Saint Petersburg Pasteur Institute

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Currently, Saint Petersburg Pasteur Institute is one of the largest institutes of epidemiology and microbiology in Russia, especially in the Northwestern region of Russia.

The Institute has a modern equipment and a quality scientific staff that are united in four departments:

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

The Institute has Centers for Surveillance of Infectious Diseases:

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

Collaboration with WHO:

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

Contents

Department of Microbiology

LABORATORY OF ENTERIC INFECTIONS

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The main field of the laboratory's research in recent years has been the study of the aerobic component of the intestinal microbiota of adults and children without infectious pathology of the digestive organs. The aim of the study was to characterize opportunistic microorganisms and to determine virulence factors and antibiotic resistance of isolated strains.

Methods: bacteriological: species identification (Vitek 2 Compact automatic analyzer, EnteroTest Lachema test systems); Determination of antibiotic sensitivity by various methods (disc diffusion test, gradient concentrations, serial dilutions); Molecular: PCR with electrophoretic and fluorescence in situ hybridization for detection of virulence and antimicrobial resistance genes); statistical.

16 (6.9%) of the studied residents of St. Petersburg were found to have gut microbiota within the normal limits, without dysbiotic disorders. 216 out of 233 people (93.1%) had dysbiotic disorders of varying severity (Tables 1 and 2).

No statistically reliable differences were found between the group of children under the age of 1 year and older children in terms of the ratio of samples without pathological changes and with dysbiotic disorders of the first and second degree. More pronounced deviations from the norm (grade 3) were reliably more often detected in children under one year of age (Table 1).

Minor deviations from the normal status (the first degree of microbiological disorders) were found in 18.0% (95% CI: 13.6–23.5) of the studied patients. The changes were characterized by a decrease in the amount of *Bifidobacterium* spp. (to 10^8 CFU/g), *Lactobacillus* spp. and "typical" *E. coli* (to 10^6 CFU/g).

Microbiocenosis disorders corresponding to the second degree occurred in 67.8% (95% CI: 61.6–73.5) of the examined individuals. Deviations from the norm were characterized by a decrease in the content of *Bifidobacterium* spp. (to 10^7 CFU/g) and *Lactobacillus* spp. (to 10^5 CFU/g), an imbalance in the quantitative and qualitative composition of *Escherichia coli* (the presence of strains with hemolytic activity and unable to ferment lactose), the appearance of opportunistic pathogens in amounts exceeding 10^5 CFU/g.

The third degree of microbiocinosis disorders occurred in 7.3% (95% CI: 4.6–11.4) of the examined residents of St. Petersburg. Violations were characterized by a marked decrease in the amount of *Bifidobacterium* spp., to less than 10^7 CFU/g, *Lactobacillus* spp. to less than 10^5 CFU/g, an increase in the content of opportunistic microorganisms to 10^6 CFU/g and above, the emergence of associations of opportunistic pathogens.

Salmonella pathogens (serological variants of *Salmonella* enterica serovar Enteritidis and Typhimurium in the amount of 8.0×10^3 CFU/g) were isolated from stool samples of two patients (0.86%) without clinical manifestations of diarrhea or a history of acute intestinal infection and contact with infectious patients. The *S.* Typhimurium strain

Table 1. Dysbiotic disorders in people of different age groups

Degree of dysbiosis	Age						Total N = 233		
	< 1 year N = 48			≥ 1 year N = 185					
	abs.	%	95% CI	abs.	%	95% CI	abs.	%	95% CI
0	1	2.1	0.4–10.9	15	8.1	5.0–12.9	16	6.9	4.3–10.9
1	4	8.3	3.3–19.6	38	20.5	15.3–26.9	42	18.0	13.6–23.5
2	34	70.8	56.8–81.8	124	67.0	60.0–73.4	158	67.8	61.6–73.5
3	9	18.8	10.2–31.9	8	4.3	2.2–8.3	17	7.3	4.6–11.4

Table 2. Characteristics of dysbiotic disorders

Degree of dysbiosis	Number of samples 233		Characteristics of changes
	abs./%	95% CI	
0	16/6.9	4.3–10.9	no changes
1	42/18.0	13.6–23.5	- reduction: *Bifidobacterium* spp. (to 10^8 CFU/g), *Lactobacillus* spp., "typical" *E. coli* (to 10^6 CFU/g)
2	158/67.8	61.6–73.5	- reduction: *Bifidobacterium* spp. (to 10^7 CFU/g), *Lactobacillus* spp. (to 10^5 CFU/g); - an imbalance in the quantitative and qualitative composition of *Escherichia coli* (the presence of strains with hemolytic activity and unable to ferment lactose); - the appearance of opportunistic pathogens in amounts exceeding 10^5 CFU/g
3	17/7.3	4.6–11.4	- marked decrease: *Bifidobacterium* spp. (less than 10^7 CFU/g), *Lactobacillus* spp. (less than 10^5 CFU/g); - the content of opportunistic microorganisms increased to 10^6 CFU/g and above; - the emergence of their associations

was sensitive to cephalosporins and carbapenems, resistant to aminoglycosides and fluoroquinolones. A strain of *S.* Enteritidis isolated from an adult patient (43 y.o.) was found to be resistant to fluoroquinolones.

Opportunistic pathogens microorganisms and their associations were found in 61.2% of all tested individuals, and in 87.8% of children under the age of 1 year.

22.8% of the samples included associations of *Staphylococcus aureus* with various members of Enterobacteria family (hemolytic and lactose-negative *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp., *Morganella morganii*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Candida* spp.).

Staphylococcus aureus was detected in 40.8% of the subjects. In children under the age of 1 year, it was detected in 70.8% of cases, in residents older than 1 year, in 33.0% of cases (Table 3). The amount of *Staphylococcus aureus* in stool samples ranged from 10^2 to 10^6 CFU/g.

Lactose-negative *Escherichia coli* were detected in 17.6% of the samples studied. The amount of *E. coli* in the samples varied from 10^2 to 10^{10} CFU/g and in most cases (82.9%) was within the range of 10^7–10^9 CFU/g.

Escherichia coli with hemolytic activity were detected in 16.7% of the subjects. Their quantity in the samples varied from 10^2 to 10^{10} CFU/g and in most cases (82.1%) was within the range of 10^7–10^9 CFU/g. The proportion of samples containing *E. coli* of these phenotypes did not significantly differ in children under the age of 1 year and older children.

Klebsiella — bacteria of two species (*Klebsiella pneumoniae* and *Klebsiella oxytoca*) — were found in 40.3% of the examined individuals, while in fecal samples of children under the age of 1 year they were detected in 83.3% of cases, and in 29.2% of older subjects. *Klebsiella pneumoniae* and *Klebsiella oxytoca* were more often detected in samples in children under 1 year old than in those older than 1 year (17.0% and 8.0%, respectively). The amount of *Klebsiella pneumoniae* in the samples varied from 10^5 to 10^{10} CFU/g and in most cases (82.1%) was within the range of 10^6–10^8 CFU/g. The amount of *Klebsiella oxytoca* in the samples ranged from 10^2 to 10^8 CFU/g and in most cases (93.3%) was within the range of 10^5–10^8 CFU/g.

According to the total data, opportunistic *Enterobacteriaceae* were detected in 44.7% of samples, while in children under 1 year old they were found in 75.5% of cases.

Among 95 studied *S. aureus* strains, 18 (18.9%) MRSA strains (methicillin-resistant *S. aureus*) were identified. 29.5% of the strains were resistant to erythromycin (Table 4).

Bacteria of the genus *Citrobacter* were detected in the studied samples in 8.2% of cases. In children under the age of 1 year, they were detected in 14.6% of samples, and in 6.5% of samples from those older than 1 year. Their number in the samples varied from 10^2 to 10^9 CFU/g and in most cases (89.5%) was within the range of 10^5–10^8 CFU/g. 5.3% of *Citrobacter* spp. strains were resistant to third-generation cephalosporins. No strains resistant to fluoroquinolones have been identified.

Enterobacter were detected in 8.6% of samples, and in children under the age of 1 year in 12.5% of cases. 45.0% of the strains had resistance to third-generation cephalosporins, and all were sensitive to fluoroquinolones.

Table 3. Opportunistic pathogens isolated during the study of the gut microbiota of St. Petersburg residents

Microorganisms	Age					
	< 1 year N = 48		≥ 1 year N = 185		Total N = 233	
	abs.	%	abs.	%	abs.	%
Escherichia coli: hem. (+)*	8	16.7	31	16.8	39	16.7
Escherichia coli: lac. (–)**	12	25.0	29	15.7	41	17.6
Klebsiella spp.	40	83.3	54	29.2	94	40.3
Citrobacter spp.	7	14.6	12	6.5	19	8.2
Enterobacter spp.	6	12.5	14	7.6	20	8.6
Proteus spp.	2	4.2	6	3.2	8	3.4
Morganella morganii	0	0	3	1.6	3	1.3
Pseudomonas aeruginosa	0	0	4	2.1	4	1.7
Acinetobacter spp.	0	0	3	1.6	3	1.3
Staphylococcus aureus	34	70.8	61	33.0	95	40.8
Candida spp.	6	12.5	26	14.1	32	13.7

*cause hemolysis of erythrocytes, hem+; **do not ferment lactose, lac–.

Table 4. Opportunistic pathogen strains resistant to antimicrobial drugs

Microorganisms	Total	Cephalosporins III generation		Fluoroquinolones		Cefoxitin		Erythromycin	
		abs.	%	abs.	%	abs.	%	abs.	%
Citrobacter spp.	19	1	5.3	0	0	–		–	
Enterobacter spp.	20	9	45.0	0	0	–		–	
Proteus spp.	8	1	12.5	0	0	–		–	
Morganella morganii	3	1	33.3	1	33.3	–		–	
S. aureus	95	–	–	–	–	18	18.9	28	29.5

"–" sensitivity to these antibiotics was not determined.

Table 5. Sensitivity to antimicrobial drugs of *Klebsiella* spp. strains

No.	Opportunistic pathogen	Sensitive			Resistant		
		abs.	%	CI	abs.	%	95% CI
1	Amoxicillin/clavulanate	78	82.98	74.13–89.24	16	17.02	10.76–25.87
2	Ceftazidime	86	91.49	84.10–95.62	8	8.51	4.38–15.90
3	Cefotaxime	86	91.49	84.10–95.62	8	8.51	4.38–15.90
4	Cefepime	85	90.43	82.80–94.88	9	9.57	5.12–17.20
5	Meropenem	93	98.94	94.22–99.81	1	1.06	0.19–5.78
6	Nalidixic acid	89	94.68	88.15–97.71	5	5.32	2.29–11.85
7	Ciprofloxacin	88	93.62	86.77–97.04	6	6.38	2.96–13.23
8	Tetracycline	89	94.68	88.15–97.71	5	5.32	2.29–11.85
9	Chloramphenicol	93	98.94	94.22–99.81	1	1.06	0.19–5.78
10	Nitrofurantoin	86	91.49	84.10–95.62	8	8.51	4.38–15.90
11	Trimethoprim/sulfamethoxazole	89	94.68	88.15–97.71	5	5.32	2.29–11.85
12	Gentamicin	89	94.68	88.15–97.71	5	5.32	2.29–11.85
13	Tobramycin	88	93.62	86.77–97.04	6	6, 38	2.96–13.23
14	Amikacin	93	98.94	94.22–99.81	1	1.06	0.19–5.78

The microorganisms that were extremely rarely detected in samples included *Proteus* spp. (3.4%), *Morganella morganii* (1.3%), *Pseudomonas aeruginosa* (1.7%), and *Acinetobacter* spp. (1.3%).

Candida were detected in 13.7% of the samples, including 3.8% with the quantity that exceeded the normal level (10^4 CFU/g).

Associations of opportunistic microorganisms in various combinations were found in the gut microbiota in 28.3% of the studied residents of the city. In children under the age of 1, associations of opportunistic microorganisms were detected in 61.2% of samples, and in 19.7% of samples from those older than 1 year. Associations of opportunistic pathogens were more often represented by two microorganisms (20.7%), with associations of three (7.2%) and four (0.4%) types of opportunistic microorganisms being less common.

According to the total data, among the strains of *Klebsiella* spp. (*Klebsiella pneumoniae*, and *Klebsiella oxytoca*), 17 (2.0%) were resistant to amoxicillin/clavulanate, 8.5% to third-generation cephalosporins, and 6.4% to fluoroquinolones. 10.7% of *Klebsiella pneumoniae* strains were resistant to amoxicillin/clavulanate, 21.4% to third-generation cephalosporins, and 8.9% to fluoroquinolones (Table 4).

Further, a detailed study was carried out to determine the sensitivity to antimicrobial agents, genes encoding resistance to cephalosporins of 3rd and 4th generations, and virulence (11 genes) of 94 strains of *Klebsiella* spp. (76 strains of *Klebsiella pneumoniae* and 18 strains of *Klebsiella oxytoca*).

The share of *Klebsiella* spp. sensitive to all the antimicrobial agents used in the tests was reliably higher in the population of strains isolated from the gut microbiota of apparently healthy individuals (children and adults). Their share for each of the 14 antimicrobial agents was more than 80% (Table 5). The proportions of strains resistant to each antimicrobial agent ranged from 1.06% (meropenem, chloramphenicol and amikacin) to 17.02% (amoxicillin/clavulanate). This trend was observed in the populations of *Klebsiella pneumoniae* and *Klebsiella oxytoca* strains.

Eight strains (8.51%) of *Klebsiella* spp. were characterized by the phenotype of multiple resistance to antimicrobial agents. Resistance to cephalosporins of the 3rd/4th generation (ceftazidime, cefotaxime and cefepime) was due to the production of broad-spectrum β-lactamases, which belonged to the epidemiologically significant cephalosporinases of three genetic families: $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{OXA}.

Two strains contained the genes of two ESBL producers ($bla_{\text{CTX-M}}$ + bla_{TEM}).

One strain containing the genes of three ESBL producers ($bla_{\text{CTX-M}}$ + bla_{TEM} + bla_{OXA}) was characterized by resistance to meropenem due to the production of metallo-β-lactamase (NDM) (Table 6).

Table 6. Phenotypes of drug resistance in ESBL producing *K. pneumoniae* and *K. oxytoca*

Resistance phenotypes	abs.	%
Amoxicillin/clavulanate + ceftazidime + cefotaxime + cefepime + nalidixic acid + ciprofloxacin + tetracycline + trimethoprim/sulfamethoxazole + gentamicin + tobramycin	2	2.13
Amoxicillin/clavulanate + ceftazidime + cefotaxime + cefepime + nalidixic acid + ciprofloxacin + tetracycline + trimethoprim/sulfamethoxazole + tobramycin	1	1.06
Amoxicillin/clavulanate + ceftazidime + cefotaxime + cefepime + gentamicin + tobramycin	1	1.06
Ceftazidime + cefotaxime + cefepime + nalidixic acid + ciprofloxacin + trimethoprim/sulfamethoxazole + gentamicin + tobramycin	1	1.06
Amoxicillin/clavulanate + ceftazidim + cefotaxime + cefepime + chloramphenicol	1	1.06
Amoxicillin/clavulanate + ceftazidime + cefotaxime + cefepime + ciprofloxacin + co-trimoxazole	1	1.06
Amoxicillin/clavulanate + ceftazidime + cefotaxime + cefepime + meropenem + nalidixic acid + ciprofloxacin + nitrofurantoin + gentamicin + tobramycin + amikacin	1	1.06

Detection of genes encoding the synthesis of 11 virulence factors: six capsular antigens *(magA, K2, K5, K20, K54, K57)*, production of toxins *(hly, cnf)*, adhesin *(FimH)*, siderophore *(iutA)*, and mucoid phenotype regulator *(rmpA)*, has shown that the *Klebsiella* strains population is heterogeneous in terms of specific virulence genes presence. The gene responsible for the regulation of the mucoid phenotype *(rmpA)* was detected in 4 strains (4.30%). Genes responsible for capsular synthesis were found in 11 strains (11.83%), of which the *magA* gene, or *K1*, was found in 5 (5.38%), the *K2* gene in 4 (4.30%), the *K54* gene in 1 (1.08%). The *fimH* gene encoding mannose-sensitive type I fimbriae was found in 3 strains (3.23%). The *iutA* gene responsible for the synthesis of the aerobactin sideraphore was found in 9 strains (9.68%). The *hly* gene encoding the synthesis of the α-hemolysin toxin was detected in 4 strains (4.30%). The *cnf* genes responsible for the synthesis of cytonecrotic factor and coding capsules *K20* and *K57* were not found in the studied strains.

According to the total findings, 20 of the 94 studied strains (21.51%) in the *Klebsiella* spp. population had virulence genes, while 78.49% of the strains did not have them (Table 7).

Table 7. Genes encoding the main virulence factors of *K. pneumoniae* and *K. oxytoca*

Genes encoding various virulence factors		abs.	%	95% CI
Virulence is present		20	21.51	14.38–30.90
Virulence is absent		74	78.49	69.10–85.62
Regulator of mucoid phenotype	*rmpA*	4	4.30	1.68–10.54
Responsible for capsule synthesis	*magA*	5	5.38	2.32–11.98
	K2	4	4.30	1.68–10.54
	K5	1	1.08	0.19–5.85
	K20	0	0.00	0.00–3.97
	K54	1	1.08	0.19–5.85
	K57	0	0.00	0.00–3.97
Adhesin	*fimH*	3	3.23	1.11–9.07
Siderophore	*iutA*	9	9.68	5.18–17.38
Toxins	*hly*	4	4.30	1.68–10.54
	cnf	0	0.00	0.00–3.97

Conclusion

In 2021–2022, the study of intestinal microbiocenosis of healthy individuals revealed the following:

1. A high level of resistance to antimicrobial agents of microorganisms that are part of the normal intestinal biota.
2. Modern molecular genetic methods must be used to detect genes encoding virulence factors and resistance to antimicrobial agents in strains of *Klebsiella* spp., the leading causative agent of healthcare-associated infections in children and adults.
3. The revealed patterns correspond to global trends that create a threat for the security of the world's population and require solutions at the global level.
4. New data have been obtained on the pathogenic potential of opportunistic microorganisms, which are part of the intestinal microbiota of apparently healthy children and adults and are characterized by resistance to antimicrobial agents, including the multiple resistance phenotype characteristic of healthcare-associated infections.
5. Opportunistic pathogens and their associations were found in every second examined patient, and in 87.8% of examined children under 1 year. In the intestinal normobiota of young children (up to two years old), opportunistic microorganisms (*Klebsiella* spp., *Staphylococcus aureus*, etc.) have a significant role, and they can cause dysbiotic disorders and diarrhea in persons with diminished resistance to infections. They can be a common cause of nosocomial infections and healthcare-associated infections, often leading to the death of patients in pediatric infectious and obstetric departments.

Publications

Articles

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8. *Suzhaeva L.V., Egorova S.A. Strains of Escherichia coli resistant to antimicrobial drugs in the intestinal microbiota of young children // Unresolved issues of etiotropic therapy: Proceedings of the All-Russian Annual Research to Practice Conference (St. Petersburg, December 5–6, 2022). St. Petersburg, 2022: 178–187.*
9. *Zabrovskaia A.V., Egorova S.A., Antipova N.A., Smirnova E.V., Semchenkova L.I., Bystraya T.E., Sokolnik S.E., Utkina N.P., Sikhando L.Y., Satosova N.V. Antimicrobial susceptibility of dominant Salmonella serovars, isolated in North-West federal district in 2004–2018 from different sourses // Journal Infectology. 2022; 14 (1): 131–139. (In Russ.) doi: 10.22625/2072-6732-2022-14-1-131-139*
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Patents

1. *Patent No. 2744203 C1, Int. Cl. C12Q 1/04 (2020.08); C12R 1/42 (2020.08). Strain of bacteria Salmonella enterica sbsp_ enterica serovar Kentucky B-9045 of the international multiresistant clone of Salmonella Kentucky ST198 used as a control strain for phenotypic and molecular studies in diagnosing salmonellosis. No. 2020122129; application: 29.06.2020: date of publication 03.03.2021 / Egorova S.A., Kaftyreva L.A. Proprietors: Federalnoe byudzhetnoe uchrezhdenie nauki "Sankt-Peterburgskij nauchno-issledovatelskij institut epidemiologii i mikrobiologii im. Pastera Federalnoj sluzhby po nadzoru v sfere zashchity prav potrebitelej i blagopoluchiya cheloveka" (FBUN NII epidemiologii i mikrobiologii imeni Pastera) (RU)*
2. *Patent No. 2744205 C1, Int. Cl. C12N 1/20(2006.01); C12Q 1/04(2006.01); C12R 1/42(2006.01). Strain of bacteria Salmonella enterica subsp. enterica serovar Newport B-9044 of the international multiresistant clone of Salmonella newport MDR-AMPC/CMY-2, used as a control strain for phenotypic and molecular studies in diagnosing salmonellosis. No. 2020122130; application: 29.06.2020: date of publication 03.03.2021 / Egorova S.A., Kaftyreva L.A. Proprietors: Federalnoe byudzhetnoe uchrezhdenie nauki "Sankt-Peterburgskij nauchno-issledovatelskij institut epidemiologii i mikrobiologii im. Pastera Federalnoj sluzhby po nadzoru v sfere zashchity prav potrebitelej i blagopoluchiya cheloveka" (FBUN NII epidemiologii i mikrobiologii imeni Pastera) (RU)*
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Software

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LABORATORY OF MEDICAL BACTERIOLOGY

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Recent trends in laboratory diagnostics of vaccine-preventable respiratory tract infections

Relevance of the study

Despite the ongoing vaccination against pertussis infection, the disease remains an urgent health problem in many countries of the world, including in the Russian Federation. There are also cases of pertussis in persons who had the disease in the past, i.e., we cannot count on lifelong immunity against this infection. Therefore, the scientific justification for the introduction of additional revaccination against pertussis in the Russian Federation is relevant for determining the strategy of vaccination.

The incidence of diphtheria has decreased in recent years thanks to vaccination. However, during the pandemic of the novel coronavirus infection, the vaccination campaign against this infection became dramatically less active. At the same time, cases of diphtheria are still being registered. Thus, in 2020, 1 case of diphtheria was detected in a vaccinated child, and in 2021, as many as 4 cases (also in children). Two cases of carriage of toxigenic strains of *C. diphtheria* have also been identified. It is necessary, therefore, to monitor the protection of the population from diphtheria all the time and thoroughly, as well as to conduct in-depth studies of pathogen strains to understand the epidemiology of diphtheria infection. It is especially important to study the dynamics of the spread of various bacterial clones, taking into account their pathogenicity factors and resistance to antibiotics prescribed as etiological therapy for diphtheria and pertussis.

As the spread of resistant strains of the ESKAPE group accelerates, including those associated with nosocomial infections, it is necessary to study the alternative ways to combat these bacteria, e.g., by using bacteriophages or new synthesized antibacterial compounds.

Study objective. The study of seroprevalence to the causative agent of pertussis among the population of St. Petersburg; the study of genetic and phenotypic markers of virulence and antibiotic resistance in strains of *C. diphtheria*, *Bordetella spp.* and strains of the ESKAPE group isolated in the Northwestern region over the past 25 years; the study of alternative ways to combat resistant strains.

Materials and methods

To assess seropevalence to the causative agent of pertussis, ELISA kits were used to determine antibodies to pertussis toxin (IgG, IgA). The following values were considered to be the positive level of antibodies: IgG ≥ 40 IU/ml, IgA ≥ 12 IU/ml. The detection of positive IgG levels was regarded as a marker of contact with the pathogen (as a result of the disease or "latent immunization") in the last 1–2 years, including high IgG levels (≥ 100 IU/ml), while a combination of positive IgG and IgA levels was regarded as a sign of pertussis suffered 6 to 12 months prior to the examination. To screen the strains of bordetella resistant to macrolides, the disc diffusion test and the determination of Minimal Inhibitory Concentration using E-tests were used, with casein-carbon agar as a growth medium.

The toxigenic properties of *C. diphtheria* strains were studied with genetic and phenotypic methods. To detect the toxigenic gene, we tested the new Lamp-PCR kit (manufacturer: Innova-plus, St. Petersburg) with a certified kit for detecting the toxigenic gene with Real-time method used for control. Phenotypic toxin production was detected in the Elek test. The ability to biofilm formation was studied with a fluorescent microscope.

Antibiotic sensitivity was determined using the disc diffusion method according to MUK 4.12.1890-04 (Moscow, 2004) and the Guidance on the assessment of bacterial susceptibility to antimicrobials (EUCAST, 2018). Sensitivity to bacteriophages was determined according to the Federal Clinical Guidelines "Rational use of bacteriophages in therapeutic and antiepidemic practice" (2014). This was done with the preparations of bacteriophages produced by AO NPO Microgen.

Key results

1. Study of seroprevalence of the population of St. Petersburg to *Bordetella pertussis*. To assess seroprevalence to the causative agent of pertussis, 297 adults and 90 children were examined in 2022. Among adults, the proportion of seropositive individuals was 9.4%, of which 1.3% had serological signs of a recent infection. The largest number of seropositive participants was revealed in the group of 18–29 year olds (12.9%), while in older age groups the proportion of people who came into contact with the causative agent of pertussis decreased, and in the group of 60 years and older, not a single seropositive participant was identified.

Of the 90 examined children and adolescents aged 3–17 years, 14.4% were found to have antibodies to pertussis toxin, including 2.2% who had serological signs of a recent infection.

2. The genomes of 20 strains of *B. pertussis* and *B. parapertussis* have been sequenced, the results are being processed.

3. Evaluation of anti-diphtheria immunity in the population of St. Petersburg. When examining the material from 160 children for antitoxic anti-diphtheria immunity, it turned out that the least protected group is the age category of 11–12 years, in which only 70% of children are protected. The indicators are slightly higher in the age group of 14 years (86%), 90% of children aged 16 have protective levels of antibodies, and 99% of children aged 17. This is primarily due to vaccination.

4. Sensitivity of corynebacteria to antibacterial drugs. The drugs of choice in the treatment of diphtheria and sanitation of carriers of *C. diphtheria* are benzylpenicillin, erythromycin, tetracycline, rifampicin, clarithromycin, and azithromycin. We determined the sensitivity of the isolated strains to these drugs. The greatest decrease in the sensitivity of the strains was noted with respect to benzylpenicillin and tetracycline, which were most often used when *C. diphtheria* strains were isolating from a patient.

