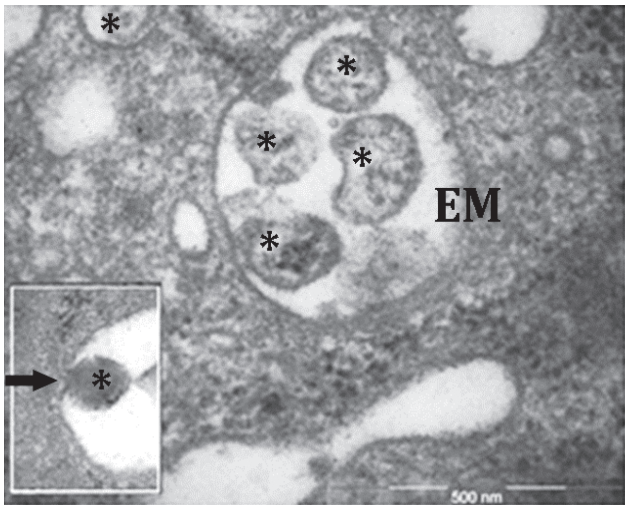

Figure 15. Influenza virions within endosomes at early stages of the viral cycle. Cells were infected with virus (m.o.i. 100) and incubated for 1.5 hours, following by processing for and analysis by transmission electron microscopy. Presented are virions within endosome in the cell cultivated with camphecene and virion in the process of membrane fusion (insert) in the endosome of the cell cultivated without camphecene. Virions are marked by asterisks; the site of membrane fusion by arrow; EM: endosomal membrane

and ligand were subjected to restrained minimization using the OPLS3 force field (Small-Molecule Drug Discovery Suite 2017–3).

Two binding sites were considered. The first was located in the binding region of tert-butyl hydroquinone (TBHQ), a well-known HA inhibitor (Russell at al., 2008). The second was found near the fusion peptide and contained valine at position 458 (615). Residue numbering here and thereafter corresponds to the 1RU7 PDB code indicated in brackets.

All docking procedures were carried out using the Schrödinger Suite 2017–3 software package (Small-Molecule Drug Discovery Suite 2017–3). Docking was performed under the following conditions: a flexible ligand and protein; standard prediction accuracy (IFD protocol and QM/MM-docking approach).

In order to dock the ligand to the hydrophobic space near V458, a grid-box was centered on the selected residue. Amino acids were refined within 5 Å of the ligand position. The binding energy (ΔGMM GBSA) of the ligand-protein complex was estimated using the variable-dielectric generalized Born model, which incorporates residue-dependent effects. Water was used as the solvent.

The V458L mutation in the HA2 (PDB code 1RU7) subunit of hemagglutinin was modeled using MM and QM methods. We estimated the total energy values of the HA2 of wild-type and mutant viruses, and we modeled the homodesmic replacement reaction of valine to leucine (at position 458) using the DFT method B3LYP with the 6–311G (d,p) basis set.

Table 12. Titer and IC_{50} of camphecene-susceptible and camphecene-resistant influenza viruses

Virus variant	Titer without compounds, $log_{10}ID_{50}$	Camphecene Cl_{50}, μM
Initial virus (PR8)	7.0±0.0	1.9±0.5
Control (CF–)	6.3±0.3	5.0±0.6
Camphecene-treated (CF+)	6.0±0.0	477.4±44.2

1.2. Results

1.2.1. Camphecene suppresses the fusion of viral and endosomal membranes

In order to visualize the effect of camphecene on viral morphogenesis, we performed an analysis of the ultrastructure of infected cells at early stages of the viral cycle, when the compound demonstrates the greatest activity. At this stage in both placebo-treated cells, numerous endosomal vacuoles were observed within the cytoplasm. Virions were localized within endosomes. They were seen in formations featuring attachment to the inner endosomal membrane or in structures showing fusion between viral and endosomal membranes. These typical formations are shown in Fig. 15.

These virions represented 15.9% of all viral particles analyzed (n = 82). Similarly, camphecene-treated cells contained numerous endosomes with virions. In contrast, the percentage of the virions in the process of membrane fusion was three-fold lower (5.4%, n = 74, p = 0.0032, t-test). However, the number of virions per endosome did not significantly differ (4.1±2.1 and 3.7±1.7 in placebo-treated and camphecene-treated culture, respectively; p = 0.5080, t-test). These results indirectly confirm that camphecene suppresses the process of membrane fusion in influenza virus-infected cells.

1.2.2. Camphecene-resistant virus can be selected after six passages in cell culture

The A/Puerto Rico/8/34 (PR8) influenza virus, which has previously been shown to be camphecene-susceptible, was serially passaged in the presence of increasing concentrations of camphecene (six passages in total) in order to obtain drug-resistant virus (CF+). The corresponding control virus (CF–) was serially passaged six times without drug. We then measured the sensitivity of all three viruses (initial PR8 virus, CF–, and CF+) to camphecene by titration in MDCK cells in the presence of various concentrations of camphecene. The IC_{50} values of camphecene were then calculated for each virus (Tabl. 12).

According to the results, all three viruses grew in MDCK cells with similar efficiency, while the camphecene-resistant virus was slightly less productive. Six passages in MDCK cells without additives did not change the camphecene susceptibility of the virus. At the same time, however, cultivation of influenza virus in the presence of camphecene induced, after six passages, an approximately 160-fold increase in resistance to the drug compared to the parent strain or virus passaged without the drug.

1.2.3. Camphecene-resistant virus acquires an V458L mutation in the HA2 subunit of hemagglutinin

After plaque purification, segment 4 from three clones of each virus variant (PR8, CF–, or CF+) were sequenced, and their amino acid compositions were compared with each other and with HA reference sequence from the A/Puerto Rico/8/34 influenza virus (Pub Med P03452). Sequence analysis results showed that variability existed among the initial virus (PR8) clones. Specifically, the changes seen (in relation to the reference strain) were: one of three clones contained an N104S substitution; one clone contained an A240T and I353V substitutions; and one clone contained an

L345I substitution. All three of the initial PR8 virus clones bore a P199S substitution. This discrepancy may reflect changes in the strain due to its long-term cultivation, under specific conditions, in our laboratory.

Variability was also detected among clones of the virus that was passaged without drug. One clone featured a D455E mutation, and one featured a G475R mutation. Like the initial PR8 virus, all three clones had P199S substitutions, compared to the P03452 reference sequence.

In contrast to the reference strain and all three clones of the control virus passaged without drug, all three clones of the camphecene-resistant virus contained leucine instead of valine at position 458 (G→T substitution, Fig. 16A). Thus, resistance of the influenza virus to camphecene is associated with the V458L mutation in hemagglutinin. For clarity, the location of the mutation is presented in a 3D model of viral hemagglutinin (Fig. 16B). According to the model, the V458L mutation is localized in the stem region of the HA2 subunit close to the fusion peptide and the site of HA proteolytic cleavage.

1.2.4. Molecular modeling results

In order to elucidate the structural mechanism behind camphecene-resistance in influenza, we performed molecular modeling experiments. In this part of the study, we examined the affinity of camphecene to wild-type HA binding sites as well as those featuring the V458L mutation. The total energies of both HA molecules types were also evaluated. According to theoretical calculations, the TBHQ inhibitor binding site located close to the fusion peptide is more favorable for camphecene binding. The docking score values are lower than values characterizing the binding between native ligand (TBHQ) and protein (Tabl. 13, entries 1–4). However, this active site is located at a distance of about 20 Å from amino acid V458. It seems improbable that camphecene would be the cause of the V458L mutation if it bound at this region of HA2 (Fig. 17).

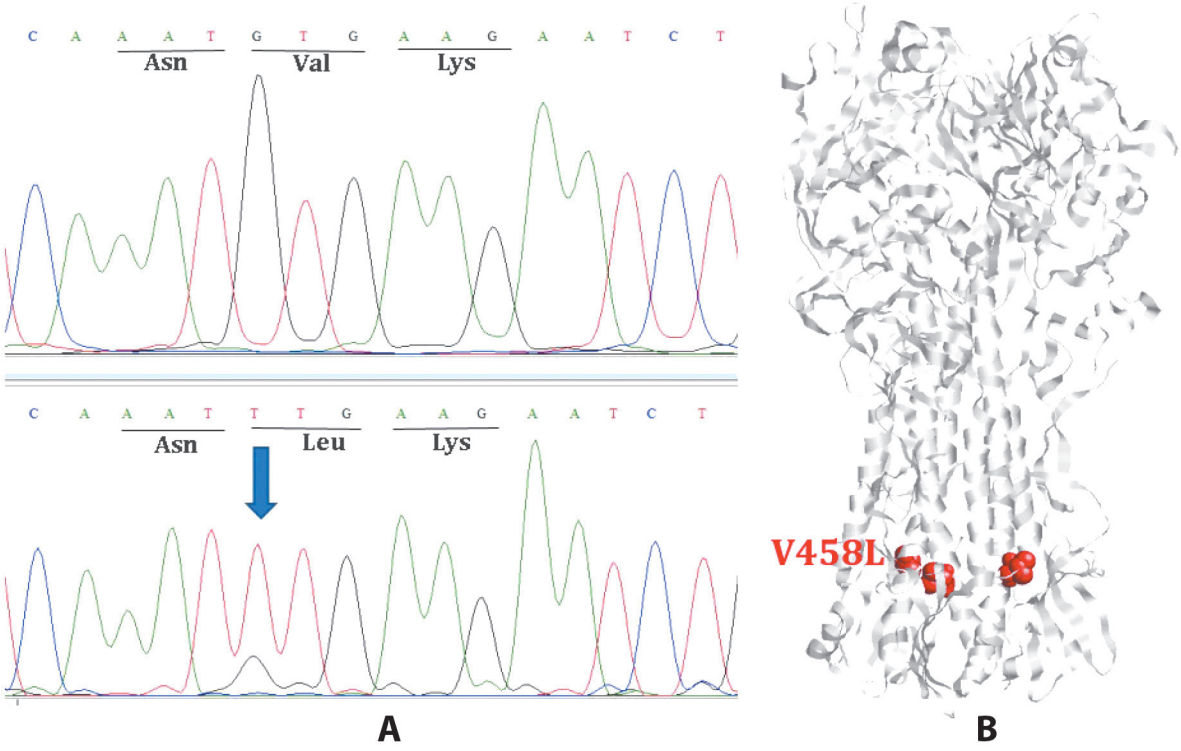

Figure 16. Influenza virus resistance to camphecene is associated with a mutation in HA gene. (A) Sequence analysis of the passaged viruses revealed a mutation, at amino acid position 458 in the hemagglutinin, that changes valine to leucine. (B) Amino acid substitution (marked red) in the hemagglutinin of camphecene-resistant mutant virus CF+ is localized near fusion peptide and proteolytic cleavage site. The substitution was found in three of three clones of resistant virus comparing to parent strain A/Puerto Rico/8/34 (H1N1) (PDB access number 1RU7)

Table 13. The results of the analysis of interaction of camphecene with influenza virus hemagglutinin in the binding sites of HA

Entry	Ligand[a]	QM docking score	GMM GBSA, kcal/mol	H-bond bridges between ligand and amino acids[b]
TBHQ binding site				
1	TBQH	–6.7±0.2	–35,3	none
2	Camphecene	–8.1±0.3	–45,0	Tyr295, Lys558
V458 binding site				
3	Camphecene	–8.0±0.4	–29.	Ile9, Val615
V458L binding site				
4	Camphecene	–5.7±0.5	–16,9	Thr31

[a]TBQH — tert-butyl hydroquinone. [b]The residue numbering corresponds to the PDB codes.

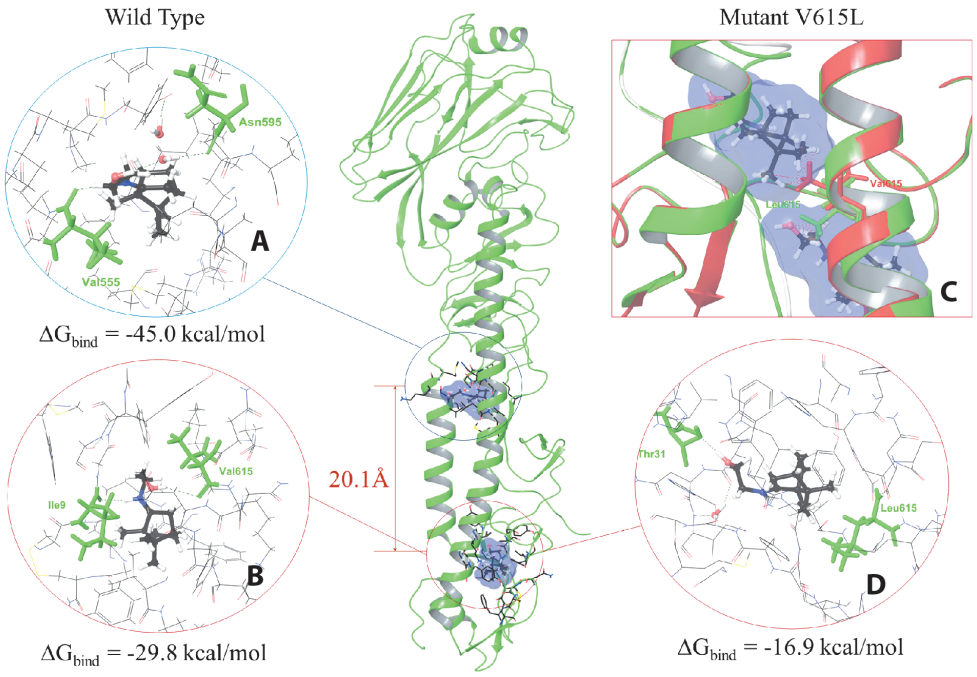

Figure 17. Binding sites for camphecene within influenza A virus HA. Two binding sites are labeled on the general model of HA (in the center). Orientation of camphecene molecule in first (A) and second (B) binding sites of wild type HA. Interaction of camphecene with first (C) and second (D) binding sites of V458L mutant. Binding energy for each site and distance between two sites are indicated. Amino acids numbering here corresponds to the 1RU7 PDB code

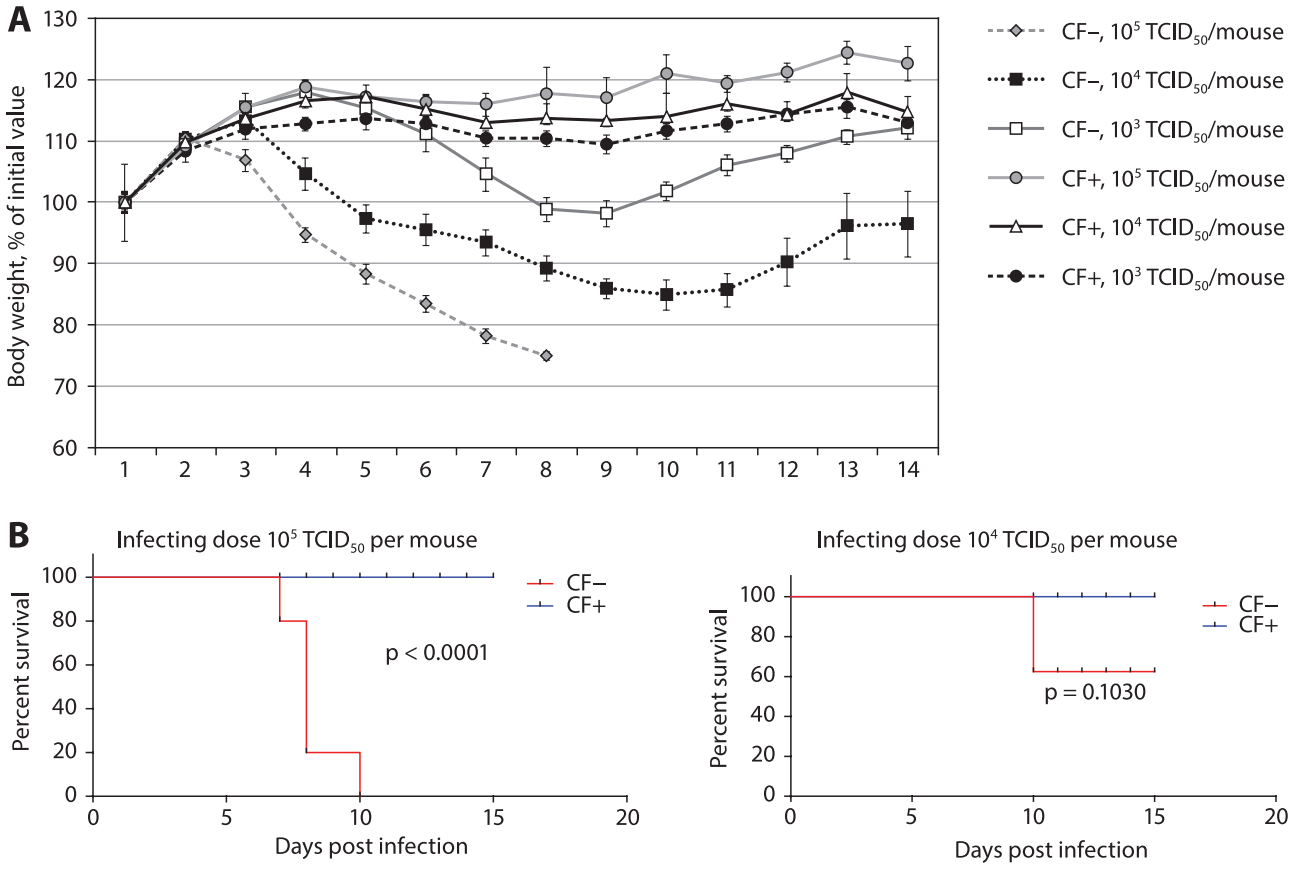

Figure 18. Serial passaging of influenza virus A/Puerto Rico/8/34 in presence of camphecene results in virus attenuation on the model of mouse influenza infection. Presented are the weight data (A) and survival curves (B) from mice infected with influenza virus A/Puerto Rico/8/34 (H1N1) that was previously passaged six times in MDCK cells with camphecene (camphecene-resistant, CF+) or with virus without camphecene pre-selection (camphecene-susceptible, CF–)

The second binding site was found in the stem region close to the proteolytic cleavage site. At this site, camphecene is surrounded by amino acid residues of two polypeptide chains and forms H-bonds with V458 and I9 (Fig. 17B). The QM-docking score values were comparable for ligand binding at both active sites (Tabl. 13, entries 3 and 4), but the binding energy (ΔGMM_GBSA) of camphecene and HA at the second site was higher by 15.2 kcal/mol compared to the ΔGMM_GBSA of ligand binding at the TBHQ active site.

The geometric parameters of the protein were slightly changed after V458L mutation (Fig. 17C), and the binding site of the ligand was shifted compared to its position in wild-type hemagglutinin. The binding of camphecene at L458 was unfavorable after mutation due to steric effects (these are shown as red dotted lines in Fig. 17). The ligand was located higher along the stem above leucine and formed an H-bond with T31 (Fig. 17D). The binding energy was higher by 12.9 kcal/mol than the camphecene binding energy with wild-type HA (Tabl. 13, entries 3 and 4). The binding of camphecene with mutant HA is therefore weaker than with wild-type HA that can serve as structural basis of drug-resistance.

It should be noted that the value of the total energy of the HA2 mutant was lower than that of the wild type (ΔE 28.4 kcal/mol, in favor of the V458L mutant). According to the quantum-chemical calculations, the homodesmic replacement process of valine to leucine is characterized by a negative energy value (data not shown). This allowed us to assume that the V458L mutation leads to a decrease in the total energy of this subunit of the HA molecule. This may have resulted in stabilization of the HA monomer and/or multi-subunit complex of the protein. Since the mutation is located near the cleavage site, it might have an impact on the proteolysis of HA in the course of the viral cycle.

1.2.5. The camphecene-resistant virus possesses lower pathogenicity in animals compared to the control strain

To assess the effect of camphecene resistance on the pathogenic properties of the virus, we inoculated Balb/c mice (10 per group) with three different doses of each virus and tracked weight changes and mortality in each group. Virus production in lung tissue was monitored as well.

Mice infected with the wild-type (camphecene-susceptible) virus showed dose-dependent mortality and weight loss. In contrast, infection of mice with camphecene-resistant virus did not result in either mortality or weight loss (Fig. 18). Moreover, when mice were infected with 5×10^5 pfu of either virus, the replication efficacy in lung tissue was about 1.5–2.0 orders of magnitude lower for the camphecene-resistant virus than for the camphecene-susceptible one (6.1±0.3 vs 4.2±0.2 $\log_{10}TCID_{50}$ on day 3 ($p = 0.0003$); 4.9±0.3 vs 3.4±0.2 $\log_{10}TCID_{50}$ on day 6 ($p = 0.0017$) (Fig. 19). Thus, acquiring resistance to camphecene is associated with a significant decrease in viral pathogenicity and attenuation of growth fitness in mouse lungs.

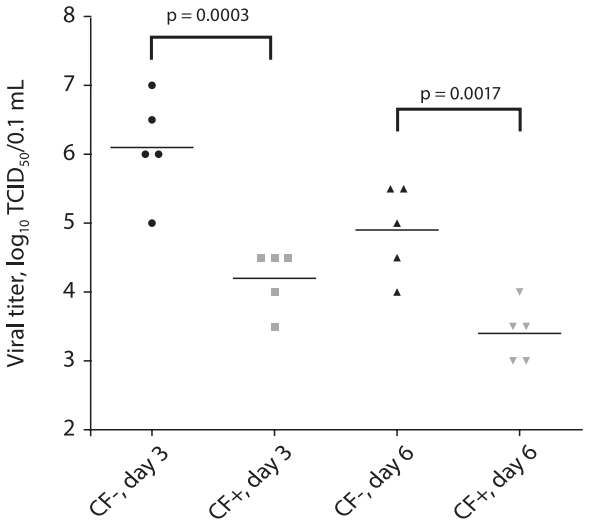

Figure 19. Lung viral titers of mice infected with camphecene-susceptible (CF–) and camphecene-resistant (CF+) influenza viruses. Mice (ten per group) were infected intranasally with 5×10^5 pfu of either virus. On days 3 and 6 p.i., five mice in each group were euthanized, their lungs isolated and homogenized in ten volumes of saline, and viral activity was measured in homogenates by titration in MDCK cells. Viral titers were compared between groups by one-way ANOVA

Stage 2

2.1. Materials and methods

2.1.1. Chemistry

Target compounds bearing a heterocyclic moiety were synthesised as shown in Fig. 20. The starting material camphecene was prepared as previously described. Subsequently, camphecene was treated with PBr_3 to produce key intermediate 1 that was used immediately used in further reactions. Key compounds 2–16 were prepared by nucleophilic substitution of secondary amines or thiols with bromide **1** in the presence of potassium carbonate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The structures of target compounds were confirmed using various spectroscopic methods, including ^1H and ^{13}C NMR (see ESI). As a result of synthetic modifications of camphecene, we obtained compounds containing the key pharmacophore structural block imine camphor's and saturated N-heterocycles: pyrrolidine **2**, piperidine **3**, 4-methylpiperidine **4**, piperidin-4-ol **5**, piperazine **6**, N-methyl- **7**, and N-ethylpiperazine **8** fragments. We synthesised agents containing five-membered heterocyclic fragments (4,5-dihydrothiazole **9**, N-methyl-imidazole **10**, and 1,2,4-triazole fragments **11**), separated from the imino group by the sulphur atom via an aliphatic linker. We have described compounds containing six-membered aromatic fragments — 4-chlorobenzene **12**, pyridine **13**, and pyrimidine **14** fragments and compounds with benzothiazole **15** and benzoimidazole **16** aromatic heterocyclic scaffolds.