5. Sensitivity of ESKAPE pathogens to antibiotics. 40 strains of bacteria of the ESKAPE group isolated in various medical institutions of St. Petersburg were studied, and their sensitivity to antibiotics at the phenotypic and

genetic levels was evaluated. Most often, the studied isolates showed resistance to cephalosporins and fluoroquinolones, as well as to inhibitor-protected penicillins recommended as antibacterial therapy for complicated forms of infection according to the Temporary Guidelines "Prevention, diagnosis and treatment of the novel coronavirus infection (COVID-19), version 16 (18. 08.2022).

6. Investigation of the antibacterial effect of chemical compounds against the ESKAPE group bacteria. The antibacterial effect of synthesized preantibiotics belonging to different chemical groups was studied using the example of bacterial strains of the ESKAPE group. It was found that 5-amino-1,2,4-thiadiazoles have pronounced antibacterial activity against *Enterobacter cloacae* and *Pseudomonas aeruginosa* strains. The MIC of this compound was lower than the MIC of the officinal antibiotic pefloxacin, which is used to eliminate these bacteria. Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecium* were found to be sensitive to azirine-containing dipeptides and depsipeptides. The MICs of the compounds were comparable to those of the fluoroquinolone group.

7. Investigation of the sensitivity of current bacteria to bacteriophages. The sensitivity of 120 bacteria, which are causative agents of nosocomial infections and bacterial complications in COVID-19, to the corresponding bacteriophages was studied. It was shown that more than 40% of antibiotic-resistant bacterial strains are sensitive to the corresponding bacteriophages. An express method for determining the sensitivity of gram-negative bacteria to bacteriophages has been developed (patent No. 2022106346/10 (013228)), which provides a quick answer the question about the sensitivity of bacteria to phages.

Conclusion

The data obtained indicate a wide circulation of the causative agent of pertussis among children and adolescents. Thus, 14.4% of children and adolescents aged 3–17 years were found to have antibodies to pertussis toxin. Therefore, revaccination of adults aged over 60 against pertussis is necessary due to a decrease in herd immunity level and an increased risk of severe and complicated course of infections in this age group.

It is also necessary to constantly monitor the protection of the population from diphtheria and the carriage of pathogenic corynebacteria. The spread of *C. diphtheria* strains resistant to antibiotics used for the treatment of patients and sanitation of carriers indicates the need for timely determination of the sensitivity of strains of these bacteria to antibiotics and their constant monitoring.

Due to the steady growth of antibiotic resistance of strains isolated from nosocomial infections, alternative ways to combat resistant strains must be found.

Diversity of CRISPR system in *Yersinia pseudotuberculosis* strains and their association with pathogenicity factors

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Introduction

Gram-negative *Yersinia pseudotuberculosis* bacteria are classified as psychrotrophic microorganisms capable of causing pseudotuberculosis, an acute sapro-zoonotic disease. Cases of pseudotuberculosis are registered in many subjects of the Russian Federation, but the regions with the highest incidence are the Far Eastern, Northwestern and Siberian Federal Okrugs. There are numerous factors that determine the virulence of *Y. pseudotuberculosis* strains and the severity of the disease they cause. The leading chromosomal pathogenicity factors of *Y. pseudotuberculosis* include the superantigen genes *ypmA, ypmC*, HPI ("high pathogenicity island") and YAPI ("Yersinia adhesion pathogenicity island"). The pYV and pVM82 plasmids contain genes associated with facultative intracellular parasitism of the bacterium: type III secretion system, adhesins and type IVB secretion system.

The constant exchange of genetical information with the participation of extra-chromosomal and transposable elements is one of the factors that leads to the existence of heterogeneous populations of microorganisms in certain environmental conditions. Some clusters of genes encoding pathogenicity determinants are associated with prophage integrases or insertion elements controlling genome rearrangements; therefore, the systems aimed at regulating horizontal gene transfer affect the evolution of bacteria. Such structures include the CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats), which is capable of targeted recognition and destruction of alien genetic material.

In the databases of nucleotide sequences, there are relatively few strains isolated in the Russian Federation, and there is also no information about the place of isolation of the strain and other datasheet specifications. Thus, the purpose of this research was to study the molecular genetic characteristics of CRISPR systems of *Y. pseudotuberculosis* strains circulating in the Russian Federation and the relationship between these characteristics and pathogenicity factors.

Materials and methods

The work studied 56 strains of *Y. pseudotuberculosis* isolated during 1935–2014 in Eastern and Western Siberia, the Far East, the North-West Region and other regions of Russia, as well as in Mongolia, Ukraine, Belarus, Turkmenistan and Abkhazia, from various sources.

The serotype of the studied strains was determined with the use of diagnostic serums produced by St. Petersburg Pasteur Institute and by multiplex PCR (Bogdanovich T. et al., 2003).

The collection strains were analysed with PCR, and chromosomal genes of *ypmA*/C superantigen, genes localized on the "pathogenicity islands" (HPI) *(fyuA, irp2)*, YAPI *(pilPQ, api74, tcp)*, and pVM82 plasmid genes *(dotO, mucAB)* were detected.

The amplification and sequencing of CRISPR system loci was performed with the primers presented in Table 8. Internal primers for sequencing fragments of > 1000 bps were selected by experiment.

CRISPR loci sequencing was performed by Sanger on a capillary sequencer Applied Biosystems 3500 (Thermo Scientific, USA). Genome-wide sequencing was performed for 10 strains on a MiSeq sequencer (Illumina, USA). Sample preparation and sequencing were performed in accordance with the manufacturer's instructions.

The fragments were assembled and analyzed with Vector NTI Advance 10.3 and BioEdit 7.2. Assembly to the contig level was carried out with SPAdes 3.15.4 genomic

assembler and the CONTIGuator 2.7.4. tool based on the reference genome. The search and decoding of CRISPR systems in the DNA sequences of strains was performed with CRISPRone (https://omics.informatics.indiana.edu/CRISPRone), CRISPRCasFinder (https://crisprcas.i2bc.paris-saclay.fr) and CRISPRDetect (http://brabtools./CRISPRDetect). The search for homologous protospacer sequences in the genomes of bacteriophages and plasmids was carried out with BLASTn search algorithm in the GenBank, EMBL, DDBJ, PDB, and RefSeq databases, as well as with CRISPRTarget online application in the GenBank-Phage, RefSeq-Plasmid and Islandviewer databases. The search for prophages in genomes was performed with PHASTER online tool.

Data analysis was performed in the RStudio programming environment 2022.07.1+554 using R 4.2.2 language and environment for statistical computing and graphics. Permutational multivariate analysis of variance (PERMANOVA) was used to search for statistically significant differences in the structure of loci and their relationship with various characteristics of strains in vegan 2.6–4 package. The clustering of strains and the construction of dendrograms based on the presence of spacers were carried out with pvclust 2.2–0 and ComplexHeatmap 2.14.0 packages using the unweighted pairwise mean (UPGMA) method with bootstrap 1000.

Results

As a result of the analysis of the quality and assembly of sequences, 78 fragments were selected. It was shown that the studied *Y. pseudotuberculosis* strains contain a CRISPR system that includes one to three loci: YP1, YP2, and YP3. According to the structure of repeats (5'-GTT CAC TGC CGC ACA GGC AGC TTA GAA A-3'), all loci belong to the IF type. The total number of spacers was 1311. In the YP1 locus, the number of spacers varies from 3 to 38 (810 spacers in total), in the YP3 locus, from 1 to 44 spacers (466 in total), while the YP2 locus is short and consists of one or two spacers.

Spacer composition of CRISPR loci

To analyze the spacer composition of CRISPR loci, a database of *Y. pseudotuberculosis* strain spacers was created (Database "Spacer sequences of CRISPR-Cas systems of *Yersinia pseudotuberculosis* strains"/State Register of databases, 25.12.2020, No. 2020622813).

Statistically reliable differences are present in the spacer composition of CRISPR systems differing in serotype, their set of pathogenicity factors, and those isolated in different territories (Table 9). In the study of individual loci, the compositions of the YP1 and YP3 loci were found to be reliably different in terms of statistics, whereas the composition of the YP2 locus is quite homogeneous. The spacer composition of the YP1 locus differs in strains of different serotypes with various determinants of pathogenicity, in particular, in strains with different plasmid composition (pVM82+ or pVM82–). The composition of the YP3 locus is reliably different in strains having YAPI and pVM82 plasmid in the genome. There were no statistically reliable differences in the groups of strains differing in other pathogenicity factors (superantigen YPM, HPI pathogenicity island).

Cluster analysis of strains based on the presence of spacers in loci makes it possible to divide strains into 2 large groups (Fig. 1).

Table 8. Primer sequences to CRISPR-loci of *Y. pseudotuberculosis*

CRISPR locus	Primers sequence
YP1 forward	ACATTGTGGTTATCGGTGGTCT
YP1 reverse	CAGTAATAAAAGATGCCATTCTCCC
YP2 forward	TTGCGCTGCTAAAAGCGTTG
YP2 reverse	CCCGATTCTTGTGACCCTCT
YP3 forward	GGATTCTTAGCCTATTCACA
YP3 reverse	CGATCTCTGTTTTGTGGTGA

Cluster 1 includes 28 strains of *Y. pseudotuberculosis* isolated mainly in the territories of the Siberian Federal Okrug (86%): three strains of serotype O:1a, 23 strains of serotype O:1b (for two strains, the serotype was not determined). The strains are characterized by the presence of YPM superantigen (89%). Strains are also divided into two subclusters within the cluster according to the presence of YAPI and pVM82 plasmid: subcluster 1a (YPM+YAPI–pVM82–) has 16 strains (57%), and subcluster 1b (YPM+YAPI+pVM82+) has 12 strains (43%).

Cluster 2 consists of 23 strains of *Y. pseudotuberculosis* of different serotypes, with almost equal shares of those isolated in various regions of Russia (52%) and in other countries (48%). Most strains are characterized by the presence of an island of pathogenicity (HPI, 83%), five strains (22%) have YPM superantigen, one strain (4%) has the YAPI, one strain (4%) has pVM82 plasmid.

Five of the 56 strains studied (9%, No. 14, 1375, 37n, 1380, and 87) are outside the main clusters. The spacers present in these strains are practically absent in other CRISPR systems.

A comparative analysis of the obtained sequences with genome sequences deposited in NCBI databases (GenBank and RefSeq) showed that strains are divided into Asian (1) and European (2) clusters (Fig. 2).

Cluster 1 included 52 strains isolated mainly in Russia and Japan (73%). There are also strains (27%) isolated in various countries: Germany, South Africa, Turkmenistan, Sweden, Ukraine, and New Zealand. Most strains of this group (87%) are characterized by the presence of ypm superantigen gene. About half of the strains (58%) have YAPI (including the *tcp* gene), and pVM82 plasmid was also detected in some strains (23%).

Cluster 2 includes 54 strains isolated in Europe (48%) and in a number of other countries of the world: China, Russia, Canada, New Zealand, and Australia (13%). Most strains of this group (59%) are characterized by the presence of an "island of pathogenicity" (HPI). Another predominant pathogenicity factor is YAPI (20%).

Thus, the CRISPR systems of *Y. pseudotuberculosis* strains circulating in the Russian Federation and a number of other countries cannot be clearly divided into Asian and European clades depending on the type of CRISPR locus, which is due to the high variability of strains. At the same time, the comparison of the spacer composition of CRISPR systems with data on the presence of the main pathogenicity factors made it possible to identify genetic variants of *Y. pseudotuberculosis* strains dominating in certain territories. It can be concluded that most of the strains circulating in the Russian Federation differ in these molecular markers from strains from other countries.

Table 9. Differences of spacer content in CRISPR-loci of *Y. pseudotuberculosis* strains (p < 0,05)

YP1 locus				
Strain characteristics	Df	MS	Pseudo-F	P-value
Region of detection	22	0.4668	3.9483	0.001
Serotype	1	0.6831	5.7779	0.001
Combination of pathogenicity determinants	3	0.554	4.6857	0.001
pVM	1	0.4659	3.9408	0.002
YAPI	1	0.2125	1.7976	0.089
Other factors	10	0.1182		
YP2 locus				
Region of detection	13	0.1869	3.3647	0.215
Serotype	3	0.0664	1.1944	0.420
Other factors	3	0.0556		
YP3 locus				
Region of detection	20	0.4439	36.117	0.001
Serotype	3	0.4352	35.402	0.001
Combination of pathogenicity determinants	3	0.5478	44.567	0.001
pVM	1	0.1984	16.145	0.001
YAPI	1	0.3016	24.537	0.001
Other factors	7	0.0123		
All loci				
Region of detection	27	0.555	2.6799	0.001
Serotype	4	0.3906	1.8858	0.001
Combination of pathogenicity determinants	3	0.5049	2.4379	0.001
pVM	1	0.5279	2.5487	0.007
YAPI	1	0.2454	1.185	0.276
Other factors	19	0.2071		

Abréviations: Df — degree of freedom; MS — mean square; Pseudo-F — Fisher criterion; P-value — significance level.

The analysis of protospacer sequences in the genomes of phages and plasmids

Based on the immune function of the CRISPR system, homologous sequences were searched in the genomes of the main mobile genetic elements (MGE) — bacteriophages and plasmids.

20 spacer sequences are homologous to fragments of the genomes of bacteriophages *Yersinia* YeP3, *Yersinia* YeP2, *Yersinia* YeP1, *Yersinia* vB_YenM, *Yersinia* vB_YenS_P400 and Klebsiella 6991.

70 spacer sequences are homologous to chromosome sequences of various *Yersinia* species: *Y. pseudotuberculosis, Y. pestis, Y. similis, Y. massiliensis, Y. intermedia, Y. kristensenii, Y. enterocolitica, Y. aldovae*. According to the results of genome analysis with PHASTER online tool, fragments of genomes containing protospacer sequences correspond to full-fledged prophages. The same spacer may be homologous to fragments of the genome of prophages found in different strains. In addition, spacers represented at the same locus are able to recognize different fragments of the genome of the same prophage.

The four spacers are homologous to the sequence of the cryptic plasmid pYptb32953 (27.7 kb), which is quite rare in strains of pathogenic yersinia. The main function of this plasmid is to participate in conjugation, as a cluster of genes encoding proteins necessary for plasmid conjugation is located in the plasmid genome.

Four spacer sequences are homologous to the pVM82 plasmid genome region of *Y. pseudotuberculosis* IP31758 and *Y. pseudotuberculosis* 598 strains (access numbers in NCBI RefSeq: NC_009705 and NZ_CP071946), with the exception of 4 nucleotides (Fig. 3). The plasmid genome contains phage-like integrase genes, which may indicate the acquisition of icm/dot genes cluster of the IVB type secretion system through horizontal transfer mechanisms.

Discussion

The main function of CRISPR systems is the specific recognition and destruction of foreign genetic elements that have entered a bacterial cell and pose a potential danger to the existence of the bacterial population. Spacer sequences are homologous to fragments of MGE genomes characteristic of microorganisms circulating in certain territories. The ability to acquire spacers in chronological order and a high proportion of polymorphisms makes it possible to use CRISPR systems as a tool for high-resolution molecular typing of strains, which was demonstrated by the example of *Streptococcus thermophilus, Campylobacter jejuni, Corynebacterium diphtheriae* and *Y. pestis*. It is known that CRISPR loci are a dynamic structure where new spacer sequences are joined from the side of the leader sequence. Some of the spacers located in the middle of the cassette may be lost as a result of deletion during the acquisition of new MGE sequences. Spacers located near the last polymorphic repeat are usually preserved.

Thus, the typing of strains by the presence of spacers located at the end of the YP1 and YP3 loci allows us to con-

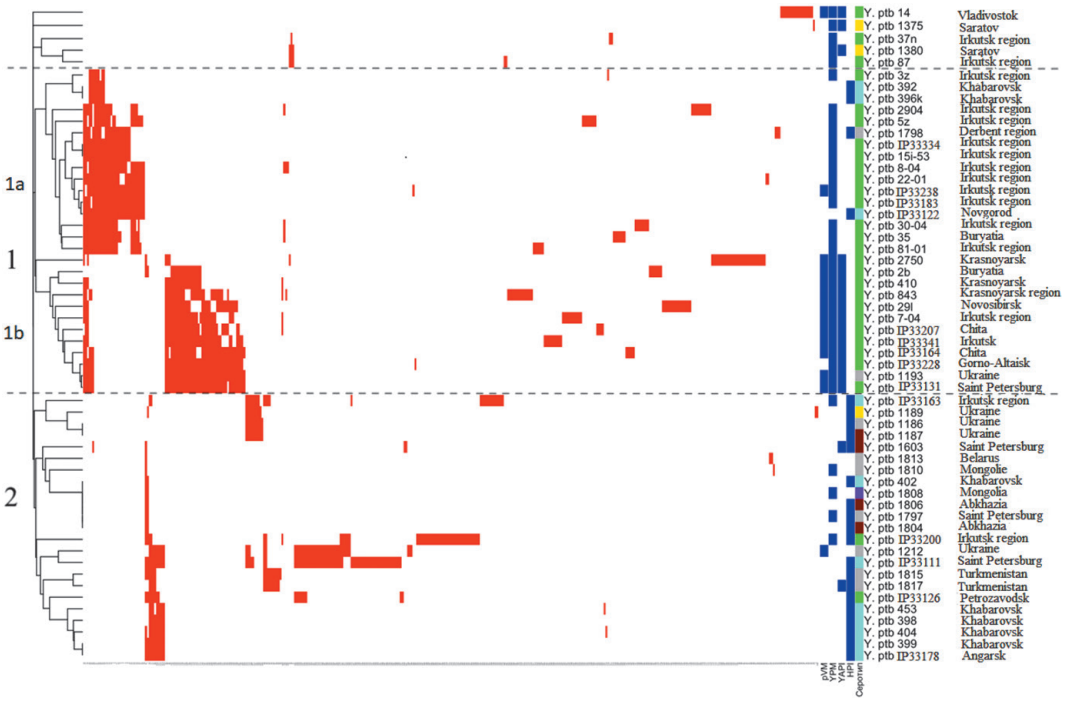

Figure 1. Clusterization of *Y. pseudotuberculosis* strains (n = 56) by presence of spacer in loci YP1–YP3

Symbols: presence of spacer — red, presence of factor — blue, serotype 1 — darkred, serotype 1a — cyan, serotype 1b — green, serotype 3 — yellow, serotype 4 — violet, serotype is not defined — grey.

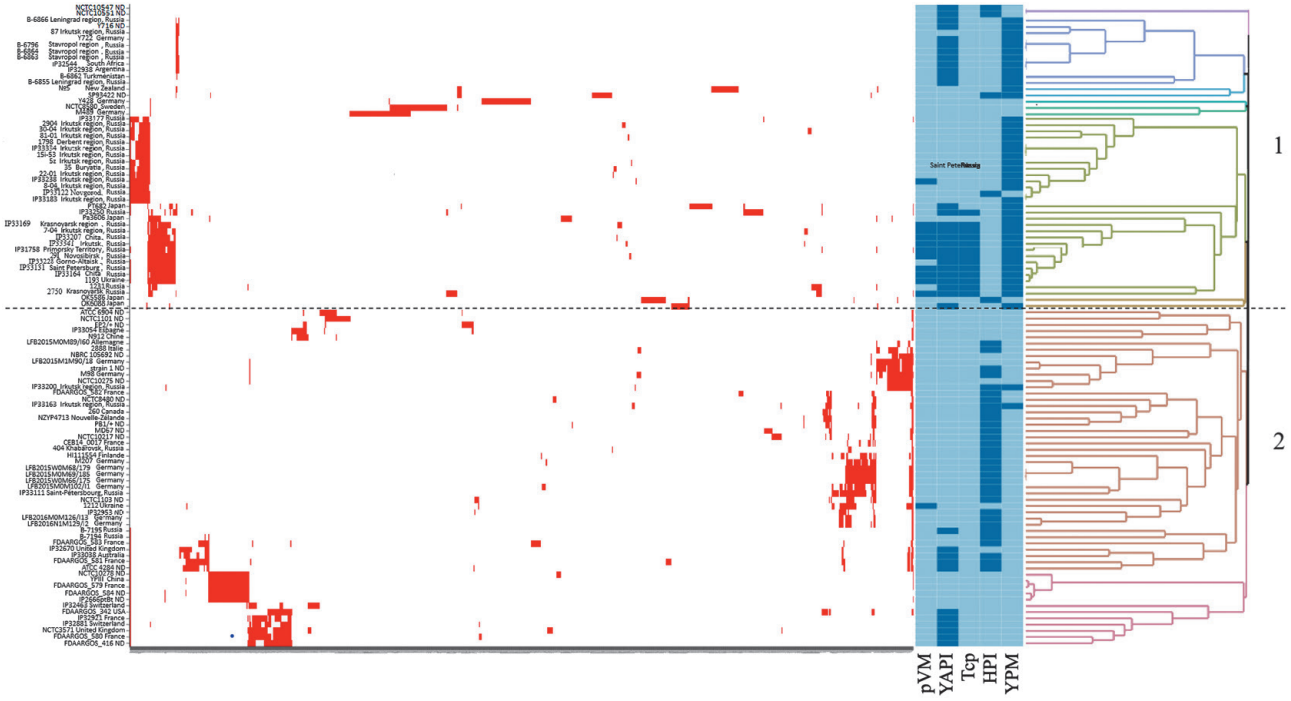

Figure 2. Clusterization of *Y. pseudotuberculosis* strains (n = 56) by presence of spacer. CRISPR-system sequenced data (n = 31) and NCBI data (n = 75)

Symbols: presence of spacer — red, presence of factor — blue.

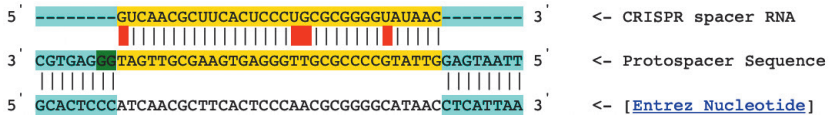

Figure 3. Protospacer sequence of *Y. pseudotuberculosis* plasmid IP31758 pVM82 (153 kb) to spacer 114 of YP3 locus

ventionally divide strains differing in a set of virulence determinants into two clusters: European and Asian (Fig. 2). 35 of the studied 56 strains were isolated in the territories of the Far East, Eastern and Western Siberia. The strains are also divided into two subgroups by the presence of pVM82 plasmid (pVM82+ and pVM82–), which determines the IVB type secretion apparatus, and, along with other pathogenicity factors, mediates the development of generalized forms of pseudotuberculosis (Fig. 1). Strains isolated in Japan, which are also capable of causing a complex of manifestations of a systemic infection called Far Eastern scarlet-like fever, have a similar pattern of distribution of CRISPR cassettes (Fig. 2, cluster 1).

According to the results of the PERMANOVA analysis, the composition of the YP1 and YP3 loci correlates with the presence of such pathogenicity factors as the pVM82 plasmid and the YAPI in the strains. The high variability of strains within the species is determined by the ability to acquire new genetic elements (genomic islands) by horizontal gene transfer using plasmids, bacteriophages, integrons and transposons. CRISPR as a system of "immunity" of bacteria against MGE can participate in the formation of the genotype of *Y. pseudotuberculosis* strains that determines the clinical manifestations of pseudotuberculosis.

The analysis of protospacers in the genomes of bacteria, bacteriophages and plasmids revealed spacers corresponding to the genome region of plasmid pVM82 in strains *Y. pseudotuberculosis* IP31758 and *Y. pseudotuberculosis* 598. It is possible that the acquisition of these spacers may be the reason for the absence of pVM82 plasmid in strains belonging to the European cluster (Fig. 2). But a spacer capable of recognizing a plasmid site is found in the YP3 locus of the *Y. pseudotuberculosis* 2750 strain of the O:1b serotype containing the pVM82 plasmid and the icm/dot gene cluster (Fig. 2, cluster 1). It is probably the large number of mutations in the protospacer sequence that prevents CRISPR interference, or this sequence may be present in the genome of a bacteriophage that is absent from the databases. In addition, the acquisition of spacers to their own sequences can serve as a trigger for the emergence of new mutations and the expression of a new phenotype, manifested in a change in the virulent properties of the microorganism. According to another hypothesis, spacers that recognize their own sequences are a mechanism for breeding strains that do not contain this factor. Thus, the CRISPR system can be one of the factors determining the emergence and circulation of strains with different pathogenic potential in the same territory. In combination with classical methods of identification and typing of the causative agent of pseudotuberculosis, the analysis of CRISPR systems can be used for phylogenetic studies, monitoring of the circulation of *Y. pseudotuberculosis* strains in territories, and epidemiological investigation of group diseases.

Conclusion

As a result of the analysis of CRISPR systems of *Y. pseudotuberculosis* strains, it was found that the spacer composition of loci differs in strains differing in serotype, a set of pathogenicity factors, and the region of isolation. The most variable loci are YP1 and YP3, whereas the YP2 locus is homogeneous. The composition of the YP1 locus differs in groups of strains that have the pVM82 plasmid and that do not. The composition of the YP3 locus correlates with the presence of pVM82 plasmid and YAPI.

Thus, *Y. pseudotuberculosis* strains circulating in the Russian Federation have different sets of virulence determinants and different spacer composition of CRISPR systems. Most Russian strains differ in these molecular markers from strains from other countries. The high variability of strains within the species is determined by the ability to acquire new genetic elements by horizontal gene transfer. The CRISPR system, as a system of "immunity" of bacteria against MGE, can contribute to the pathogenic potential of *Y. pseudotuberculosis* strains.

The results obtained indicate the expediency of using CRISPR typing when monitoring *Y. pseudotuberculosis* strains circulating in specific territories and identifying a possible connection between the structural and functional characteristics of CRISPR system sites with clinical manifestations of pseudotuberculosis. The available data determine the prospect of using CRISPR loci as specific molecular markers in the study of intraspecific diversity and evolution of *Y. pseudotuberculosis*.

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LABORATORY OF ZOOANTHROPONOSES

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The study of the biological (including genetic) characteristics of leptospira

Monitoring studies of the spread of leptospirosis infection in the conditions of the metropolis, St. Petersburg, continue. For this purpose, samples from 94 domestic dogs were tested in the microagglutination (MA) reaction. Antibodies to leptospira were detected in 24.5% of the animals. Positive findings by serogroup were distributed as follows: *Icterohaemorrhagiae* (47.8), *Canicola* (34.8), *Grippotyphosa* (13.1), and 4.3% were found to have both leptospira *Icterohaemorrhagiae* and *Canicola*. In the study conducted together with the North-Western Anti-plague Station, 87 common rats were caught in St. Petersburg, and leptospira cultures identified as *L. Icterohaemorrhagiae* were isolated from the kidneys of 6.7% of the animals.

Their specifics were determined with the use of the findings of the study of the biological properties of *Leptospira interrogans* strain of *Grippotyphosa* serogroup of grippotyphosa serovar No. 353 "Red-backed mouse-339" isolated from the kidney of a red-backed mouse (Clethrionomis glareolus) caught in the Leningrad Region, and the strain of *Leptospira interrogans* of *Icterohaemorrhagiae* serogroup of the copenhageni serovar No. 352 "Taganrog 2018 ", isolated from the organs of a person who died of leptospirosis. The strain "red-backed mouse-339" provides the most effective detection of antibodies to *L. grippotyphosa* compared with the prototype and can be used for serological diagnosis of leptospirosis. The Taganrog 2018 strain has pronounced virulent properties. We have received patents for invention for these strains.

The authors have studied the biological properties of leptospirosis strains isolated in the Kaliningrad region of the Russian Federation and identified some biological specifics of these strains, which made it possible to deposit 3 strains (as part of the patent procedure) in the State Collection of Pathogens of Infectious Diseases of Pathogenicity Groups II–IV of the Gamaleya National Research Center for Epidemiology and Microbiology under No. 528, 529, and 530.

Work continued on the selection of genetic markers for the study of leptospira. We evaluated the effectiveness of 16S rRNA gene sequencing. A comparative analysis of the nucleotide sequences obtained during this study and presented in the database showed a 99–100% similarity of the 16S rRNA gene. High similarity of 16S rRNA fragments of various *Leptospira* spp. species makes this technique unsuitable for genotyping, but it is effective as an initial screening. Based on the results of these studies, 4 sequences assigned registration numbers OP861645-OP861648 were deposited in NCBI GenBank. To solve the issues of leptospira genotyping, we used the "housekeeping genes" used in MLST schemes, which are species-specific and stable. The *adk* gene encoding the enzyme adenylate kinase was chosen as the main object of study. The obtained sequences of this gene were compared with the data in NCBI GenBank, and deposited in this database under numbers OP605965-OP605967.

To differentiate Leptospira into pathogenic, intermediate and saprophytic strains, the PCR conditions were optimized for *flab* gene fragment, encoding flagellin (a class B polypeptide subunit (FlaB) of the periplasmic flagella of leptospira), which is a virulence factor.

To control the purity and stability of the antigenic compositions of reference strains used for the serological diagnosis of leptospirosis, we continue the genetic analysis of strains from the collection of the Pasteur Institute. 16s rRNA genes of 31 strains of pathogenic leptospira were sequenced. The obtained results were compared with the data of NCBI GenBank international database. This made it possible to deposit the 16s rRNA gene sequences of the Russian strains into this database under registration numbers OL703050-OL703069 and OL825727-OL825737. A comparative analysis with the nucleotide sequences available in the GenBank international database showed that the results obtained matched the data, despite the presence of differences in some nucleotides.

Study of the genetic characteristics of *C. burnetii*

Studies continue on the genetic characteristics of *C. burnetii*, in order to improve the detection of these microorganisms. To solve this problem, we studied 96 Ixodes ticks of *Ixodes persulcatus*, *Dermacentor marginatus*, and *D. reticulatus* species. By PCR with genus-specific primers Cox1 F–Cox1 R (blast.ncbi.nlm.nih.gov, blastn algorithm), five positive samples were revealed, on which PCR was performed to obtain an extended nucleotide sequence of 16S rRNA gene. The obtained amplicons were sequenced in two directions, and the homology of the nucleotide sequence with previously published sequences of strains and isolates of *C. burnetii* and *Coxiella*-like microorganisms was analyzed. The analyzis of the nucleotide sequences showed that one of the studied samples contained the DNA of *C. burnetii*, and the other contained the DNA of a *Coxiella*-like microorganism. DNA of genetically distant microorganisms was found in three samples: *Pseudomonas* sp. in two of them and *Stenotrophomonas* sp. in one. *Coxiella burnetii* and *Coxiella* sp. were found in ticks of *Dermacentor marginatus* species. For a *C. burnetii*-positive sample, the homology of the 16S rRNA gene when compared with other strains was 99.16–99.83%, and the maximum degree of similarity was noted with Western European strains (Henzerling and Heizberg, originating from Italy and Greece, respectively). The degree of sequence similarity in a *Coxiella*-like microorganism with *C. burnetii* in the extended sequence of the 16S rRNA gene was less than 96%. When analyzing the gene sequences of other ticks-associated *Coxiella*-like bacteria, this sample was clustered with bacteria found in *D. marginatus* ticks in Western Europe (sequence homology 99.33%). The obtained results of genetic characterization show that *Coxiella* sp. bacteria associated with *Dermacentor* sp. ticks are candidate species genetically distanced

from *C. burnetii*. Thus, on the basis of molecular screening and analysis of nucleotide sequences of the 16S rRNA gene, uncultivated isolates related to *C. burnetii* and *Coxiella* sp. were studied. *Coxiella*-like microorganisms were first discovered in the European part of Russia; Our results justify the expediency of determining the taxonomic position of *Coxiella* sp., based on the comparison of the nucleotide sequences of the 16S rRNA gene, to take into account the percentage of gene homology and the ratio of polymorphisms in the group of closely related bacteria. The results of these studies were used in the preparation of the guidelines Epidemiological Surveillance, Laboratory Diagnostics and Prevention of Q Fever (MR 3.1.0281-22).

Thus, as a result of the performed genetic studies, new data on the genetic characteristics of *Coxiella burnetii* and pathogenic leptospira were obtained. Our results justify the expediency of determining the taxonomic position of *Coxiella* sp., based on the comparison of the nucleotide sequences of the 16S rRNA gene, to take into account the percentage of gene homology and the ratio of polymorphisms in the group of closely related bacteria.

The new data on the biological, including genetic, characteristics of the causative agents of Q fever and leptospir fever can be used to develop improved products for the diagnosis of these infections.

Study of epidemiological features of leptospirosis in Vietnam (according to literature data)

Within the framework of research and methodology cooperation and joint research work on the diagnosis and study of epidemiological specifics of leptospirosis in the Socialist Republic of Vietnam, more than 100 research papers devoted to this problem were analyzed, which made it possible to publish a review of the literature, "Leptospirosis in Vietnam", in English in Russian Journal of Infection and Immunity. It is shown that the main sources of leptospirosis infection in Vietnam are synanthropic rodents (rats), farm animals (oxen, cattle, pigs) and pets (cats and dogs). An essential factor in the infection of Vietnamese residents is the fact that their diet includes cats, dogs, and rats. Vietnam has an extremely wide variety of pathogenic leptospira serovars. The landscape of pathogenic leptospira differs significantly from region to region, which can be attributed to the landscape and climatic peculiarities of territories, as well as due to anthropogenic activities. Geographical and social differences in the northern, central and southern parts of Vietnam largely determine the epidemiological features of leptospirosis and justify the development of specific measures to prevent this infection for each territory with due account for its specifics. Leptospirosis remains largely underdiagnosed in Vietnam. On average, about 10% of Vietnamese residents are infected with leptospira. Working in agriculture is probably the dominant risk factor for infection. The highest rates of seroprevalence to leptospira were found in farmers. More women had the disease than men. About two thirds of patients with leptospirosis were persons of working age (24 to 60 y.o.); 12.8% of tested children were found to have antibodies to leptospira. A pronounced diversification of leptospira serovars circulating in Vietnam was established.

Studies to identify tick-borne pathogens circulating in the Northwestern Federal District of the Russian Federation

To detect spotted fever group rickettsiae (SFGR) in St. Petersburg, carriers were tested for the presence of rickettsia DNA using real-time PCR (RT-PCR). 55 pools of blood-sucking mite larvae collected from rodents were analyzed. *Rickettsia* DNA was detected in 12% of the pools. 100 adult hungry ticks collected from vegetation on a flag were studied. *Rickettsia* DNA was detected in 10% of carriers. Verification of the RT-PCR results with primers for standard PCR flanking fragments of the *gltA* gene of rickettsia of the tick-borne spotted fever group produced a negative result, which presumably indicates the existing genetic polymorphisms at primer entry sites. The results obtained may indicate the genetic distancing between the rickettsia population circulating in the Russian Federation and the rickettsia population abroad.

Detection of DNA of *Coxiella burnetii*, *Borrelia burgdorferi* sensu lato, *Rickettsia* species, and tick-borne encephalitis virus RNA was done by polymerase chain reaction in real time using commercial reagent kits RealBest, produced by Vector State Research Center of Virology and Biotechnology. The study of 347 hungry ticks collected in St. Petersburg revealed genetic markers of *Coxiella burnetii* in 16 samples (4.6%). Ticks infected with *Coxiella burnetii* were found in two administrative districts of the city, Primorsky and Kurortny. Ticks collected in the Arkhangelsk region and in the Republic of Karelia were also examined to determine their infection with *C. burnetii*. Genetic markers of this pathogen were detected in 2.9% of ticks in the Arkhangelsk region and 4.5% in Karelia.

To identify the DNA of borrelia of the *Borrelia burgdorferi* sensu lato complex, we analyzed 792 individual adult hungry ticks collected from vegetation on a flag in the Leningrad Region (a total of 540 samples, including 493 *Ixodes persulcatus* and 47 *Ixodes ricinus*) and in the Kurortny district of St. Petersburg (a total of 252 samples, 3 *Ixodes persulcatus* and 249 *Ixodes ricinus*), as well as 203 ticks collected in the Arkhangelsk region (137) and in the Republic of Karelia (66). Borrelia DNA was detected in 165 (20.8%) samples, including 42 *Ixodes ricinus* collected in both territories (8 in Leningrad region and 34 in St. Petersburg), and 123 *Ixodes persulcatus* from the Leningrad region. The infection rate of *Borrelia burgdorferi* s.l. ticks was 19.71% for those collected in the Arkhangelsk region and 15.15% for those in the Republic of Karelia.

Hungry ticks were tested for *Rickettsia* species DNA. A total of 548 samples collected from vegetation on the flag were examined in the Leningrad region (a total of 348 samples, of which 322 *Ixodes persulcatus* and 26 *Ixodes ricinus*) and in Kurortny district of St. Petersburg (a total of 200 samples, 3 *Ixodes persulcatus* and 197 *Ixodes ricinus*). Genetic markers of *Rickettsia* species were found in 38 samples (10.9%), including in 5 *Ixodes persulcatus* collected in the Leningrad region, and 33 *Ixodes ricinus* collected in both territories.

The study of the spread of tick-borne encephalitis (TBE) in the natural foci of this infection in St. Petersburg and its environs, as well as in the Arkhangelsk region and the Republic of Karelia, continues. The RNA of the tick-borne encephalitis virus was not detected in 138 *Ixodes ricinus* and 329 *Ixodes persulcatus* collected in St. Petersburg and its

Table 10. The results of the detection of antibodies to the pathogens of tick-borne infections in residents of the NWFD

Territory of residence	Number of samples	IgG-antibody-positive samples (number of positive/seroprevalence, %)						
		Antibody-positive to any TBD pathogens	Tick-borne encephalitis virus	*Borrelia burgdorferi* sensu lato	*C. burnetii*	*Anaplasma phagocytophilum*	*E. chaffeensis/ E. muris*	Antibodies against 2 or more TBD pathogens
Republic of Komi	659	149/22.6	83/126	21/3.2	23/3.5	15/2.3	7/1.1*	15/2.3
Republic of Karelia	292	59/20.2	38/13.0	9/3.1	9/3.1	1/0.3	2/0.7	5/1.7
AO	103	15/14.6	7/6.8	2/1.9*	1/1.0	0	5/4.9*	1/1.0
PO	98	19/19.4	11/11.2	5/5.1	0	0	3/3.1	2/2.0
LO	92	28/30.4	13/14.1	6/6.5*	3/3.3	4/4.3	2/2.2	3/3.3
Total	1244	270/21.7	152/12.2	43/3.5	36/2.9	20/1.6	19/1.5	26/2.1

When comparing the seroprevalence rates of IgG antibodies against TBD pathogens significant differences were found: *in relation to *Borrelia burgdorferi* sensu lato in AO and LO: the corresponding value of Fisher's criterion is 1.651 (while for p = 0.01 the critical value is 1.450); **in relation to *E. chaffeensis/E. muris* in Komi and AO: the corresponding Fisher's criterion is 2.244 (while the critical value for p = 0.01 is 1.450).

suburbs. The infection rate of 67 *Ixodes persulcatus* collected on the flag in Karelia was 3.0%. In the Arkhangelsk region, 123 *Ixodes persulcatus* were collected on the flag and 19 from large mammals. Their infection rate with tick-borne encephalitis virus was 3.25% and 5.26%, respectively.

The study of seroprevalence of the population of the Northwestern Federal District of Russia in relation to tick-borne infections

The research involved blood serums of apparently healthy residents of 6 administrative territories of the Northwestern Federal District of Russia: The Republics of Komi and Karelia; Arkhangelsk, Leningrad, Pskov Regions, and St. Petersburg. Blood serums of donors were examined by the ELISA method with the use of commercially available test systems (Table 10).

The detection of antibodies to tick-borne pathogens in almost 22% of the population of the Northwestern Federal District of Russia indicates a wide spread of infections caused by these pathogens in the territory.

We analyzed the incidence of tick-borne infections in the European Arctic region of Russia. It has been established that against the background of a decrease in the incidence of tick-borne encephalitis (TBE) in Russia as a whole, from the late 1990s to the present, periods of growth and decline in the incidence of TBE have been recorded in the European north of the country. In the Republic of Karelia, the peak incidence was observed in 2003, reaching an indicator of 15.3 per 100 000 inhabitants, while in the Arkhangelsk region, the maximum incidence rates were detected in 2009–2013. In the Komi Republic, the six-year peak of average annual morbidity rates was observed in 2009–2014 (1.98 per 100 000 inhabitants). There has been a significant decrease in this indicator in the Republic in recent years. Thus, in 2020, it was 0.85 per 100 000 inhabitants, which is comparable to the same indicator in Russia as a whole (0.66 per 100 000 inhabitants).

Based on the analysis of the data obtained during the study of TBE incidence and the tick-bite incidence rate in the European north of Russia, we came to the following conclusions: the incidence rate and the number of TBE cases remain high, above the national average, but have been decreasing over the last decade; the number of victims and the indicator of tick-bite incidence rate (TBIR) remain at a consistently high level, and no tendency to decrease is observed; in the Arkhangelsk region and Karelia, the TBIR has apparently reached saturation and will not grow in the future; morbidity and TBIR are spreading northward, to new territories in the Arctic zone; the main driving force of the observed epidemiological situation in the European North of Russia is the natural processes of climate change.

Publications

Articles

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Surveillance of poliomyelitis and enterovirus infection

The St. Petersburg Subnational Laboratory for the Diagnosis of Polio on the basis of Pasteur Institute is part of the network of Polio laboratories of the World Health Organization (WHO). As part of the Global Polio Eradication Program, it oversees polio and acute flaccid paralysis in 14 constituent entities of the Russian Federation, being a regional polio surveillance center for these territories. The laboratory receives samples of primary material from children under 15 years of age with acute flaccid paralysis syndrome (AFP), as well as from people in contact with them, patients diagnosed with enterovirus infection (EVI) and children from risk groups, including children from migrant families who arrived from polio-affected territories. The laboratory monitors the circulation of polioviruses and non-poliomyelitic enteroviruses (NPEV) among various population groups, and also studies samples from the environment in order to detect polioviruses and non-poliomyelitic enteroviruses.

All virological studies, which are the gold standard of the Polio Eradication Program, and molecular studies are carried out in accordance with WHO protocols in compliance with the containment requirements. Every year, the laboratory performs professional tests received from the WHO and undergoes the WHO accreditation procedure. Every year, the laboratory personnel prepares generalized reports on maintaining the polio-free status of 14 administrative territories of Russia. These reports are sent to the Federal Service of Rospotrebnadzor (Federal State Agency for Health and Consumer Rights), where a report of the Russian Federation to the WHO is prepared for certification of the Russian Federation as a polio-free country.

In the period from 2021 to 2022, 186 samples from patients with acute flaccid paralysis syndrome were examined. In 2021, 4 polioviruses (mixtures of polioviruses of type 1 and 3 from one patient) were isolated, which, according to the results of intra-type differentiation, were vaccine-derived viruses. In 2022, 2 Coxsackievirus A16 (NPEV) were isolated from one case of AFP. There were no cases of vaccine-associated paralytic polio in the territories monitored by the Center.

Also, the laboratory continued to study material from children from migrant families in order to search for polioviruses and non-polio enteroviruses. In 2020–2021, due to the unfavorable epidemic situation with polio in neighboring countries (polio outbreak associated with circulating vaccine-derived poliovirus type 2 (cVVVP2) in Tajikistan; a case of AFP caused by cVVVP2, and isolation of cVVVP2 from contact persons in Ukraine), significantly more samples of this category were examined in the laboratory than usual. In 2021, among the total number of healthy children from migrant families who underwent testing (1176), the majority, 1018 children (86.6%), were from Tajikistan. 3 polioviruses of type 1, 5 polioviruses of type 2, 5 polioviruses of type 3, and 3 mixtures of polioviruses of type 1 and 3 were isolated from the newly arrived. All polioviruses of type 2 were isolated from children from Tajikistan and were vaccine strains from the new oral poliovirus vaccine nOPV2 used in Tajikistan to stop the outbreak of polio caused by circulating vaccine-derived poliovirus 2. In addition, 104 non-polio enteroviruses belonging to different types were isolated from samples from migrant children.

In 2022, a total of 890 samples from children from migrant families were tested. 11 polioviruses of type 1, 11 polioviruses of type 3, 5 mixtures of polioviruses of type 1 and 3, as well as 35 non-poliomyelitic enteroviruses were isolated. No polioviruses of type 2 were isolated in the 14 territories monitored by the Center. The children whose samples were studied mainly came from Tajikistan (597), the Republics of Chechnya and Dagestan (149), and Ukraine (97). In 2021, the coverage of children with polio vaccination in Ukraine was 53%, so much attention was paid to the examination of children from Ukraine, the LPR and the DPR. The difference between the number of isolated non-polio enteroviruses in 2021 and 2022 is explained by the sampling time: in 2021, most of the samples were taken in September-December (1074 samples, 91%), whereas in 2022, most of the samples, on the contrary, were taken in January-June (650 samples, 73%), when in a moderate climate zone the circulation of enteroviruses is at its low.

An important component of surveillance to confirm the polio-free status of the Russian Federation is the assessment of the state of immunity to polioviruses in the population (in accordance with Guidelines 3.1.2943-11). A sequential polio vaccination scheme is currently used in the Russian Federation in accordance with the National Vaccination Calendar, with the first two vaccinations with an inactivated poliovirus vaccine, and oral poliovirus vaccine used for the next four vaccinations.

Research is continuing on the intensity of immunity to polioviruses among children in one of the 14 territories. Over four years (2017–2021), the blood sera of children from two indicator groups, 3–4 y.o. and 16–17 y.o., were examined (Table 11). Data on the absence of antibodies to poliovirus type 2 in some children vaccinated with a divalent polio vaccine indicate a continued decrease in herd immunity to poliovirus type 2 after its withdrawal from the oral poliovirus vaccine. The absence of antibodies to poliovirus type 2 in vaccinated children is an alarming signal, and the laboratory will continue to monitor the intensity of immunity to this type of virus in the indicator groups.

In 2021–2022, more than 5000 samples from patients with enterovirus infection were examined in 14 territories monitored by the center. The incidence of various forms of enterovirus infection in 2021 remained low compared to 2019, at 0.5 and 9.06 per 100 000 population, respectively. In 2021, most patients who had the disease were children

(97.6%). Exanthemic forms of the disease prevailed in most territories. Only in two territories, the Vologda and Saratov regions, the proportion of enterovirus meningitis, the most severe form of enterovirus infection, exceeded 40%. Non-polio enteroviruses were isolated in 1.5% of the total number of samples studied. Vaccine-derived polioviruses were rarely isolated (0.1%) from children recently vaccinated with oral poliovirus vaccine. In most cases (54.1% of the total number of isolated viruses), Coxsackievirus A enteroviruses of different types were isolated from EVI patients. Coxsackievirus B1–6 enteroviruses were isolated in 24.6% of cases. The remaining isolated enteroviruses belonged to various ECHO types (6, 11, 25), two strains belonged to EV71 and 99 types.

In 2022, the incidence of enterovirus infection began to grow again, reaching 9.9 per 100 000 population. The highest incidence rates were noted in the Murmansk region and the Komi Republic. Exanthemic forms of EVI still prevailed in the territories monitored by the Center. The percentage of NPEV isolation increased to 2.5% of the total number of samples studied in 2022, the share of poliovirus isolation being 0.5%. Among the isolated enteroviruses, the majority were EV Coxsackievirus A of different types (62.9%), while EV Coxsackievirus B, ECHO 6, 15 were also isolated. Two isolates of Coxsackievirus A10 from EVI patients from the same territory, had a cytopathogenic effect characteristic of polioviruses as early as in primary infection on cells of a specialized L20B line (with a built-in poliovirus receptor), which is used to differentiate non-polio enteroviruses and polioviruses. Other isolates of Coxsackievirus A10, which were previously isolated in the laboratory, had virtually no cytopathogenic effect on L20B cells. Isolates from patients from different families were completely identical in terms of the decoded section of the VP1 genome. In diagnostic PCR with type-specific primers to polioviruses they behaved like non-polio enteroviruses, and, therefore, laboratories can use PCR diagnostics to correctly identify such viruses as non-polio. Nevertheless, such phenomena require further study.

One of the key elements of monitoring the circulation of polioviruses and non-polio enteroviruses among the population during the final phase of the Polio Eradication Program is the supervision of environmental areas by testing wastewater samples. The WHO Subnational Polio Laboratory has been detecting polioviruses and non-polio enteroviruses in environmental samples for many years.

In 2021–2022, 3590 wastewater samples were examined by virological method in 14 territories of the region. The structure of enteroviruses isolated from wastewater samples was dominated by polioviruses of type 3 (42% and 55.3% in 2021 and 2022, respectively). A high proportion of polioviruses in wastewater samples is associated with the ongoing routine vaccination of children with oral poliovirus vaccine (Fig. 4).

According to the results of intra-type differentiation, all polioviruses were classified as vaccine-derived. No type 2 polioviruses have been isolated. Coxsackievirus B dominated among non-polio enteroviruses: their share was 20% and 14.9%. These viruses may be an etiological factor of enterovirus meningitis, while it is typical for them to be found in asymptomatic carriers. Unlike in samples from EVI patients, Coxsackievirus A enteroviruses were isolated from wastewater samples in insignificant amounts (6% in 2021 and 2.1% in 2022). ECHO enteroviruses of various types accounted for 22% in 2021 and 14.9% in 2022. No enteroviruses of ECHO 30 type were isolated from wastewater samples in 2022.