2.1.2. Virus inhibition assay

The compounds were dissolved in 0.1 mL DMSO to prepare stock solutions, and final solutions (1000.0–4.0 μM) were prepared by adding MEM with 1 μg/mL trypsin. Compounds were incubated with MDCK cells for 1 h at 36°C. Each concentration of the compounds was tested in triplicate. The cell culture was then infected with influenza virus A/Puerto Rico/8/34 (H1N1) (moi 0.01) for 24 h at 36°C in the presence of 5% CO_2. A virus titer in the supernatant was determined by hemagglutination test after cultivating of the virus in MDCK cells for 48 h at 36°C in the presence of 5% CO_2. Rimantadine, Amantadine, Deitiforin and Ribavirin were used as reference drugs. For calculations, virus titer was expressed as per cent of the titer in control wells without compounds. The 50% inhibiting concentrations (IC_{50}) and the selectivity index (SI, the ratio of CC_{50} to IC_{50}) were calculated from the data obtained.

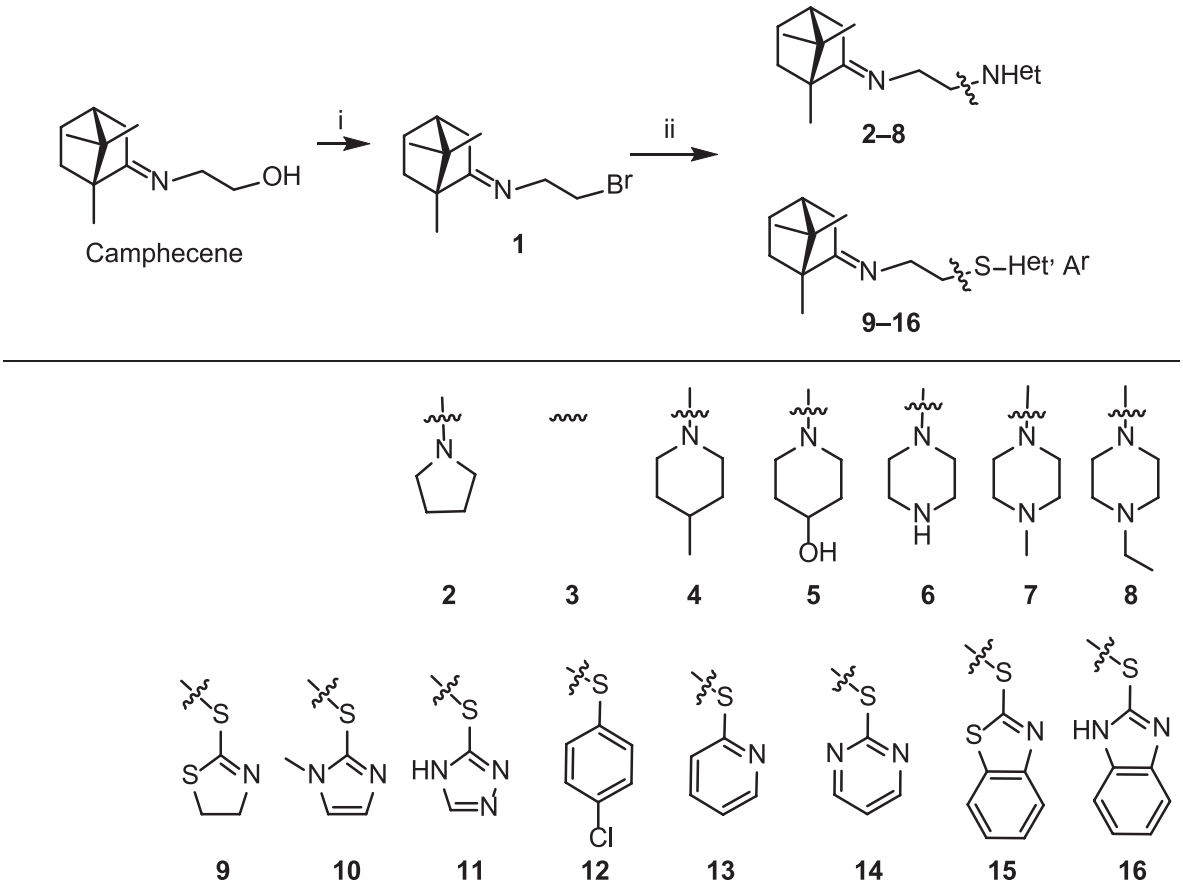

Figure 20. (i) PBr_3, Et_2O, room temperature (r.t.); (ii) appropriate amine or thiol, K_2CO_3, DBU, CH_3CN, r.t.

Table 14. Antiviral activity of camphecene-based compounds 2–16 against influenza virus A/Puerto Rico/8/34 (H1N1) in MDCK cells

Compound	CTD_{50}[a], μM	IC_{50}[b], μM	SI[c]
2	> 1260	12±2	106
3	> 1141	27±4	43
4	294±14	25±2	12
5	> 1031	NA	1
6	1196±90	37±5	33
7	1079±82	45±6	24
8	222±19	101±12	2
9	753±62	33±4	23
10	1126±63	341±46	3
11	197±15	24±3	8
12	170±12	47±6	4
13	503±38	6±1	91
14	> 1025	6±1	183
15	383±22	19±2	20
16	406±29	21±3	20
Rimantadine	335±27	67.0±4.9	5
Amantadine	284±21	64±5	4
Deitiforin	1266±82	209±15	6
Ribavirin	> 2000	25	> 81.0

[a]CTD_{50}, cytotoxic concentration; the concentration resulting in 50% death of cells.
[b]IC_{50}, effective concentration; the concentration resulting in 50% inhibition of virus replication.
[c]SI, selectivity index, ratio CC_{50}/IC_{50}.

2.1.3. Cytotoxicity assay

The microtetrazolium test (MTT) was used to study the cytotoxicity of the compounds. Briefly, series of three-fold dilutions of each compound in MEM were prepared. MDCK cells were incubated for 48 h at 36°C in 5% CO_2 in the presence of the dissolved substances. The cells were washed twice with phosphate-buffered saline (PBS), and a solution of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (ICN Biochemicals Inc. Aurora, Ohio) (0.5 μg/mL) in PBS was added to the wells. After 1 h incubation, the wells were washed and the formazan residue was dissolved in DMSO (0.1 mL per well). The optical density in the wells was then measured on a Victor2 1440 multifunctional reader (Perkin Elmer, Finland) at wavelength of 535 nm and plotted against the concentration of compounds. Each concentration was tested in three parallels. The 50% cytotoxic concentration (CC_{50}) of each compound was calculated from the data obtained.

2.1.4. Time-of-addition experiments

To determine the stage of the viral life cycle that is affected by the compound **14**, cells were seeded in 24-well plates and incubated with influenza virus A/Puerto Rico/8/34 (H1N1) (moi.10) for 1 h at 4°C. After washing for 5 min with 0.5 mL of cold MEM (+4°C) per well to remove unabsorbed virions, plates were incubated for 8 h at 36°C at 5% CO_2. The starting point of this incubation was referred as to 0 h. Compound **14** (final concentration 300 μmol/L) was dissolved in MEM and added to the cells at the following time points: –2 and –1 hpi (before infecting); 0 (simultaneously with infecting); 2, 4 and 6 hpi (after infecting). After 8 h of growth, the infectious titer of the virus in the culture medium and cells was determined as described above.

2.2. Results

2.2.1. Biological activity of derivatives

Among 15 tested compounds, 11 (73%) demonstrated a selectivity index (SI) higher than 10 and IC_{50} values in the micromolar range. The antiviral activity and toxicity were shown to strongly depend on the nature of the heterocyclic substituent. Compounds **2** and **14** demonstrated the highest virus-inhibiting activity with SIs of 106 and 183, and bearing pyrrolidine and piperidine moieties, correspondingly. Compound **14** was shown to interfere with viral reproduction at early stages of the viral life cycle (0–2 h post-infection). Taken together, our data suggest potential of camphecene derivatives in particular and camphor-based imine derivatives in general as effective anti-influenza compounds (Tabl. 14).

It should be noted that direct comparison of the values of SIs for compounds of different structural types, as a rule, does not make sense, since they can have fundamentally different pharmacokinetic properties *in vivo*. When determining promising compounds for further research, the most active compounds of each structural type should be selected, the SIs of which exceed 10.

Among the nitrogen-containing heterocyclic derivatives, compound **2** with the pyrrolidine fragment was the most efficacious against influenza virus (IC_{50} = 12 μM). Derivative **2** exhibited potent viral inhibitory activity together with low toxicity, which indicates a high therapeutic index (SI = 106). The antiviral activities of compounds with piperidine **3** and 4-methyl-piperidine **4** fragments were almost the same; however, compound **3** was much less toxic. The presence of a hydroxyl group in the piperine ring of compound **5** resulted in a loss of antiviral activity, although the compound remained non-toxic. Substances with piperazine **6** and N-methylperazine **7** fragments showed comparable antiviral activity and exhibited low toxicity.

Among compounds containing a five-membered nitrogen-containing moiety linked via a sulphur atom to the natural camphor backbone **9–11**, agents **9** and **11** exhibited antiviral activity, although compound **9** was significantly less toxic than **11**. The highest activity was exhibited by agents containing pyridine **13** (IC_{50} = 6 μM) and pyrimidine fragment **14** (IC_{50} = 6 μM). It should be noted that replacement of the pyridine-2-thiol fragment for the pyrimidine-2-thiol radical in compound **14** significantly reduced toxicity, while antiviral activity remained high. In the series of sulphides bearing benzothiazole **15** and benzoimidazole **16** rings, the observed toxicity and antiviral activity were the same.

To determine the suggestive target for virus-inhibiting activity of the most active compound **(14)** in the virus life cycle, time-of-addition experiments were performed. The results are summarised in Fig. 21. As suggested from the data, compound **14** appeared the most effective when added 0–2 h post-infection. Over time, the efficacy of the drug decreased, and starting from 4 h after infection, the infectious activity of the virus did not differ statistically from the control values. Based on these results, it can be assumed that derivative **14** acts in the initial stage of the life cycle of the influenza virus, which involves adsorption of virions on the surface of the target cell and penetration of the virion inside the host cell. At this stage, two viral

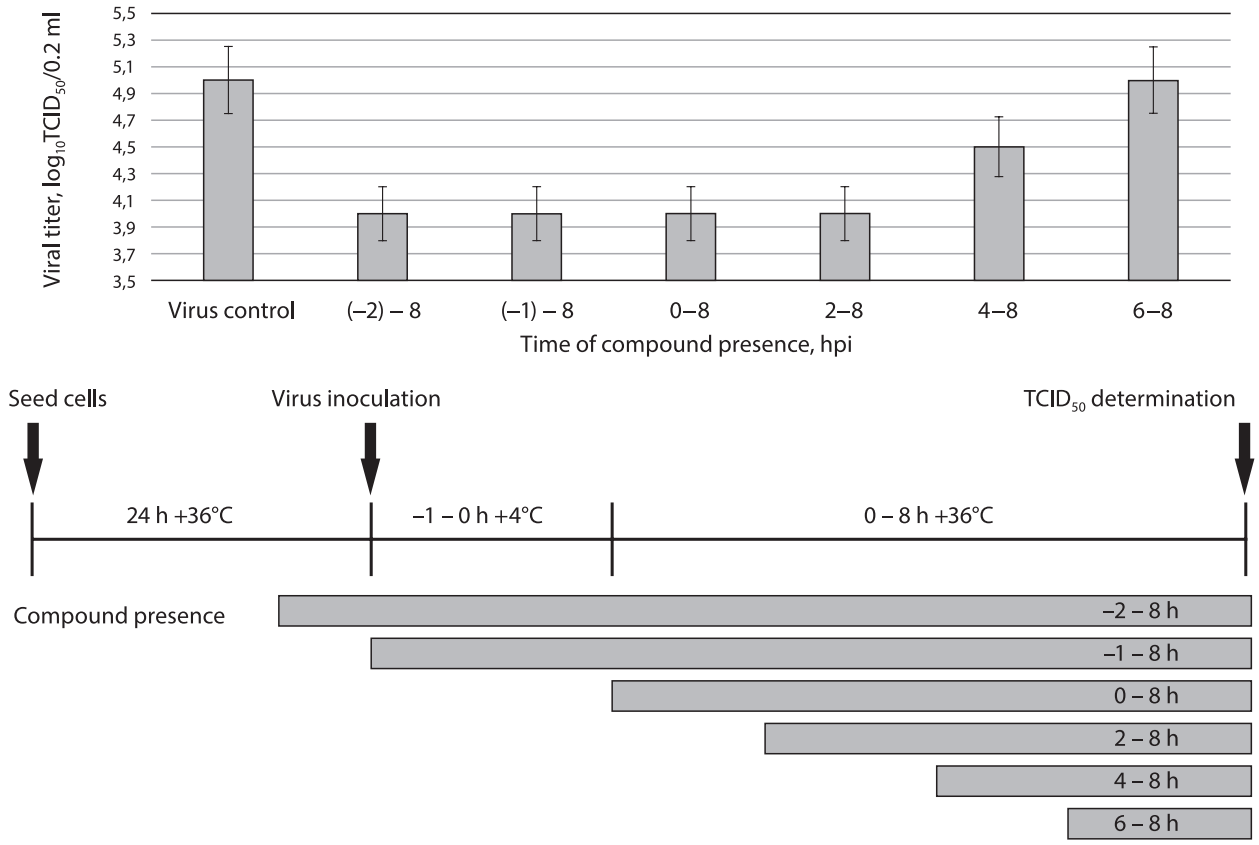

Figure 21. Time-of-addition activity of 14 against influenza virus A/Puerto Rico/8/34 (H1N1)pdm09. Influenza virus was absorbed onto MDCK cells for 1 h at +4°C, unbound virions were removed by washing, and the plate was transferred to CO_2-incubator (36°C) (t = 0). Compound 14 was added at the indicated time points. The infectious activity of viral progeny was tested by further titration in MDCK cells. hpi, hours post-infection

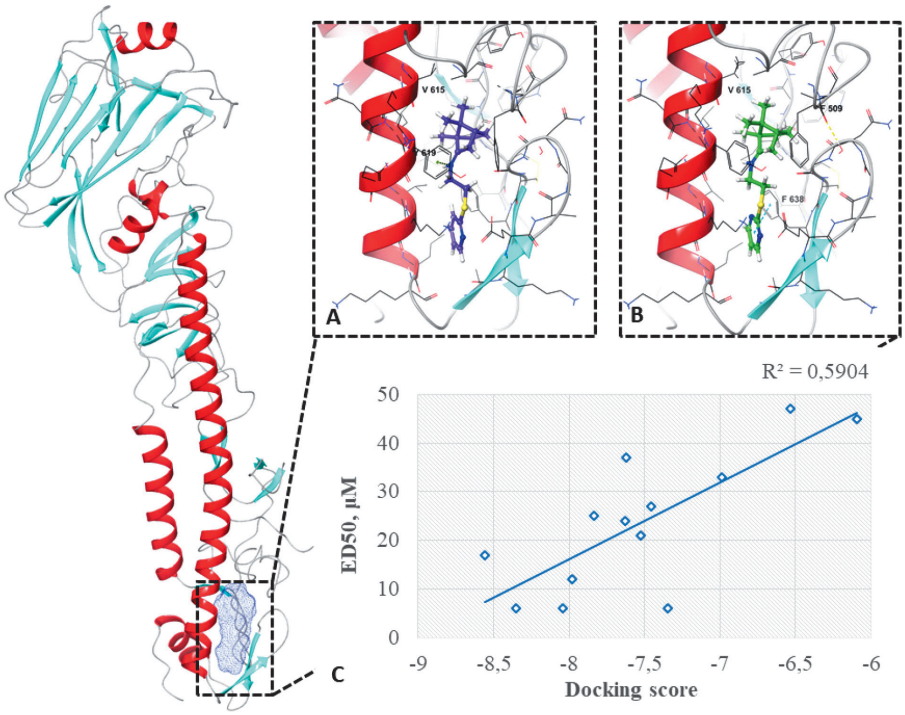

Figure 22. Molecular docking. A and B, locations of compounds 13 and 14 in the active site of HA2, respectively: π-cation interaction is shown as a green dotted line and π-π stacking in blue. C, correlation between ED_{50} values and the results of docking of the active compound

proteins are essential. First, viral HA allows for attachment of virions to the cell surface and fusion of the viral envelope with the endosomal membrane. Second, the virus-specific proton channel M2 conducts protons into the virion interior, thus providing acidification of the core and allowing dissociation of viral ribonucleoproteins from envelope structures. Further studies will be necessary to clarify the exact mechanism of anti-influenza activity of camphecene-related molecules.

2.2.2. Molecular docking study

Based on the results of the biological studies, HA may be a molecular target for compounds **2–16**. We considered a binding site located near the fusion peptide with valine at position 615 (PDB code 1RU7 [(2004) Science 303: 1838–1842, 10.1126/science.1093155]). We performed docking analysis of the most active compounds **2–16** (**8** and **10** were excluded as inactive) into the above-described active site and compared the results of the molecular docking and biological tests using a regression model. The dependence of the docking score on the ED_{50} value was considered. All stages of theoretical calculations (ligand and protein preparation, docking procedure) were carried out using Small-Molecule Drug Discovery Suite 2018–1 (Schrödinger, LLC, New York, NY, 2018).

Molecular docking demonstrated good correlation between the experimental data and theoretical calculations. The correlation index did not exceed 59%. This value can be considered as evidence that compounds **2–16** may be located in the active site of HA2 near the peptide fusion. The most active compounds **13** and **14** form π-cation and π-π stacking interactions with tyrosine at position 619 (Fig. 22).

Conclusion

In summary, we have assessed the ability of influenza virus to develop camphecene-resistance and studied the properties of resistant strain. We demonstrated that the selection for camphecene resistance is accompanied with dramatic decrease of viral fitness and pathogenicity for mice. We synthesized the set of camphecene analogs and demonstrated that some of derivatives, similar to camphecene itself, possess high anti-viral activity thus confirming the potent virus-inhibiting properties of camphor derivatives in general.

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Department of Immunology

LABORATORY FOR PATHOGEN IDENTIFICATION

Head of the Laboratory: Natalia Roschina
Researchers: L. Kouliasheva, A. Zakrevskaya, A. Svarval, K. Ermolenko

In 2017–2018, the Laboratory for pathogen identification analysed the seroepidemiological features of *H. pylori* infection in the population in 2007–2016. The prevalence of *H. pylori* infection among adults in 2007–2016 was 68.00±0.9%; the prevalence of CagA+ strains infection was 57.21±1.0%. A decade of research in the adults showed the trend towards *H. pylori* seroprevalence stabilising at a rather high level of 60.22–72%. The same is true for CagA+ *H. pylori* seropositivity rate, which is 48.15–61.91%. The prevalence of *H. pylori* infection among children and teenagers was 30.28±1.1%; infection with CagA+ strains was found in 27.81±1.1%. A decade of research in the pediatric population showed the trend towards *H. pylori* seroprevalence rate decrease from 45.74±4.4% in 2008 to 20.9±3.8% in 2016. The same is true for CagA+ *H. pylori* seropositivity rate, which decreased from 37.21±4.4% to 16.36±3.5% in 2008 and 2016, respectively. These high values of *H. pylori* infection seroprevalence in St. Petersburg in the last decade is evidence of the need for further prevalence monitoring that will help to justify strategies of treatment and prevention of *H. pylori*-associated diseases.

Research into genotypic features of *H. pylori* showed that the circulation of toxigenic strains consisting *cagA* in their genome remains high. For example, among the patients with stomach diseases the proportion of *cagA* is 75.59%, in the case of ulcer disease it is up to 87.23%. Together with the Institute of Experimental Medicine (St. Petersburg) the presence of pathogenic factor genes of *H. pylori* isolated from the stomach and duodenum was studied. The presence of pathogenic genes *vacA, cagA, cagE, cagH, ureC*, and urel was investigated. *UreC, urel,* and *vacA* genes were more often found in the samples of gastric mucosa (100%, 67%, and 89%, respectively) as compared to the samples from the duodenum (44%, 14%, and 43%, respectively). The *cagA* gene was found only in the samples from the gastric mucosa (33%). In two samples of *H. pylori* from the stomach 3 genes of the pathogenicity island were found at the same time, i. e. *cagA, cagI,* and *cagH*. On the whole, no direct correlation between gastrointestinal diseases and the set of pathogenic factor genes of *H. pylori* was found. The presence of the large number of pathogenicity genes in gastric samples can contribute to the development of oncological diseases in this area which requires case follow-up of the patients with this set of genes.

Together with the specialists of the Kirov Military Medical Academy the influence of *H. pylori* pathogenicity factors *cagA* and *vacA* on the activity of gastric mucosa inflammation as well as on the development of atrophic processes in it. 40 patients with chronic gastritis aged 30 to 60 (mean age 44.1±7.9 years) were examined. To verify the process activity in the gastric mucosa light microscopy with the count of polymorphonuclear leukocytes was used. The presence of determinants of *H. pylori* pathogenicity factors *cagA* and *vacA* in the gastric mucosa bioptates was found using the RT-PCR with the test systems produced by ZAO "Sintol", Moscow. Determinants of *H. pylori* pathogenicity factors *cagA* and *vacA* were found in the majority of patients with chronic gastritis (in 72.5%); at the same time active gastric mucosa inflammation was mainly associated with these factors (86.7%). It was also found that the presence of *H. pylori cagA* and *vacA* had no impact on the presence and severity of atrophic changes of the gastric mucosa.