At the stage of post-certification of Global Polio Eradication, improving the surveillance of polio and acute flaccid paralysis plays a crucial role. Enhanced virologi-

Table 11. Results of a study of children's blood sera for the presence of antibodies to polioviruses in the territory in 2017–2021

Type of vaccine	Years	Number of children	Percentage of sera negative to PV					
			Children aged 3–4 y.o.			16–17 y.o.		
			PV 1	PV 2	PV 3	PV 1	PV 2	PV 3
bOPV	2017	204	0	0	2.0	0	0	13.0
	2018	202	0	1	2	1	2	8
	2019	200	0	2	11	0	6	19
	2021	175	0	8	2.7	0	3	20

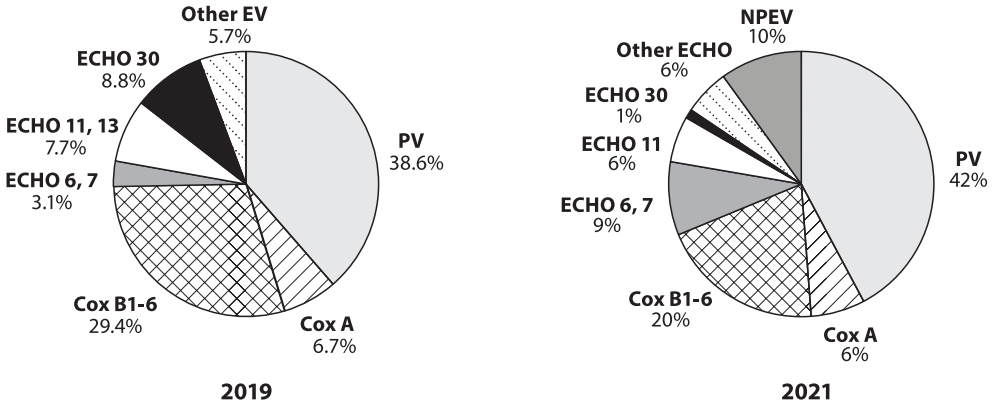

Figure 4. Isolation of enteroviruses from wastewater samples in 14 territories of the Center (2019, 2021)

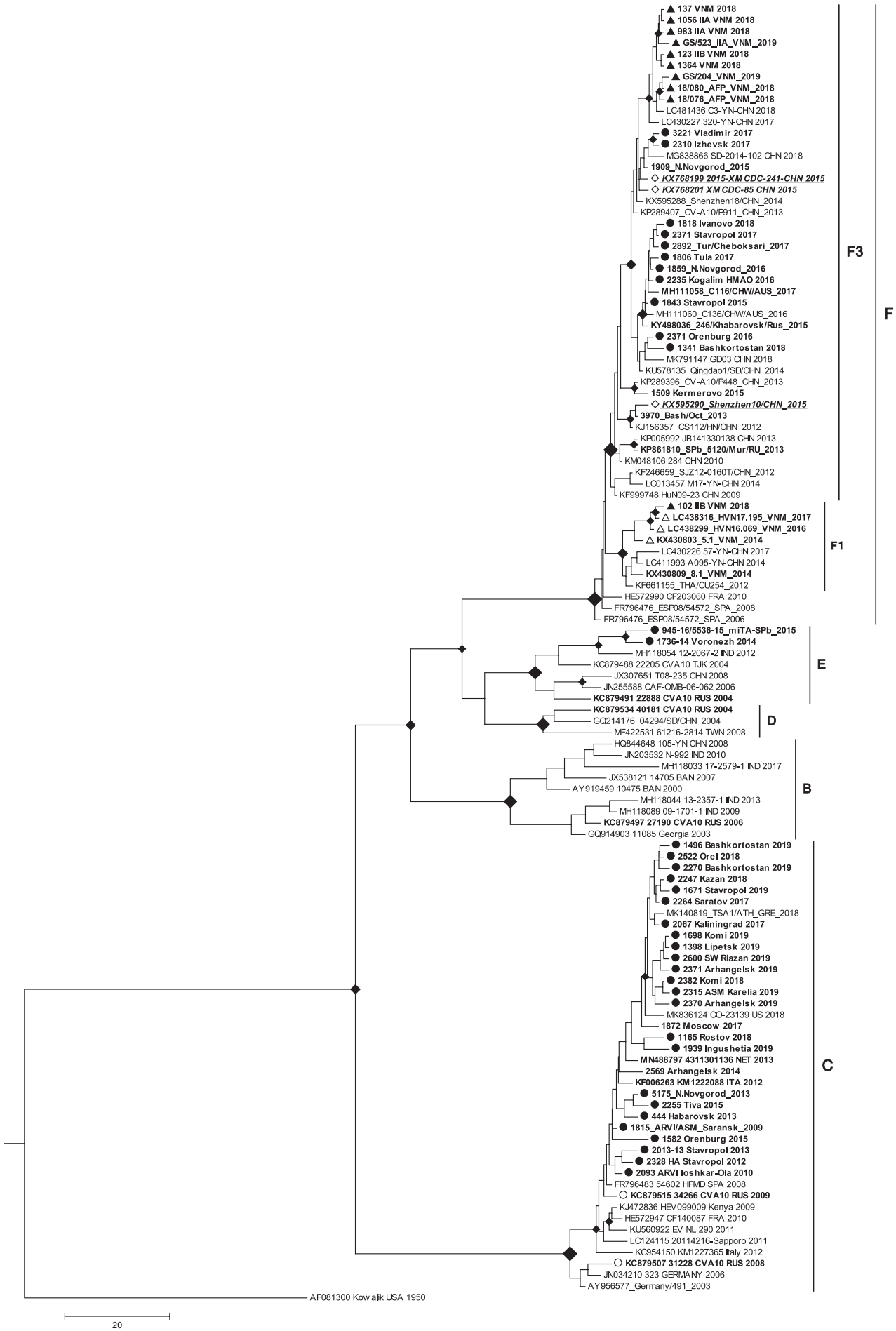

Figure 5. A phylogenetic tree based on the analysis of the VP1 fragment of the Coxsackievirus A10 genome

cal supervision of patients with AFP in combination with the most important types of additional supervision, such as supervision of children from risk groups, enterovirus infection and the environment will preserve the polio-free status of individual territories of Russia and the country as a whole and ensure the implementation of the Global Polio Eradication Program. Knowledge about the peculiarities of the circulation of non-polio enteroviruses among the population in certain territories in different time periods and about the evolution of enteroviruses will make it possible to understand the patterns and specific features of the epidemic process development for this infection, as well as contribute to improving the prevention of enterovirus infection to reduce its incidence.

Within the framework of cooperation with institutes of the Pasteur Network, the laboratory actively cooperates with Institut Pasteur in Ho Chi Minh City (Vietnam). As part of the project "Detection of non-poliomyelitic enteroviruses in the material from patients with enterovirus infection", together with colleagues from Vietnam, we studied enteroviruses isolated from patients with enterovirus infection and the specifics of the circulation of enteroviruses of different types in Russia and Vietnam.

Hand-Foot-and-Mouth Disease (HFMD) has been the most common form of enterovirus infection in Southeast Asia for many years. Its main symptoms are rash on the hands and feet, as well as sores on the oral mucosa. In hot countries, the infection is often complicated by pathological phenomena in the nervous or cardiovascular systems, so the diagnosis and prevention of enterovirus infection is an urgent task for Vietnam.

87 strains of enteroviruses isolated from EVI patients in South Vietnam were studied with the use of virological and molecular research methods. The nucleotide sequences of the VP1 genome region of 23 strains of Coxsackie A10 enterovirus and 2 strains of Coxsackie A2 virus were decoded. The sequences were deposited in GenBank database. A comparative analysis of Coxsackievirus A10 viruses circulating in the two countries was carried out. Enteroviruses of this type account for 23% of all enteroviruses isolated from patients with HFMD clinical manifestation in south Vietnam. Most of the isolated Vietnamese virus strains belonged to genotype F, mainly to subgenotype F3, while one strain isolated from a patient with a severe course of the disease belonged to subgenotype F1 (Fig. 5).

A comparison of Russian and Vietnamese strains of Coxsackievirus A10 by phylogenetic analysis revealed that the strains isolated in the Murmansk region belonged to genotype F, and the strains isolated in the Arkhangelsk region belonged to genotype C. Russian strains isolated in different time periods showed kinship with various foreign isolates. Strains related to Russian strains have been identified in the countries of Southeast Asia and the Pacific region. Such genetic heterogeneity of Russian CV-A10 strains may indicate the repeated introduction of this virus into the territory of the Russian Federation over the past decade.

26 strains of enteroviruses isolated in South Vietnam from patients with acute flaccid paralysis syndrome were also studied. 1 CVA4, 1 CVB1, 3 CVB3, 15 EVA-71 strains of non-polio enteroviruses were isolated. The types of viruses were determined by sequencing. Sequencing of Coxsackievirus A2 strains isolated from sick children with acute flaccid paralysis syndrome was performed. The connection of Coxsackievirus A2 strains with the clinical presentation of acute flaccid paralysis had not been recorded in Russia before. Work continues on comparing Russian and Vietnamese strains of non-polio enteroviruses. Over two years, nucleotide sequences of 39 strains of enteroviruses Coxsackievirus A, Coxsackievirus B, ECHO, EVA-71 (OL381925-OL381924 and OR948136-OR948149) were deposited in GenBank, which can be used by other researchers working on the issue of enterovirus infection.

From November 29 to December 3, 2021, a training seminar "Virological and molecular methods of detection of non-polio enteroviruses in samples from patients with enterovirus infection and in samples from the environment" was held. Forty seminar participants joined online from Hanoi and Ho Chi Minh City, and six participants from the Pasteur Institute in Ho Chi Minh City came to St. Petersburg to participate personally in a seminar on a problem of interest to them.

In 2021–2022, Dr. T.T.T. Nguyen from Institut Pasteur in Ho Chi Minh City worked on her thesis "Prevalence and etiology of enterovirus infection in South Vietnam". During her visits to St. Petersburg, the author of the thesis consulted with the academic supervisor. The structure of the thesis has been discussed, and tables and other illustrative material have been prepared. The "Materials and methods" chapter of the thesis has been written. The epidemic situation with AFP from 2010 to 2021 was analyzed. 22 strains of polioviruses and 249 strains of non-polio enteroviruses were isolated, and patient data were analyzed in terms of demographic indicators and vaccination status. We analyzed cases of enterovirus infection belonging to two clinical forms from 2012 to 2021. Cases of EVI with clinical manifestations of enterovirus meningitis and/or meningoencephalitis were analyzed separately; 244 strains of NPEV were isolated from such patients and were found to belong to three kinds (A, B and D) and 23 types of EV. From EVI patients with the clinical presentations of a viral exanthem, 3700 NPEV were isolated, classified into three kinds (A, B and C) and 13 types. Enterovirus EVA-71 was most often isolated in both clinical forms of EVI. Two genotypes of the EVA-71 virus circulate most widely among the population of South Vietnam, causing diseases with various clinical symptoms (Fig. 6).

It was found that in addition to EVA-71, enteroviruses of type B (CVB3, B4, B5, ECHO 11, 9, 6 and 30) prevailed in patients with clinical manifestations of meningitis, while viruses of type A (CVA10, A16, A4) dominated in the exanthemic form of EVI. We conducted a multifactorial analysis of the influence of climatic, demographic, socio-hygienic and geographical factors on the incidence of EVI, in particular, the fact that about a quarter of the population of Vietnam reside in the Mekong Delta. The Mekong flows through the territory of five Southeast Asian countries and carries wastewater from all these countries to South Vietnam, which causes the highest incidence of EVI in the southern provinces of the country.

In 2022, a textbook "Improving epidemiological surveillance and laboratory diagnostics of enterovirus infection in the Socialist Republic of Vietnam" was published in Russian and Vietnamese languages. The book provides data on the analysis of the incidence of enterovirus infection in South Vietnam and describes the etiological agents of EVI. Recommendations have been given on improving epidemiological surveillance and laboratory diagnostics

in the Socialist Republic of Vietnam. The educational material is useful for a wide range of medical specialists of different specialist areas.

Epidemiological surveillance of measles and rubella in the post-pandemic period

The St. Petersburg Regional Center for Measles and Rubella Surveillance of the Pasteur Institute monitors the situation in 11 regions of the Northwestern Federal District. The subnational Virological Laboratory of the Center, functioning at the Laboratory of Etiology and Control of Viral Infections and accredited by WHO, performs routine studies of blood sera from patients with suspected measles and rubella and exanthemic diseases supervision.

In 2021, the laboratory examined 24 samples of blood sera from measles patients, 21 samples of blood sera from rubella patients, and 239 samples of blood sera from patients with exanthemic diseases. In the conditions of a difficult epidemiological situation with COVID-19, as well as due to restrictive measures, no measles cases were registered in the territories of the Northwestern Federal District in 2021 (Fig. 7).

In 2022, the laboratory examined 23 samples of blood sera from measles patients, 15 samples of blood sera from rubella patients, and 244 samples of blood sera from patients with exanthemic diseases. After the lifting of COVID-19 restrictions, 7 cases of measles were registered in 2022, the incidence rate being 0.04 per 100 000 population. Cases of measles were registered in two territories of the NWFD: one case in St. Petersburg, and six cases in the Leningrad region.

In all cases, the infection was brought to the Northwestern Federal District by residents of Tajikistan who arrived from May to November 2022. As a result of the import, a focus of infection was formed, spreading to 3 children from Tajikistan and 2 children in contact with them, residents of the Leningrad region, who were not vaccinated for medical reasons.

In the period from 2019 to 2022, the shares of adults and children (under the age of 18) who had the disease were almost equal, with 54% of adults. In terms of vaccination status, 92.65% were unvaccinated or with an unknown vaccination history; 7.35% of patients with the disease were previously vaccinated and revaccinated persons.

All identified cases of measles had laboratory confirmation; in all cases, isolates of measles viruses and genotyping results were obtained. In 2020, one outbreak in St. Petersburg was caused by genotype D8 Gir Somnath measles virus, and all other cases of measles in St. Petersburg and the Leningrad region were caused by the other genotype, B3 Kabul. The last case of measles in the region was registered in May 2020. Since May 2022, all cases of measles in the territories of St. Petersburg and the Leningrad region have been caused by measles virus genotype D8 (MeaNS sequence code 8248), which continues to circulate in the Republic of Tajikistan.

Monitoring of the incidence of rubella in the territories of the NWFD

From 2021 to 2022, no cases of rubella were registered in the NWFD (Fig. 8).

Work is done on identifying rubella in pregnant women and cases of congenital rubella syndrome. During the period 2021–2022, 3 pregnant women with suspected infection or who were in contact with a laboratory-confirmed cases of rubella were examined. In all women, the diagno-

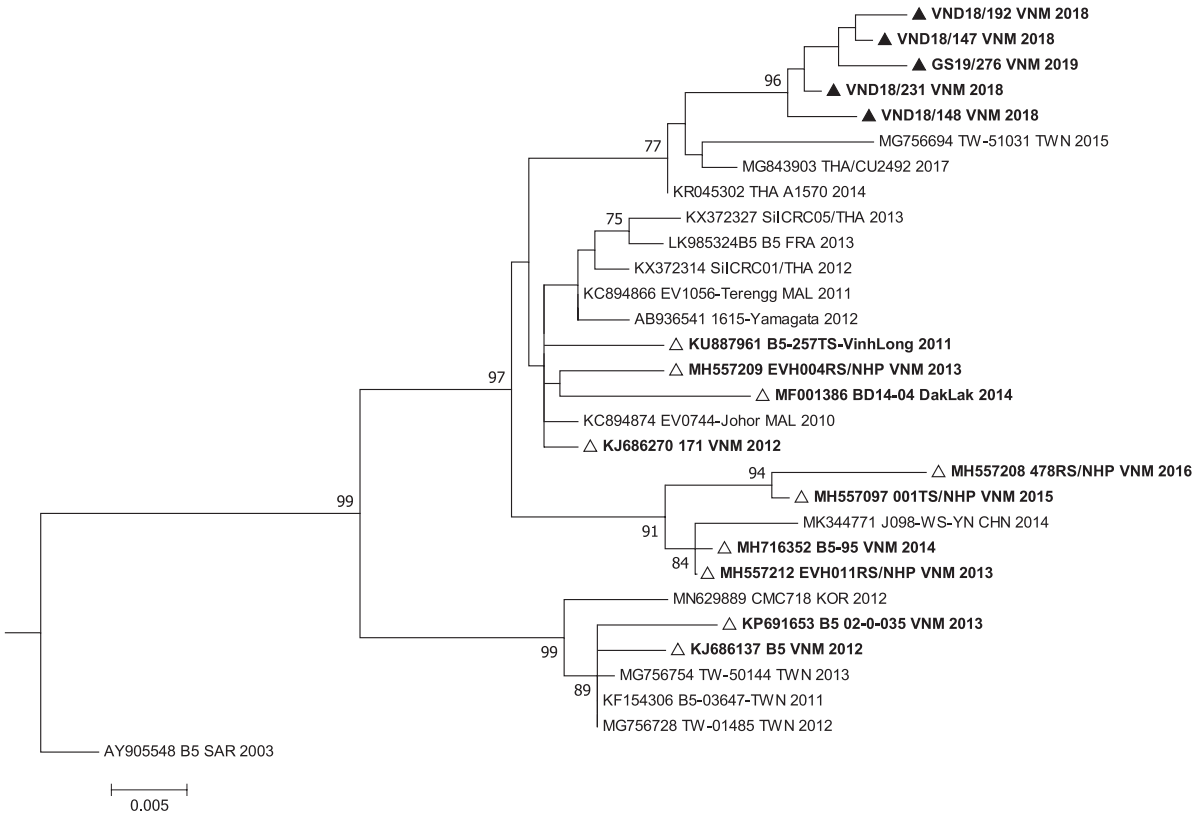

Figure 6. A phylogenetic tree constructed based on the analysis of VP1 fragment of EVA-71 strains genome of B5 genotype
The strains isolated in Vietnam are marked with ▲.

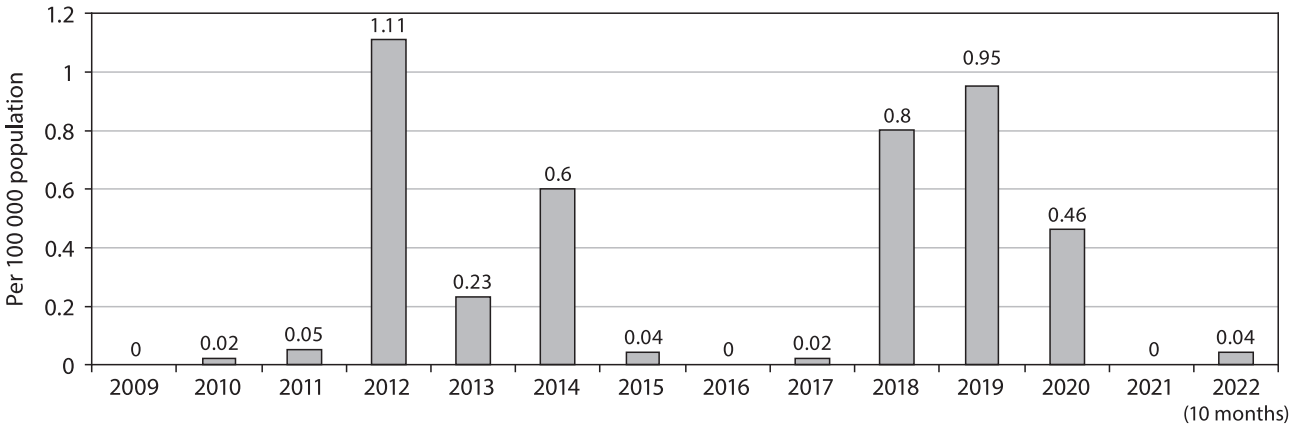

Figure 7. The incidence of measles in the territories of the Northwestern Federal District in 2009–2022 (per 100 000 population)

sis of rubella was excluded (absence of IgM dynamics, presence of high grade IgG).

In general, there is no endemic circulation of the measles virus in the territories of the NWFD. The low incidence of rubella in the NWFD during 2017–2022 and the data of an sufficient molecular study of the material from patients confirm that the NWFD has achieved the phase of rubella elimination.

Ethical commentary on COVID-19 vaccination (the ethical component of epidemic process control)

A systematic analysis of the ethical aspect of COVID-19 vaccination was performed. The acuteness of the problem lies in the fact that the concept of vaccination ethics has been introduced into legislative circulation and the terminological palette of normative documents, discussed in scientific publications and the media; it has become the subject of discussion between the civil and scientific community, secular authorities and religious denominations and is in the field of constant attention of leading international organizations, including UNESCO, UNAIDS, WHO, CDC, and others.

The assessment of the ethical component of various stages of vaccination revealed a number of critical points, which include a lack of scientific knowledge on COVID-19, limited data on the technological conditions of vaccine production, their effectiveness and safety, and the practical base of their application. The promotion of vaccines became an ethical compromise, accompanied by unregulated discrediting of competitors' vaccines, the creation of a stereotype of distrust and skepticism and a false interpretation of the concept of rights and freedoms, which excluded from consideration the objective nature of "double loyalty" state and the constitutionally enshrined social responsibility of the state to limit the rights and freedoms of an individual to protect the rights and legitimate interests of society.

The paper proposes to innovatively adapt the ethical concept of vaccination against COVID-19 to ensure the right balance of trust and justice. The practice of vaccination should be based on the following ethical principles: necessity, proportionality, diversity, legality, limitation, humanity, tracking, prevention, correction and responsibility for an offense.

The principles of necessity and proportionality provide for the determination of the scale, duration, and intensity of the use of specific vaccines, taking into account the actual threat and the dynamics of epidemiological and social consequences. The principle of diversity is intended to establish objective criteria for the personalized appointment of vaccines with different mechanisms of action to different groups, depending on clinical, epidemic, social and occupational risks.

The principles of legality and restrictions require comp liance with the norms of the current law on the organization of vaccination, minimizing the restrictions of rights and freedoms that are not provided for by law. The principle of humanity is about respecting and protecting persons who are considered to be vulnerable due to their social, age, psychological, administrative, and political characteris-

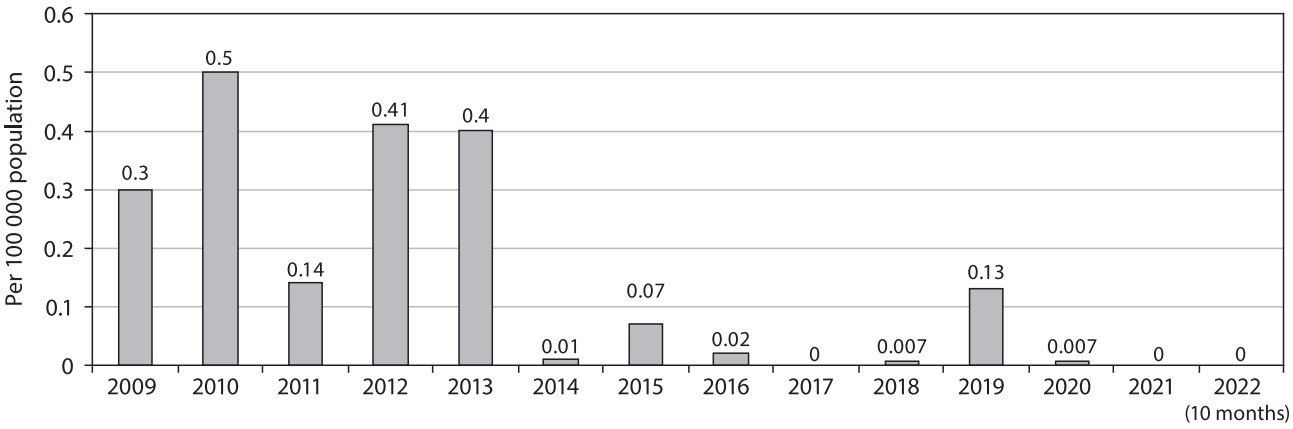

Figure 8. The incidence of rubella in the Northwestern Federal District in 2009–2022

tics. The principles of tracking, prevention, correction and responsibility involve dynamic monitoring of the consequences of vaccination for the purpose of prompt information support and logistics adjustments, including the possibility of replacement/suspension to prevent and minimize unforeseen risks and promote social responsibility.

As a general conclusion, it should be emphasized that achieving the harmonious implementation of the proposed system of ethical guidelines should directly contribute to the creation of a scientifically sound and morally acceptable platform for the development of public trust and voluntary commitment to vaccination.

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LABORATORY OF EXPERIMENTAL VIROLOGY

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Researchers: V. Zarubaev, A. Slita, A. Antipova, A. Volobueva, E. Sinegubova, S. Belyaevskaya, A. Garshinina, Ya. Esaulkova, R. Kadyrova, Ya. Orshanskaya

In 2021–2022, two research topics were studied in the laboratory of experimental virology:

I. Topic: "Development of new preventive and therapeutic drugs against socially significant viral diseases"

II. Topic: "Optimization of the surveillance system for measles and rubella at the stage of their elimination".

Development of new preventive and therapeutic drugs against socially significant viral diseases

Researchers: V. Zarubaev, A. Slita, Ya. Esaulkova, A. Volobueva, E. Sinegubova, S. Belyaevskaya, A. Garshinina, Ya. Orshanskaya

During the reporting period, research continued on the search and development of new synthetic low-molecular-weight inhibitors of influenza viruses. The high potential of derivatives of annelated and non-annelated tetrazoles has been shown. Of the 21 tested substances, 7 (33%) had a selectivity index (SI) of 10 or more, which indicates high potential of this group and the possibility of optimizing chemical structures in order to obtain compounds with optimal pharmacological characteristics (Fig. 9).

The search for effective inhibitors of influenza virus reproduction among chemical libraries of compounds of other structural classes continues. Thus, in the course of research, a high anti-influenza potential of polyfluorinated derivatives of heterocyclic compounds, as well as derivatives of natural terpenoid compounds, was shown. 32 of the 93 studied compounds of this group (34.4%) showed the required level of anti-influenza activity (SI 10 and higher). 4 compounds had SI above 100 (Fig. 10).

For a number of compounds in this library, their ability to block viral hemagglutinin was studied. As a result of experiments, it was demonstrated that the antiviral properties of oxadiazole frame derivatives are due to their ability to interfere with the fusogenic activity of viral hemagglutinin (Fig. 11), binding to the proximal portion of the stem part (Fig. 12).

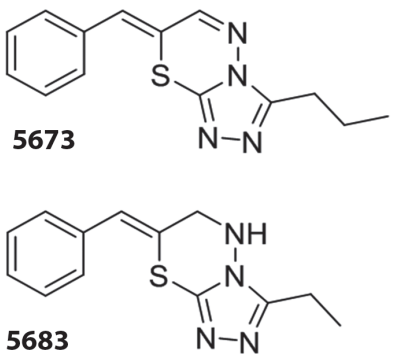

Figure 9. Derivatives of annelated tetrazoles 5673 (SI = 33) and 5683 (SI = 27), active against influenza virus A/Puerto Rico/8/34 (H1N1) in *in vitro* experiments

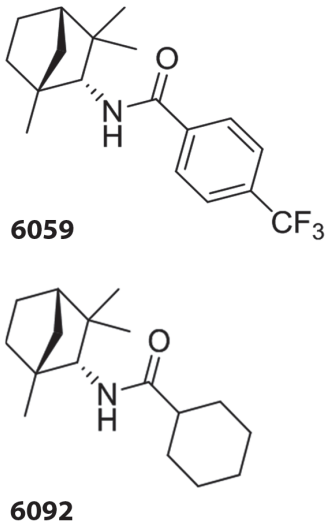

Figure 10. Derivatives of fluorinated cyclic derivatives 6679 and 6300, active against influenza virus A/Puerto Rico/8/34 (H1N1) in *in vitro* experiments

We continued studying the antiviral activity mechanism of ginsamide, a derivative of ginsenol, a natural polycyclic alcohol. With the help of selection in the presence of this compound, a strain of influenza virus resistant to it was obtained and mutations associated with resistance were localized in the hemagglutinin molecule (Fig. 13).

With the help of molecular modeling, the binding site of the ginsamide molecule with viral hemagglutinin was localized (Fig. 14). The analysis of the data obtained showed that it is in this site that the V458L resistance mutation is located (Fig. 13D). Thus, the data obtained by two independent methods explain the mechanisms of antiviral activity of ginsenol derivatives in general and the ginsamide leader compound in particular.

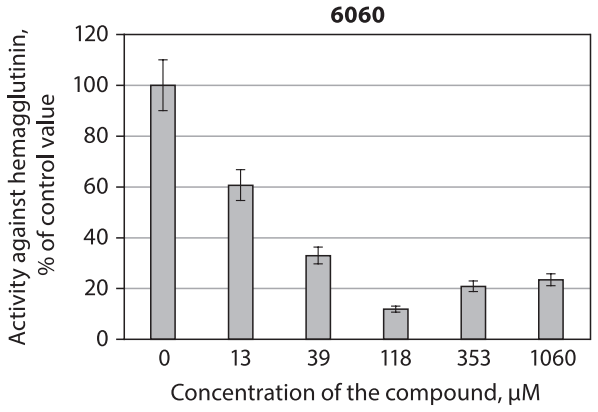

Figure 11. Anti-fusogenic activity of compound 6060 against hemagglutinin of influenza virus A/Puerto Rico/8/34 (H1N1)

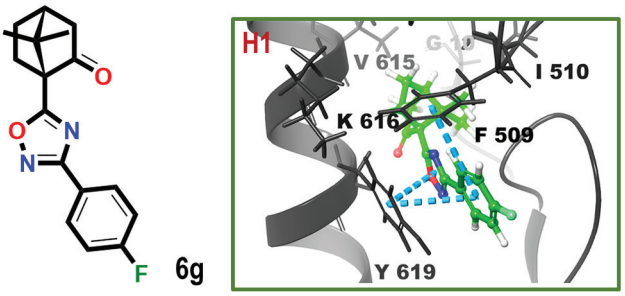

Figure 12. Model of binding of compound 6060 with hemagglutinin of influenza virus A/Puerto Rico/8/34 (H1N1)

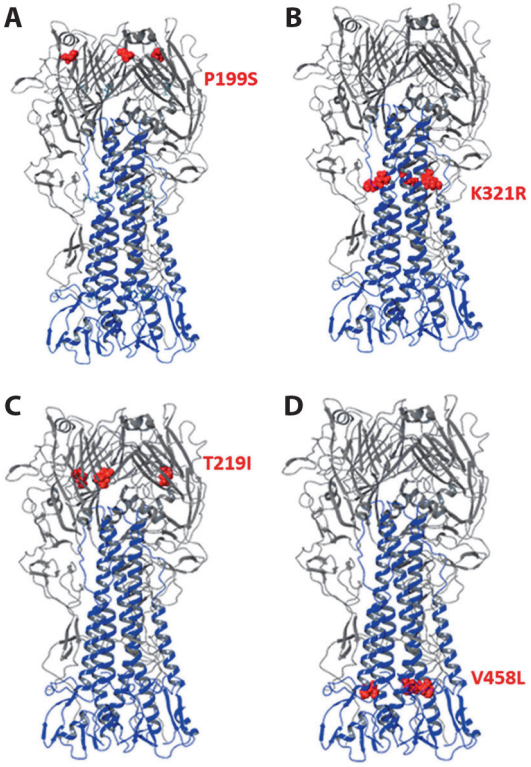

Figure 13. Mutations of resistance to ginsamide in the hemagglutinin molecule of influenza virus A/Puerto Rico/8/34 (H1N1)

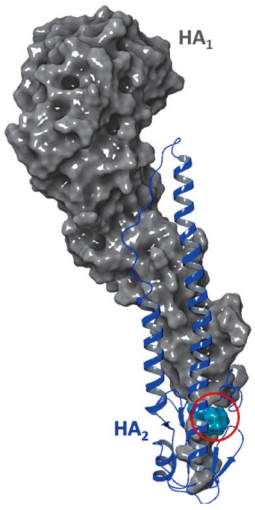

Figure 14. Localization of the binding site of the ginsamide molecule with viral hemagglutinin (indicated in red), based on molecular modeling data

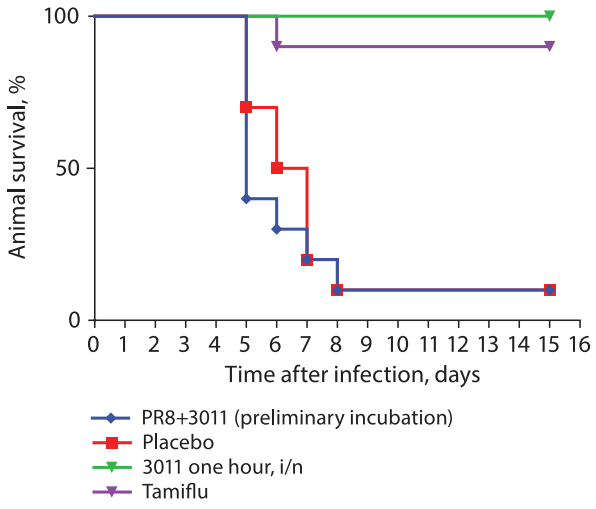

Figure 15. Dynamics of animal deaths from influenza pneumonia in the conditions of polymer compounds use

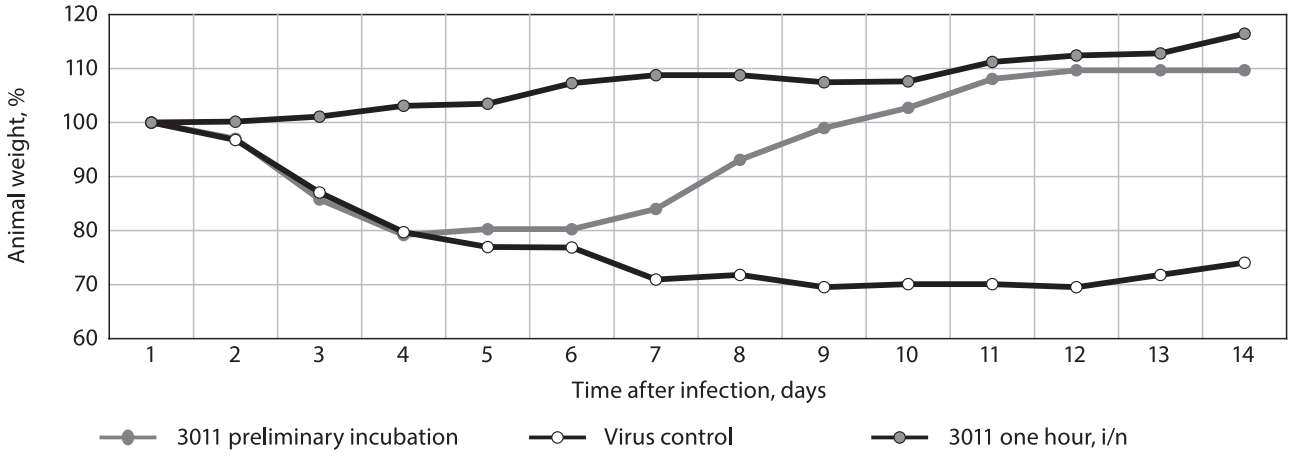

Figure 16. Dynamics of weight indicators of animals during influenza pneumonia in the conditions of polymer compounds use

The protective activity of the copolymer of vinylphosphonic acid with 4-acryloylmorpholine was studied in experiments on an animal model of influenza infection. It was shown that intranasal administration of the polymer prevents the death of animals and normalizes their weight indicators (Fig. 15, 16). All animals that received the polymer 1 hour after infection survived. At the same time, 1 mouse died in the group of animals receiving Tamiflu, the comparison drug. This makes the studied polymer a very effective means of preventing influenza infection, which affects not the virus directly, but possibly reduces the body's susceptibility to viral infection at the level of the portal of entry of infection.

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Optimization of the surveillance system for measles and rubella at the stage of their elimination

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Relevance

Despite the successful development of the Measles, Rubella and Congenital Rubella Syndrome Elimination Program, these infections still require attention due to the worsened epidemic situation for measles in Europe, Asia, Central and South America. The spread of the measles virus has not been interrupted in the Russian Federation, too. The COVID-19 pandemic and related anti-epidemic restrictions in a number of countries, including Russia, could have an impact on the circulation of the measles virus. The study of this issue is important for the surveillance and control of the infection.

The epidemiological situation of rubella in Russia is quite trouble-free. In particular, only isolated cases of rubella or their absence have been registered in the Northwestern Federal District for a number of years. However, in the conditions of sporadic morbidity, the importance of screening studies of herd immunity to the rubella virus increases.

The Russian Federation does not register the incidence of parvovirus infection (PVI), which is particularly dangerous for people from risk groups. However, according to previously obtained data, this infection is widespread in the Russian Federation.

Therefore, studies devoted to improving epidemiological and virological surveillance of measles, rubella and parvovirus infection remain relevant.

In 2020–2021, research on this topic was conducted in the following main areas:

1. The impact of the COVID-19 pandemic on the spread of measles in a number of geographical regions in 2020;
2. Herd immunity to rubella virus in some geographical regions;
3. Study of the spread of parvovirus infection markers among the population of the NWFD, including in the risk group;
4. Characteristics of isolates of parvovirus B19 (PVB19) circulating in the Republic of Guinea (GR).

Materials and methods

The study involved a total of 3136 samples of blood sera obtained in 2020–2022 from a number of territories of the Russian Federation, South Vietnam and the Republic of Guinea from persons aged 7 months to 89 years, healthy for the purposes of the study, as well as patients with exanthemic manifestations of the infectious process. Samples were provided by virological laboratories of regional centers for the supervision of measles and rubella in the Northwestern Federal District of the Russian Federation, in the Republic of Serbia, in South Vietnam, and in the Republic of Guinea. The study also included patients of the hemodialysis department in one of the medical institutions of St. Petersburg.

Enzyme immunoassay. Determination of IgM antibodies to measles virus and parvovirus B19 (PVB19) was carried out with diagnostic kits Anti-Measles Virus ELISA (IgM) (EUROIMMUN, Germany), Anti-Parvovirus B19 ELISA IdM (EUROIMMUN, Germany), according to the manufacturer's instructions. The presence of IgM antibodies in blood sera was qualified as an acute infection. Quantitative and qualitative determination of IgG antibodies to rubella virus and PVB19 was carried out with diagnostic kits Anti-Rubella Virus ELISA(IgG) and Anti-Parvovirus B19 ELISA IgG manufactured by EUROIMMUN (Germany), according to the manufacturer's instructions.

Molecular genetics methods. The extraction of nucleic acids (DNA) from blood plasma was carried out with a commercial kit Ampli Prime Ribot-prep (FBRI Central Research Institute of Epidemiology, Moscow), according to the manufacturer's instructions. Detection and/or quantitative determination of RVB19 DNA was carried out by real-time PCR with hybridization-fluorescence detection with a commercial kit AmpliSens® Parvovirus B19-FL (FSUE Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow) according to the manufacturer's instructions in a qualitative or quantitative format.

The amplification and sequencing of the product was performed with specific primers (Syntol, Russia). The sequence of primers and fluorescent probes was taken from literature sources, and was also selected using the NCBI/

Table 12. Nucleotide sequences of primers additionally used for sequencing the NS1/VP1 PVB19 region

Primer	Nucleotide sequence 5'→3'
PVB19 1F	CAATTGTCACAGACACCAGTA
PVB19 1R	ACTTAGCCAGTTGGCTATACCT
PVB19 2F	CCCGCGCTCTAGTACGCCCA
PVB19 2R	TTGCGGGGGCCCAGCTTGTA

Primer-BLAST software according to general recommendations (Table 12). Purification of amplification and sequencing reaction products was carried out with Qiaquick PCR Purification kit, a commercial set reagents (Qiagen, Germany) according to the manufacturer's instructions, or by alcohol precipitation in the presence of sodium acetate.

PVB19 DNA Sequencing. Sequencing was performed on plasma samples with a viral load of at least 10^2 IU/ml. A purified DNA fragment with a concentration of 50–100 ng was used to perform sequencing reactions with forward and reverse primers in three repetitions for each pair of primers of each sample. PVB19 primers were used for the reaction, allowing to analyze the NS1/VP1 region (NS1–VP1u locus), recommended for geno- and subgenotyping of PVB19, with a length of about 994 base pairs (bp), according to the isolate J35 (AY386330) present in in GenBank database, together with additional primers. For additional control of samples, two analytical systems were used with the the reagents used according to the manufacturer's instructions, in GenomeLab GeXP genetic analyzer (Beckman Coulter Inc., USA) and the ABI PRISM 3500 genetic analyzer (Applied Biosystems, USA).

Phylogenetic analysis. To determine the belonging of PVB19 isolates to genotypes, a fragment of the genome was selected, including a conservative fragment for the NS1 and VP1 gene sites (NS1–VP1u locus), 994 bp. The primary analysis of fragments obtained during sequencing was carried out with NCBI Blast in comparison with the nucleotide sequences of reference samples in GenBank database.

The alignment of nucleotide sequences was done in MEGA 7.0 application with ClustalW algorithm. To construct phylogenetic trees and perform subsequent phylogenetic analysis, the distances between sequences were considered by the Neighbor-Joining method, which allows optimizing the tree in accordance with the criterion of "balanced minimal evolution" using the "Maximum Composite Likelihood" model, with bootstrap for 1000 repetitions carried out to assess the reliability of the constructed trees.

Statistical processing of the results was carried out in MS Excel software package and Prizm 5.0 (GraphPad Software Inc). The comparison of nominal data was carried out using the t-test. The reliability threshold for the differences: with a probability of $p < 0.05$.

Results

2.1. The impact of the COVID 19 pandemic on the spread of measles in a number of geographical regions

According to the WHO strategic plan, by 2020, five out of six geographical regions (except the Southeast Asia region, SEAR) were to achieve the status of measles elimination. These are the American (AMR), European (EUR), Western Pacific (WPR), Eastern Mediterranean (EMR), and African (AFR) regions. However, until recently, measles has been actively spreading in the world. In 2019, 524 718 cases of the disease were detected. Measles was recorded in all WHO regions: AFR: 288 364, EUR: 104 420, WPR: 62 165, SEAR: 29 599, EMR: 20 629, and AMR: 19541 measles cases. In the European region, the highest incidence of measles was found in Ukraine (57 282 cases), Kazakhstan (13 326 cases), and Georgia (3920 cases). In the Southeast Asia region, it was registered in India (76 588 cases) and in the Western Pacific region, with the largest number of cases in 2019 was observed in the Philippines (46 689). On the African continent, it was Madagascar (151 032 cases), Nigeria (27 195 cases), and some other countries.

In 2019, 4491 cases of measles were detected in the Russian Federation, of which 132 cases were detected in the NWFD; 14 156 cases of measles were detected in Vietnam, and 1301 in the Republic of Guinea, which is the incidence that exceeds the level required to achieve elimination (established as less than 1 case per 1 million population). The age structure of cases in Vietnam and RG was dominated by children under three years old, whereas in the Russian Federation, by persons over 18 years old. The infection spread mainly among people who were not vaccinated against measles.

In 2020, during the spread of COVID-19, the circulation of the measles virus in the surveyed regions continued. Thus, in the Northwestern Federal District, the incidence rate in 2020 was 0.45 per 100 000 population, and the total number of cases was 63. Most of the patients were adults. In South Vietnam, 463 cases of measles were detected in 2020; 512 cases of measles were registered in the Republic of Guinea in 2020. In the latter two, measles spread mainly among children under the age of three.

However, in March-April 2020, a lockdown was introduced both in the Northwestern Federal District of the Russian Federation and in South Vietnam. Since that time, the number of measles cases has decreased to sporadic levels in both territories.

This trend was not observed in the Republic of Guinea, which did not have any anti-epidemic measures related to the pandemic of the novel coronavirus infection. Measles in the GR was registered throughout 2020, and the twofold decrease in the number of cases in relation to 2019 is apparently due to a natural decrease in the incidence after the rise of 2018–2019.

Thus, a sharp decrease in the intensity of the measles virus circulation both in the Northwestern Federal District of the Russian Federation and in South Vietnam in 2020 is, in our opinion, due to anti-epidemic measures introduced in the Russian Federation and Vietnam in order to limit the spread of COVID-19. Therefore, after the restrictions are lifted, an increase in the incidence of measles in various geographical regions can be expected due to a decrease in measles vaccination coverage, which is due to an increased number of exemptions from routine immunization during the pandemic and the diversion of resources to COVID-19 vaccination.

For instance, in 2022, the resumption of the circulation of the measles virus was found in the North-West of Russia: 6 cases of the disease were registered, including 1 case in St. Petersburg and 5 cases in the Leningrad region. The incidence rate was 0.04 per 100 000 population. Most of the patients (83.0%) were either not vaccinated against measles or had uncertain vaccination history. A focus of infection was registered, from where the disease spread, and a case of measles was detected in a previously vaccinated

person. All patients were found to have the virus of D8 genotype, sequence code MeaNS 8248, endemic to the territory of Tajikistan, from where the patients came.

2.2. Herd immunity to rubella virus in some geographical regions

In the context of globalization, it is important to constantly monitor the level of herd immunity to pathogens of certain infections, including rubella, in order to identify epidemiologically significant groups of the population. It is especially important to carry out such monitoring in countries where vaccination against rubella is not carried out, or where the control by specific prevention methods is insufficient.

In the Russian Federation in 2020–2022, the seroprevalence of the population of the NWFD to the rubella virus was 96.6–97.7% and fluctuated only slightly between age groups. Consistently high rates indicate a high coverage of rubella vaccination and the effectiveness of specific infection prevention.

In the Republic of Serbia, the overall seroprevalence index was 86.8%, which is significantly lower than in the Russian Federation. However, the lowest number of IgG-positive sera (72.0%) was recorded in the age group of 2 to 4 year olds. Further, the proportion of seropositive individuals consistently increased with age, reaching a maximum (90.8%) among the 50 years and older group. Such an age distribution of persons protected from infection is more typical for the formation of herd immunity through the natural spread of rubella. In Serbia, according to the National Calendar, the first rubella vaccination is given to children aged 12–15 months, and the second at the age of 7 years. The fact that among young children (2–4 y.o.) the share of those protected from the infection is the lowest shows insufficient vaccination against rubella. The same thesis is confirmed by the low proportion of seropositive persons in the age groups of 8–14 y.o. (49%) and 15–25 y.o. (57.3%), i.e., among people who, according to the National Calendar, should have received not only the first rubella vaccination, but also a revaccination, which had to be quite recently. The insufficient level of MMR vaccination coverage, including the rubella component, was revealed by us earlier, when studying the humoral immunity of the Serbian population to the measles virus.

A similar trend was found in South Vietnam when we analyzed rubella cases that occurred in 2019–2020. There was a large share of children aged 1–3 years (41.9%) among those who had the disease, i.e., the age cohort that should have the highest vaccination protection. The second age group with a large number of cases detected were children of primary and secondary school age (22.6%). Thus, it can be assumed that herd immunity to rubella in Vietnam is most actively formed due to the involvement of children and adolescents in the infectious process. Therefore, routine vaccination against rubella in Vietnam does not provide full control of the infection.

In the Republic of Guinea, unlike in other regions included in this study, no specific rubella prevention measures are in force. In the conditions of natural spread of rubella, 75.2% of the examined individuals had IgG antibodies to the virus, which corresponds to the characteristic of the infection as a widespread disease with low contagiousness. The proportion of seropositive persons both under 20 years of age and from 20 to 49 years of age was 73%, and a higher share, 95%, was only in the age group of 50 years and older. I. e., herd immunity to rubella virus in Guinea is formed mainly among children and adolescents and remains at the same level in the population. The exception is the elderly: a higher proportion of seropositive persons in this group is probably due to closer contacts with children in families. Among the examined women, 30% of unprotected persons of the most active reproductive age (20–29 y.o.) were identified, which shows the potential for their infection during pregnancy and resulting cases of congenital rubella infection.

2.3. Study of the spread of parvovirus infection markers among the population of the NWFD, including in the risk group

2.3.1. Characteristics of the spread of parvovirus infection in the territories of the Northwestern Federal District in 2021–2022

During the implementation of the WHO program for the elimination of measles and rubella in different regions of the world, including in the Russian Federation, it is necessary to perform differential laboratory diagnostics of measles, rubella and other exanthemic diseases, among which parvovirus infection (PVI) has a high medical significance.

In 2021–2022, new results were obtained to characterize the spread of parvovirus infection among healthy individuals and at risk groups in the Northwestern Federal District of the Russian Federation. In general, 62 out of 503 examined samples were IgM-positive, 12.3% of the total.

The Northwestern Federal District includes 11 administrative territories. Cases of parvovirus infection were detected in seven territories in 2021 and in ten in 2022.

For a number of years, the infection has been spreading most actively, with the emergence of family foci, in St. Petersburg (30–35% of all cases), which can be explained by the high population density in the metropolis, a large number of educational, medical and other institutions where long-term close contacts between people take place.

The winter-spring seasonality of the disease was confirmed: 64% of cases occurred in the period from January to May 2021–2022.

The analyzis of the clinical diagnoses of patients with laboratory-confirmed PVI, it was found that in none of the cases was parvovirus infection suspected by clinicians at the initial treatment. The most common erroneous clinical diagnosis was ARVI, established in respect 21.0% of all laboratory-detected cases of PVI. In 8% of cases, patients were mistakenly diagnosed with the primary diagnosis of measles or rubella. Also, parvovirus B19 infection was detected in 10% of cases with suspected herpesvirus infections (infectious mononucleosis and varicella); in the same proportion of cases, PVI was detected in patients with a clinical diagnosis of infectious exanthem. IgM-PV B19 antibodies were detected in 30% of the samples for which the doctor's reference stated "exanthem of unclear etiology" and "allergic rash/allergic dermatitis". That means that in about a third of cases PVI was seen by clinicians as a somatic rather than infectious disease, which does not require any anti-epidemic measures (Fig. 17).

The results obtained indicate the continued circulation of parvovirus B19 in the North-Western region with possible infection of persons from risk groups.

2.3.2. Identification of laboratory markers of parvovirus infection in patients of the hemodialysis department

Patients with kidney diseases undergoing hemodialysis are at risk for parvovirus B19 infection. The spread of PVI among these patients may be facilitated by factors such as

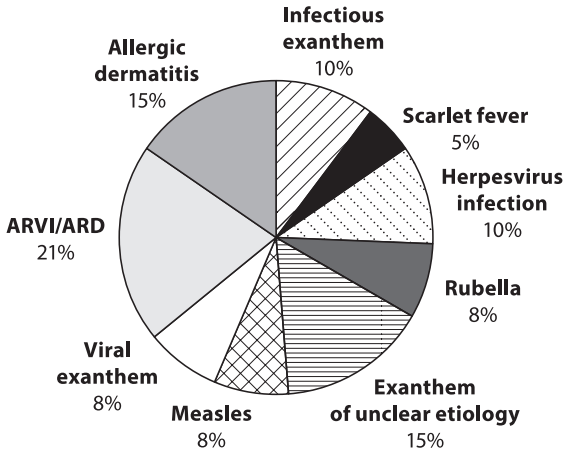

Figure 17. Primary clinical diagnoses in patients with parvovirus B19 infection established by laboratory tests

an invasive method of treatment; secondary immunodeficiency conditions; short life span of erythrocytes; violation of erythropoietin synthesis. Moreover, all patients of hemodialysis centers are potential recipients of donor organs and tissues that may be infected with parvovirus B19.

In 2022, a laboratory testing was performed of samples from 78 patients of the hemodialysis department (23–89 years old, average age 57.4 years) for laboratory markers of parvovirus B19 infection. Blood sampling and testing for PVI markers were performed three to four times. The control group consisted of 64 patients of the laboratory department of the diagnostic and treatment center who did not receive renal replacement therapy (23–89 y.o., average age 60.5 y.o.). Blood sampling and PVI markers detection in this group was carried out once. Informed consent was obtained from all patients.

76.9% (60 out of 78) of patients at the dialysis center and 75.0% (48 out of 64) of those examined in the control group were found to have IgG antibodies. IgM antibodies (a marker of acute PVI) were detected in one person from the hemodialysis center and were absent in the samples of the control group (Table 13). At the same time, there were no IgG and virus DNA in the patient's blood sample, i.e., the infection with parvovirus B19 was recent. The patients had no clinical manifestations of PVI. PVB19 DNA was found in four patients of the dialysis center. The viral load in all cases was less than 720 IU of Parvovirus B19 DNA/ml. Three DNA-positive samples contained a low titer of IgG antibodies, from 50 to 77 IU/ml, which may indicate a progredient course of infection. In one case, a high titer of IgG antibodies was found, indicating a recent disease. The results obtained indicate the spread of PVI among patients of the hemodialysis department and the latent circulation of the virus.

The interferon status of hemodialysis patients was significantly lower than that of patients in the comparison group. The analysis of clinical and hematological indicators of patients of the hemodialysis department and the control group demonstrate significant differences between the groups: a decrease in the amount of hemoglobin, erythrocytes and platelets among patients on hemodialysis compared to the control group (Table 14).

Thus, laboratory markers of IgM and DNA indicating acute PVI were found in 4 out of 79 (5%) patients of the hemodialysis department and in 1 out of 64 patients of the clinical and laboratory department (1.6%). It has been shown that patients of the hemodialysis department can be virus carriers even in the presence of IgG class antibodies in high titers. Two out of four patients with laboratory-confirmed PVI undergoing hemodialysis had persistent, non-correctable anemia, which may indicate the aggravation of the underlying disease in patients with renal insufficiency infected with PVB19.

2.4. Characteristics of isolates of parvovirus B19 circulating in the Republic of Guinea (GR)

In 2021–2022, research continued on the study of the spread of parvovirus infection in certain geographical regions, including the Republic of Guinea.

In the reporting period, isolates of parvovirus B19 circulating in the territory of the GR were isolated and characterized. For this purpose, 924 blood plasma samples from residents of the Republic of Guinea were tested by PCR. In 84 out of 924 blood samples of persons healthy for the purposes of the study and patients with acute PVI, PVB19 DNA was detected, which was 9.1±0.95% of the number of blood samples examined.

Table 13. Cases of PVI in patients of the dialysis cente

Patient No.	Diagnosis	Age, years	PVI markers			Interferon status*		
			IgM	DNA (UI/ml)	IgG (UI/ml)	Se	INFαβ	INFγ
1	Vesicoureteral reflux	29	–	0	–	16	80	80
2	Hypertensive disease	48	–	584	76.98	8	80	80
3	IgA-nephropathy	40	–	462	50.49	16	80	160
4	Chronic glomerulonephritis	25	–	190	> Max	Not examined		
5	Chronic glomerulonephritis	26	–	290	60.89	8	160	160

*Reference values: Se < 10; INFαβ — 320–160; INFγ — 160–80.

Table 14. Complete blood count findings in patients receiving renal replacement therapy and patients who do not need hemodialysis

Patients	Hemoglobin, g/l M±m	Red blood cells, 10^{12}/l M±m	Platelets, 10^{9}/l M±m
Diagnostic Treatment and Department	142.4±2.51	4.76±0.071	264.4±19.94
Hemodialysis Department	115.3±1.79	3.79±0.068	228.4±9.15

For sequencing, samples with the highest viral load (Ct < 30) were used, which for most samples did not exceed 10^3 IU DNA/ml. For genetic characterization, 26 blood samples were selected from Guinean citizens, some of them healthy for the purposes of the study and some with acute PVI, regardless of gender and age, living in different regions of the country. A positive result of PCR with different primers was obtained for 9 out of 26 tested samples. Only 5 samples contained a sufficient quantity of the material for sequencing (Table 15). The region of detection is specified in the table as follows: Région, Préfecture, S/Préfecture, District. In some cases, the name of S/Préfecture is not specified. The name of the nucleotide sequences was given according to WHO recommendations for designation of new isolates of the measles virus. All isolates were obtained from blood samples of patients with acute PVI.