Together with the Federal state state-financed scientific institution "Institute of Experimental Medicine", St. Petersburg State University, and Sokolov Clinical Hospital No. 122 we studied the features of stomach microbiocenosis associated with the Helicobacter infection. The objective of our work was to compare stomach microbiota in patients with dyspepsia associated and not associated with Helicobacter infection. The clinical examination was carried out on 21 patients with dyspepsia. All patients underwent gastroscopy with biopsy collection from the gastric body and antrum. The presence of *H. pylori* was confirmed by genetical and bacteriological methods. Gastric microbiota was analyzed using RT-PCR. Herpes virus was identified using Amplisens® Multiplex Real-Time kits. The patients were divided into two groups: H.p. (+) — 10 patients with confirmed *H. pylori* infection; H.p. (–) — 11 patients without *H. pylori* infection. The average quantitative content of *Enterobacter, Proteus, Klebsiella,* and *Staphylococcus* was higher in the samples of the H.p. (+) patients. The quantity of *Lactobacillus* spp., *Bacteroides* spp., and *Feacalibacterium* spp. was lower in this group. Cytomegalovirus and herpes simplex virus were not found in either group. Epstein–Barr virus was found only in patients with *H. pylori* (3 samples). Human herpes virus 6 was found in 1 case both in the group of H.p. (+) and H.p (–) patients. It may be noted that in the case of Helicobacter infection gastric dysbiosis usually develops manifesting in the increased number of opportunistic pathogenic bacteria and decreased number of beneficial bacteria. The presence of herpes virus in gastric bioptates is more typical of the patients with *Helicobacter pylori* infection.

In 2017–2018, we continued to study the seroepidemiological features of infection with sexually transmitted disease (STDs) causing agents in the population of St. Petersburg and the Northwestern Federal District (NWFD) including children and teenagers of St. Petersburg, and to research into molecular and biological properties of the strains of STD-causing agents in the NWFD.

The genotype spectrum of the human papilloma virus (HPV) DNA (types 16, 18, 26, 31, 33, 35, 38, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, and 82), their prevalence and viral load of patients from St. Petersburg was studied. The age of the probands ranged from 17 to 50 years. 351 patients including 279 females and 72 males were examined. When examining patients with history of HPV or suspected HPV the genotype and viral load were determined. Analysing the prevalence of HPV genotypes among women of childbearing age showed that HPV type 16 was predominant (32.8%). It was followed by: type 51 — 23.7%, type 56 —

22.3%, type 31 — 17.2%; 53, 73 and 68 type HPV was found with the same prevalence of 15.6% each, type 33 — 14.8%, type 66 — 13.1%, type 18 — 13.1%, type 39 — 11.5%, types 52 and 45 — 9.8%, type 59 — 9%, other genotypes (3, 82 and 26) were found in less than 5% of cases. The prevalence of oncogenic types in men was as followed: genotype 59 (10%), then 31 (8%), types 16, 18 and 56 were found with the same prevalence of 6.8% each, the other genotypes were found in 5% of cases or less.

Monoinfection among female patients with HPV was found in 52.8% of cases, multi-infection with 2 virus types in 24.7%, with 3 and more genotypes — in 22.47% of cases. In men, monoinfection was found in 65% of examined patients, in 23.5% of cases 2 genotypes of oncogenic HPV were found. HPV virus load in infected women ranged between 1.8 and 7.8 log of HPV per 10^5 cells. The maximum viral load in men was 1.2 to 4.7 log of HPV per 10^5 cells.

The prevalence of asymptomatic shedding of the Epstein–Barr virus (EBV) in women with genital herpes with a relapsing course depending on the stage of the infectious process and the composition of the vaginal microbiota was studied. In patients with severe genital herpes virus shedding was found both during disease recurrence (28% of cases) and during remission (18% of cases). In the case of moderate course during remission and against the background of bacterial vaginosis EBV was isolated in 23% of cases and after the treatment — in 8%. This is explained also by type Th2 of the immune response and insufficiency of the local antiviral immunity in the case of bacterial vaginosis. It was found that the level of the local and systemic antiviral immunity response can "switch on" the mechanism of asymptomatic EBV shedding.

Together with the Pavlov St. Petersburg state medical university the test on seropositivity of children to HHV 7 was carried out for the first time in Russia. 116 children from 10 days to 10 years old were examined. It was found that 18% of examined patients aged up to 10 years contacted the infectious agent, and the number of HHV 7 positive children increases among children above 4 years of age. The rather high frequency of identifying HHV 7 positive children at birth is possibly due to the presence of specific maternal antibodies. It is not unexpected that by 6–12 months of life the number of seropositive children decreases and remains at this level up to 4 years of age. Data on high frequency of simultaneous identification of HHV 6 and HHV 7 antibodies are of interest. Comparative analysis of HHV 7 and HHV 6 seropositivity showed higher prevalence of HHV 6 infection among the examined children of all age groups which is evidence of later contact of children with HHV 7 than with HHV 6. Taking into account different frequency of isolating (probably maternal) antibodies to HHV 6 and HHV 7 in children in their first weeks and months of life, it is fair to assume that in the adult population the prevalence of HHV 6 is also higher. On the other hand, breast-feeding and the child's receiving maternal specific antibodies is believed to be a protective factor which may cause later development of the primary HHV 7 infection as compared to HHV 6.

Together with the Institute of Experimental Medicine (St. Petersburg) research into antiviral activity of enterocins and probiotics was started. The findings show that antiviral activity of enterocins and *E. faecium* L3 strain on the reproduction of type 1 simple herpes virus is comparable with the antiviral therapy *in vitro*.

Therefore, the results of investigation of the oncogenic papilloma virus spectrum are evidence of marked viral load in the examined women which increases the risk of dysplastic changes in this group. The conducted examinations showed that the structure of HPV genotypes in the women of St. Petersburg was dominated by the genotype 16; genotypes 51, 56, and 31 were also common. In males, genotypes 59 and 31 were the most common. The total prevalence in the women of St. Petersburg of HPV types 16 and 18 that are covered by the existing vaccines is 45.9%. The findings of research into the asymptomatic EBV shedding suggest that the relapsing course of genital herpes creates the conditions for persistence and shedding of one more representative of herpes virus family, i. e. EBV, from the genitourinary tract organs against the background of secondary immune insufficiency. This phenomenon can be seen during relapse and, although less frequent, during remission. Taking into account the respective independence of the local immunity of vagina, bacterial vaginosis can be considered as one more factor possibly contributing to the development of this condition. Also, as up to 20% of urethrites in men are associated with EBV, we see it fit to assess the shedding of the infectious agent during relapse and remission of genital herpes in these patients. The obtained results show that the decrease in the level of the systemic antiviral immune response and similar changes of the local immunity can contribute to the development of conditions for asymptomatic EBV shedding as well as show the need for further investigation of this phenomenon. These findings of assessing seropositivity to HHV 7 in children are consistent with the contemporary view of HHV 7 prevalence in the population. At the same time, our data showed a significantly less seropositivity to HHV 7 among children than according to the foreign literature, meaning that further research is needed.

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LABORATORY OF MOLECULAR IMMUNOLOGY
Resource Sharing Centre

Head of the Laboratory: Areg Totolian
Researchers: N. Arsentieva, O. Batsunov, V. Drobychevskaya, E. Zueva, N. Lubimova, Yu. Ostankova, O. Petrova

The Laboratory of molecular immunology is engaged in research in clinical immunology, including immunology of infectious diseases, as well as study of infectious agents. The head of the Laboratory is director of the Institute, member of the Russian Academy of Sciences, doctor of medical sciences, professor Totolian Areg Artemovich. The laboratory personnel are highly qualified specialists in various medical and biological specialities: medical doctors, biologists, including geneticists and immunologists, and biotechnologists. A resource sharing centre works on the base of the Pasteur Institute. The centre houses equipment enabling state-of-the-art research. The devices used are: NovoCyte (ACEA Biosciences) and FACS Canto II (Beckton Dickinson) flow cytofluorimeters, MagPix (Millipore) multiplex analyzer based on xMAP technology (Luminex), MALDI-TOF (Brucker Daltonics) Microflex time-of-flight mass spectrometer with matrix laser desorption/ionization, GenomeLab XP (Beckman Coulter) genetic analyzer and Pyromark Q24 (Qiagen) genetic analysis system, Ettan IPGphor 3 IEF System and Ettan DALTsix Large Vertical System for 2D-electrophoresis (GE Healthcare), Biomek 4000 (Beckman Coulter) automated workstation etc.

The laboratory team are involved in the research projects aimed at immunological and molecular biological diagnosis of primary immune deficiencies (PID). The Centre for Diagnosis and Treatment of Primary Immune Deficiencies (PID Centre) was established on the base of the resource sharing centre. The PID Centre studies lymphocyte subpopulations, including small subpopulations of B- and T-cells, granulocyte oxidative burst and phagocytosis using flow cytofluorimetry. Molecular genetic methods are used to detect mutations in the process of PID diagnosis confirmation. *btk, rag1, rag2, cybb* gene sequencing is carried out. The diagnostic algorithm of PID now includes quantitative assessment of TRECs and KRECs (excision circles) as a screening method of PID detection. Research and diagnostic work of the PID Center is carried out along with clinical work in the follow-up polyclinic department of the Institute.

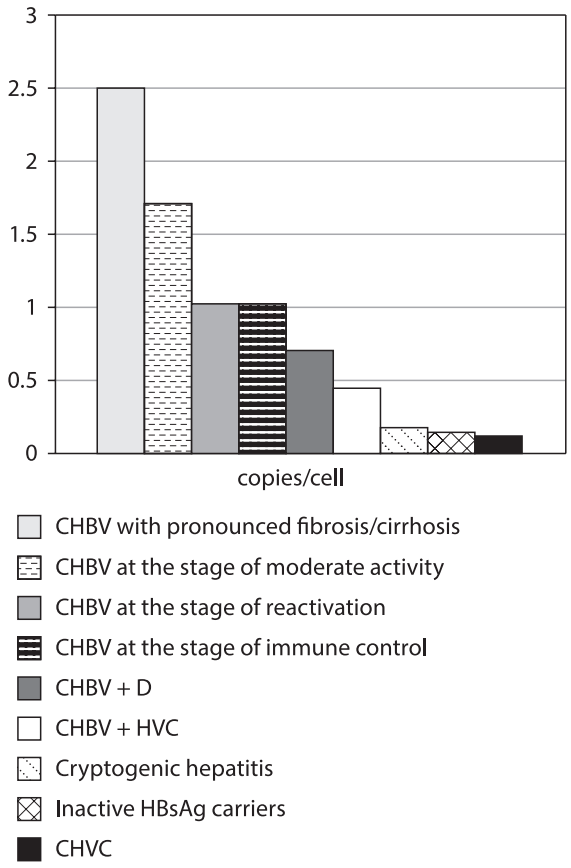

Figure 23. Quantitative assessment of the covalently closed circular DNA of HBV in the liver puncture bioptate tissue

The study and comparative analysis of the content of cytokines and chemokines involved in the antiviral immune response in the peripheral blood of patients with chronic viral hepatitis B (CHBV), including in groups of patients with different stages of liver fibrosis, are conducted. Mechanisms of chronicity and features of immune response to hepatitis B virus are investigated. This paper considers the expression of chemokine receptors on various lymphocyte subpopulations. Further study of the expression of chemokine receptors and activation markers on the surface of key cells of the immune system involved in the control of viral infection will allow a deeper understanding of the mechanisms of interaction between a microorganism and the immune system.

Also, work is in progress on the measurement the concentration of cytokines/chemokines in the blood plasma of patients with autoimmune liver lesions. The results of scientific research on this subject have been presented at various scientific conferences.

We continue to search for laboratory markers of liver damage of different origin. This research project is intended to take several years and includes two methodological approaches, i.e. the genomic and proteomic one. Test system for quantitative assessment of the covalently closed circular DNA (cccDNA) of the hepatitis B virus in the liver tissue (Fig. 23) was developed and tested. The prevalence of hepatitis B not detected by routine tests in HIV-positive persons is studied.

Studies focusing on viral hepatitis elimination assessed the significance of migration processes for the spread of viral hepatites, including HBsAg-negative HBV, with genotypes/sub-genotypes not typical of the host region.

One more line of research is the prevalence assessment of HBsAg-negative (occult) HBV both in the population and in various risk groups as well as in blood donors which is of particular importance due to high infectivity of the pathogen even with low viral loads. To enable such studies we developed and tested a method based on dual asymmetric PCR with subsequent sequencing that enables the identification of HBsAg-negative HBV with low viral load in blood plasma. A PCR-RT test system is being developed to make possible the diagnosis of HBsAg-negative HBV in clinical practice.

A doctoral thesis on molecular and immunologic markers of liver damage associated with chronic viral hepatites was written (A.V. Semyonov). The study objective was to assess the relevance and informational value of molecular-biological and immunological markers of liver damage associated with chronic viral B, B+D and C hepatitis to identify the stage of liver fibrosis and outcome prognosis. An algorithm for staging liver fibrosis in patients with chronic viral C hepatitis using three cytokines was developed (Fig. 24). The research resulted in a successful thesis defence in 2017, and A.V. Semyonov was awarded the title of the doctor of biological sciences.

A candidate's thesis on molecular-genetic features of the hepatitis B virus in HBsAg-positive and HBsAg-negative (occult) disease forms was prepared (Yu.V. Ostankova). The research resulted in a successful thesis defence in 2018, and Yu.V. Ostankova was awarded the title of the candidate of biological sciences.

The laboratory continues to study the prevalence of allelic variants of the CCR5 genotype in the population of St. Petersburg. CCR5 is an important component of the inflammation in response to infection. Identifying the deletion frequency in the CCR5 gene may contribute to more specific prognosis of AIDS incidence in the Northwestern region. Simultaneously with the studies of the CCR5 polymorphism prevalence, the polymorphism of one more protective gene, i.e. CCR2, is being studied using the same sample of the human population that was examined for CCR5 polymorphism. An original method of identifying the CCR2 allelic state using pyrosequencing was developed.

Together with the other laboratories of the Institute research has been conducted in the following projects: genome analysis of the hepatitis B strains circulating in the territory of the Northwestern Federal District of the RF (together with the Viral Hepatitis Laboratory). Sequencing and comparative phylogenetic analysis of HBV strains are carried out. Sequencing of Cor/preCor and preS/S areas of the HBV genome enables the genotyping of the isolated virus and the research into HBV resistance (primary and secondary) to antiviral drugs. Accumulation of data on HBV molecular epidemiology is part of the program for AHBV elimination in the Northwestern Federal District. Based on the results of sequencing of HBV strains a thesis for a candidate degree in biological sciences was written.

The prevalence of drug resistance mutations of the hepatitis C virus in the NS3, NS5a, and NS5b regions is assessed in patients who received antiviral therapy with direct-acting drugs.

As part of the research on "developing a method of express identification of pathogen *Leptospira* strains based on MALDI-TOF mass spectrometry" (conducted together with the Laboratory of Zooanthroponotic Infections of the St. Petersburg Pasteur Institute) studies were carried out aimed at creating a data base of reference mass-spectra of proteins from cell lysates of *Leptospira* spp. strains from the collection of the St. Petersburg Pasteur Institute. A method of identification of *Leptospira* serological variants using MALDI-TOF mass-spectrometry was developed (Fig. 25).

Multiplex analysis is used to study blood serum samples of patients with history of leptospirosis. The level of chemokines and cytokines is also measured, including the cases of severe and lethal leptospirosis. The collection of *Leptospira* sp. strains was described with the focus on 16S rRNA.

Yersinia enterocolitica-like bacteria are identified using MALDI-TOF mass-spectrometry. Species diversity of *Yersinia* makes identification based on phenotypical features more difficult, and the bacteria are often considered to be *Y. enterocolitica* because of an insufficient number of biochemical tests in commercial test systems. We created our own collection of mass-spectra of *Yersinia* spp. reference strains that are included in the microagglutination test kit, and this collection is used during collection subculturing to check that the composition of specific peaks in the mass-spectra of *Yersinia* reference strains has not changed.

Within the framework of implementation of the Russian governmental decree on Russia-Guinea scientific and technological cooperation the laboratory researchers study the prevalence of serological and molecular-biological markers of viral hepatites (A, B, C, D, E) and HIV among apparently healthy residents of the Republic of Guinea and in the risk groups. Genotypic/subgenotypic structure and mutations of the identified viruses is analyzed. The prevalence of HBsAg-negative (occult) viral hepatitis B is assessed.

Research within the framework of Russian-Vietnamese cooperation also has its primary focus on socially significant diseases such as viral hepatites and HIV. We work to assess the prevalence of markers of enteric and paren-

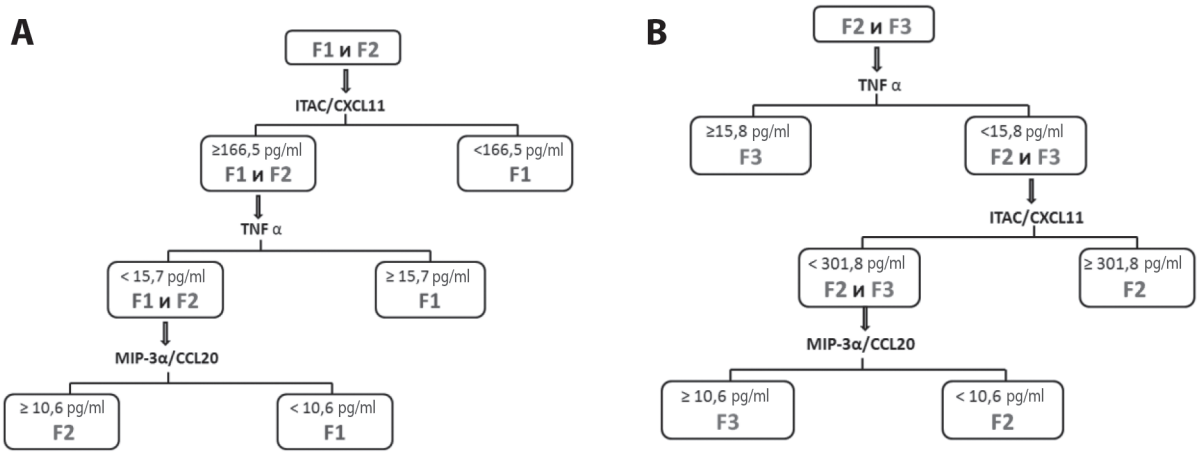

Figure 24. Decision tree for discerning between groups of patients with CHCV and different stages of liver fibrosis (F). Figure A shows the decision tree for discerning between groups of patients with CHCV and F1 or F2, while figure B shows the decision tree for discerning between groups of patients with F2 and F3

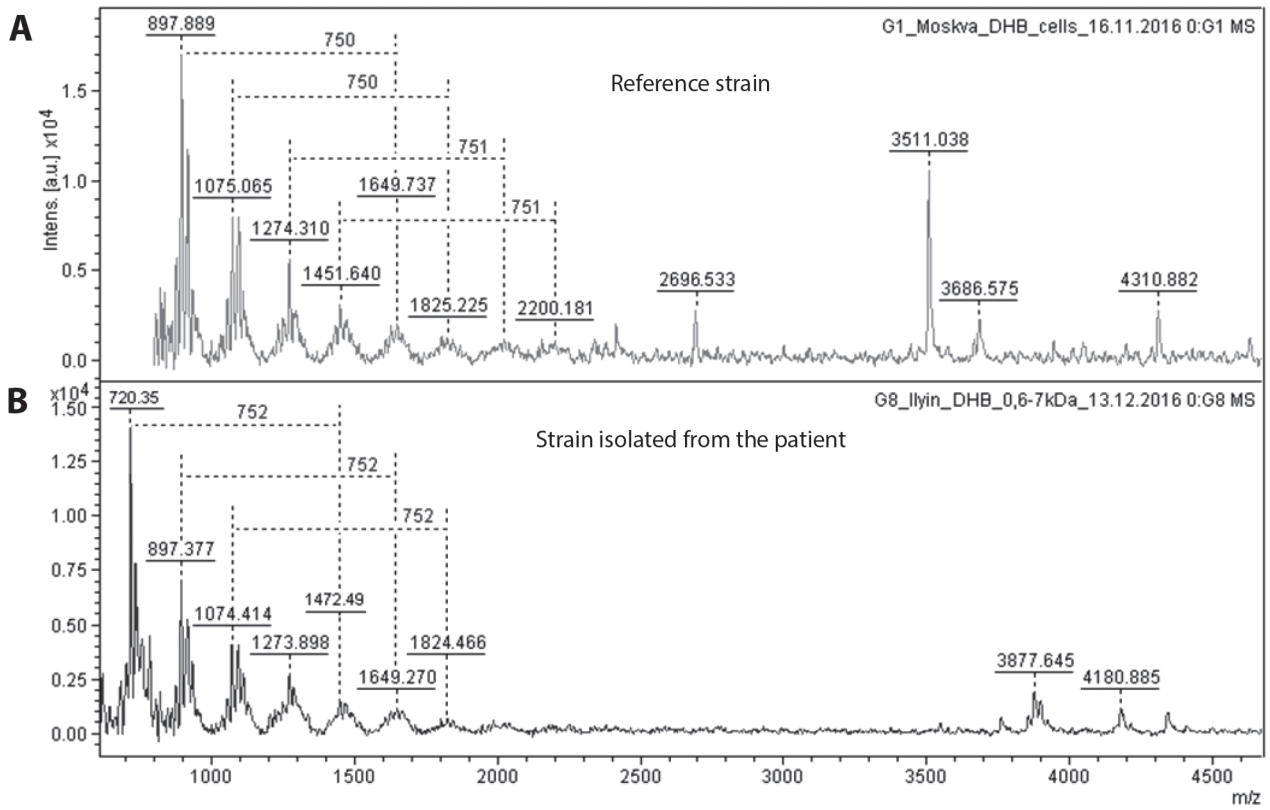

Figure 25. Comparison of spectral profiles of repeating subunits of O-antigen polysaccharide chains in a reference strain of the grippotyphosa serological variant (A) and strain isolated from a patient (B)

teral viral hepatites as well as analyze the genetic structure of hepatitis and HIV viruses in Vietnam.