The five obtained genetic sequences, aligned according to the reference isolate J35 (AY386330), were deposited in GenBank database under numbers ON076009; ON730888; OM721657; ON788002; ON911498 (Table 16). In one case, a region from 1447 to 1827 nt was sequenced, which belongs to the NS1 region of parvovirus B19 genome. In other cases, the obtained sequences were localized in the range from 2075 to 3177 nt, which corresponds to the NS1-VP1u locus.

Molecular genetic analysis was performed with Neighbor joining method. The evolutionary history was derived with Neighbor Joining method. The analysis involved 59 nucleotide sequences. All items containing gaps and missing data have been eliminated. The evolutionary analysis was performed in MEGA7. As a result of the study, it was shown that isolates of the parvovirus B19 circulating in the Republic of Guinea belong to 1A2 subgenotype.

The dendrogram shows, for all isolates, the GeneBank number, the country (the three-letter Alpha3 code was used) and the year of isolation, if these data were published by the authors. To construct a phylogenetic tree, sequences related to known genotypes and subgenotypes of parvovirus B19 were selected as reference isolates. The reference sequences are assigned GenBank codes: genotype 1A1 — M13178, KM065414; 1A2 — KM065415; 1B — DQ357064; 2B — AY064476, AY044266, AY661663; 3A — AJ249437, AX003421; 3B — AY083234, DQ408302 (Fig. 18).

The African continent is mainly represented in GeneBank by isolates from Burkina Faso (BFA), Gabon (GAB), Nigeria (NGA), and South Africa (ZAF). Parvovirus B19 identified on the African continent includes all of its known genotypes. Genotypes 2 and 3 are widely represented in Africa, as well as genotype 1A, the most common in the world.

To sum up the results obtained in 2021–2022, we can come to the following conclusion.

In 2022, 7 cases of measles were detected in the NWFD. Most of the patients (83.0%) were either not vaccinated against measles or had uncertain vaccination history. A focus of infection was registered, from which it spread. That means that after the cancellation of anti-epidemic measures to limit the spread of COVID-19, measles imports in the NWFD resumed. This indicates the need to continue targeted efforts to stop the circulation of the measles virus in Russia, including in the North-West of the country.

Insufficient level of herd immunity to rubella was found in a number of geographical regions included in this study. This can contribute to the maintenance of the infectious process of rubella and the spread of infection, and globalization contributes to the import of the virus into regions with sporadic incidence from other regions. The results obtained indicate the need for additional infection control measures at the stage of measles and rubella elimination.

Parvovirus B19 is actively circulating in the NWFD. The spread of PVI continues, including in risk groups. In the natural spread of infection, PVB19 isolates are characterized by a high degree of homology both among themselves and with other isolates in Southeast Asia, the European and African regions (as determined by WHO). Further molecular genetic studies are also needed to expand knowledge about the biological features of parvovirus B19, the peculiarities of its distribution and evolution.

Table 15. Characteristics of isolates from samples collected in GR

Sequence name	Territory were the isolates were produced	Date of material collection	Gender (m/f), age/date of the disease
B19V/Labe.GIN/2.2020/7775	Labé, Lélouma, Parawol	29.01.2020	F., 5 y.o./26.01.2020
B19V/Kankan.GIN/9.2021/8409	Kankan, Kouroussa, Kouroussakoura	03.03.2021	M., 10 y.o./03.02.2021
B19V/Labe.GIN/19.2021/8503	Labé, Lélouma, Balaya, Dar-es-Salam	05.10.2021	M., 10 months/05.01.2021
B19V/Boke.GIN/44.2021/8850	Boke, Boké, Sangaredi, Lavage	02.11.2021	F., 35 y.o./29.10.2021
B19V/Conakry.GIN/45.2021/8854	Conakry, Dixinn, Kipé	11.11.2021	M., 4 y.o./06.11.2021

Table 16. Brief description of the nucleotide sequences of DNA of parvovirus B19 deposited in the GenBank

GenBank number	Sample number	Locus of the genome	Localization	Sequence length, nt	Genotype
ON076009	7775	NS1	1447–1871	425	1A2
ON730888	8409	NS1-VP1u	2299–3047	749	1A2
OM721657	8503	NS1-VP1u	2139–3177	1039	1A2
ON788002	8850	NS1-VP1u	2075–3145	1071	1A2
ON911498	8854	NS1-VP1u	2737–3048	312	1A2

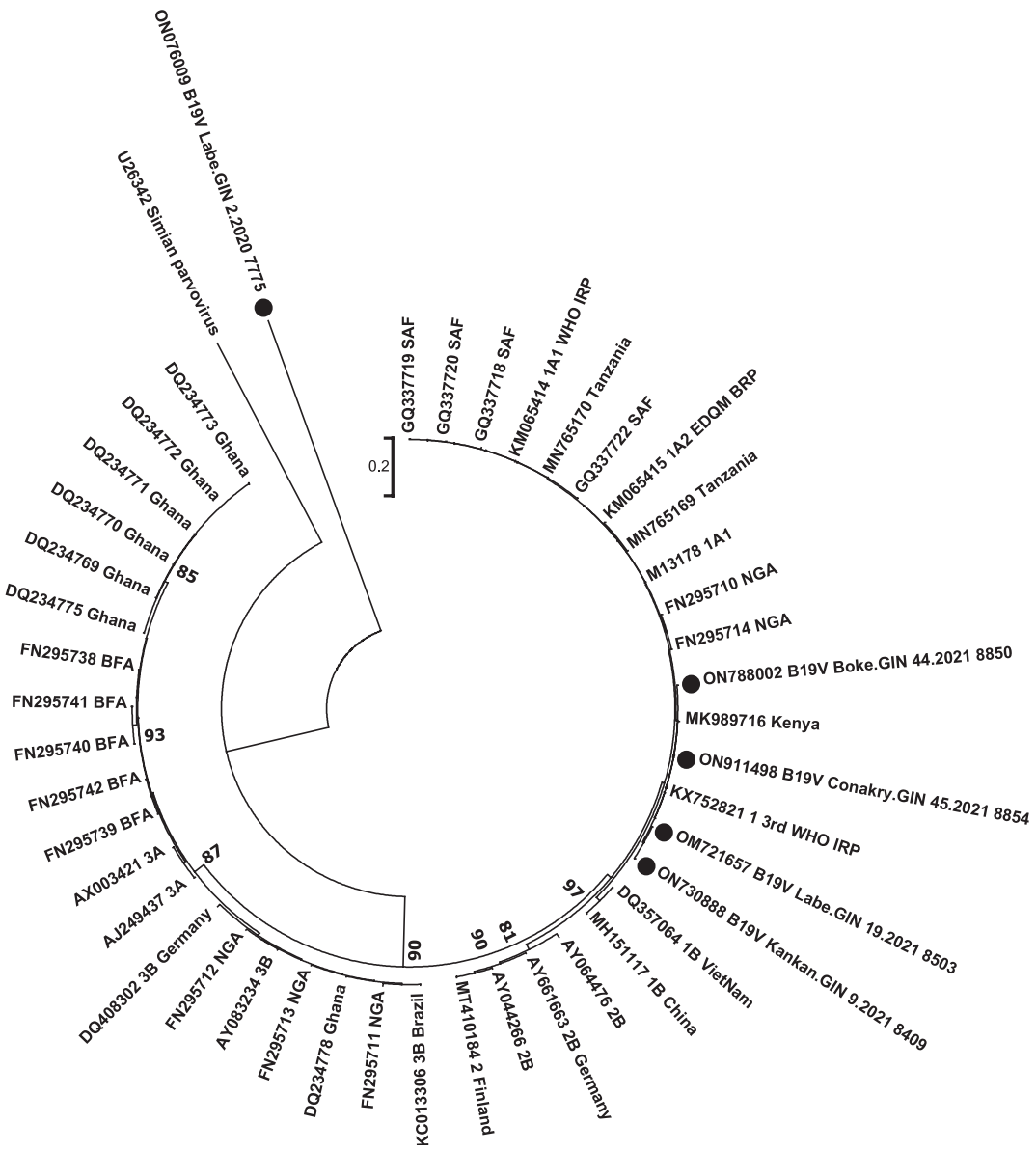

Figure 18. Phylogenetic analysis of nucleotide sequences of fragments of genomes (NS1 and VP1u) of Human parvovirus B19 isolated from blood samples of persons living in the Republic of Guinea (●) in comparison with reference sequences and sequences from African countries presented in GenBank database. Bootstrap values ≥ 70 are given. KM065415

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Patents

1. *Patent No. RU 2 753 310 C2, CPC C12Q 1/68 (2021.02). Method for detecting parvovirus B19 in DNA biological material based on two step PCR. No. 2019121500; application: , 08.07.2019: date of publication 13.08.2021 / Ostankova Yu.V., Khamitova I.V., Lavrenteva I.N., Semenov A.V. Proprietors: Federalnoe byudzhetnoe uchrezhdenie nauki "Sankt-Peterburgskij nauchno-issledovatelskij institut epidemiologii i mikrobiologii im. Pastera Federalnoj sluzhby po nadzoru v sfere zashchity prav potrebitelej i blagopoluchiya cheloveka" (FBUN NII epidemiologii i mikrobiologii imeni Pastera) (RU)*

Department of Immunology

LABORATORY FOR PATHOGEN IDENTIFICATION

Head of the Laboratory: Alyona Svarval
Researchers: D. Neronskaya, R. Ferman, N. Gladychev

Helicobacter pylori is one of the most common human pathogens. *H. pylori* infection affects about 60% of the world's population. Currently, this infection shows a downward trend in highly developed countries (20–40%), while in developing countries it remains at a high level (80–90%) [Hooi et al., 2017]. The *H. pylori* is an important factor in the etiopathogenesis of chronic gastritis, gastric ulcer and duodenal ulcer, low-grade gastric lymphoma (maltoma), as well as stomach cancer [Blaser M.J., Atherton J.C., 2004; Atherton J.C., 2006]. The increased risk of developing this pathology is associated with the expression of specific bacterial virulence factors, with variations and features of the interaction between the pathogen and the macroorganism [Mitchell H., Katelaris P., 2016]. The global population of *H. pylori* demonstrates a high degree of genetic heterogeneity due to mutations and recombinations. A number of genes associated with increased virulence of the microbe have been identified (e.g., *vacA, cagA*), and their role in the development of various forms of gastrointestinal pathology is being investigated [Cao D.M. et al.; 2016, Noto J.M., 2017]. Data are being accumuled on the uneven geographical distribution of various *H. pylori* genotypes, their clinical and epidemiological significance [Pinto Ribeiro et al., 2016].

The aim of our work was to study the clonal structure of the regional population of *H. pylori* based on the study of the phenotypic and genotypic properties complex of strains isolated from patients with gastrointestinal pathology.

We isolated and identified *H. pylori* cultures from biopsies of the gastric mucosa and duodenum of patients from St. Petersburg with gastrointestinal pathology (chronic gastritis, gastroduodenitis, gastric or duodenal ulcer, malignant neoplasms of various localization). We estimated the frequency of detection and polymorphism of genes *cagA, oipA, vacA* (*s-, m-, i*-regions) of *H. pylori* strains isolated in various pathologies of the gastroduodenal region. The spectrum of phenotypic resistance to nitroimidazole derivatives, macrolides, beta-lactams, and fluoroquinolones used in helicobacteriosis eradication treatments was determined for various *H. pylori* strains. Based on the results obtained, the Database of Phenotypes and Genotypes of Helicobacter Pylori Strains Isolated in St. Petersburg was created (registration date 16.09.2021, Certificate No. 2021621974). The database contains information on the phenotypic properties, antibiotic resistance and the presence of virulence determinants of 60 strains of *H. pylori* isolated from patients with gastroduodenal pathology in St. Petersburg and characterizes the prevalence of antibiotic resistance and genotypes of the causative agent of helicobacteriosis in this region. The data are presented using a binary code (1/0 — presence/absence of a trait, +/– — presence/absence of a gene, S/R/NA — sensitive/resistant/not assessed, respectively). The table with data from the Database of Phenotypes and Genotypes of Helicobacter Pylori Strains Isolated in St. Petersburg is presented in Microsoft Excel format (3 tabs of 62 rows, 5, 6, and 8 columns, respectively). The database is designed to store and analyze information on the prevalence of genotypes, as well as antibiotic resistance of *H. pylori* strains isolated in St. Petersburg. The database can be used to study the global diversity and regional characteristics of *H. pylori* strains, for scientific research in the field of epidemiology, gastroenterology, genetic analysis and in the epidemiological surveillance system.

babA2 gene polymorphism was evaluated by comparing the obtained sequences with the database of sequenced genomes of *H. pylori* strains with the use of BLAST resource (Basic Local Alignment Search Tool), NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The degree of genetic kinship between strains based on the polymorphism of the *babA2* gene region was evaluated using the Neighbor-Joining algorithm and graphically displayed as a dendrogram built with MEGA v X software. The analysis of amplification products with two sets of primers for the detection of *babA2* gene sites with sizes of 832 bps and 271 bps revealed differences in *H. pylori* strains of this sample. The largest proportion of *babA2*-positive strains, 90.4% (47/52), was detected with a set of primers for detecting an amplification product with a size of 271 bps. A PCR product with a size of 832 bps was detected only in 51.9% of cases (27/52). Twenty-one strains of *H. pylori* were *babA2*-negative when using 832 bps set of primers, but *babA2*-positive when using 271 bps set of primers. In one of the 832 bps positive strains of *H. pylori*, a set of primers for the detection of 271 bps gave a negative result. Our studies have demonstrated that neither of the two sets of primers (271 bps, 832 bps) is reliable enough to detect the *babA2* gene in *H. pylori* clinical isolates. The obtained data raise the question of the expediency of using sets of primers amplifying fragments of 271 bps and 832 bps to identify the *babA2* gene in order to assess the virulence of Russian strains of *H. pylori*. The association between the detection of *babA2*+/*cagA*+/*vacA*s1+ strains of *H. pylori* with various clinical manifestations of *H. pylori* infection was not statistically significant. Based on the data obtained during the work on research and development, the following nucleotide sequences of the *babA H. pylori* gene were deposited in GenBank on 22.09.2022:: BankIt2625127 Helicobacter OP499944, BankIt2625185 Helicobacter OP499945, BankIt2625676 Helicobacter OP499946, BankIt2625962 Helicobacter OP499947. The data can be used to study the global diversity and regional characteristics of *H. pylori* strains, for scientific research in the field of epidemiology, gastroenterology, genetic analysis and in the epidemiological surveillance system.

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Articles

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LABORATORY OF MOLECULAR IMMUNOLOGY (RESOURCE SHARING CENTRE)

Head of the Laboratory: Areg Totolian
Researchers: E. Zueva, N. Arsentieva, N. Lyubimova, O. Batsunov, Z. Korobova

The laboratory personnel continues to participate in many research projects.

The project **"Cytokine/chemokine system in bacterial and viral infections: pathogenesis, diagnosis and therapy"** studies and performs a comparative analysis of the content of cytokines and chemokines involved in the antiviral immune response in the peripheral blood of patients with chronic hepatitis B and chronic hepatitis C, including in groups of patients with various stages of liver fibrosis, as well as patients with the novel coronavirus infection. We investigate the mechanisms of chronization and features of the immune response against hepatitis B virus and hepatitis C virus. The research studies 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 the microorganism and the host immune system. According to the results of the study, a method for differentiating the development of the initial forms of fibrosis (F0–1) in patients with HBV or HCV has been developed, which allows separating patients with HBV and HCV based on the determination of three cytokines (CCL2/MCP-1, CCL8/MCP-2, IFNγ) in blood plasma (Fig. 19).

Work is also underway to determine the content of cytokines/chemokines in the blood plasma of patients with autoimmune liver diseases. The results of research on the topic have been presented at various scientific conferences.

Jointly with St. Petersburg State Pediatric Medical University, we are studying the content of chemokines in blood plasma of patients with hepatitis C. Patients were divided into two groups: patients with the presence of concomitant pathology (diseases of the gastrointestinal tract and pancreas, diseases of the cardiovascular system, and endocrine system and metabolism pathology) and patients without concomitant pathology. Concentrations of cytokines/chemokines TNFα, IFNγ, CCL20/MIP-3a, CXCL9/MIG, CXCL10/IP-10, CXCL11/ITAC in blood plasma were determined by multiplex analysis using xMAP technology (Luminex) with commercial test systems Milliplex MAP (Millipore, USA), based on Milliplex Mag magnetic microspheres (USA). The cytokine profile showed high concentrations of chemokines CCL20/MIP-3a and CXCL9/MIG in the group of patients with concomitant pathology compared with patients without it, and the predominance of the concentration of chemokine CCL20/MIP-3a in patients with endocrine pathology, including types 1 and 2 diabetes. The cellular component of both the innate and

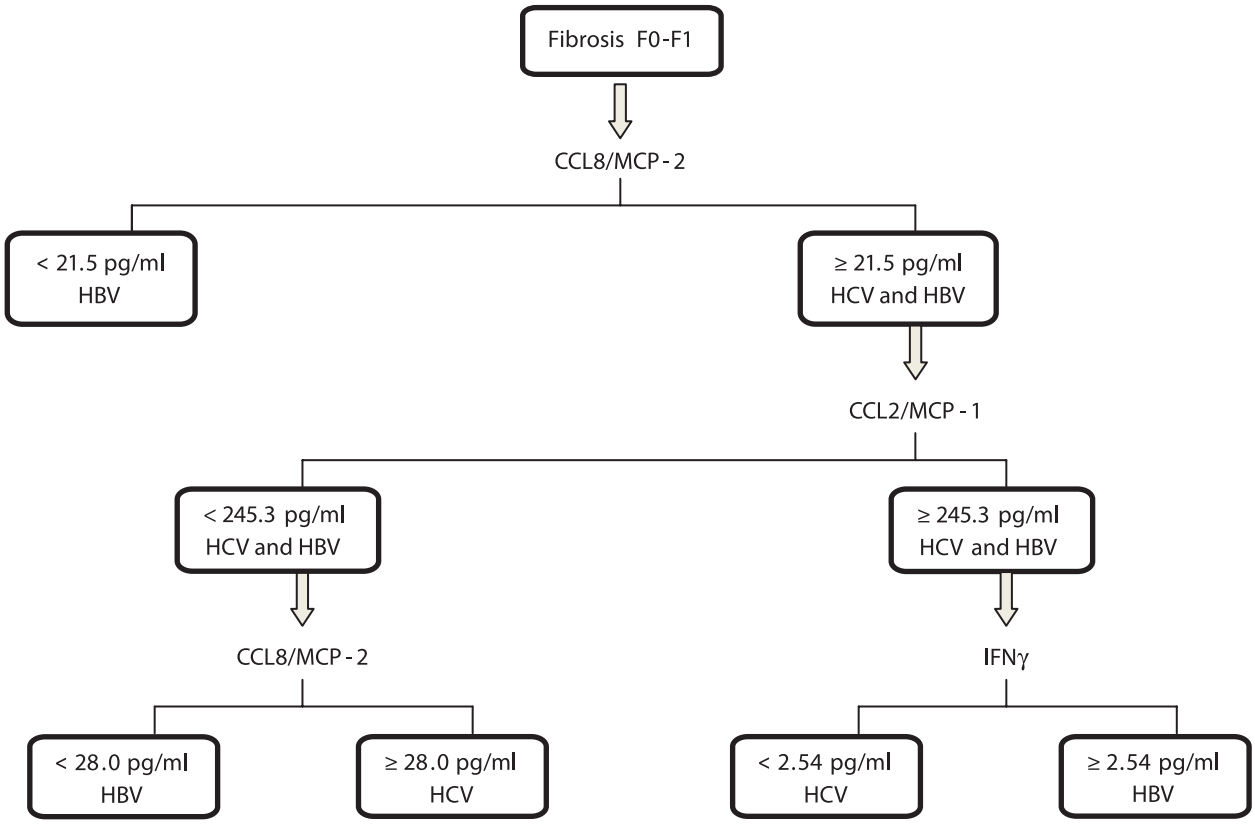

Figure 19. Algorithm for the separation of patients with HCV and HCV at F0-F1 stages of liver fibrosis according to the content of cytokines CCL2/MCP-1, CCL8/MCP-2 and IFNγ

adaptive immune response was most strongly suppressed in the group of patients who had HCV and a comorbidity.

Within the framework of Russian-Guinean research and technical cooperation, the laboratory personnel are investigating the prevalence of serological and molecular biological markers of viral hepatitis (A, B, C, D, E) and HIV among residents of the Republic of Guinea considered healthy for the purposes of the study and in risk groups. The genotypic/subgenotypic structure and mutations of the identified viruses are being analyzed.

Research conducted as part of Russian-Vietnamese cooperation is also primarily devoted to such socially significant diseases as viral hepatitis and HIV. Work continues to assess the prevalence of markers of enteral and parenteral viral hepatitis, and to analyze the genetic structure of hepatitis and HIV viruses in Vietnam.

In addition, we determined the concentrations of chemokines/cytokines in the peripheral blood of COVID-19 current and convalescent patients, as well as in patients with various variants of the disease (Wuhan, Alpha, Delta, Omicron). It was shown for the first time that the convalescence phase after SARS-CoV-2 infection is characterized by a significantly reduced level of cytokines regulating cell differentiation, hematopoiesis, especially the lymphocytic link (T-lymphocytes, NK cells), which is absent in the acute phase of the disease. I.e., the convalescence stage is characterized by a reduced level of most of the pro-inflammatory and anti-inflammatory cytokines studied in blood plasma, while the acute phase is characterized by their significant increase. Based on the data obtained on the levels of IL-6 and IL-18 cytokines in the blood plasma of COVID-19 patients, an algorithm was made for predicting the outcome of an acute stage of the disease. In addition, for the first time, differences in the cytokine profile were detected in patients infected with various variants of SARS-CoV-2 (Wuhan, Alpha, Delta, Omicron) (Fig. 20).

Thus, it is worth noting that hypercytokinemia and cytokine storm are becoming less threatening with the appearance of new mutations in the viral genome. Meanwhile, some cytokines that were studied as potential biomarkers lose their diagnostic value as the virus evolves. The spectrum of potential markers narrows as the virus evolves. However, IL-6, IL-18, IL-10, and IL-27 can still be considered as diagnostic markers of the novel coronavirus infection disease.

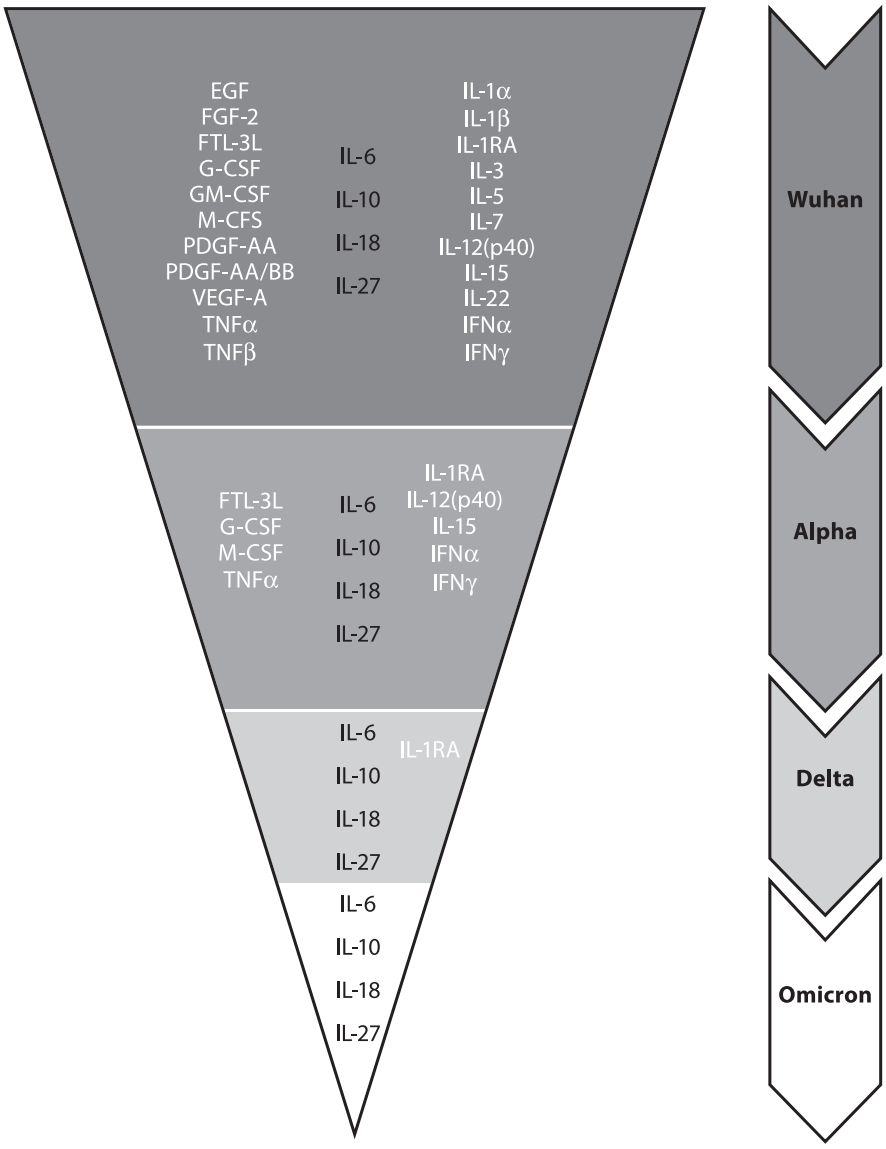

Figure 20. The spectrum of cytokines for which statistically significant ($p < 0.01$) changes were detected compared to the group of healthy donors (n = 51). Each color represents one of four variants of SARS-CoV-2

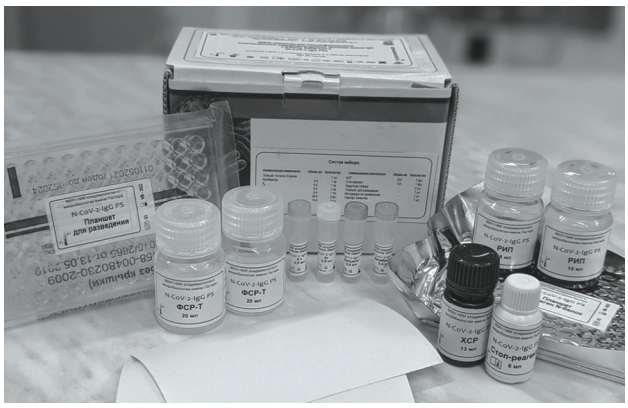

Figure 21. A set of reagents for the enzyme immunoassay quantitative determination of human IgG antibodies to the N-protein of SARS-CoV-2 (N-CoV-2-IgG PS), registration certificate No. P3H 2022/16633 issued on 02.03.2022

The St. Petersburg Pasteur Institute of Epidemiology and Microbiology has developed a set of reagents "N-CoV-2-IgG PS" for the quantitative determination of human IgG to the N protein of SARS-CoV-2 (temporary registration certificate of Roszdravnadzor No. P3H 2021/1485) (Fig. 21).