The resource sharing centre is engaged in scientific cooperation with the following organizations:

- Federal state state-financed institution Research institute of Experimental Medicine of the Northwestern branch of the Russian Academy of Medical Sciences (immunology department), St. Petersburg;
- State state-financed educational institution of higher professional education (GBOU VPO) St. Petersburg State Pediatric Medical University (subdepartment of adults' infectious diseases and epidemiology), St. Petersburg;
- GBOU VPO St. Petersburg State Pavlov-Medical University (subdepartments of infectious diseases, pneumology, otolaryngology, centre of molecular medicine), St. Petersburg;
- GBOU VPO Russian State Medical University (subdepartment of pediatrics), Moscow;
- Federal state state-owned military educational institution VPO Kirov Medical Military Academy of the Defense Ministry of the Russian Federation (subdepartment of infectious diseases), St. Petersburg.

Two theses for a candidate degree in biological sciences are planned and in progress in the laboratory.

The laboratory team take active part in presenting their scientific findings at leading Russian and international conferences. In 2017–2018, 80 oral and poster presentations were made and 123 conference papers published.

Publications

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Patent

1. *METHOD FOR IDENTIFYING SEROVARS OF LEPTOSPIRA BACTERIA USING MALDI-TOF SPECTROMETRY. Zueva Elena Viktorovna, Stoyanova Nataliya Aleksandrovna, Tokarevich Nikolaj Konstantinovich, Totolian Areg Atemovich No. 2017102864/10 (004987), 01/27/2017. Patentee: St. Petersburg Pasteur Institute.*
2. *METHOD FOR HEPATITIS B VIRUS DNA IDENTIFICATION IN BIOLOGICAL MATERIAL WITH LOW VIRAL LOAD BASED ON TWO-STAGE PCR. Ostankova Yuliya Vladimirovna, Semenov Aleksandr Vladimirovich, Totolian Areg Artemovich No. 2016144898 (2633755), 17/10/2017. Patentee: St. Petersburg Pasteur Institute.*

Department of Epidemiology

LABORATORY OF EPIDEMIOLOGY OF INFECTIOUS AND NON-INFECTIOUS DISEASES

Head of the Laboratory: Liudmila Lyalina
Researchers: J. Terentieva, V. Kaziahmedova, V. Vetrov

In 2017–2018, the Laboratory of Epidemiology of Infectious and Non-infectious Diseases took part in the research work in the following fields:

- epidemiological justification and efficacy assessment of vaccination against papillomavirus infection and malignant tumors associated with the human papillomavirus;
- epidemiology of measles and rubella at the stage of their elimination in the Northwest of Russia;
- implementation of the acute viral hepatitis B elimination program in the territory of the Northwestern Federal District of Russia; and
- epidemiology of tuberculosis and drug resistance of *M. tuberculosis* in Leningrad Region.

Papillomavirus infection

In the Russian Federation, vaccination against papillomavirus infection has been carried out since 2007; it is not, however, included in the national immunization schedule. In 2017–2018, we continued to study the spread of diseases associated with the human papillomavirus (HPV) in St. Petersburg and Leningrad Region in the setting of low HPV vaccination coverage. Vaccine was given to those aware of the disease at their sole expense. In 2017, 770 persons completed the entire vaccination course in St. Petersburg; in 2018 the figure was 268 persons (predominantly females).

The incidence rate of anogenital condylomas is an early criterion used for efficacy assessment of vaccination against human papillomavirus. Fig. 26 shows the incidence of anogenital condylomas in St. Petersburg and Leningrad Region in the context of low vaccination coverage. The incidence in the metropolitan city St. Petersburg and the neighbouring Leningrad Region differs significantly. In 2017 and 2018, for anogenital condylomas it was 52.9 and 55.4 per 100,000 of population in St. Petersburg, whereas in Leningrad Region it was 19.4 and 17.0 per 100,000. In St. Petersburg there is a trend towards growing incidence rate which means low vaccination coverage has no effect at the population level.

Anogenital condylomas have been found both in male and female patients and in all age groups; singular cases have even been recorded in children (Fig. 27). The main risk group includes young men and women aged 18–29: in 2017 and 2018, in this age group the incidence rate in Leningrad Region was 9.5 and 8.4 per 100,000 persons, respectively. In patients aged 30–39 the incidence of anogenital condylomas was several times lower (5.3–4.0 per 100,000). Among 15–17-year-old teenagers the incidence ranged from 1.6 (2017) to 1.7 (2018) per 100,000 persons of this age.

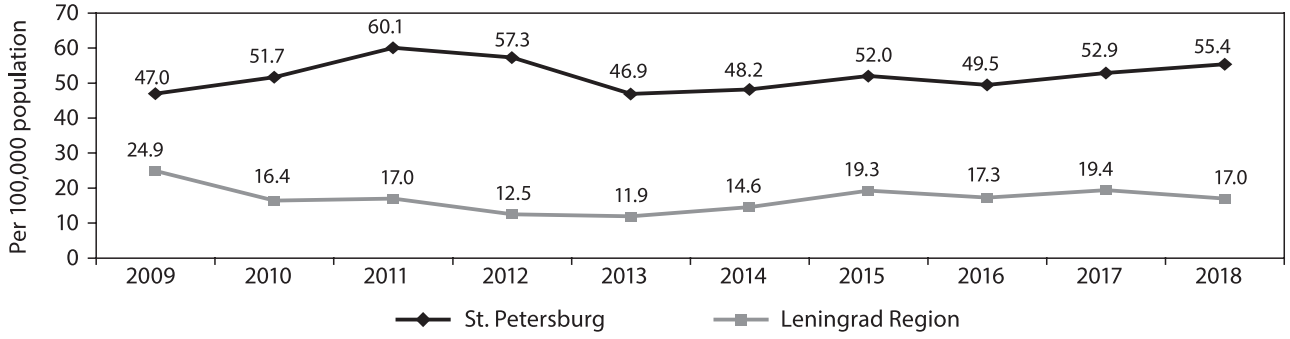

Figure 26. Incidence of anogenital condylomas in St. Petersburg and Leningrad Region in 2009–2018

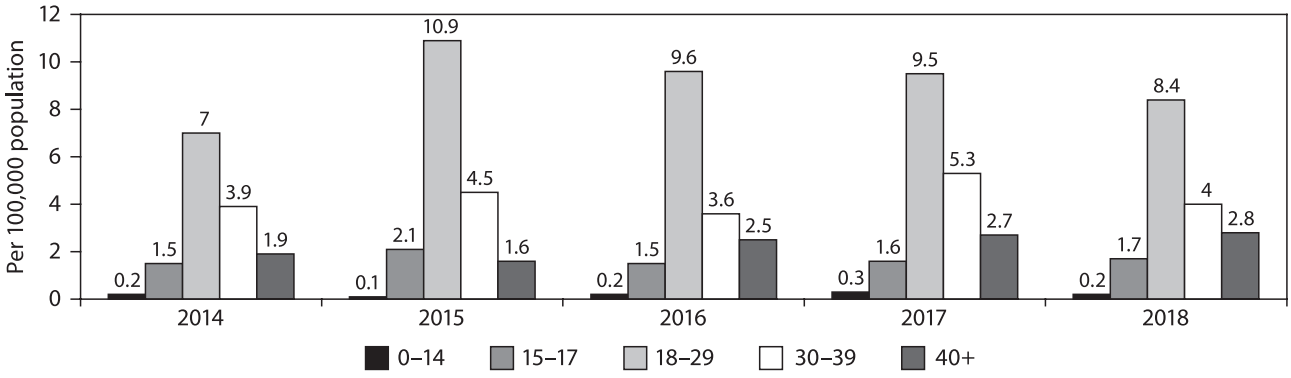

Figure 27. Incidence of anogenital condylomas in different age groups in Leningrad Region, 2014–2018

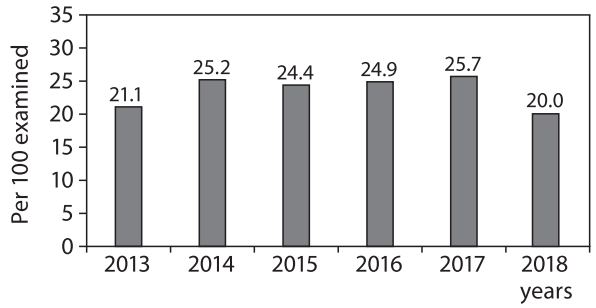

Figure 28. Prevalence of HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73 and 83) in patients of a St. Petersburg dermatology and STD clinic, 2013–2018

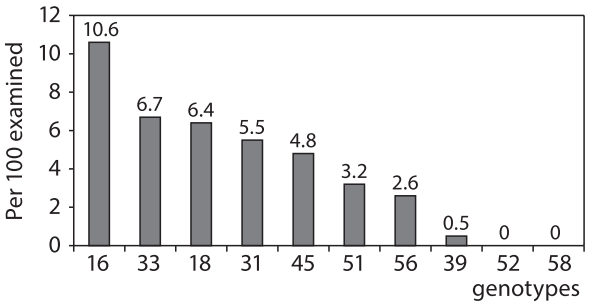

Figure 29. Prevalence of different HPV genotypes in female patients of a dermatology and STD clinic in St. Petersburg, 2014–2017 (n = 312)

One more early criterion used for efficacy assessment of vaccination against human papillomavirus is the prevalence of highly carcinogenic HPV types. Fig. 28 shows the findings of HPV prevalence study (a total of 16 genotypes) in patients of a St. Petersburg dermatology and STD clinic. The study showed that in 2013–2018 HPV detection rate ranged from 20.0 to 25.2 per 100 examined patients. In 2017–2018, HPV was found in 23.4% and 20.0% of cases, respectively. Russian test systems were used for laboratory diagnosis, test method was real-time PCR.

The prevalence of individual HPV genotypes in this category of patients is shown in Fig. 29. The most common genotype in St. Petersburg as well as in other regions of Russia and abroad is HPV 16 genotype — 10.6 per 100 examined persons. The second and third most common HPV genotypes are HPV 33 and 18: 6.7 and 6.4 per 100 examined persons, respectively. They are followed by HPV genotypes 31, 45, 51, 56, and 39. HPV genotypes 52 and 58 have not been found in this study.

In the other territorial units of the Russian Federation where such studies had been carried out the prevalence of individual genotypes differed; therefore it is necessary to study this factor in each region in order to demonstrate the urgency of the issue and its social significance and to assess the efficacy of the papillomavirus vaccine at the population level.

Cervical cancer is one of the urgent issues of the public health care in the Russian Federation and in the territorial units of the Northwestern Federal District of Russia in particular. In terms of HPV vaccination the incidence of cervical cancer is a late criterion of vaccination efficacy assessment. Fig. 30 shows the incidence of cervical cancer in the Russian Federation and in St. Petersburg in 2008–2017. There is a trend towards growing standardized incidence rate. In St. Petersburg, the incidence level was lower during the whole period of study: in 2017 in the Russian Federation on the whole the incidence was 15.76, whereas in St. Petersburg it was 12.08 per 100,000.

Measles and rubella

In 2017–2018, we continued our work at the program of measles and rubella elimination in the Russian Federation as part of activities of the St. Petersburg Regional Centre for Measles and Rubella Surveillance based at St. Petersburg Pasteur Institute. The Centre supervises 11 territorial units of the Northwestern Federal District of Russia with the population of 13.5 million persons. Duties of the research officers of the Laboratory of Epidemiology of Infectious and Non-infectious Diseases include:

- drafting monthly reports on measles and rubella cases in the region and sending these reports to the National Research Guidance Centre for Measles and Rubella Surveillance (Moscow);
- visiting the supervised territorial units with scheduled checks of activities related to the measles and rubella elimination program (2 in 2017–2018);
- consultation assistance in the event of major outbreaks of infection (2 in 2017–2018);
- entry of data from epidemiological survey records on measles and rubella foci into the Centralized Information System for Infectious Diseases (CISID);
- analysis of measles and rubella incidence in the territorial units of the Northwestern Federal District of Russia;
- making reports on the state of measles and rubella epidemiological surveillance in the supervised territorial units at annual panel meeting of experts in the Russian Federation;

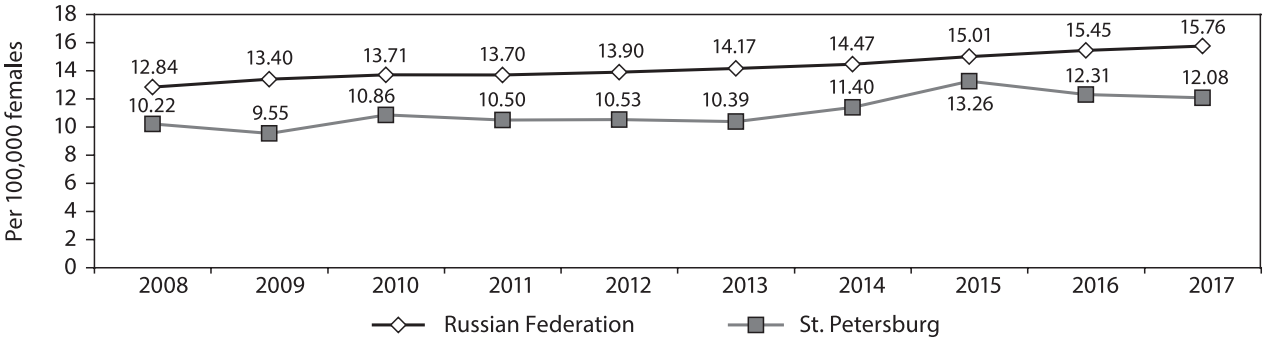

Figure 30. Incidence of cervical cancer in the Russian Federation and in St. Petersburg in 2008–2017 (standardized values, world standard of the population)

– drafting annual reports on implementation of the measles and rubella elimination program in the territorial units of the Northwestern Federal District of Russia.

In 2017, 3 cases of measles were recorded in the Northwestern Federal District; the incidence rate was 0.2 per 1 million of population. All patients belonged to the same territorial unit, i.e. St. Petersburg (incidence rate 0.6 per 1 million of population). According to the epidemiological investigation findings, two cases of measles in adult patients were deemed to be imported from Italy and one case was diagnosed in a child not vaccinated against measles who came from another Russian region.

In 2018, measles incidence in the region increased. 109 cases of measles were recorded in 6 territorial units; the incidence rate was 7.8 per 1 million of population. The majority of patients were registered in St. Petersburg — 54 cases (10.2 per 1 million), the Republic of Karelia — 29 cases (46.0 per 11 million) and Leningrad Region — 21 cases (12.2 per 1 million). The main reason for measles outbreaks was that the first cases of the disease were overlooked, the patients having been diagnosed with acute respiratory viral infection with exanthema of various origins. Insufficient alertness of medical specialists to measles in some territorial units can be explained with the fact that this disease had not been recorded there for a decade or more. In three territorial units singular cases of measles without its further spread were registered. In five territorial units of the district no cases of measles have been recorded.

Patients diagnosed with measles in the Northwestern Federal District of Russia in 2018 were predominantly adults aged 18 and older (64.2%). The percentage of children and underage teenagers was 35.8% (Fig. 31).

The majority of cases were recorded in patients not vaccinated against measles (53.2%) or in patients with unknown immunization status (21.1%). The share of patients vaccinated and revaccinated against measles was 6.4% and 18.4%, respectively.

Rubella incidence in the territorial units of the Northwestern Federal District of Russia has been less than 1 per 1 million of population since 2014 (Fig. 32). In 2017, no rubella cases were registered in the territorial units of the district. In 2018, one case was recorded in Leningrad Region in an adult not vaccinated against this disease in the age group of 20–24-year-olds. Measles and rubella vaccination coverage level of the relevant population groups in 2018 was 95.0% and higher; however, in one territorial unit in the age groups of 1 year and 6 years it was lower than prescribed in the guidelines.

Implementation of the acute viral hepatitis B elimination program in the Northwestern Federal District of the Russian Federation

In 2017–2018, we continued to implement the regional program of acute hepatitis B elimination in northwest Russia that was developed by the personnel of St. Petersburg Pasteur institute under the guidance of its director, Prof. A. Zhebrun, in 2010–2012 and approved by the head of the Federal Service for the Oversight of Consumer Protection and Welfare in August 2013.

The 2nd stage of the program implementation (2016–2018) was focused on organizational and consultative work, analysis of the incidence rate of acute and chronic hepatitis B, assessment of hepatitis B vaccination coverage data and

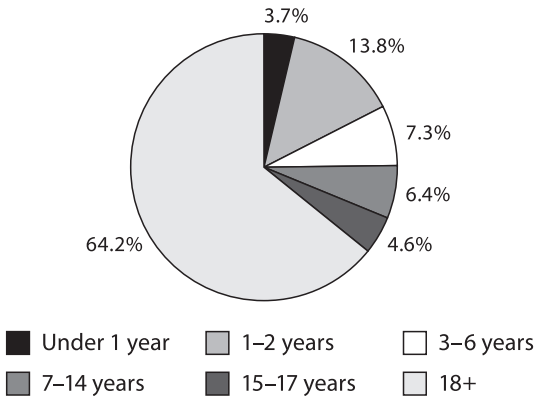

Figure 31. Breakdown of measles patients by age in the territorial units of the Northwestern Federal District in 2018

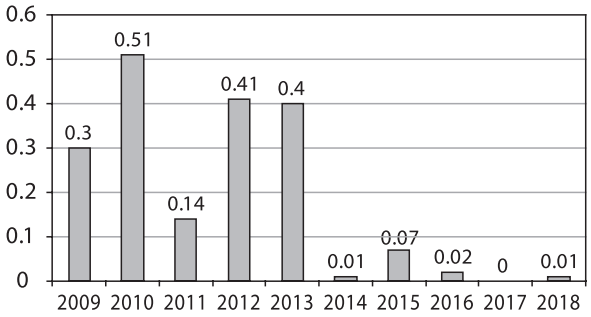

Figure 32. Rubella incidence in the Northwestern Federal District of Russia in 2009–2018

findings of the serological monitoring of immunity to hepatitis B virus. Annual panel meetings of experts taking part in the program implementation were held for progress review and defining major tasks for the next period.

Vaccination against hepatitis B contributed to considerable progress in bringing down the incidence of acute viral hepatitis B in the Russian Federation and the Northwestern Federal District in particular. In 2017 and 2018, the overall incidence rate in the district was 0.50 and 0.37 per 100,000 of population (in 2016 it was 0.58 per 100,000). In Leningrad Region no cases of acute hepatitis B were recorded (Tabl. 15).

In one region the incidence rate in 2018 was less than 1.0 per 1 million of population.

At the same time, the incidence of chronic viral hepatitis B (CHB) decreases significantly slower, and in some territorial units it does not show any tendency to decrease.

Tuberculosis

The study findings showed that in the Russian Federation and in all territorial units of the Northwestern Federal District tuberculosis incidence is on the decrease. In Leningrad Region the incidence of respiratory tuberculosis decreased from 85.3 (95% CI 80.7–89.9) to 27.2 (95% CI 24.8–29.6) per 100,000 of population in 2009–2018. At the same time, the share of patients newly diagnosed with multiple drug resistance of *M. tuberculosis* is growing (Fig. 33). This figure in 2009 and 2018 made 23.9% and 36.9%, respectively ($p < 0.05$). There is also a trend toward growing multiple drug resistance of *M. tuberculosis* in patients who received treatment with anti-tuberculosis drugs. Molecular genetic testing showed that in these patients mycobacteria of the Beijing family were predominant (76.5%).

Table 15. Incidence of acute hepatitis B in the territorial units of the Northwestern Federal District in 2016–2018

Territorial units	2016		2017		2018	
	No. of patients	Per 100,000 of population	No. of patients	Per 100,000 of population	No. of patients	Per 100,000 of population
Arkhangelsk Region	6	0.53	2	0.18	4	0.36
Vologda Region	5	0.42	10	0.84	1	0.08
Kaliningrad Region	8	0.82	9	1.32	2	0.20
Leningrad Region	1	0.06	0	0	0	0
Murmansk Region	3	0.39	2	0.26	2	0.26
Nenets Autonomous District	1	2.29	0	0	1	2.27
Novgorod Region	3	0.49	3	0.65	2	0.33
Pskov Region	0	0	1	0.16	0	0
Republic of Karelia	4	0.63	1	0.16	1	0.16
Komi Republic	8	0.93	5	0.59	3	0.35
St. Petersburg	42	0.81	24	0.61	36	0.68
NWFD	81	0.58	70	0.50	52	0.37

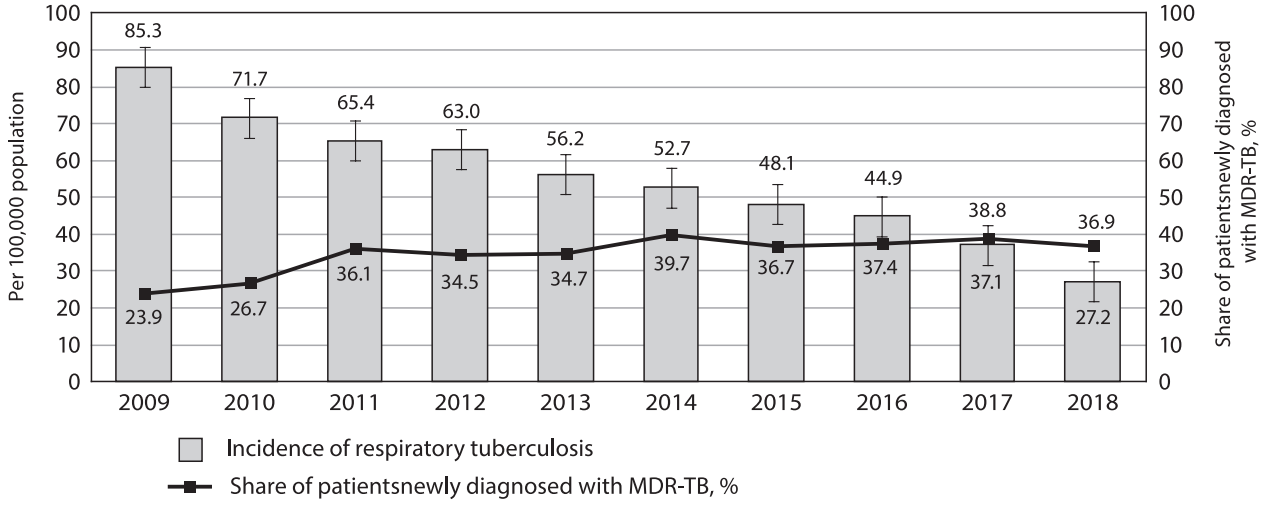

Figure 33. Incidence of respiratory tuberculosis and share of patients newly diagnosed with multi-drug resistant *M. tuberculosis* in Leningrad Region in 2009–2018

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LABORATORY OF VIRAL HEPATITIS

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Acute viral hepatitis

In 2017–2018 as well as in the previous years, the etiological structure was dominated by hepatites with enteric transmission mechanism, i.e. hepatitis A (HAV) — 64% and hepatitis E — 1.2%. Hepatitis types with parenteral transmission — acute hepatitis B (AHB) and acute hepatitis C (AHC) — account for 13.8 and 18% of cases, respectively.

From 1997 to 2018, there were significant changes in the hepatitis A (HAV) epidemic process in the Russian Federation. First of all, this refers to its intensity (there is a stable trend towards incidence decrease) and age structure of the disease (adults). This situation, however, resulted in the decreased community immunity, mainly in the adult population; together with poor housing and amenities conditions of some territorial units this can lead to the increased HAV incidence. Increased HAV incidence recorded in 2017 marks the beginning of one more periodic incidence increase.

In contrast to HA, official registration of hepatitis E (HEV) cases only started in 2013. During the period under consideration the incidence ranged from 0.06 to 0.08 per 100,000 with the trend to slight increase in 2017–2018. Although the Russian Federation (RF) is not endemic to HEV, autochthonous cases of the disease are recorded increasingly frequently.

The complex of extensive preventive measures including those performed as part of the national health care priority project, resulted in the sharp decrease in the incidence of acute hepatitis B and C (AHB, AHC) in the RF.

The long-term analysis of AHB incidence in the RF in 1999–2017 showed its drastic (more than 100-fold) decrease. AHB incidence in the Northwestern Federal District (NWFD) decreased from 1.29 per 100,000 of population in 2013 to 0.58 per 100,000 in 2016. In 2015–2018, the incidence of AHB was less than 1 per 100,000 of population. In Leningrad Region, the Republic of Karelia and Nenets Autonomous District no cases of AHB were recorded. Age groups of adult patients are predominant in AHB: adults account for 96.3 to 99.4% of patients. The share of local cases of the disease in 2016–2018 was 89.6%.

Studying of immunization status of patients with AHB in 2016 showed that they had been mainly not vaccinated against hepatitis B (72.8%), the share of persons with unknown history of immunizations was 23.5%, the share of vaccinated persons was 3.7% (3 patients). The interval between the third vaccination and the disease onset ranged from 5 to 10 years.

Improvement was also evident in the incidence of AHC: in 2017, this figure among adults in the RF was 1.48, and in 2018 it was 1.28 per 100,000 of population, whereas in 2001 it was 16.7 per 100,000 of population. In the NWFD these changes were even more obvious, and the incidence became almost 30 times lower, from 31.9 per 100,000 of population in 2001 to 1.2 in 2017).

Molecular biology opens up new possibilities for studying the clinical course of acute viral hepatites, increases the efficiency of epidemiological surveillance planning. For example, it has been found that from 2017 to 2018 two sub-genotypes of HAV circulated in St. Petersburg, i.e. 1a and 1b, with prevailing sub-genotype 1a.

In 2017–2018, phylogenetic analysis was carried out and the circulation of genotype D of hepatitis B virus circulation in the NWFD was found in 95.8% of cases. Analysis of the distribution of HBV subtypes revealed three types of circulation, i.e. D1 subtype (100%) — in Murmansk Region and Pskov Region, D2 (74.4%) — in St. Petersburg, Leningrad Region, and the Republic of Karelia, D3 (89.5%) — in Vologda Region and Komi Republic. Genotype A was found in 2 isolates (4.2%) and belongs to the A2 subtype. The obtained data on the distribution of circulating genotypes/subtypes of HBV in the NWFD units are consistent with the trend typical of the entire population and with the results of previous studies on HBV genotype circulation within the above-named territory in the case of chronic hepatitis B.

The main sources of infection with AHB and AHC in the RF and NWFD are patients with chronic hepatitis B and C; they accounted for 94.1%. of all known sources of infection.

Chronic viral hepatitis

There are no significant positive trends in the epidemic process of chronic viral hepatitis B and C (CHB, CHC). In recent years there is a stable trend towards the increase in the cumulative number of persons with chronic viral diseases of the liver.

It must be admitted that, taking into consideration the number of patients with the proven diagnosis, CHC is a more important issue for the country than the CHB. In the general etiological structure of chronic viral hepatites it accounts for almost 77% of all newly diagnosed persons.

In 2017, the cumulative number of patients with CHC in Russia was 562,622 persons (0.4% of the population). Using the standard approach of breaking down the country territorial units by CHC incidence level we revealed that in 2018 there were six districts with the incidence above the country average (35.3 per 100,000 of the population), and situation was the worst in the Northwestern Federal District (50.0 per 100,000 of the population).

There are no means of specific prophylaxis of hepatitis C, therefore the priority in its control is giving etiological treatment to patients with acute or chronic disease, including those with liver cirrhosis or after liver transplantation. Such therapy is aimed at eradication of the causing agent from the system. In the RF, the share of patients who received therapy is 3.5%, which can significantly impede progress in achieving the announced global goal of 80% treatment coverage by 2030.

In the RF the incidence of the CHB in the last decade has remained virtually unchanged. The country average in the last decade amounted to 12.1 cases per 100,000 of the population. The cumulative number of patients, however, has grown annually and reached 253,668 by 2017. In contrast to CHC, modern means of hepatitis B therapy do not enable complete virus eradication from the system, but vi-

ral load reduction to an undetectable level makes it possible to slow down the disease progression and minimize the epidemiological danger of the infection source.

Latent hepatitis B

It should be noted that the prevalence of HBsAg-negative HBV in patients with CVHB examined at different stages of specialized medical care ranges, according to our data, from 27.1% (outpatient stage) to 67.8% (hospital stage). Analysis of the findings of the clinical and laboratory examination of patients with HBsAg-negative HBV revealed the variability of the clinical course and disease stages up to the cirrhotic stage (52.1%). In the case of HBsAg-negative HBV, marked liver fibrosis (F3–F4) stages confirmed by instrumental tests accounted for 55.4%. Tests for albumin, prothrombin index, platelets, gamma-globulins as well as HBsAb titers are essential for the clinical assessment of the disease stage and severity of cirrhosis developed as an outcome of HBsAg-negative HBV. Postmortem pathomorphological examination of liver tissue of patients with cirrhosis and intravital negative blood serum HBsAg confirms the clinical diagnosis of CVHB.

Mixed infections with viral hepatites

Persistent circulation of agents causing acute viral hepatitis and annually growing number of patients with chronic viral liver diseases increase the risk of superinfection of patients with other hepatotropic viruses.

Of 241 patients with mixed viral hepatitis (acute-on-chronic), in 69.7% the disease developed against the background of CHC, in 30.3% — against the background of CHB, which corresponds to the structure of patients with chronic viral hepatites. Analysis of the etiological structure of acute hepatites in this category of patients shows that hepatites with parenteral transmission are more common: in 46.5% of cases it is AHB including the variant with the delta agent, in 16.6% it is AHC. The share of HAV is 36.9%.

Analysis of the prevalence of acute hepatites with the enteric and parenteral transmission mechanisms in patients with CHB revealed that here the prevalence was comparable (45.2 and 54.8%, respectively), whereas in patients with CHC acute hepatitis with parenteral transmission was registered twice as often as hepatitis with the enteric transmission (in 66.7 and 33.3% of cases, respectively; $p < 0.05$).

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LABORATORY OF MOLECULAR EPIDEMIOLOGY AND EVOLUTIONARY GENETICS

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TUBERCULOSIS AND MYCOBACTERIA

PROJECTS AND COLLABORATIONS

Funded Projects

- Russian Science Foundation project #14-14-00292 "Evolution of pathogenetic potential of phylogenetic lineages of Mycobacterium tuberculosis", 2014–2018 (PI — I. Mokrousov).
- Russian Foundation for Basic Research project No. 17-54-30020 "A personalized approach to fight the HIV and drug resistant TB epidemic in Irkutsk, Siberia" (project PI — O. Ogarkov, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia), 2017–2019.
- Russian Foundation for Basic Research project 17-04-00367 "Population of Mycobacterium tuberculosis in the Western Siberia region: current molecular epidemiology in the context of macroevolutionary reconstruction" (PI — I. Mokrousov), 2017–2018.
- Russian Foundation for Basic Research project 18-04-01035 "Investigation of the role of the repeat element IS6110 in the micro- and macroevolution of Mycobacterium tuberculosis phylogenetic lineage 2" (PI — E. Shitikov, Center of Physico-Chemical Medicine, Moscow), 2018–2019
- Russian Foundation for Basic Research, Project #19-04-00263 "Pathogenomic features and epidemic potential of highly resistant strains of ancient sublineage of Mycobacterium tuberculosis Beijing genotype" (PI — I. Mokrousov), 2019–2020

International collaborations

National Institute for Public Health and the Environment, RIVM (2018–2021), Aitkhozhin Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan (2015–2017), Kobe Institute of Health, Japan (2016–2018), Beijing Children's Hospital, China (2017–2021), Hospital General Universitario Gregorio Marañón (Madrid Spain), North Estonian Medical Centre (Tallinn Estonia), Biomedical Research and Study Centre, University of Riga (Latvia).

National collaborations

Central Research Institute for Epidemiology (Moscow), Omsk State Medical University, Scientific Center of Family Health and Reproductive Problems (Irkutsk), Ural Research Institute of Phthisiopulmonology (Ekaterinburg), Anti-tuberculosis dispensaries in Kaliningrad, Petrozavodsk (Karelia), Syktyvkar (Komi), Murmansk, Pskov.

MAJOR RESEARCH RESULTS

Analysis of whole-genome data for phylogenetic analysis and search of molecular markers of significant genovariants of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis Beijing 94-32-cluster dominates in Kazakhstan and Central Asia, and is one of two main subtypes of *M. tuberculosis* in Russia, often associated with MDR/XDR and marked by global distribution due to migration flows. As a result of bioinformatics genomic analysis, we identified a single nucleotide polymorphism in the sigE gene, specific for Beijing 94-32 cluster strains, and developed a method for its detection in the PCR-RFLP and real-time PCR formats. The methods were validated *in silico* (by analyzing the GMTV database) and experimentally, on the global collection of strains of different genotypes (342 isolates from Russia, Kazakhstan, China, Vietnam, Bulgaria, Estonia, Brazil). The developed methods for detecting the Beijing 94-32-cluster genotype can be used (simultaneously with the method of detecting the other significant Russian epidemic strain Beijing B0/W148) in prospective studies or for retrospective evaluation of historical DNA collections.

Mycobacterium tuberculosis population in region of West Siberia: current molecular epidemiology in the context of macroevolutionary reconstruction

New data were obtained on the trends in the prevalence of multi- and extensive-drug resistant (MDR/XDR) *M. tuberculosis* population in the Omsk region of Western Siberia. Molecular analysis of 425 *M. tuberculosis* strains isolated from patients with pulmonary tuberculosis in different years in the Omsk region revealed a diversity of the structure of genotypes and the prevalence of strains of the Beijing genotype in different time points (~ 60%) and among TB/HIV-negative and co-infected patients. 80.2% of cases with an unfavorable outcome of the disease (lethal cases) were infected with strains of the Beijing genotype. At the same time, the proportion of Beijing B0/W148 cluster increased 1.4 times (19.2% in 2017 against 13.5% in 2015–2016) and these strains were associated with MDR. The younger age of patients infected with Beijing genotype strains indicates their active circulation in the population. In general, three families make a significant contribution to the spread of drug resistant strains — Beijing, LAM (Latin American Mediterranean) and Ural.

For the first time, two clusters of the early ancient sublineage of the Beijing genotype were identified as emerging and potentially epidemic subtypes (Fig. 34). Almost all ancient Beijing strains were MDR, and half of them were XDR/pre-XDR. A comparison with the globally available data has shown that these two clones mainly circulate in the Asian part of Russia and demonstrate a certain phylogenetic affinity with strains from Japan, Korea and northeastern China. Based on phylogenetic, phylogeographic, and historical data, we put forward a hypothesis that these two clones of the early ancient sublineage of the Beijing genotype were probably brought to Russia ~ 70 years ago after World War II with Japanese prisoners of war and until recently, mostly circulated in Siberia and the Far East. Their relatively higher prevalence in Omsk, along with an extremely strong association with not only MDR, but also pre-XDR/XDR, also observed in other regions of the Russian Federation, demonstrates their epidemic potential and requires permanent monitoring.

Clonal complexes of *Mycobacterium tuberculosis* Beijing genotype in the North-West Russian regions, bordering the countries of the European Union

The high prevalence of the multidrug-resistant tuberculosis in north-west Russia may have a negative impact on tuberculosis control programs not only in Russia but in neighboring the countries of the European Union (EU). 304 isolates of *M. tuberculosis* (2013–2017) isolated from newly diagnosed tuberculosis patients living in northwestern regions of Russia (Pskov, Kaliningrad and Murmansk regions, the Republic of Karelia) were studied (Fig. 35). The prevalence of *M. tuberculosis* strains of the Beijing genotype differed and amounted to 44.9% in the Pskov region, 55.1% in Karelia, 48.4% in the Murmansk region, 63% in the Kaliningrad region. B0/W148 cluster was detected in 6.7%, 17.9%, 9.4%, and 19.2% *M. tuberculosis* strains, respectively. The prevalence of the Beijing 94-32-cluster in the four studied territories did not differ: 29.2%, 28.2%, 31.3% and 28.8%, respectively. In the Pskov and Murmansk regions, 62.9% and 60.9% of *M. tuberculosis* strains were susceptible to anti-TB drugs, and 17.9% and 26.6% of strains were MDR, respectively. MDR strains prevailed in Karelia and the Kaliningrad region — 51.3% and 43.8%; while 41.0% and 36.9% strains, respectively, were susceptible. In total, in four regions, 90.0% of *M. tuberculosis* Beijing B0/W148 isolates were MDR. The Beijing 94-32 strains had similar proportions of drug sensitive (41.6%) and MDR (34.8%) isolates. Thus, in the territories of the North-West of Russia, bordering the EU countries, the proportion of Beijing strains in the structure of *M. tuberculosis* genotypes varied from 44.9% to 63.0%. The Beijing strains of cluster 94-32 (28.2% – 31.3%) and cluster B0/W148 (6.7% – 19.2%) prevailed. However, these *M. tuberculosis* clones differed significantly in the proportion of MDR. The circulation of MDR strains of the *M. tuberculosis* Beijing B0/W148 cluster poses a serious problem for local and national tuberculosis control programs.

Dynamics of MDR-TB in Estonia: key role of immigration and National TB control program

We assessed the genetic structure of the *M. tuberculosis* population in Estonia with particular reference to the main epidemic/endemic clones and determinants of drug resistance. 39.8% of isolates are attributed to the Beijing genotype; 56.8% of them were MDR. In contrast, all three major other genotypes (LAM, Haarlem, Ural) were mainly drug sensitive. MDR was more common among Beijing B0/W148 isolates (81.8%) compared to other Beijing isolates (20.0%, P = 0.0007). The Pre-XDR phenotype was found in 8 isolates, of which 6 belonged to Beijing B0/W148. All resistant to rifampicin and ofloxacin and 97% of isoniazid-resistant isolates had mutations in *rpoB, gyrA, katG*. The most common mutations were *rpoB* S531L, *katG* S315T and *embB* M306V. The main pool of isolates of the Beijing genotype was brought to Estonia in 1945–1990 due to massive human immigration from USSR. However, the active circulation of the hazardous MDR-related cluster Beijing B0/W148 started only in the last 20 years, and represents a serious threat to the fight against TB in Estonia.

Kazakhstan: the influence of Russia and China on the structure of the *Mycobacterium tuberculosis* population

Kazakhstan is characterized by the dominance of strains of Central-Asian/Russian Beijing 94-32 cluster (probably originating in northwestern China) and the rarity of strains of the LAM family (which until recently almost completely belonged to the Russian branch of LAM-RUS). In our study, we identified the first genetically confirmed strain of the LAM RD-Rio sublineage in Kazakhstan. Molecular analysis and comparison with proprietary LAM global database showed that it belongs to a phylogenetic branch endemic to the north of European Russia/Eastern Europe. Contrary to the popular (and justified) opinion about the association of RD-Rio strains with MDR, this branch included only drug-sensitive strains. We hypothesize that this strain was brought to southern Kazakhstan not from the primary Ibero-American region of origin of RD-Rio, but from the secondary circulation zone (Russia) (Fig. 36).

Evolution and origin of significant Russian subtypes Beijing 94-32 and Beijing B0-W148/100-32 in the light of the Beijing genotype evolution in China

The study included 369 isolates that represented different provinces in all geographic parts of China. All isolates were assigned to the Beijing genotype, according to spoligotyping, and all had a deletion of RD105. All isolates were additionally analyzed by: (i) markers of large sublineages — mutations in the mutT2, mutT4 and deletion RD181 and (ii) in 24-VNTR loci. Geographically closest to China are two Eurasian epidemic strains defined by the 24-MIRU-VNTR loci: the Russian type 100-32 and the "Central Asian-Russian" type 94-32. Their profiles differ in loci MIRU26 and QUB26 (7 and 7 repeats for 100-32 and 5 and 8 repeats for 94-3217). When these profiles were placed in the Chinese tree of the Beijing genotype, they were both associated with the central and "modern" part of the network. Profile 24-MIRU-VNTR type 100-32 (B0/W148) is a single-locus variation relative to strains from Shanxi and Jilin provinces in our study. Both of these provinces represent northeast China, which has historical ties with the Russian Empire/USSR. The "Central Asian-Russian" type of Beijing 94-32 is widely distributed in the former Soviet Central Asia and is one of the two largest and most significant subtypes of Beijing in Russia. This profile is separated by a single-focus polymorphism from a strain from Qinghai province, a neighboring province with Xinjiang Uygur district and Central Asia as a whole. The revealed phylogeographic patterns indicate that large-scale (but not medium/small-scale) migration remains one of the decisive factors in the genetic divergence of *M. tuberculosis* populations in China. Analysis of the diversity and topology of local collection networks seems to confirm the recent hypothesis about the South China origin of the Beijing genotype. A review of the results obtained in the Eurasian context suggests that two significant Russian epidemic clones of the Beijing genotype could have their origin in the northeast and northwest regions of China, respectively.

Emerging resistant clone of *Mycobacterium tuberculosis* in Western Asia

M. tuberculosis strains of the genotype NEW-1/SIT127, are endemic for Western Asia (Iran, Afghanistan), are found in Central Asia and sporadically in Russia. Phylogenetic analysis of the Lineage 4 isolates confirmed the separate position of the NEW-1 family, which we provisionally designated L4.5.1/Iran. The hypothesis of the evolutionary migration scenario was developed: origin of L4.5 1000–1300 years in China, subsequent emergence of the intermediate genotype pre-NEW-1 in Tibet, further migration to Xinjiang and, finally, to Iran 800 years ago (emergence of NEW-1/SIT127)

Figure 34. Geographical distribution of the *M. tuberculosis* strains of ancient Beijing subtypes in different locations in northern Eurasia and different districts of the Omsk region. Reproduced from Mokrousov et al. (2019). Copyright Elsevier NV.

Figure 35. Distribution of *M. tuberculosis* Beijing genotype subtypes in northwestern Russia. Reproduced from Vyazovaya et al. (2018). Copyright "Russian Journal Infection and Immunity".

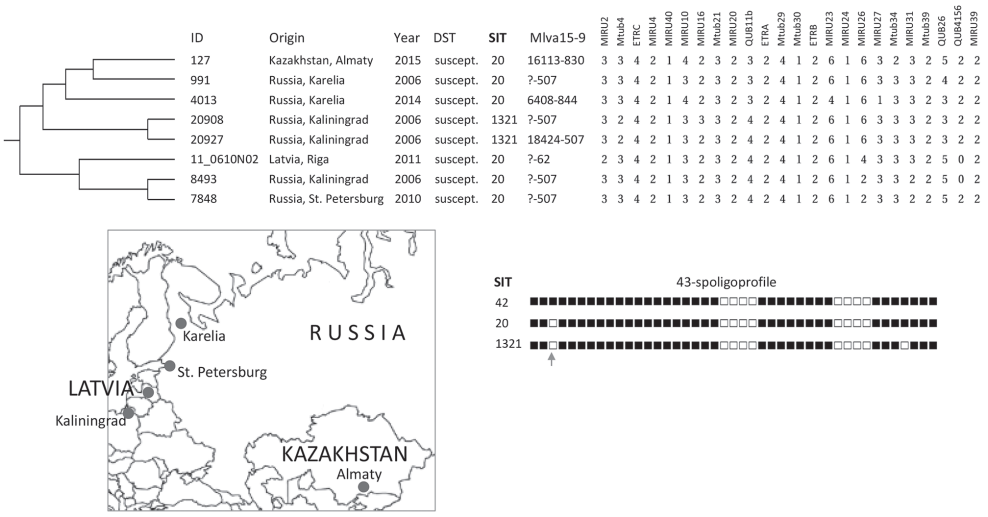

Figure 36. The part of the MIRU-VNTR-based dendrogram of the *M. tuberculosis* LAM family with enlarged branch including SIT20 strain from Kazakhstan, locations of isolation of the studied strains and their binary spoligoprofiles. Reproduced from Skiba et al. (2019). Copyright CDC, Atlanta, USA.