The N-CoV-2-IgG PS set has the following functional characteristics: its analytical sensitivity, with 1:100 dilution, was 3 BAU/ml, the concentrations of IgG antibodies in three blood serum samples with their proportional dilution with blank samples were linear within the range of 837–17 BAU/ml, the measurement accuracy was 90–110%, and the limit of quantitative determination was 16.0 BAU/ml. When testing the kit, it was demonstrated that there was a good correlation between N-protein-binding antibodies and the comparability of the developed kit with the SARS-CoV-2 virus neutralization test. The verification for test consistency provided evidence of the informativeness and effectiveness of the developed kit, which confirmed its potential for IgG screening in people who have had COVID-19 and for using it in large-scale population studies to assess seroprevalence to COVID-19 in different population groups. The effectiveness of the developed set was 95%, and the positive and negative predictive values were 97% and 87%.

In addition, by using a special technique for enzyme immunoassay in the laboratory, the dynamics of the concentration of IgG subclasses (IgG1, IgG2, IgG3, IgG4) in the blood plasma of patients with the novel coronavirus infection was compared for the first time. Statistically reliable differences were revealed in the concentration of different IgG subclasses between mild and more severe forms of infection. The correlation analysis revealed a positive relationship between the concentrations of IgG subclasses and the severity of the disease. The most noticeable change of concentration in response to COVID-19 was observed for IgG3 (Fig. 22).

In the project "Studying the mechanisms of formation of an immune response to the novel coronavirus infection caused by SARS-CoV-2 in the population of the Northwestern Federal District", seroprevalence has been studied in 26 model territories of the Russian Federation for two years of the spread of the COVID-19 infection according to a unified methodology developed by Rospotrebnadzor with the participation of St. Petersburg Pasteur Institute of Epidemiology and Microbiology (Fig. 23).

The method involved forming a group of volunteers in the model region of the Russian Federation, and their venous blood plasma samples were tested for the presence of antibodies to the nucleocapsid (Nc) of SARS-CoV2 virus by ELISA method. For the first time, the level and structure of population immunity to SARS-CoV-2 was determined among the population of the Russian Federation, including the population of St. Petersburg and Leningrad Region, during the period of intensive spread of COVID-19. Among children and adults, there is a tendency of Nc seropositivity increase in the first stages of seromonitoring, with a decrease in subsequent stages. The proportion of RBD-seropositive persons steadily increased throughout all stages of seromonitoring. Monitoring revealed a statistically significant increase in antibodies to RBD along with a statistically significant decrease in the proportion of Nc-seropositive individuals. This pattern was particularly characteristic of individuals vaccinated with Gam-COVID-Vac. Prior to the use of specific vaccines, seroprevalence of antibodies against Nc was observed. After the introduction of the Gam-COVID-Vac vaccine in adults, a decrease in the level of anti-Nc antibodies was noted due to an increase in the proportion of RBD-seropositive individuals. It was demonstrated that children living in the Russian Federation have a statistically significant predominance of antibodies to RBD over antibodies to Nc, so that antibodies to RBD provide the main contribution to the level of humoral immunity to SARS-CoV-2. The presented results confirm the important role of vaccination in herd immunity development.

We conduct studies of population immunity to COVID-19 in the conditions of the pandemic in the population of the Republic of Kazakhstan, Turkmenistan, the Kyrgyz Republic, the Republic of Uzbekistan, and the Republic of Belarus (Fig. 24).

Together with the Institute of Experimental Medicine, we studied a subpopulation of memory B cells and T cells by flow cytometry in patients with COVID-19 and COVID-19 convalescents. In patients with the acute phase of COVID-19, there was a decrease in the level of memory B-cell subpopulations and an increase in the proportion of plasma cell precursors compared with recovering patients. Nevertheless, the level of circulating plasmoblasts/plasma cells was increased. In addition, patients with COVID-19 had a higher percentage of activated B cell subpopulations — circulating plasmoblasts and transitional B cells — compared to healthy donors. During the acute phase of COVID-19, the number of Tfh1-like cells (CXCR3+CCR6–) decreased, and the number of Tfh17-like cells (CXCR3–CCR6+) increased compared to the cells of control group and recovering patients (Fig. 25). Both groups of patients had an aberrant distribution of circulating subsets of Tfh memory cells, which may be associated with an abnormal distribution of B cells in peripheral blood.

In addition, the laboratory continues to study the states of immunity in patients with primary and secondary immunodeficiency. A highly sensitive method has been developed for laboratory assessment of the state of immunity of patients using real-time PCR based on the determination of the concentration of TREC and KREC excision rings in children and adults. Reference intervals were determined for TREC and KREC content in peripheral blood of St. Petersburg residents. For this purpose, whole blood samples obtained from 717 volunteers considered healthy

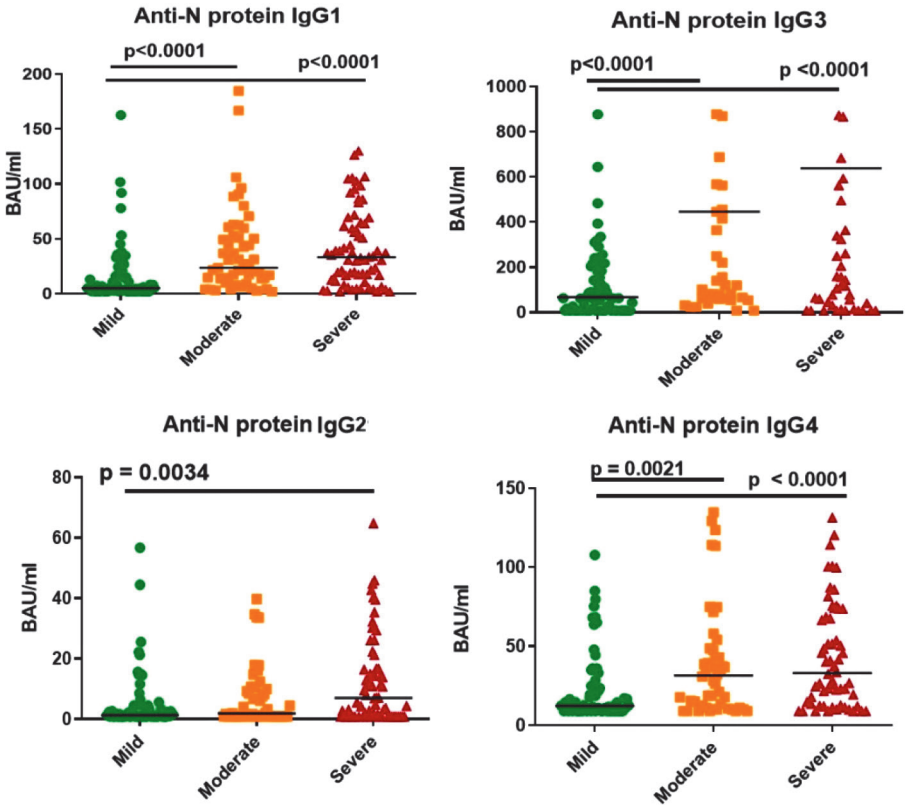

Figure 22. Differences in the concentrations of subclasses of specific IgG (IgG1, IgG2, IgG3, IgG4) to N-protein SARS-CoV-2 depending on the severity of the disease

for the purposes of the study aged 0 to 108 years were used as part of the program for assessing the population immunity of residents of St. Petersburg. The quantitative content of the target DNA fragments, TREC and KREC, was determined with a set of reagents for the quantitative determination of excision circles TREC and KREC by real-time PCR. Reference intervals were found by a direct method according to the recommendations of the International Federation of Clinical Chemistry and the State Standard (GOST) P 53022.3-2008. The volunteers in the study were divided into six age groups: 18–29 y.o., 30–39 y.o., 40–49 y.o., 50–59 y.o., 60–69 y.o. and over 70 y.o. Correlation analysis allowed us to establish a negative dependence of the concentration of TREC molecules in blood samples with

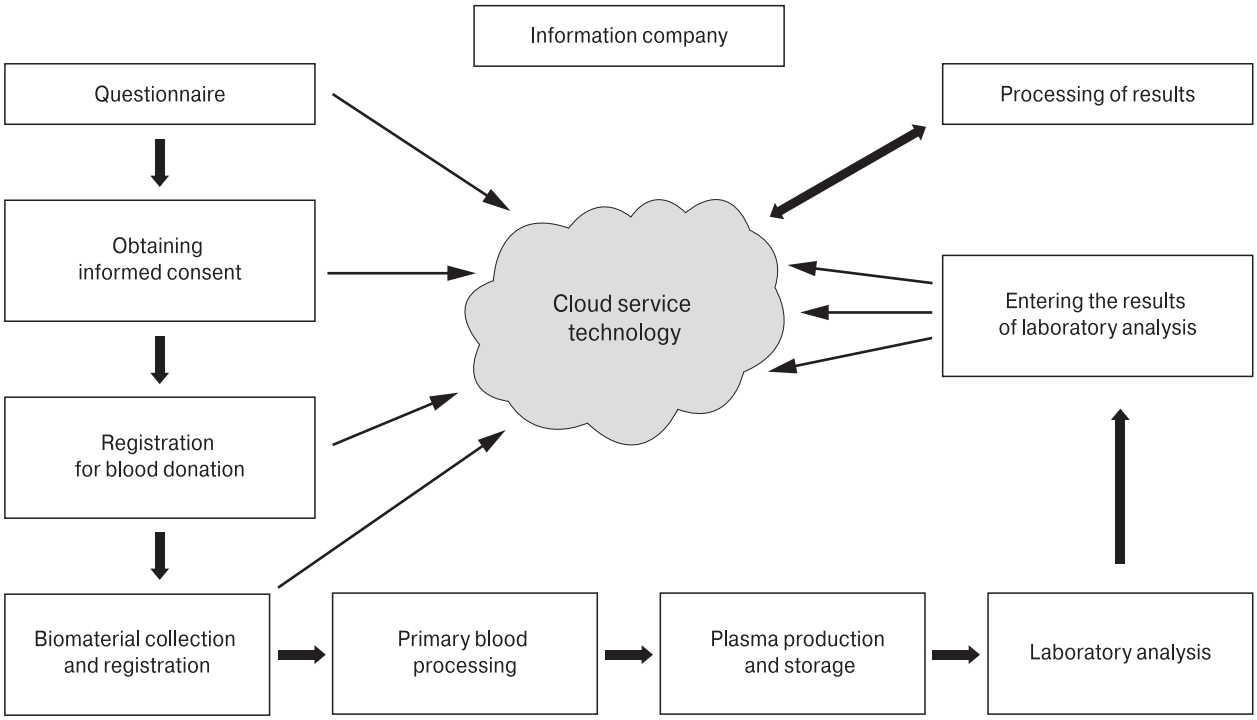

Figure 23. An algorithm for implementing the stages of population immunity assessing using a cloud service

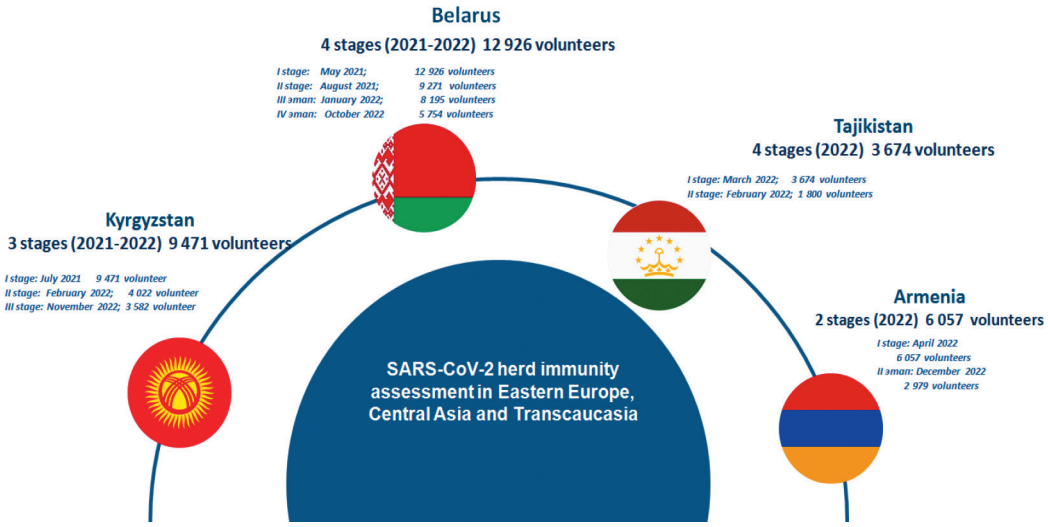

Figure 24. Brief description of the study of herd immunity in Eastern Europe, Central Asia and Transcaucasia

the age of the study participants. Significant differences in TREC levels were revealed between different age groups. No correlation was established between the content of KREC molecules in blood samples and age, and no differences between age groups. Reference intervals of the TREC level were determined for each selected age group. A single reference interval of KREC molecule levels was established for all groups. The obtained results, which made it possible to establish reference intervals of TREC and KREC levels for adults, will contribute to effective personalized laboratory diagnostics of immunodeficiency conditions of various genesis.

The laboratory continues to study the genetic nature of primary immunodeficiency. There is an obvious need to identify candidate genes that can potentially lead to the development of a certain type of primary immunodeficiency. Hereditary angioedema (HAE) is a rare genetic disorder characterized by recurrent swelling of soft and submucosal tissue that poses a threat to the patient's life. Bioinformatic analysis of candidate genes for the development/pathogenesis of HAE was carried out. The basis for the analysis was a group of genes with mutations reliably associated with HAE: SERPING1, F12, PLG, ANGPT1, KNG1, MYOF, HS3ST6. To build genetic and protein-protein

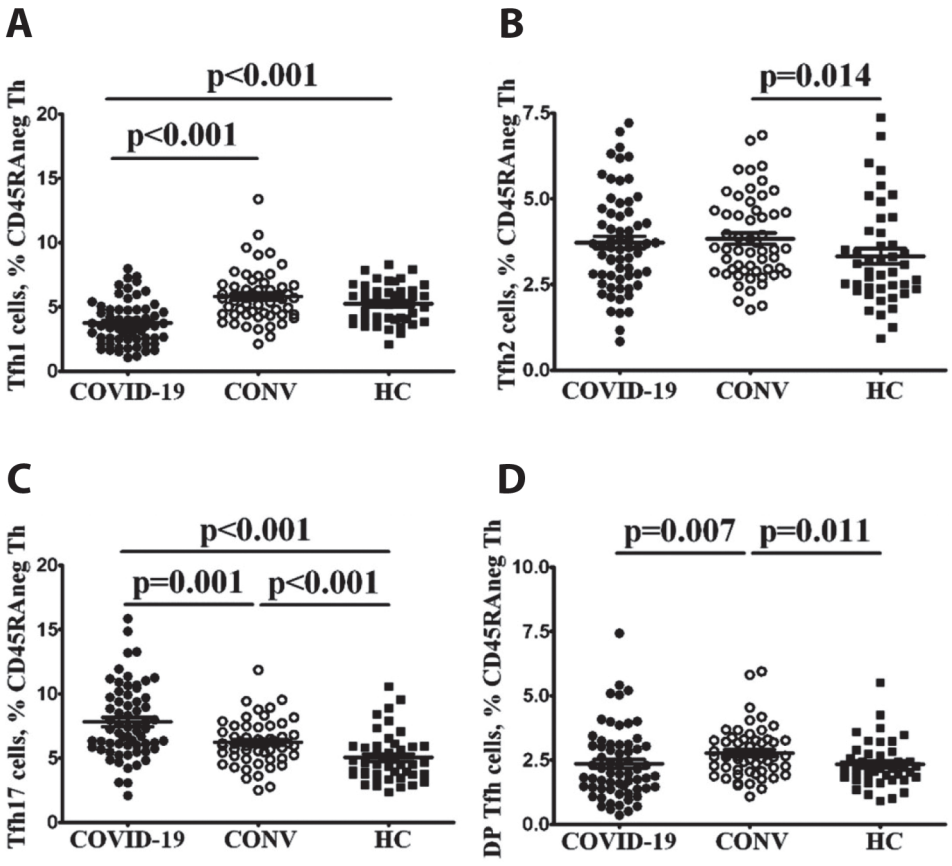

Figure 25. Imbalance in the Tfh cell system in patients with acute coronavirus infection, in convalescents and in healthy donors, depending on the cell subpopulation (Tfh1, Thf2, Thf17, twice positive Tfh)

networks and identify the biological context of selected candidate genes, a number of web resources were used: HumanNetv3, GeneMania, and FUMA GWAS in GENE2FUNC mode. Data on localization of a particular protein in cellular components were obtained, from extracellular location (HSPG2, SDC3, F2, SERPING1, KRT1, GPC5, GPC6, THBS1, IGFALS, SERPINF2, GP1BA, APOH, LAMA3, ELANE, GPC1, CLEC3B, HRG, GPC2, PCOLCE, SERPINE1, SDC2, MATN2, GPC4, GPC3) to the location in specific cell granules (PLAU, ELANE, PLAUR, CD93). Proteins widely represented in most cellular components are SERPING1, KNG1, PLG, F12, and their mutations are associated with the most common types of HAE. For the first time, one hundred potential candidate genes have been identified, mutations in which may be associated with HAE. The biological context of the identified genes has been determined. The data of the biological context, genetic and protein-protein interactions made it possible to exclude a number of genes from the list of the most likely participants in the pathogenesis and classify the remaining ones into groups with greater or lesser involvement potential.

The Cytometry and Biomarkers Resource Sharing Center, based at the Laboratory of Molecular Immunology, carries out research cooperation with the following organizations of St. Petersburg:

- Institute of Experimental Medicine (Department of Immunology);
- St. Petersburg State Pediatric Medical University (Department of Infectious Diseases of Adults and Epidemiology);
- Pavlov First Saint Petersburg State Medical University (Departments of Infectious Diseases, Pulmonology, Otolaryngology, Center for Molecular Medicine) (Pavlov University);
- Military Medical Academy named after S.M. Kirov (Department of Infectious Diseases).

Two thesis works for the degree of Candidate of Sciences (Biology) have been planned and are being carried out in the laboratory.

The laboratory personnel takes an active part in presenting the scientific findings at leading Russian and international conferences.

Publications

Articles

1. *Arsentieva N., Liubimova N., Batsunov O., Korobova Z., Kuznetsova R., Rubinstein A., Stanevich O., Lebedeva A., Vorobyov E., Vorobyova S., Kulikov A., Gavrilova E., Pevtcov D., Polushin Yu., Shlyk I., Totolian A. Predictive value of specific cytokines for lethal COVID-19 outcome // Russian Journal of Infection and Immunity. 2022; 12 (5): 859–868. doi: 10.15789/2220-7619-PVO-2043*
2. *Arsentieva N.A., Liubimova N.E., Batsunov O.K., Korobova Z.R., Stanevich O.V., Lebedeva A.A., Vorobyov E.A., Vorobyova S.V., Kulikov A.N., Lioznov D.A., Sharapova M.A., Pevtcov D.E., Totolian A.A. Plasma cytokines in patients with COVID-19 during acute phase of the disease and following complete recovery // Medical Immunology (Russia). 2021; 23 (2): 311–326. (In Russ.) doi: 10.15789/1563-0625-PCI-2312*
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6. *Korobova Z.R., Arsentieva N.A., Liubimova N.E., Dedkov V.G., Gladkikh A.S., Sharova A.A., Chernykh E.I., Kashchenko V.A., Ratnikov V.A., Gorelov V.P., Stanevich O.V., Kulikov A.N., Pevtsov D.E., Totolian A.A. A Comparative Study of the Plasma Chemokine Profile in COVID-19 Patients Infected with Different SARS-CoV-2 Variants // Int. J. Mol. Sci. 2022; 23 (16): 9058. doi: 10.3390/ijms23169058*
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Patents

1. *Arsentieva N.A., Lyubimova N.E., Batsunov O.K., Korobova Z.R., Kuznetsova R.N., Rubinstein A.A., Stanevich O.V., Lebedeva A.A., Vorobyev E.A., Vorobyeva S.V., Kulikov A.N., Gavrilova E.G., Polushin Y.S., Shlyk I.V., Totolian A. A. Method of predicting the outcome of acute illness caused by a new coronavirus infection COVID-19. Application: 2021122630, 07/28/2021. Patent No. 2766347. Patentee: Saint-Petersburg Pasteur Institute. The date of the start of the patent validity period: 8.07.2021. Registration date: 03/15/2022. Published: 03/15/2022*
2. *Batsunov O.K., Semenov A.V., Arsentieva N.A., Lyubimova N.E., Ostankova Yu.V., Esaulenko E.V., Totolian A.A. Method of differentiation of the causes of liver fibrosis of the initial level in chronic viral Hepatitis B and C. Application No. 2019141709/10(081287). Patentee: Saint-Petersburg Pasteur Institute. The application submission date is 12.12.2019. The decision to grant a patent 15.07.2021*
3. *Ostankova Yu.V., Saitgalina M.A., Lyubimova N.E., Totolian A.A. A method of laboratory personalized diagnostics of the state of immunity of patients and a set of oligodeoxyribonucleotide primers and fluorescently labeled probes, and reference samples. Patentee: Saint-Petersburg Pasteur Institute. Application: 2022107106. Issued on 1.11.22.*

Department of Epidemiology

LABORATORY OF EPIDEMIOLOGY OF INFECTIOUS AND NON-INFECTIOUS DISEASES

Head of the Laboratory: Liudmila Lyalina
Researchers: V. Zagouzov, E. Gorziy, V. Vetrov, S. Golubkova

In 2021–2022, the Laboratory of Epidemiology of Infectious and Non-Communicable Diseases participated in research in the following areas:

- Molecular genetics characteristics and prevalence of human papillomavirus of high carcinogenic risk in the context of HPV vaccination
- COVID-19 epidemiology and preventive vaccination in the regions of the North-West of Russia
- Implementation of the measles and rubella elimination program and reduction of incidence of epidemic parotitis to sporadic in the territories of the North-West of Russia
- Polio prevention measures and organization of epidemiological surveillance of (non-polio) enterovirus infection in 14 territories of Russia supervised by the St. Petersburg Regional Center
- Implementation of the acute viral hepatitis B elimination program in the NWFD (NWFD) of Russia

Papillomavirus infection

Papillomavirus infection is one of the urgent, socially significant problems in the world and the Russian Federation. The peculiarity of this infection is a large number of human papillomavirus (HPV) genotypes and the presence of carcinogenic potential in a number of genotypes, wide distribution, a variety of sources and transmission routes of the pathogen, the predominance of asymptomatic forms and polymorphism of clinical manifestations, including the occurrence of malignant neoplasms of a certain localization. In 2020, the World Health Organization (WHO) adopted the global strategy to eliminate cervical cancer as a public health problem based on a strategy that included having 90% of girls fully vaccinated by the age of 15 years.

On September 18, 2020, the Russian Federation approved a Strategy for the development of immunoprophylaxis of infectious diseases for the period up to 2035, which provides for the improvement of the National Vaccination Calendar, which is to include a vaccine for the prevention of papillomavirus infection. In this regard, the study of the prevalence of various HPV genotypes and an in-depth study of the genetic heterogeneity of the population of relevant genotypes in the context of the existing vaccination coverage indicators is important for the development of HPV screening, monitoring, epidemiological surveillance, and vaccination against diseases associated with the human papillomavirus.

The results of epidemiological monitoring of the prevalence of high risk HPV genotypes among gynecological, urological, dermatovenerological and oncological patients in two regions of the NWFD in 2021–2022 showed the high relevance of the problem. The study included male and female patients who applied to medical organizations in St. Petersburg (medical center of a research organization — 606 people, dermatovenerologic center — 695 people, family planning and reproduction center — 2296 people, city polyclinic No. 107 — 14 451 people, city clinical oncology center — 80 people) and Kaliningrad Region (Center for specialized types of medical care: 238 people). The material for the study was taken with a cytoprep brush from the cervical canal, urethra, and tumor tissue. We studied the results of cytological tests of 158 patients with a positive result for high risk HPV.

Evaluation of the results of the study in 2021–2022 showed a high intensity of the latently developing epidemic process of papillomavirus infection.The frequency of detection of high risk HPV among the examined patients of gynecological, urological and dermatovenerological departments in St. Petersburg was 16.1% and 18.5% at the medical center of the Research Institute (MC RI), 16.7% and 19.3% at the Center for Family Planning and Reproduction (CFPR), 18.9% and 19.9% in the Dermatovenerologic center (DVC) No. 8, and 21.8% and 23.6% in the city polyclinic (CP) No. 107 (Fig. 26). In most cases, the differences in the prevalence of high risk HPV are not statistically significant ($p > 0.05$). The exception was the results of the examination of patients in CP No. 107, where the frequency of HPV detection was significantly higher compared to the Medical Center of the Research Institute and the Center for Family Planning and Reproduction. None of the patients included in the study were vaccinated against papillomavirus infection.