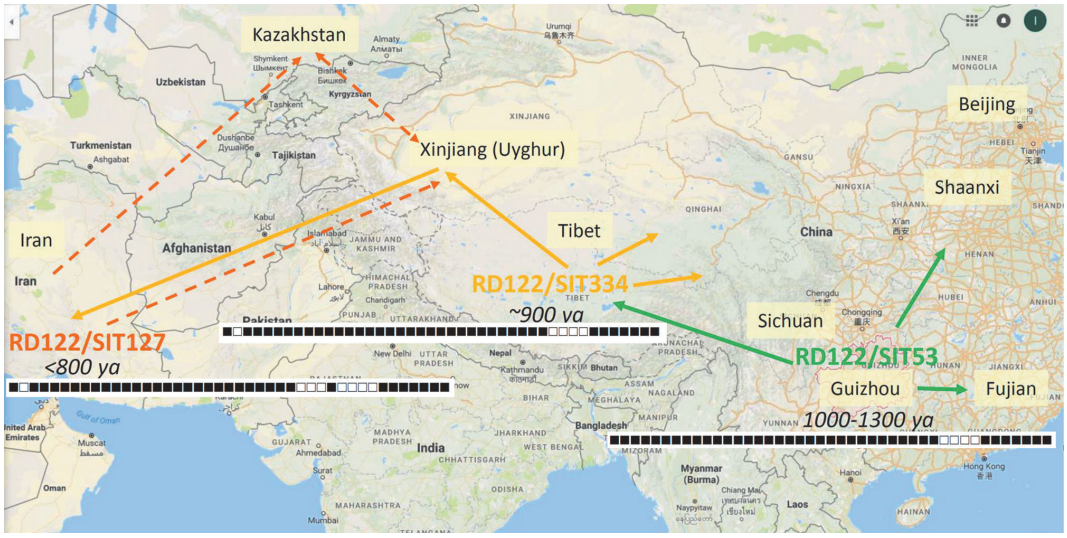

Figure 37. Evolution/migration scenario of RD122/SIT127 lineage of *M. tuberculosis*. Reproduced from Mokrousov et al. (2017). Copyright Elsevier NV.

Origin of L4.5/RD122 1000–1300 ya in China. Origin of intermediate SIT334 in Tibet, further migration to Xinjiang/Uyghur, and Iran 800 ya, possibly, via expansion of the Mongol Yuan empire. Origin of NEW-1 founding type SIT127 in south Iran. Dispersal of SIT127 from Iran, eastwards: via Iranian languages, trade exchange, Islamic world.

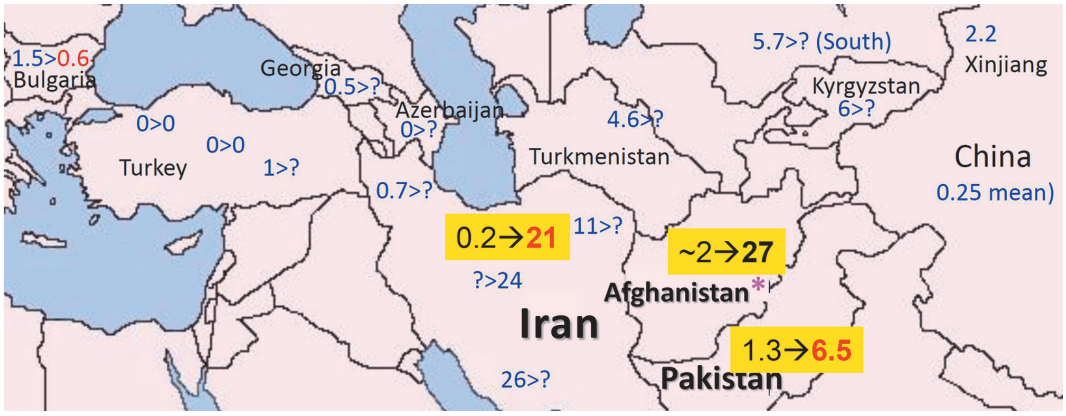

Figure 38. Longitudinal data since 1990s reveal an increase of *M. tuberculosis* SIT127/NEW-1 in Iran and neighbors, along with association with MDR. Modified from Mokrousov et al. (2017). Copyright Elsevier NV.

Arrows: transition from earlier (2005–08) to recent studies (2014–16). Red: significant association with multidrug resistance. Data on Afghanistan are proxied by Afghan refugees in Iran.

(Fig. 37). After this, SIT127 slowly spread eastward from Iran, possibly as a result of a continuum of Iranian languages, trade exchange and interaction in the Islamic world. Data analysis for the last 20–25 years has shown a sharp increase in the prevalence of NEW-1 strains in Iran, Afghanistan and Pakistan, moreover, accompanied by a significant association with multidrug resistance (Fig. 38). Migrations of the population, especially the flows of Afghan refugees, can lead to a wider spread of the resistant subtype NEW-1, which we named an emerging resistant clone of *M. tuberculosis* in Western Asia.

Conclusions on the role of migrations in the spread of epidemic strains of *Mycobacterium tuberculosis*.

1. The usual exchange (sporadic contacts, tourism) is insufficient to bring and settle the imported strain in a new population. 2. A new emerging strain becomes epidemic in the region of its origin, where the ancestral strain have been circulating. 3. Massive immigration of the population is a critical factor leading to a change in the local population of the tuberculosis pathogen. 4. The imported strain must be not only epidemic, but also sufficiently prevalent in its country of origin.

Contagiosity, virulence of an *M. tuberculosis* strain is conditional, not absolute. Speculatively, a kind of human resistance is developed in local population through its co-existence with historical local clones, and acting against imported clones: hence role of host genetics.

Even within "uninteresting" *M. tuberculosis* families, such as NEW-1/Iran and Ural, more hazardous clones may emerge. The notorious Beijing and LAM genotypes are not an exception. We do not know where new emerging *M. tuberculosis* strain will emerge. Once it emerges, it is advised to look at their genomics and phenotype and no less closely at relevant migration flows, to predict its spread.

Reasons of success of different emerging clones may be different in each particular case and be related to strain properties.

Single nucleotide polymorphisms in hsp65 and MACPPE12 genes of *Mycobacterium avium* subsp. *hominissuis*

Mycobacterium avium subsp. *hominissuis* (MAH) is the typical inhabitants of the environment, which are known as opportunistic pathogens of animals and humans. The aim of our study was to analyze single-nucleotide polymorphisms (SNPs) in the *hsp65* and *MACPPE12* genes to characterize the Russian population of MAH in the context of studying phylogenetic relationships and the evolution of geographically distant populations of *M. avium* subsp. *hominissuis*. The sequence analysis of the *hsp65* and *MACPPE12* genes was applied for 40 MAH strains isolated from humans (patients with mycobacteriosis) (Fig. 39). The nucleotide sequences were aligned to the reference genome of *M. avium* subsp. *hominissuis* 104 (accession no. NC_008595.1.). The mutational profiles of Russian strains were compared with those isolated in other countries. In total, the 40 MAH strains were classified into three different *hsp65* sequevars: code 1, code 2 and code 3. The majority of MAH strains (72.5%) belonged to code 1, the same sequevar as for MAH strain 104. The sequence analysis of the *MACPPE12* gene revealed 20 SNPs grouped into nine sequevars at the nucleic acid level: NA01, NA02, NA03, NA06, NA10, NA13, NA14, NA19, and NA_Rus01. Among 20 SNPs eight were nonsynonymous resulting in seven sequevars at the amino acid level: AA01, AA02, AA04, AA07, AA08, AA13, and AA_Rus01. The sequevar AA02 consisted of three different NA variants with syno-

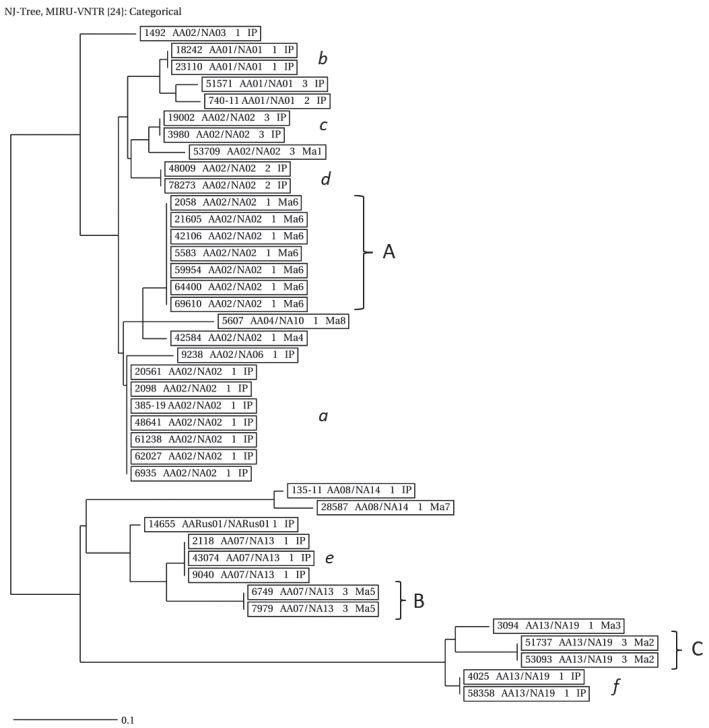

Figure 39. Dendrogram of VNTR profiles of 40 *M. avium* subsp. *hominissuis* isolates with information on their SNP types *(MACPPE12, hsp65).* Reproduced from Starkova et al. (2019). Copyright MAIK Nauka-Interperiodika

The following information is provided for each isolates in boxes: strain ID, *MACPPE12/hsp65* sequence types and MATR-VNTR type (IP stands for individual profile). A to C clusters combine MAH isolates with identical MATR-VNTR and sequence type profiles. a to f clusters combined isolates with identical *MACPPE12* and *hsp65* SNP types, but individual MATR-VNTR profiles.

nymous SNPs profiles: NA02, NA03, and NA06. Half of the MAH strains belonged to the sequevar AA02 (type NA02). Presently, the predominant cluster AA02 (type NA02)/code 1 and the unique variant AA_Rus01 (NA_Rus01) were identified among MAH strains from Russia. Thus, we confirmed the relative conservativeness of the nucleotide sequence of the *hsp65* gene but the polymorphism of the *MACPPE12* gene. At the same time, a comparative analysis of the SNPs profiles of the *hsp65* and *MACPPE12* genes allowed to identify differences and similarities between geographically distant populations of MAH, which highlighted the variability of the global population of *M. avium* species.

Publications in peer-reviewed journals (Pubmed, Web of Science)

IN ENGLISH. 18 articles: 12 in Q1; cumulative IF = 92.453; mean IF = 5.136

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2. *Jagielski T.; Partnership to fight against TB in Central and Eastern Europe (Aleksa A., Bachiyska E., Bakos Á., Crudu V., Dziadek J., Homolka J., Homorodean D., Jansone I., Katalinic-Jankovic V., Krenke R., Kuzmic U., Mokrousov I., Nikolayevskyy V., Nikolenka A., Osmani G.M., Papaventsis D., Pole I., Porvazník I., Savic B., Shubladze N., Solovic I., Szabó N., Tafaj S., Ustamujic A., van Ingen J., Vasiliauskiene E., Yablonsky P.K., Zemanova I., Zhuravlev V., Žolnir-Dovc M.). FATE: the new partnership to fight Against TB in Central and Eastern Europe // Lancet Infect. Dis. 2017 Apr; 17 (4): 363. doi: 10.1016/S1473–3099(17)30120–2. Impact factor 19.864. Q1.*
3. *Li Q.J., Jiao W.W., Yin Q.Q., Li Y.J., Li J.Q., Xu F., Sun L., Xiao J., Qi H., Wang T., Mokrousov I., Huang H.R., Shen A.D. Positive epistasis of major low-cost drug resistance mutations rpoB531-TTG and katG315-ACC depends on the phylogenetic background of Mycobacterium tuberculosis strains // Int. J. Antimicrob. Agents. 2017 Jun; 49 (6): 757–762. Impact factor 4.307. Q1.*
4. *Shitikov E., Kolchenko S., Mokrousov I., Bespyatykh J., Ischenko D., Ilina E., Govorun V. Evolutionary pathway analysis and unified classification of East Asian lineage of Mycobacterium tuberculosis // Sci. Rep. 2017 Aug 23; 7 (1): 9227. Impact factor 4.259. Q1.*
5. *Mokrousov I., Shitikov E., Skiba Y., Kolchenko S., Chernyaeva E., Vyazovaya A. Emerging peak on the phylogeographic landscape of Mycobacterium tuberculosis in West Asia: Definitely smoke, likely fire // Mol. Phylogenet. Evol. 2017 Nov; 116: 202–212. Impact factor 4.419. Q1.*
6. *Ioannidis P., van Soolingen D., Mokrousov I., Papaventsis D., Karabela S., Konstantinidou E., Marinou I., Nikolaou S., Kanavaki S., Mantadakis E., Samonis G., Anthony R., Vogiatzakis E. Multidrug-resistant/extensively drug-resistant tuberculosis in Greece: predominance of Mycobacterium tuberculosis genotypes endemic in the Former Soviet Union countries // Clin. Microbiol. Infect. 2017 Dec; 23 (12): 1002–1004. Impact factor 5.292. Q1.*
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9. *Mokrousov I., Chernyaeva E., Vyazovaya A., Skiba Y., Solovieva N., Valcheva V., Levina K., Malakhova N., Jiao W.W., Gomes L.L., Suffys P.N., Kütt M., Aitkhozhina N., Shen A.D., Narvskaya O., Zhuravlev V. Rapid Assay for Detection of the Epidemiologically Important Central Asian/Russian Strain of the Mycobacterium tuberculosis Beijing Genotype // J. Clin. Microbiol. 2018 Jan 24; 56 (2). pii: e01551–17. Impact factor 3.712*
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11. *Chernyaeva E., Rotkevich M., Krasheninnikova K., Yurchenko A., Vyazovaya A., Mokrousov I., Solovieva N., Zhuravlev V., Yablonsky P., O'Brien S.J. Whole-Genome Analysis of Mycobacterium tuberculosis from Patients with Tuberculous Spondylitis, Russia // Emerg. Infect. Dis. 2018 Mar; 24 (3): 579–583. Impact factor 8.222. Q1.*
12. *Mokrousov I. On sunspots, click science and molecular iconography // Tuberculosis (Edinb). 2018 May; 110: 91–95. Impact factor 2.873*
13. *Sun L., Zhang L., Wang T., Jiao W., Li Q., Yin Q., Li J., Qi H., Xu F., Shen C., Xiao J., Liu S., Mokrousov I., Huang H., Shen A. Mutations of Mycobacterium tuberculosis induced by anti-tuberculosis treatment result in metabolism changes and elevation of ethambutol resistance // Infect. Genet. Evol. 2018 Oct 4. pii: S1567-1348(18)30759-7. Impact factor 2.885*
14. *Perdigão J., Silva C., Diniz J., Pereira C., Machado D., Ramos J., Silva H., Abilleira F., Brum C., Reis A.J., Macedo M., Scaini J.L., Silva A.B., Esteves L., Macedo R., Maltez F., Clemente S., Coelho E., Viegas S., Rabna P., Rodrigues A., Taveira N., Jordao L., Kritski A., Lapa E., Silva J.R., Mokrousov I., Couvin D., Rastogi N., Couto I., Pain A., McNerney R., Clark T.G., von Groll A., Dalla-Costa E.R., Rossetti M.L., Silva P.E.A., Viveiros M., Portugal I. Clonal expansion across the seas as seen through CPLP-TB database: A joint effort in cataloguing Mycobacterium tuberculosis genetic diversity in Portuguese-speaking countries // Infect. Genet. Evol. 2018 Mar 17. pii: S1567-1348(18)30102-3. Impact factor 2.885*
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IN RUSSIAN: 1 monographie, 4 articles

1. *Shulgina M.V., Narvskaya O.V., Mokrousov I.V., Vasilieva I.A. Pathogenic and conditionally pathogenic mycobacteria. Moscow, NEW TERRA, 2018: 104. ISBN 978–5-9907505–7-9. Russian.*

2. *Mokrousov I., Pasechnik O., Vyazovaya A., Stasenko V., Blokh A. On importance of evolutionary robust markers for detection of M. tuberculosis strains of LAM family // Zhurnal mikrobiol. epidemiol. immunobiol. 2018. (3): 60–66.*
3. *Pasechnik O., Blokh A., Vyazovaya A., Stasenko V. Meta-analysis of prevalence of Mycobacterium tuberculosis genotypes Beijing and Latin-American Mediterranean in Russia and neighboring countries // Zhurnal infektologii. 2018. 10 (3): 97–107. Russian.*
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5. *Vyazovaya A.A., Akhmedova G.M., Solovieva N.S., Gerasimova A.A., Starkova D.A., Turkin E.N., Zhuravlev V.Y., Narvskaya O.V., Mokrousov I.V. Molecular epidemiology of tuberculosis in the Kaliningrad Region of Russia: 10 years after // Russian Journal of Infection and Immunity. 2017; 7 (4): 367–374. Russian.*

Patents

1. *Patent (Mokrousov I., Vyazovaya A., Zhuravlev V., Solovieva N., Vishnevsky B., Narvskaya O. Method of detection of Mycobacterium tuberculosis Beijing genotype B0-cluster by real-time PCR). Priority: No. 2017123302 of 30.06.2017. Approved on 09.01.2019.*
2. *Patent pending (Mokrousov I., Vyazovaya A., Chernyaeva E., Solovieva N., Narvskaya O., Zhuravlev V. Method of detection of Mycobacterium tuberculosis Beijing genotype 94-32-cluster by real-time PCR). Priority: No. 2017142885 of 11.12.2017.*
3. *Patent pending (Mokrousov I., Vyazovaya A., Solovieva N., Mushkin A.Y., Vishnevsky B.I., Narvskaya O., Zhuravlev V. Method of detection of Mycobacterium bovis BCG strains by real-time PCR). Priority: No. 2018120630 of 06.06.2018.*

VIRAL HEPATITES

PROJECTS et COLLABORATIONS

Projects

- "Molecular-seroepidemiological monitoring and genetic characteristics of parenteral hepatitis viruses in Vietnam", 2012–2017, funded by Russian Vietnamese Tropical Center, Hanoi and by Rospotrenbadzor (Russian PI — O. Kalinina).
- "Laboratory Diagnosis of Hepatitis C virus in Guinea", funded by Rospotrenbadzor (Federal Service for sanitary and epidemiological Surveillance, Moscow), 2015–2017; Russian PI — O. Kalinina.

National collaborations

– Saint-Petersburg Botkin Infectious Disease Hospital.
– St. Petersburg Center for the Prevention and Control of AIDS and Infectious Diseases.
– Moscow Central Research Institute for Epidemiology.
– Moscow State University of Medicine and Dentistry.
– Saratov Russian Anti-Plague Research Institute "Microbe".

International collaborations

– Baltic Network for control and prevention of life-threated disease.
– Vietnam: Russian Vietnamese Tropical Center (Hanoi).
– Guinea: Institut de Recherche en Biologie Appliquée de Guinée (Kindia).

MAJOR RESEARCH RESULTS

The occurrence of Hepatitis C markers among residents of the Republic of Guinea

The burden of HCV infection continues to be significant in low- and middle-income countries, especially in Asia and Africa. The study of the hepatitis C virus evolution indicates that most of its subtypes occurred more than 300 years ago on the African continent, which is now considered a potential pool of "future" epidemic variants of HCV. At the same time, due to economic and social reasons, the prevalence and genetic diversity of HCV, the route of the pathogen transmission, as well as the clinical course of the infection in many countries of sub-Saharan Africa, including the Republic of Guinea, still remain understudied. The global elimination of HCV by 2030 is possible with the advent of effective diagnostic methods available to the majority of the population The aim of this study was to evaluate the occurrence of serological and molecular markers of hepatitis C among residents of the Republic of Guinea.

The 562 serum samples, obtained in 2016–2017 years from residents at the age 1–90 years (mean age 38.91±1,6) of two prefectures, Mamou and Kindia, were studied. All samples of serum and plasma were obtained for scientific research under the code names according to the Declaration of Helsinki. The presence of total anti-HCV and the specific antibodies to the core, NS3, NS4, NS5 HCV proteins were determined using ELISA-kits (Diagnostic Test Systems LLC, Russia). RNA HCV was detected by real-time PCR using the "AmpliSens HCV-FL" kit (FBIS "CRIE", Russia) and by nested-PCR "in-house". HCV genotypes were determined by phylogenetic analysis based on 5'UTR/core gene. The confidence interval (95% CI) was calculated by the Wilson method.

The anti-HCV was detected in 5,16% (95%, CI 3.62–7.31) adults, RNA HCV was detected only in 0,71%, (95%, CI 0.28–1,82) (Tabl. 16). Phylogenetic analysis revealed the circulation of two epidemically significant HCV variants: subtype 1b and subtype 2c. Regardless of the region, no HCV markers were detected in the age group 0–17 years, as well as no significant difference in the occurrence of anti-HCV among different age groups in was not revealed. In the same time the uncertain results of the anti-HCV were obtained in 3,20%

Table 16. The results of HCV markers detection in samples obtained from residents of Mamou and Kindia prefectures

Regions	Total	anti-HCV (confirmed) n (%, 95% CI)	anti-HCV (uncertain) n (%, 95% CI)	RNA HCV n (%, 95% CI)
Mamou_HR*	232	17 (7.33%, 4.63–11.42)	14 (6.03%, 3.63–9.87)	2 (0.86%, 0.24–3.09)
Kindia_**	242	9 (3.72%, 1.97–6.92)	2 (0.83%, 0.23–2.96)	0
Kindia_HR*	55	3 (5.45%, 1.87–14.85)	2 (3.64%, 1.00–12.32)	2 (3.64%, 1.00–12.32)
Total	562	29 (5.16%, 3.62–7.31)	18 (3.20%, 2.04–5.01)	4 (0.71%, 0.28–1.82)

*HR — fever patients with clinical symptoms of various diseases; **practically healthy residents.

(95%, CI 2.04–5,01). The presence of a large number of anti-HCV false-positive results is an actual problem on the African continent, as noted in a number of studies. One of the reason of false-positive results can be a significant infectious pressure on the host's immune system, which is caused by a huge variety of pathogens of various nature that are widespread in Africa. In our study the most uncertain anti-HCV positive results were in samples obtained from fever patients with clinical symptoms of various diseases in the Mamou and Kindia hospitals (Tabl. 16).

The obtained results indicate a significant burden of hepatitis C in the Republic of Guinea, and point to the need both to develop an algorithm of diagnostic criteria and to improve the diagnostic test systems for mass screening among the African population to establish the proportion of people involved in the epidemic process.

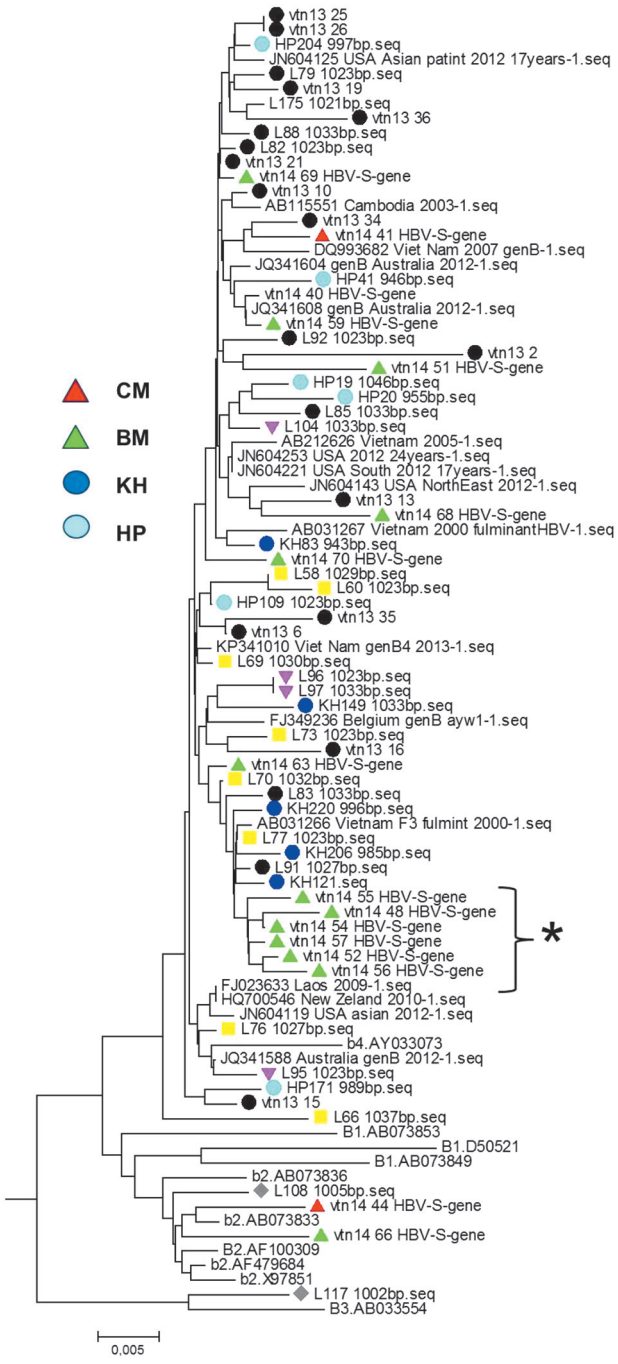

Figure 40. Dendrogram, representing the branch formed by genotype B isolates (obtained by Maximum Likelihood method). Subcluster consisting of HBV strains isolated from workers of the same farm is indicated by asterisk

Burden of parenteral viral hepatitis in Vietnam

South-East Asia and Western Pacific Region are endemic area for hepatitis B virus (HBV), where it is successfully transmitted perinatally and horizontally. In Viet Nam, 8.4 million individuals were estimated to live with HBV infection. Currently the adult Vietnamese population is covered not enough by universal HBV vaccine. This study was performed to analyze the current prevalence of HBV, HCV and HDV in general population in Viet Nam and to investigate the genetic heterogeneity of HBV population.

A total of 1041 adults of 18–79 years old (mean age 43.38±11.97) from four regions Binh Duong (BD) (two communities Binh My (BM) and Chanh My (CM)); Khanh Hoa (KH); Quang Tri (QT), Hai Phong (HP)) were enrolled in the study in 2013–2014. The presence of HBsAg were analyzed with Monolisa® HBsAg kit (Bio-Rad, USA). The presence of total anti-HCV and the specific antibodies to the core, NS3, NS4, NS5 HCV proteins, the anti-HDV were determined using ELISA-kits (Diagnostic Systems, Russia). DNA HBV, RNA HCV, RNA HDV were detected by real-time PCR using the "AmpliSens HCV-FL", "AmpliSens HBV-FL", "AmpliSens HDV-FL" kits (FBIS "CRIE", Russia). HBV genotypes were determined by phylogenetic analysis based on S gene. The confidence interval (95% CI) was calculated by the Wilson method.

The high prevalence of HBsAg was revealed in all four regions: 9.9% (95% CI 6.6–14.1) in BD; 14.1% (95% CI 10.1–18.9) — in KH, 8.9% (95% CI 5.7–13.0) — in QT, and 12.3% (95% CI 8.5–16.9) — in HP. At the same time within BD region the prevalence of HBsAg between BM and CM communities was significantly different: 16.0% — in BM (95% CI 10.0–23.6) and 4.8% — in CM (95% CI 1.9–9.6). The rate of residents who had total anti-HBc was 61.1% (95% CI 57.6–64.5). The anti-HCV prevalence was low 1.33% (95% CI 0.73–2.44), RNA HCV was detected in 0.66% (95% CI 0.28–1.53). No markers of HDV infection were detected in residents of BD, JH, HP regions. A total of 60 HBV strains were genotyped. Four HBV subgenotypes (B2, B3, B4, and C2) and three subtypes (ayw1, adr, adw2) were identified. Subgenotype B4 was found to be the predominant affecting 71.7% (43/60) of the adults. Vietnamese HBV strains belonging to subgenotype B4 formed a distinct branch separated from other strains isolated in Asia (Fig. 40). Within this branch the subcluster consisting of six HBV strains from men of 38–42 years old who worked in the same farm in BM community, was revealed. This finding indicates the intra-farm transmission of HBV among the workers, and explains the highest HBsAg prevalence in BM community compared to CM. No amino acid substitutions, which may be responsible for vaccine escape, as well as no mutations associated with resistance to nucleoside analogs were found.

The high genetic diversity of HBV viral population highlights the multiple sources of infection, successful spreading of a variety of viral variants and provides insight into the driving force of the HBV epidemic process in Viet Nam. The intra-farm HBV transmission among the workers demonstrates that horizontal transmission is still important route of HBV infection in Viet Nam and indicates the expansion of the national vaccination strategy against HBV among adults of 18–40 years old as an important measure to prevent the horizontal spreading of HBV infection in Viet Nam.

Hepatitis C virus core+1/f protein as a possible factor in the progression of chronic process

Alternative reading frame encoding a single F protein is located in the core region of the hepatitis C virus (HCV) genome. The length of F protein vary depending on the viral genotype, its role in the viral morphogenesis and the pathogenesis of the infectious process actively studied in the last decade. The aim of this study to develop the ELISA "in house" based on the synthetic peptides corresponding to the antigenic determinant of the F protein of HCV subtype 1b and 3a and to evaluate the presence of the anti-F in the HCV positive patients with different stage of fibrosis.

Synthetic peptide F10 corresponding to the antigenic determinant of the F protein of HCV subtype 1b was obtained by solid-phase synthesis. The immunogenicity and immunochemical specificity of F10 peptide were demonstrated in laboratory animals (mice). The optimal condition of the ELISA test was worked out. The specificity of the developed ELISA was tested on the five panels of 165 serum samples (Tabl. 17). The antibodies to protein F were detected in samples obtained from anti-HCV(+) or anti-HCV(+)/anti-HIV(+) patients. No statistically significant differences were found between the presence of anti-F in panel 1 and 2 ($\chi^2 = 2.1876$; $p = 0.139$).

The presence of the anti-F was evaluated in the serum samples of the 105 patients chronically infected by HCV with different stage of fibrosis. 42 (40%) were infected by HCV subtype 1b, 63 (60%) by subtype 3a. Liver fibrosis F0-F1 was in 72 (68.57%) patients. The anti-F was revealed in 27 (25.71%) patients: 12 of them infected by 1b subtype and 15 by 3a subtype. Among 12 patients infected by 1b subtype 24.1% had F0-F1, 50.0% — F2, 33.3% — F3, 25.0% — F4. Among 15 patients infected by 3a subtype 20.9% had F0-F1, 18.2% — F2, 25.0% — F3, 60.0% — F4. No statistically significant differences were found between the presence of anti-F in patients infected by 1b and 3a subtypes with different stage of fibrosis ($p = 0.6397$ and $p = 0.2612$, respectively).

Table 17. The detection of antibodies to HCV F protein in different panels

Panels	N	Anti-F (+), n (%)
No. 1 — anti-HCV(+)	72	7 (9.7)
No. 2 — anti-HIV(+)/anti-HCV(+)	35	7 (20.0)
No. 3 — anti-HIV(+)/antiHCV(–)	7	0
No. 4 — HBsAg(+)/anti-HCV(–)	31	0
No. 5 — anti-HCV(–)/anti-HIV(–)/HBsAg(–)	20	0
Total	165	14 (8.5)

The results showed the possibility of using synthetic peptides for detection anti-F in serum samples. The presence of anti-F in the anti-HCV(+) patients as well as anti-HCV(+)/anti-HIV(+) patients show expression of the HCV core+1/F protein in the natural course of hepatitis C. Anti-F protein were detected in patients with HCV, regardless of the severity of fibrosis and HCV subtype.

Publications

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HUMAN HERPESVIRUS 6

(In collaboration with Vega Ltd. (St. Petersburg) and Pavlov First St. Petersburg State Medical University)

The invention relates to the field of biotechnology, in particular the clinical laboratory diagnosis of viral infections, and can be used for the genotyping of human herpesvirus 6 (HHV-6). The method allows rapid and reliable identification of HHV-6A and HHV-6B viruses. The method makes it possible to determine the HHV-6A and HHV-6B specific mononucleotide polymorphisms in the U67 gene of HHV-6 by real-time PCR using primers and two fluorescently labeled probes. The invention can be used for genotyping the HHV-6 virus.

Patent

1. *PATENT RUS 2627607: Vedernikov V.E., Nikolsky M.A., Vyazovaya A.A., Lioznov D.A., Narvskaya O.V. Method of identification of variants A and B of human herpes virus 6. Priority 28.09.2016. https://elibrary.ru/item.asp?id=36895519*

Northwestern District Centre for AIDS Prevention and Control

LABORATORY OF HIV IMMUNOLOGY AND VIROLOGY

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Continued study of HIV epidemic features in the Northwestern Federal District of the Russian Federation in 2017–2018

The Northwestern District Centre has the same tasks as before: studying and compiling data on HIV epidemic process in the Northwestern Federal District of the Russian Federation (NWFD). The epidemic process analysis is based on the official statistics concerning the cases of HIV infection detected in the administrative territories of the NWFD during serological screening and registered in the accounting forms of the State federal statistical monitoring.

The Northwestern federal district includes 11 entities of the Russian Federation with the total population of 13.7 million (9.5% of the Russian population).

In 2017, the population of the NWFD as compared to 2016 increased by 0.3% (or 52,693 persons). In St. Petersburg and Leningrad Region the number of residents started to grow due to increased birth rate (in St. Petersburg) and population migration (+1.3% and +1.2%, respectively). In Murmansk Region, the population in 2017 as compared to 2016 decreased by 0.5%, in Vologda Region by 0.8%, in Arkhangelsk Region by 0.9%, in Pskov Region by 0.9%, in Novgorod Region by 1.1%, in Komi Republic by 1.1%. In these regions outbound migration is the main cause of population decline. Overall in the District, the number of deaths was more than that of births 1.1 times; the index of natural population decline was –1.8 per 1000 persons, whereas in the Russian Federation as a whole this figure was 0.9. Birth rate situation is least favourable in Leningrad and Pskov Region.

The overall screening coverage of the Russian Federation (RF) citizens for HIV antibodies in 2017 as compared to 2016 increased by 15.7%. The number of persons who underwent HIV tests in the NWFD amounted to: RF citizens — 3,025,246 persons, foreign citizens — 395,388 persons. The number of tested foreign citizens in 2017 (395,388 persons) as compared to 2016 (371,035 persons) increased by 6.6%. Of the overall number of tested persons, in 2017 foreign citizens accounted for 11.6%, whereas in 2005 it was 2.1%.

In the NWFD in general, the testing coverage in 2017 as compared to 2016 increased by 5.2% (Fig. 41) in the vulnerable, or the so called risk groups (drug abusers, sexual minorities, persons with STDs, and persons deprived of liberty). When compared to the same value in 2007, however, we will see a decrease of 40%.

The average detection rate in the District in general (code 100) in 2017 was 218.2 per 100,000 of tested persons (in 2016 it was 261.8), which makes a decrease by –16.7%. If we consider the detection rate for individual territories of the NWFD, we will see the increase in this value in 2017 in Vologda Region (+9.8%), in the Republic of Karelia (+8.9%), in Novgorod Region (+5.2%) and in Komi Republic (+4.4%). The other NWFD territories showed a decrease in detection rate.

Screening efficiency analysis for individual groups (Form 4 codes) showed that in the NWFD in general in 2017 the detection rate among drug abusers (code 102) was 2.6% (in 2008 it was 5.2%). In the group of persons examined for epidemiological reasons according to code

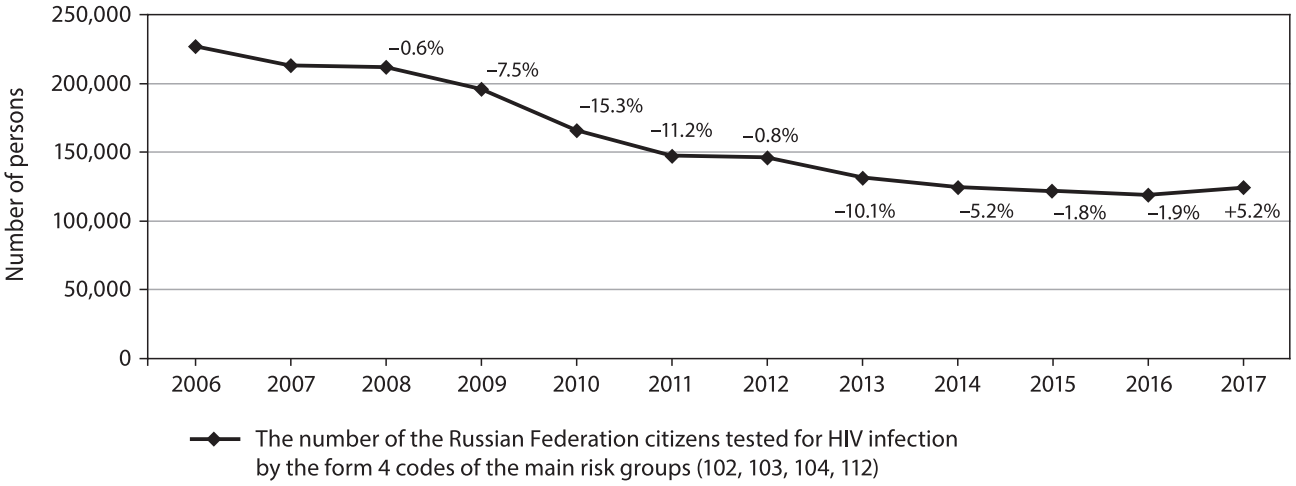


Figure 41. Changes in the number of persons tested for HIV in the NWFD broken down by years, in the most prominent risk groups (codes 102, 103, 104, 112) in 2006–2017

Table 18. Explanation of codes used in HIV testing in the population

Cohort of tested persons	Code of the cohort
Citizens of the Russian Federation	100
Donors (of blood, biological liquids, organs and tissues)	108
Health specialists working with HIV-positive patients or infected material	115
Drug abusers	102
Homosexuals and bisexuals	103
Persons with STDs	104
Persons deprived of liberty	112
Persons examined for clinical reasons	113
Pregnant women (donors of placenta and miscarriage-derived blood)	109
Others	118
Persons examined within the framework of an epidemiological investigation	120
Foreign citizens	200

Table 19. Number of recorded new HIV-cases in the territorial units of the NWFD in 2018 as compared to 2017

Territorial unit	2017	2018	Increase/ decrease (%)
Republic of Karelia	185	244	+31.9
Vologda Region	389	451	+15,9
Murmansk Region	381	437	+14,7
Arkhangelsk Region	296	336	+13,5
Pskov Region	90	102	+13,3
Leningrad Region	1164	1243	+6,8
Novgorod Region	306	325	+6,2
Komi Republic	448	475	+6,0
Kaliningrad Region	557	433	–22,3
St. Petersburg	2778	2628	–5,4
Nenets Autonomous District (NAD)	5	9	+80
NWFD	6599	6683	+1,3
RF	104 402	101 345	–2,9

120 it was 1.9% (in 2008 — 7.4). The detection rate in the group of persons deprived of liberty (code 112) was 1.8% (in 2008 — 3.6%) (Tabl. 18).

The detection rate was more than 5% and more than the district average in the group of drug abusers (code 102) in Komi Republic — 6.8%. In 2017 in the NWFD the detection rate in the group of homo- and bisexual persons (code 103) was at the similar level of 3.5% (in 2009 — 3.2%). The detection rate was more than 5% and more than the district average in Murmansk Region (22.2%), Vologda (5.0%), and Pskov (8.3%) Region. HIV detection rate among foreign citizens in 2017 decreased by 13.6% and made 97.6 per 100,000 of the examined persons (in 2010 it was 273.2).

In total, during the whole period of registration as of 31.12.2018 in 11 territorial units of the NWFD 136,359 cases of HIV infection were recorded. In general it makes 11.0% of the total number of HIV-positive persons in the RF (as of the state date 1,326,329 persons). Not taking into account the deceased (29,343 persons), by the end of 2018 in the NWFD there were 107,016 HIV-positive persons.

In 2018, 6683 new cases of HIV were recorded in 11 territorial units of the NWFD among the citizens of the RF, which is 1.3% more as compared to 2017 (Fig. 42, Tabl. 19)

Increased incidence of HIV infection was observed in 9 territorial units of the NWFD: the Republic of Karelia (31.9%), Vologda Region (15.9%), Murmansk Region (14.7%), Arkhangelsk Region (13.5%), Pskov Region (13.3%), Leningrad Region (6.8%), Novgorod Region (6.2%), Komi Republic (6.0%) and Nenets Autonomous District (9 persons in 2018 as compared to 5 in 2017).

For many follow-up years, HIV incidence in the Northwestern Federal District of the RF was more than the country average (up to 1.5 times). Since 2009, however, there has been an emerging trend towards incidence decrease, and in 2013 the incidence level was already lower than the country average (44.7 and 54.3 per 100,000 of population, respectively). In 2018, the incidence in the NWFD among persons newly diagnosed with HIV increased as compared to 2017 (40.4) and made 47.9 per 100,000 of population, which is still less than the country average (69.0 per 100,000 of population) (Fig. 43).

Based on the monitoring of the HIV epidemic situation in 11 territorial units of the NWFD, the following tendencies can be seen in the District.

When considering age groups broken down by age we found out that in 2017, the highest incidence in males was in the age group 35–39 years (174.5 per 100,000 of popula-

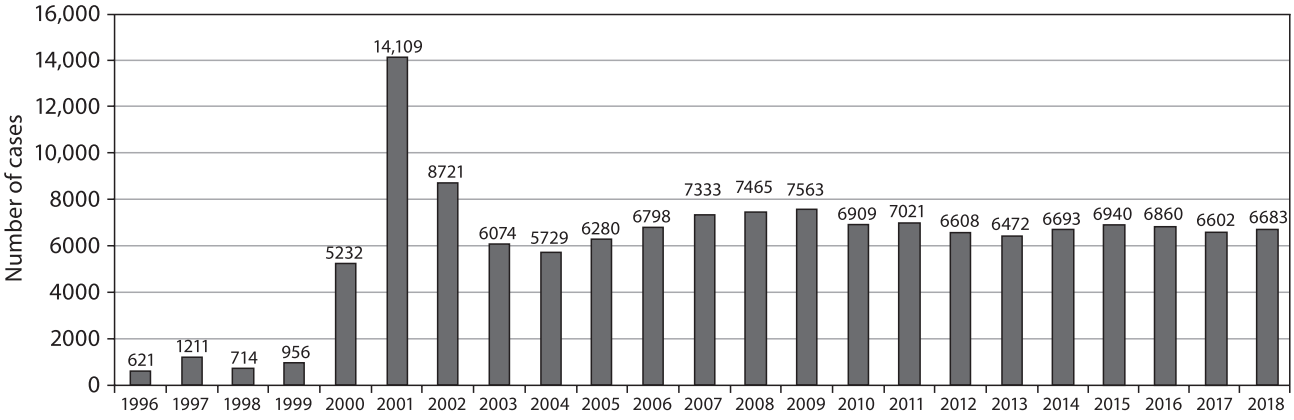
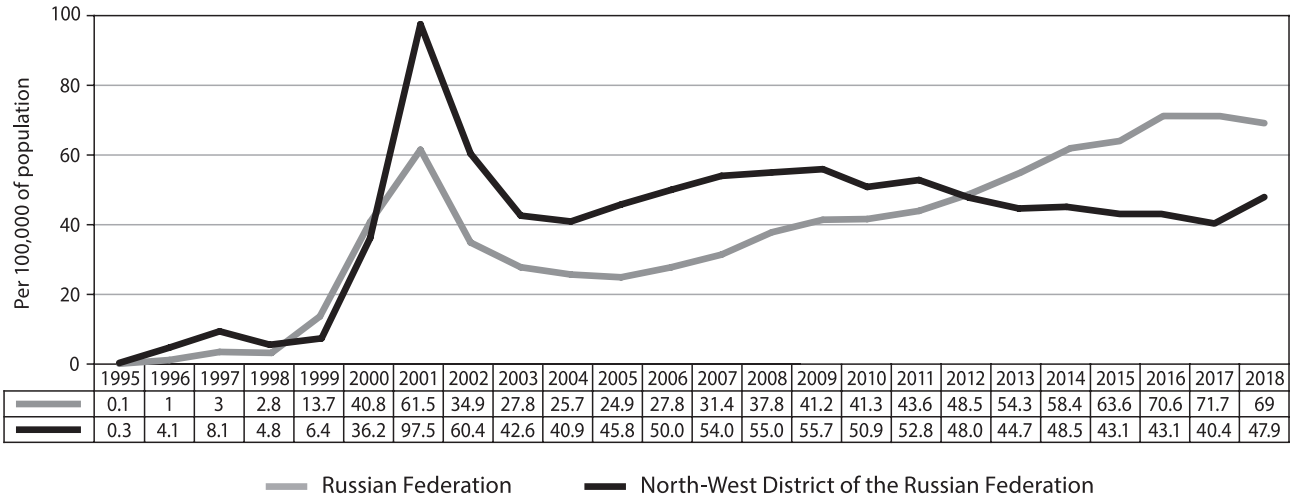


Figure 42. Annual changes in HIV incidence in the NWFD (1996–2018)



	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Russian Federation	0.1	1	3	2.8	13.7	40.8	61.5	34.9	27.8	25.7	24.9	27.8	31.4	37.8	41.2	41.3	43.6	48.5	54.3	58.4	63.6	70.6	71.7	69
North-West District of the Russian Federation	0.3	4.1	8.1	4.8	6.4	36.2	97.5	60.4	42.6	40.9	45.8	50.0	54.0	55.0	55.7	50.9	52.8	48.0	44.7	48.5	43.1	43.1	40.4	47.9

Figure 43. HIV incidence in the Russian Federation and the NWFD, 1995–2018

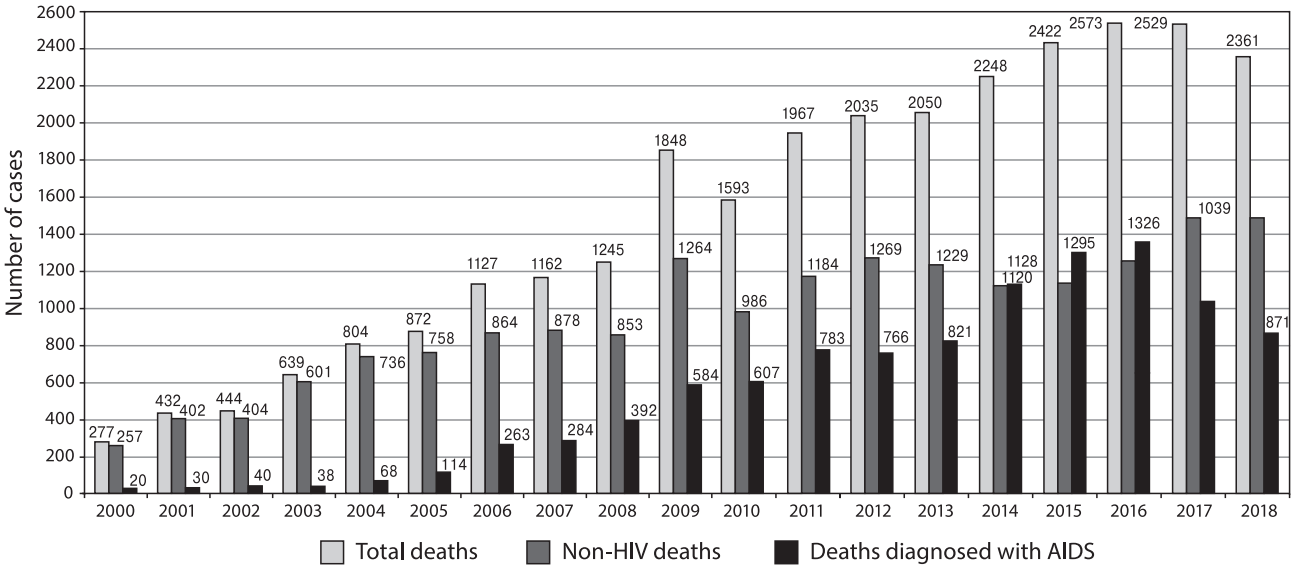


Figure 44. Number of deaths in HIV-positive patients and those with AIDS in the territorial units of the NWFD, 2000–2018

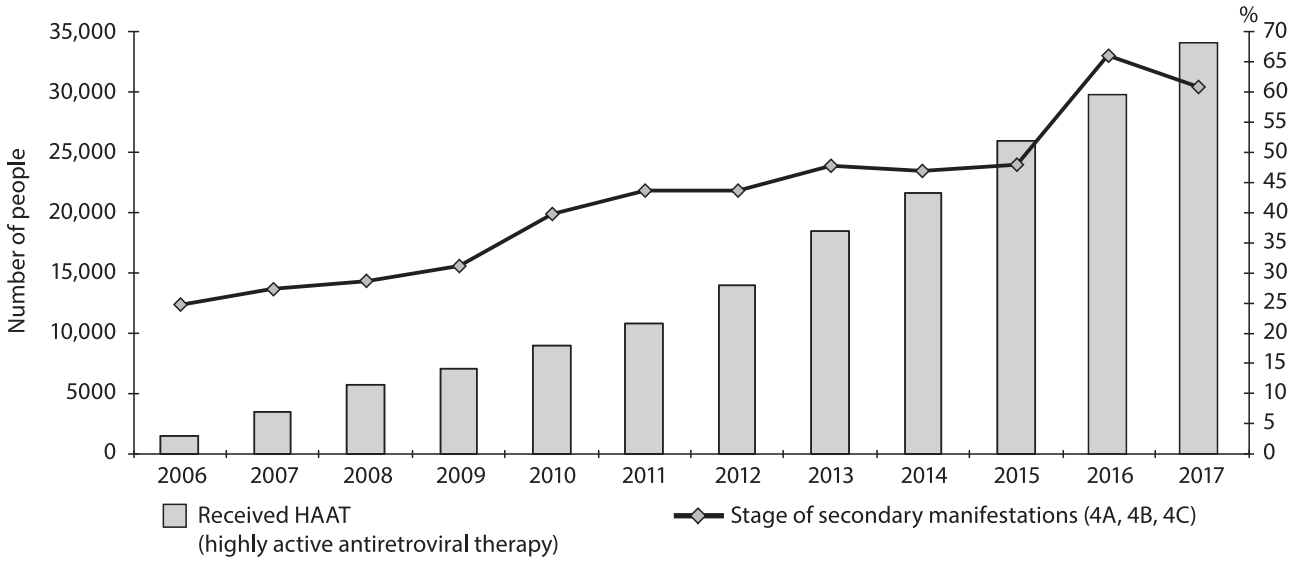


Figure 45. Number of HIV-positive patients receiving HAART and the share of patients with advanced disease in the territorial units of the NWFD from 2006 to 2017

tion), in females — in the age group of 30–34 years (89.4 per 100,000 of population). In spite of relatively low incidence rate, teenage girls and young women remain the group most vulnerable to HIV infection.

In 2017, the share of young people among persons newly diagnosed with HIV continued to decrease. In 2005, the age group of 15–19-year-olds accounted for 8.4%, in 2016 — for 1.0%; the age group of 20–24-year-olds in 2005 accounted for 30.6%, in 2017 — for 5.3%.

The total cohort of HIV-positive NWFD residents in 2017 was composed predominantly of males (60.7%). The percentage of women in the population of HIV-positive persons in on the increase: from 18.9% in 1995 and 26.2% in 2000 to 39.3% in 2017.

In 2017, virus transmission through heterosexual contacts was recorded in 65.3% (in the RF — in 53.5%), through injection drug use — in 29.1% (in the RF — in 43.6%). Only in the Arkhangelsk Region virus transmission through injection drug use prevailed (51.0%).

Both in Russia in general and in the NWFD the last five years of monitoring the HIV epidemic showed the growing share of the sexual minorities group, which in 2017 made 4.5% (in 2011 it was 1.3%). When looking at separate territorial units, the share of the sexual minorities group in 2017 in St. Petersburg (13.8%) was more that the district average.

With regard to the distribution of HIV cases according to risk factors in men and women separately it should be noted that heterosexual pathogen transmission is the leading way in women. In 2017, among HIV-positive women sexual transmission was recorded as the main risk factor of infection in 81.9% (in 2005 — in 45.4%). In men in 2017 sexual transmission as a risk factor of infection was recorded in 54.2% of cases (in 2005 — 13.5%), injection drug use — in 37.3% of cases.

Due to late detection and seeking medical health as well as the increased number of HIV-positive persons with accompanying diseases, the death rate increased, including those diagnosed with AIDS, although in 2017 and 2018 this figure decreased, whereas the number of persons who died due to reasons not associated with HIV increased (Fig. 44).

In 2018, 1490 HIV-positive persons died in the NWFD due to reasons not associated with HIV. 871 deaths with the AIDS diagnosis were recorded. Main causes of death in persons with AIDS were tuberculosis, pneumonias, lymphomas, meningoencephalites.

In total, from the start of HIV recording in 1987 to 2018 29,343 HIV-positive persons died, including 10,590 diagnosed with AIDS.

Active involvement of women in the epidemic process resulted in the increased number of children born from HIV-positive mothers. Since the beginning of HIV recording in the district in 1987, 17,370 children have had perinatal HIV exposure. For example, in 2000 101 cases of perinatal exposure were registered, in 2012 — 1476, in 2013 — 1353, in 2014 — 1367, in 2015 — 1379, in 2016 — 1347, in 2017 — 1270. The cumulative number of children with confirmed HIV diagnosis due to perinatal transmission as of 31.12.2017 was 844. It should be noted that over half of children diagnosed with HIV in 2017 lived in St. Petersburg and Leningrad Region. Perinatal infection in the NWFD decreased from 25 to 1.3%, in some territorial units it ranged from 0 to 3.3%. The coverage with three-stage chemoprophylaxis to prevent HIV transmission from mother to child has increased. In 2017, the complete course of three-stage chemoprophylaxis of HIV transmission from mother to child was received by 88.9% mother-baby pairs, whereas in 2006 the figure was 72.5%.

The number of HIV-positive persons who are under regular medical check-up at AIDS centres in the NWFD grows every year. As of 31.12.2017, 68,264 HIV-positive persons were under regular medical check-up in 11 territorial AIDS centres.

In 34.1% of the examined persons HIV infection was at the subclinical (latent) stage 3. The stage of secondary manifestations (4A, 4B, 4C) was diagnosed in 61.1% of patients (in 2009 the figure was 31.5%, in 2005 — 11.3%) (Fig. 45).

In 2017, specific antiretroviral therapy was received by 34,180 HIV-positive persons which makes 87.9% of those in need of the treatment, 46.3% of persons under regular medical check-up and 33.3% of persons diagnosed with HIV infection.

Laboratory of HIV Immunology and Virology

The Laboratory of HIV Immunology and Virology carries out research into HIV genotyping as well as detecting mutations associated with drug resistance. These studies have been carried out since 2009 upon request of territorial Centres for AIDS Prevention and Control of the Northwestern Federal District.

In 2017–2018, 323 studies were conducted aimed at detecting resistance to antiretroviral drugs. A number of mutations causing multiple resistance to NRTI, NNRTI and PI drugs were revealed. The findings analysis of the study of HIV drug resistance revealed some of the most common mutations: M184V (51.08%), K103N (18.71%), L74V (12.95%), K101E (11.51%), A62V and G190S (10.79%). Other mutations are found in less than 10% of cases. They all emerged against the background of given treatment with antiretroviral drugs.

The findings of the study in the population of HIV-positive persons in the NWFD are put together in a uniform database for retrospective analysis of trends in primary and secondary HIV resistance to antiretroviral drugs in nine years from 2009 to 2018.

A promising line of research seems to be the identification of genetic markers of resistance to the infection with HIV and markers of HIV progression. In 2017–2018, the research aimed at detecting markers of genetic resistance to HIV — CCR5delta32 and CCR2 64I mutation by pyrosequencing was continued. Studying these laboratory markers is of special interest for the NWFD, since genetically this region belongs to the areas of maximum spread of the CCR5delta32 mutation. According to preliminary study findings, the prevalence of carriage of the CCR5delta32 allele in St. Petersburg is 17.9% (n = 586), which is similar to the prevalence values for this allele in the southern Sweden and Finland. A test kit for the detection of CCR2 64I mutations, which is of epidemiological interest as a genetic marker of HIV replication inhibition, has been developed and is now tested on the population of the NWFD.

The significance of detecting HIV resistance markers (example of discordant pairs) was proved through the clinical practice.

Conclusion

1. In recent years, in the NWFD there is a trend towards the decrease in HIV incidence both as absolute values and with reference to the general population.
2. These trends suggest that the NWFD stands in a better position in terms of HIV incidence as compared to the neighbouring Ural and Central Federal Districts as well as the Volga and Siberia Federal Districts.
3. Of all new cases registered in 2017–2018, St. Petersburg and Leningrad Region account for 60%; however, in recent years the trend towards incidence decrease in these areas has been registered. This is typical of all the regions where metropolis cities provide the majority of HIV-positive patients.
4. The main NWFD units have had a rather low incidence level for a number of years. At various times, Kaliningrad Region, Komi Republic, and Vologda Region were an exception, where the values were slightly higher. In 2017, a significant increase in incidence was registered in Vologda (28.0%), Kaliningrad (12.8%), Novgorod (4.4%) regions and in the Republic of Karelia (7.6%). In 2018, an increase in HIV incidence was seen in nine territorial units of the NWFD, i.e. the Republic of Karelia (31.9%), Vologda Region (15.9%), Murmansk Region (14.7%), Arkhangelsk Region (13.5%), Pskov Region (13.3), Leningrad Region (6.8%), Novgorod Region (6.2), Komi Republic (6.0%) and NAD (9 persons in 2018 as compared to 5 in 2017). However, epidemiologic data and laboratory findings show that these newly diagnosed cases were associated with infection in the previous years. The average time between the possible infection transmission to the patient and HIV detection is 3 to 6 years and more.
5. Particular attention should be paid to HIV prevalence in the region (number of persons living with the virus except those who died earlier). It is this value that reflects general trends in virus spread in the society and makes it evident that prevention, diagnosis and treatment expenditures are inevitable. This value has constantly grown since 2000, and by the end of 2018 the number of HIV-positive persons is 107,016. In recent years St. Petersburg is not the most affected region anymore taking into account its population; nevertheless, the prevalence is high and constantly growing both throughout the country and in the region.
6. The number of HIV isolates with multiple resistance to antiretroviral drugs is on the increase, which can require changes in the laboratory testing algorithm for patients newly diagnosed with HIV in the nearest future.
7. Therefore, according to HIV preliminary analysis of the two main values (incidence and cumulative prevalence) for 2017 and 2018, the HIV epidemic is an unstable one with a tendency towards growing HIV incidence and prevalence.

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Department of New Technologies

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The Department of New Technologies includes the laboratory of biological products (head Vyacheslav Verbov), the laboratory of molecular and biological technologies (head Olga Freilikhman), and the laboratory of immunochemical technologies (head Olga Zarucheinova).

In 2017–2018, the work in the laboratories was carried out along several lines. Each of the directions included both the development of new diagnostic preparations and the commercial output of products developed previously.

1. Enzyme-linked immunoassay (EIA)

1.1. Scientific developments

Assessment of cell-mediated immunity in patients with tuberculosis and HIV gave the following results. The QuantiFERON-TB Gold In-Tube kit based on quantitative measurement of antigen-induced gamma interferon cannot be used for the examination of patients with tuberculosis + HIV co-infection. This is due to the decreased amount of CD4 T-lymphocytes. At the same time, it has been shown that chemokine IP-10 can be better suited as a biomarker.

1.2. Commercial products

Based on its own developments, the department produces the following EIA test kits:

- immunoenzyme test kit for the identification of IgG to the tuberculosis-causing agent (EIA-anti-TUB);
- immunoenzyme test kit for the identification of IgM to the leptospirosis-causing agent (EIA-anti-LER-M);
- immunoenzyme test kit for the identification of *C. burnetii Rickettsia* antigens (EIA-Q-ANTIGEN);
- immunoenzyme test kit for the identification of IgG to the Q fever-causing agent (EIA-anti-Q).

2. Polymerase chain reaction method

2.1. Scientific developments

2.1.1. Development of RT-PCR test kits for the identification of mycoplasma and ureaplasma DNA in cattle

Reproductive system disorders of the cattle caused by *Mycoplasma bovis, M. bovigenitalium,* and *Ureaplasma diversum* result in substantial economic damage in cattle-breeding. These diseases often have only mild symptoms or are asymptomatic. Therefore, the development of methods of laboratory diagnosis of these diseases is an important and relevant task.

In 2018, the officers of the department developed test kits based on real-time PCR for the identification of *M. bovis, M. bovigenitalium,* and *U. diversum* DNA in the cattle biological material. Tests showed that the prevalence of *U. diversum* carriage differs depending on the age group. Most frequently *U. diversum* was found in calves.

Novelty of the developed test kit for *U. diversum* DNA identification was confirmed by the patent No. 2 683 029 Method of identification and quantitative assessment of *U. diversum* DNA in the material from adult cattle using real-time PCR. The test kit received the gold medal of the Bioindustry-2018 exhibition.

2.1.2. Development of the RT-PCR test kit for the identification of *Coxiella burnetii* DNA

The test kit (a set of primers and a fluorescent probe) for the identification of *C. burnetii* in the biological material based on real-time PCR amplification (RT-PCR) according to the TaqMan technique of the *higA* gene fragment coding the virulence-associated protein of *C. burnetii*. The developed test kit underwent comparative estimation, optimisation, and assessment of its specificity and sensitivity. The obtained results showed that TaqMan modification of RT-PCR using *higA* primers and the designed detection probe labelled with fluorescein is an efficient method of identification and quantitative measurement of *C. burnetii* in the biological material.

2.1.3. Research into the resistance of human-pathogenic mycoplasma species to fluoroquinolones

Currently, macrolides and fluoroquinolones are the main drugs used to treat the diseases caused by *M. hominis*. At the same time, resistance to these drugs is also common. The conducted study made it possible to get into pure culture and describe the *M. hominis* isolate resistant to second- and third-generation fluoroquinolones. Through the sequencing of fluoroquinolone target genes, subunits of gyrase *(gyrA/B)* and topoisomerase IV *(parC/E)*, we were able to find two mutations. In ParC protein we found a new, previously not described replacement of arginine with lysine in 144; we also discovered a new mutation causing replacement of glycine with valine in 409 residue of the GyrB protein; these are new data in the study of molecular fundamentals of mycoplasma resistance to fluoroquinolones.

2.1.4. Adaptation de la méthode d'amplification isotherme à boucle (LAMP) pour la détection de *Neisseria gonorrhoeae*

The optimal genetic targets (*porA* gene) have been selected for the amplification of *N. gonorrhoeae* DNA from the clinical material using loop-mediated isothermal amplification.

3. Nephelometric analysis

3.1. Scientific developments

The following test kits have been developed:

- test kit for urine bacteriuria screening using coherent fluctuation nephelometry;
- test kit for detecting antibiotic residues in the urine using coherent fluctuation nephelometry.

The method of coherent fluctuation nephelometry has a number of advantages over conventional nephelometry. The main advantage is that standard glass round 5 ml bottles can be used as cuvettes. It has been shown that with the developed test kits bacterial growth can be reliably registered from the concentration of 10^3–10^4 CFU/ml.

4. Culture diagnosis

4.1. Scientific developments

The test kit for determining *Candida* fungi susceptibility to antifungal agents has been developed. The kit is centered around an original chromogenic culture medium. It allows for visual assessment of findings. Examination of *Candida albicans* clinical isolates showed their high susceptibility to voriconazole (100%), ketoconazole (96%), fluconazole (94%) miconazole (78%), amphotericin B, and itraconazole (78%) and lower susceptibility to clotrimazole (54%).

4.2. Commercial products

Based on its own scientific developments, the department produces the following commercial test kits for the cultural diagnosis:

- test kit for visual identification of *Mycoplasma hominis* (MYCOPLASMA-50);
- test kit for visual identification of *Ureaplasma urealyticum* (UREAPLASMA-50);
- test kit for the determination of *Mycoplasma hominis* antibiotic susceptibility (MYCOPLASMA-AS);
- test kit for the determination of *Ureaplasma urealyticum* antibiotic susceptibility (UREAPLASMA-AS);
- test kit for the simultaneous visual identification of *Ureaplasma urealyticum* and *Mycoplasma hominis* (UREA/MYCO-SCREEN-2);
- test kit for the simultaneous identification, semi-quantification of the titer and determination of susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* to 8 antibiotics (UREA/MYCO-SCREEN-AS);
- test kit for the visual identification of *Trichomonas vaginalis* (SVT);
- test kit for gonococci identification in culture (SVG);
- test kit for single-stage isolation and identification of *P. putida* and *P. aeruginosa* (PSEUDOMONAS APS);
- test kits for isolation and simultaneous identification of bacteria of the genera groups *Proteus, Providencia,* and *Morganella* (PROTEUS PPM);
- test kits for isolation and simultaneous identification of bacteria of the *Klebsiella* genus: *K. oxitoca, K. pneumoniae,* and *K. mobilis*.

5. Passive hemagglutination test (PHA)

5.1. Scientific developments

Dry tetanus erythrocytic diagnosticum for the passive hemagglutination test has been developed. The work was based on the purified tetanus toxoid. Main physical and chemical parameters have been optimised for the sensitization stage of chick erythrocytes.

5.2. Commercial products

Based on the previous scientific developments, the department produces the following commercial passive hemagglutination test kits:

- antigen dry measles erythrocytic diagnosticum for passive hemagglutination test (KED-90);
- Vi-antigen dry erythrocytic diagnosticum for passive hemagglutination test (SED).

6. Disk-diffusion method

6.1. Scientific developments

The set of discs with antibiotics in cartridges has been developed using modern criteria for the assessment of findings according to the Antimicrobial susceptibility test clinical practice guidelines dated 02.05.2015 and EUCAST, Version 7.1, 2017.

6.2. Commercial products

Based on the previous scientific developments, the department produces discs with antimicrobial and antifungal drugs as well as indicator discs:

- disc kit for determining susceptibility to antimicrobial agents (ND-PMP-1) — total of 66 antibiotic drugs.

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The Testing Laboratory Centre

The Testing Laboratory Centre is a structural unit of our research institute. Along with the research, it is also engaged in commercial activities. The Testing Center includes a vivarium and specialized (virological and microbiological) laboratories of the Institute.

Scientific research of the Testing Centre deals with applied clinical microbiology and parasitology, i.e. with the development and production of various means for infection prevention and treatment, including wound infection, in order to further improve the epidemic prevention system. In this regard, the Centre is working at new drugs and medical products for the prevention and treatment of wound infection as well as for the control of multi-drug resistant pathogens causing infectious complications in patients.

Commercial activity of the Testing Center includes preclinical and clinical studies of a wide range of products, i.e.

- pharmaceutical products;
- medical products;
- immunobiological drugs;
- disinfectants;
- antiseptic drugs;
- personal protective equipment;
- cosmetic agents;
- oral hygiene products, and
- household products.

Equipping the Testing Centre in accordance with the requirements of the GLP (good laboratory practice) may contribute to its further development.