A comparative assessment of the prevalence of HPV of high carcinogenic risk and confidence intervals (CI) indicators among males and females in St. Petersburg did not reveal statistically reliable differences ($p > 0.05$). As an example, Table 17 shows the results of patients testing at the Family Planning and Reproduction Center. Among women and men examined at the MC of the Research Institute in 2022, high-risk HPV was detected in 13.2% and 20.4% ($p > 0.05$), in CP No. 107, the detection rate of high risk HPV was also approximately the same: 23.6% and 19.6%, the differences not being statistically significant ($p > 0.05$). The highest percentage of HPV detection in women was in the age group of 20 to 29, with HPV detected in 30% of the persons. HPV 16 (24.8%) and HPV 31 (12.4%) genotypes were the most common. In the examined female and male patients of Dermatovenerologic center No. 8, the prevalence rates of high-risk HPV were 20.2% and 19.5%, respectively ($p > 0.05$).

In the Kaliningrad Region, the prevalence of high risk HPV was significantly higher and reached 32.8% in general, 22.97% among men, and 32.2% among women in the routine testing system. It should be noted that the results of the study of the incidence of cervical cancer in 2011–2021 showed that the incidence rates in the Kaliningrad Region were also significantly higher compared to the levels in St. Petersburg during the entire follow-up period,

and among women aged 30–39 years, an increase of incidence was observed, which is consistent with higher HPV prevalence rates in the age group of 18–29 years: 38.5%. The detection rate of HPV among women of the specified age in St. Petersburg in 2022 was 20.4%.

The analyzis of the results of cytological examination (by liquid cytology) in St. Petersburg in 2022 shows that various types of the pathology were detected in 6.6% of cases. Two patients (1.3%) were diagnosed with cervical cancer, one of them under the age of 40. Atypical squamous epithelial cells, indicating intraepithelial neoplasia of varying severity, were found in 43.5% of cases. The average age of patients with high-grade intraepithelial lesions of the squamous epithelium in 50% of cases was under 40 years.

The study of the prevalence of specific genotypes of the high-risk HPV by the example of patients of the Family Planning and Reproduction Center in 2022 showed that the most prevalent is HPV genotype 16 (21.1%, 95% CI: 15.88–27.12), while closely related genotypes determined with the test systems used (31, 33, 35, 52, 58) accounted for 39.9%. Genotype 18 was detected much less frequently, in 6.4% of cases (95% CI: 3.56–10.54), the differences with the detection frequency of genotype 16 being statistically reliable ($p < 0.05$).

The results of the examination of 80 patients of the St. Petersburg City Clinical Oncology Center showed that the detection rate of high risk HPV in patients with cancer varied depending on the localization of the neoplasm and amounted to 100% for anal cancer, 94.6% for cancer of female genital organs, 76.2% for cancer of head and neck. In patients with severe cervical dysplasia, High-Risk HPV was detected in 83.3% of cases (Fig. 27).

HPV genotype 16 was detected in all patients with HPV-associated malignancies, mixed infection was diagnosed in 14.1% of cases, with virus genotypes 18, 31, 35, 39 and 45 in various combinations detected simultaneously with HPV 16. None of the patients were vaccinated against HPV. As a result of studying the physical status of the virus DNA in the samples of malignant growth tissues, the episomal form was not detected, the partially integrated ("mixed") form of the virus DNA was found in 86.4%, the integrated form, in 13.4% of cases.

The results of the study of the prevalence of High-Risk HPV in the framework of the first organized epidemiological monitoring system allowed us to obtain new data on the frequency of detection and circulating genotypes of the virus in conditions of low vaccination coverage against papillomavirus infection. The results obtained characterize the high intensity of the latently developing epidemic process of papillomavirus infection and the polymorphism of clinical manifestations of the disease. The data on approximately the same prevalence of high-risk human papillomavirus among men and women indicate the feasibility of a gender-neutral vaccination strategy against this infection.

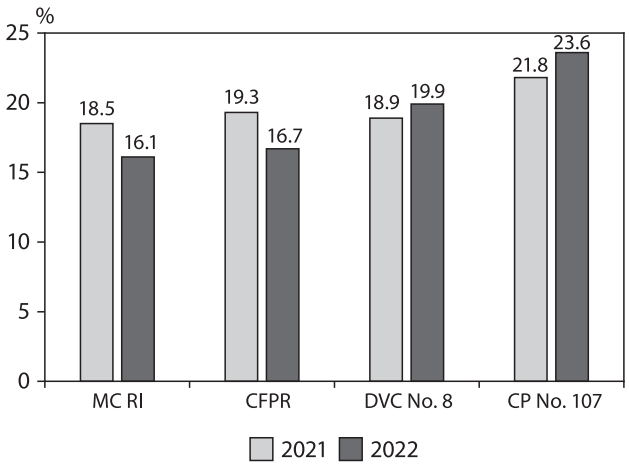

Figure 26. Prevalence of human papillomavirus of high carcinogenic risk in St. Petersburg in 2021–2022

Table 17. Prevalence of High-Risk HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 genotypes among patients of the Center for Family Planning and Reproduction in St. Petersburg, 2021–2022

Year	Sex	Number of tested individuals	Of these, HPV positive	% (95% CI)
2021	Women	932	178	19.1 (16.6–21.8)
	Men	39	9	23.1 (11.1–39.3)
	Total	971	187	19.3 (16.8–21.9)
2022	Women	1250	209	16.7 (14.7–18.9)
	Men	75	12	16.0 (8.6–23.6)
	Total	1325	221	16.7 (14.7–18.8)

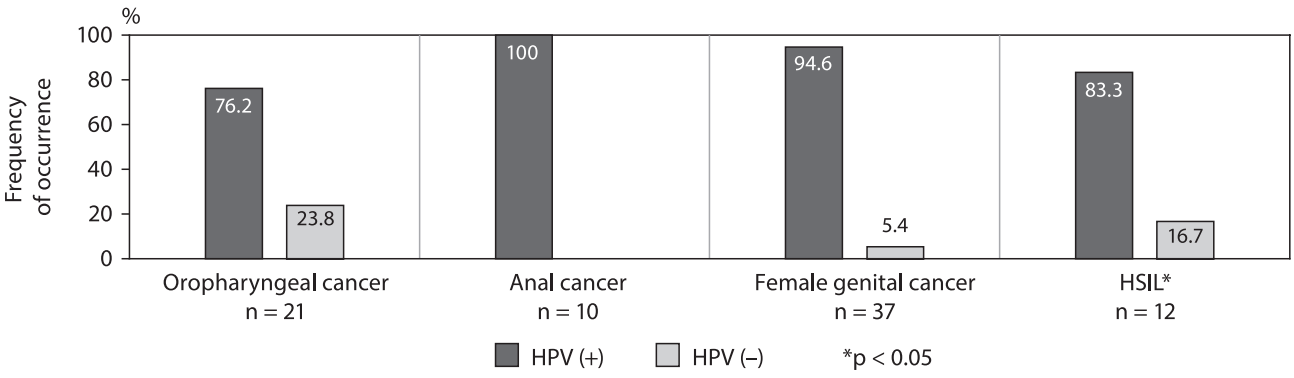

Figure 27. HPV detection in tissue samples of malignant neoplasms of various localizations and severe cervical dysplasia in St. Petersburg, 2022

The results of studying the physical status of HPV DNA in patients diagnosed with HPV-associated malignant neoplasms of various localization showed the degree of integration of the virus into the genome of human cells; these data can be used to predict the severity of the clinical course of the disease, determine treatment tactics and risk groups for vaccination against papillomavirus infection according to epidemic indications.

Coronavirus infection

At the end of 2019, a new type of coronavirus was identified, which was named SARS-CoV-2 by the International Committee on Taxonomy of Viruses. It is an RNA-containing virus of the *Coronaviridae* family, Beta-CoV B lineage. The virus is assigned to group II of pathogenicity, as are some other representatives of this family (SARS-CoV virus, MERS-CoV). The genetic sequence of SARS-CoV-2 is at least 79% similar to the sequence of SARS-CoV.

Studies on SARS-CoV-2 have shown that this virus is not only capable of causing severe forms of infection in certain groups of people, often leading to fatal outcomes, but spreads fast, which was the reason for the WHO declaration of a public health emergency and a pandemic.

Due to the rapid variability of the causative agent of the novel coronavirus infection (COVID-19), the assessment of the dynamics of the spread of known and new strains of the virus is an extremely important element of the monitoring system, and to achieve this goal, specialists of research organizations use bioinformatic data analysis tools to identify epidemiologically significant genovariants and their sublines.

The first case of the novel coronavirus infection caused by SARS-CoV-2 in the Russian Federation was registered on March 1, 2020. The results of the analysis of COVID-19 incidence in the territories of the NWFD in 2021–2022 showed that the highest levels were observed in the metropolis, in St. Petersburg (Fig. 28).

In 2022, an increase in the incidence of COVID-19 compared to 2021 was noted in 10 regions of the NWFD. The incidence rate per 100 000 population was the following: St. Petersburg: 18 120.64 (95% CI: 18 088.1–18 153.2), Arkhangelsk Region: 13 190.97 (95% CI: 13 128.6–13 253.6), Republic of Karelia: 11 895.82 (95% CI: 11 814.6–11 977.4), Komi Republic: 11 712.78 (95% CI: 11 643.0–11 782.9), Kaliningrad Region: 10 909.32 (95% CI: 10 848.8–10 970.0), Nenets Autonomous Okrug: 10 908.11 (95% CI: 10 619.5–11 201.8), Vologda Region: 10 130.91 (95% CI: 10 075.8–10 186.2), Pskov Region: 8812.11 (95% CI: 8741.7–8882.9), Novgorod Region — 8671.62 (95% CI: 8600.1–8743.6), Leningrad Region: 7340.37 (95% CI: 7303.3–7377.6), Murmansk Region: 7337.38 (95% CI: 7277.8–7397.3).

The results of the analysis of COVID-19 mortality showed that during the three years of the epidemic (2020–2022), 59 223 deaths from this disease were registered in the NWFD. There was a statistically reliable decrease in mortality in 2022 compared with 2021 in all territories of the NWFD (Fig. 29). In 2022, the mortality rate from COVID-19 in the was 0.75% (95% CI: 0.74–0.76). In the regions of the NWFD, mortality rates are the following: Pskov Region: 1.28% (95% CI: 1.19–1.38), Murmansk Region: 1.16% (95% CI: 1.08–1.26), St. Petersburg: 0.88% (95% CI: 0.86–0.89), Vologda Region: 0.80% (95% CI: 0.75–0.85), Kaliningrad Region: 0.71% (95% CI: 0.66–0.76), Komi Republic: 0.65% (95% CI: 0.60–0.71), Novgorod Region: 0.62% (95% CI: 0.55–0.69), Republic of Karelia: 0.46% (95% CI: 0.41–0.51), Leningrad Region: 0.36% (95% CI: 0.33–0.40), Nenets Autonomous Okrug: 0.33% (95% CI: 0.21–0.50), Arkhangelsk Region: 0.20% (95% CI: 0.17–0.22).

The decrease in mortality may be due to a change in the circulating genetic variant of the virus. The Delta strain was first detected in the NWFD in May 2021. 194 of the positive samples examined in the Kaliningrad Region in 2021 had mutations (186 Delta, 6 Alpha, 1 northwestern, and 1 Beta). In 2021, this gene variant was dominant, whereas in 2022 its share was only 1.0%.

At the beginning of 2022, the Omicron strain was discovered in the NWFD, which subsequently took a leading place in the structure of circulating variants of the virus. In the Kaliningrad Region, among 488 positive samples with mutations studied in 2022, Omicron strain was detected in 483, and Delta in 5 samples. From January to June 2022, BA.1 and BA.2 sublines were identified, which were subsequently superseded by variants BA.4 and BA.5. The comparison of the

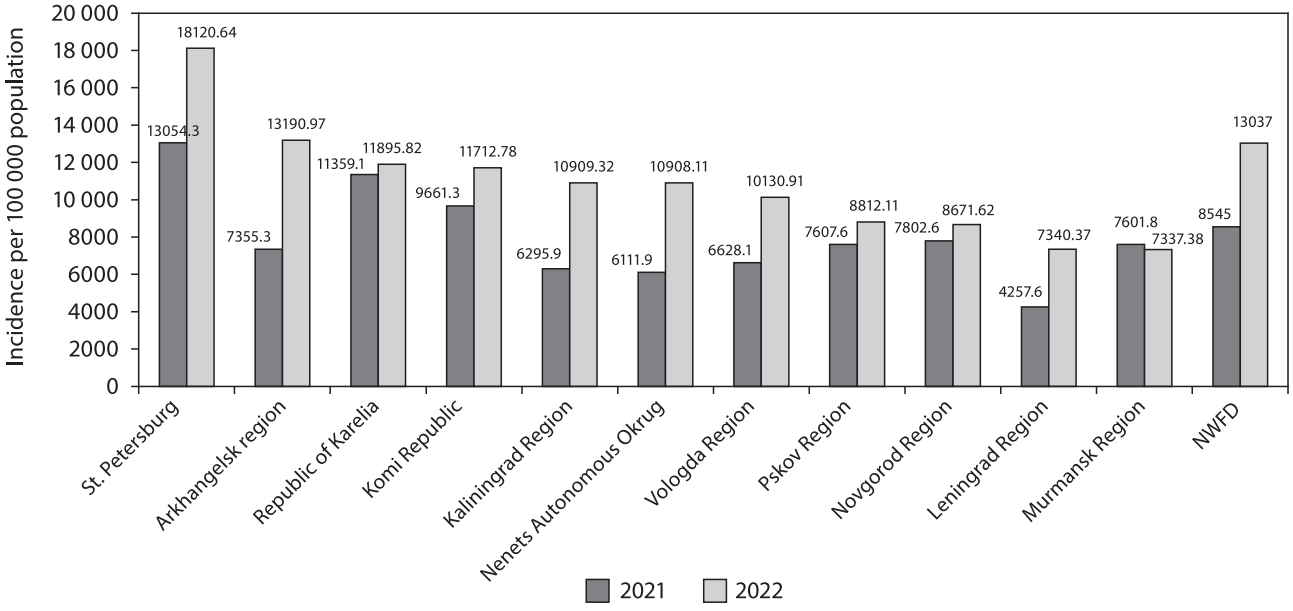

Figure 28. Incidence of COVID-19 in the territories of the NWFD in 2021–2022

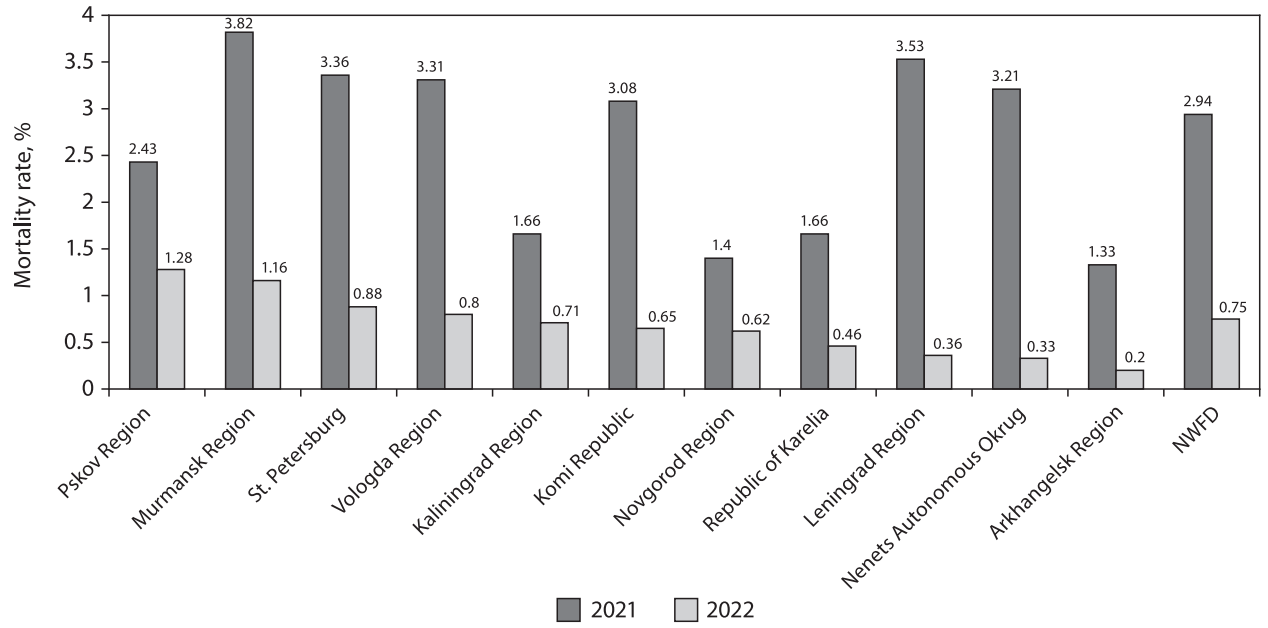

Figure 29. Mortality from COVID-19 in the territories of the NWFD in 2021–2022

monthly incidence rate with the monthly structure of circulating variants in the Kaliningrad Region shows the coincidence of the periods of rising incidence with the periods of the emergence of new circulating strains of SARS-CoV-2.

Vaccination, being an important tool for managing the COVID-19 epidemic process, started in Russia in December 2021. Evaluation of the effectiveness of vaccination as a means of specific prevention of this infection is of great scientific and practical importance. Our study assessed the epidemiological effectiveness of vaccination against COVID-19 in three regions of the NWFD of Russia.

In 2022, in most territories of the NWFD, the vaccination coverage reached 80% or more. In 2021 and 2022, the incidence among vaccinated and unvaccinated patients in the Leningrad Region was 1008.3 (95% CI: 991.6–1025) and 26 510.6 (95% CI: 26381.9–26639.3), in Pskov Region: 5486.0 (95% CI: 5421.8–5550.3) and 54 656.0 (95% CI: 54 366.0–54 946.0), in Kaliningrad Region: 6266.3 (95% CI: 6207.5–6325) and 84 335.0 (95% CI: 84 161.2–84 508.7) per 100 000 people, respectively, the differences being statistically reliable (Fig. 30).

The mortality rates from COVID-19 among vaccinated and unvaccinated persons in 2021–2022 also differed significantly (Fig. 31): 1.06% (95% CI: 0.87–1.25) and 1.91% (95% CI: 1.83–1.98) in the Leningrad Region, 0.16% (95% CI: 0.12–0.2) and 1.31% (95% CI: 1.25–1.38) in the Kaliningrad Region, 0.63 (95% CI: 0.55–0.71) and 2.02 (95% CI: 1.92–2.12) in the Pskov Region.

In 2022, compared with 2021, a decrease in the proportion of pneumonia was noted in the Leningrad Region from 6.5% (95% CI: 6.03–6.97) to 2.0% (95% CI: 1.91–2.09), in the Kaliningrad Region from 9.2% (95% CI: 8.97–9.43) to 1.0% (95% CI: 0.94–1.06), in the Pskov Region from 18.1% (95% CI: 17.75–18.45) to 3.5% (95% CI: 3.34–3.66). Among the total number of cases of pneumonia associated with COVID-19 in the Kaliningrad Region, the proportion of vaccinated persons was 5.7% (95% CI: 5.15–6.25), and of unvaccinated, 94.3% (95% CI: 93.75–94.85).

As a result of the research, the regional features of the COVID-19 epidemic in the territories of the NWFD of Russia were determined, and the high effectiveness of vaccination against the infection was confirmed.

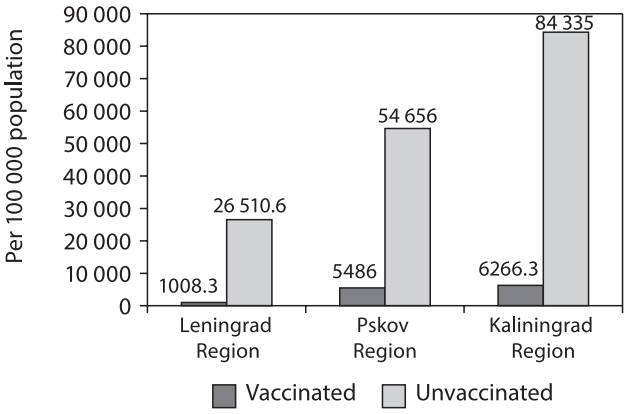

Figure 30. Incidence of COVID-19 among vaccinated and unvaccinated persons in the Leningrad, Pskov and Kaliningrad regions in 2021–2022

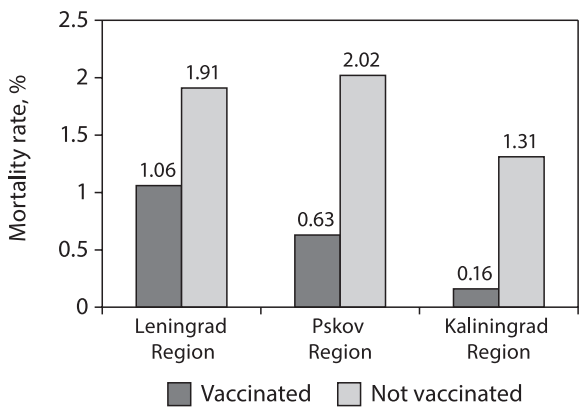

Figure 31. COVID-19 mortality among vaccinated and unvaccinated persons in the Leningrad, Pskov and Kaliningrad regions in 2021–2022

Implementation of the Measles and Rubella Elimination Program, achievement of sporadic incidence of epidemic parotitis in the NWFD

In 2021–2022, work continued on the measles and rubella elimination program in the Russian Federation, and in 2021, epidemic parotitis was included in the program. Research and practical work is carried out as part of the activities of St. Petersburg Regional Center for the Supervision of Measles, Rubella and Epidemic Parotitis, which operates at St. Petersburg Pasteur Institute. The Center oversees 11 territories of the NWFD of Russia with a population of 13.5 million people. The responsibilities of the research staff of the Laboratory of Epidemiology of Infectious and Non-Communicable Diseases include:

- epidemiological monitoring of the incidence of measles, rubella and epidemic parotitis, preparation of monthly reports on the registration of these infections in the regions of the district, and sending the reports to the National Research and Expertise Center for the Supervision of Measles, Rubella and Epidemic Parotitis (Moscow);
- visits to supervised territories to provide advisory and methodological assistance and monitor the implementation of the Program for the elimination of measles, rubella and the achievement of sporadic morbidity of epidemic parotitis; in 2021, there were no visits to supervised territories due to the COVID-19 epidemic, in June 2022, an inspection of Rospotrebnadzor institutions of the Novgorod Region was held in connection with epidemiological surveillance and prevention of measles, rubella and epidemic parotitis;
- participation in the preparation of regulatory and methodological documents; methodological recommendations "Organization of internal quality control in laboratories performing serological tests for measles" were prepared in cooperation with the specialists of G.N. Gabrichevsky Moscow Research Institute for Epidemiology & Microbiology (approved by the Federal State Agency for Health and Consumer Rights in 2022);
- entering information from the epidemiological survey data of measles and rubella foci into the Centralized Information System for Infectious Diseases (CISID);
- analysis of the incidence of measles, rubella and epidemic parotitis in the territories of the NWFD of Russia;
- preparation of reports on the results of epidemiological surveillance of measles, rubella and epidemic parotitis in the supervised territories at annual meetings of specialists of the Russian Federation and CIS countries;
- preparation of annual reports on the implementation of the measles and rubella elimination program in the territories of the NWFD of Russia.

In 2021, no cases of measles and rubella were registered in the NWFD (Fig. 32). In 2022, 7 cases of measles were reported in two regions. The measles incidence rate in the NWFD was 0.04 per 100 000 population. The largest number of patients was registered in the Leningrad Region: 6 cases (0.3 per 100 000).

All measles cases were imported from other countries or other regions of Russia. Measles virus of D8 MeaNS 8248 genotype was detected in all patients. This type of virus was imported to Russia from Tajikistan.

The majority of measles patients registered in the NWFD of Russia in 2019–2022 were adults aged 18 years and older (54%). Children and adolescents accounted for 46% of cases (Fig. 33).

The majority of cases were in patients not vaccinated against measles (70.59%), as well as in patients with unknown vaccination status (22.06%). The share of persons revaccinated against measles was 7.35% (Fig. 34).

The incidence of rubella in the territories of the NWFD of Russia since 2014 is less than 1.0 per 1 million population, with the exception of 2019. (Fig. 35). In 2021–2022, no cases of rubella were detected in the territories of the NWFD. Monitoring is performed in respect of rubella in pregnant women and congenital rubella syndrome (CRS). No cases of CRS have been identified in the NWFD since 2008. The level of vaccination coverage against measles and rubella of target population groups in 2022 was 95.0% and higher; only in 2 territories it was below the established level in age groups of 1 year and 6 years. Serological monitoring of immunity to rubella virus shows better results than in case of measles virus. In all regions of the NWFD and all age groups, the proportion of individuals who were seronegative to the rubella virus in 2021–2022 was below 7.0%.

Figure 36 shows the incidence of epidemic parotitis in the NWFD of Russia. In 2021–2022, cases of the disease were registered only in St. Petersburg. Nine cases of epidemic parotitis were detected in 2021 (0.16 per 100 000 population), and 11 cases in 2022 (0.2 per 100 000 population). In 2014–2022, not a single case of epidemic parotitis was registered in the Komi Republic, the Nenets Autonomous Okrug, the Arkhangelsk, Vologda, Kaliningrad, and Pskov Regions.

Epidemiology surveillance over polio and enterovirus (non-polio) infection

In accordance with the action plan to maintain the polio-free status of the Russian Federation, The St. Petersburg Regional Center (St. Petersburg RC), operating on the basis of the Pasteur Research Institute of Epidemiology and Microbiology since 1998, oversees 14 territories (11 territories of the NWFD; 1 territory of the Central Federal Dictrict (Kostroma Region), 2 territories of Volga Federal Dictrict (Nizhny Novgorod and Saratov Regions)). The main tasks of the Laboratory of Epidemiology of Infectious and Non-Communicable Diseases include epidemiological surveillance of diseases with acute flaccid paralysis syndrome (AFP) and enterovirus (non-polio) infection (EVI), visits to the territories supervised by the center to provide advisory and methodological assistance and quality control of polio prevention, participation in regional meetings of specialists with reports on the results of the work, preparation of annual reports with the results of the implementation of action plan to maintain polio-free status in the supervised territories.

In 2021, the incidence rates of AFP among children under 15 years of age of 1.0 and higher per 100 000 children of the specified age were recorded in 6 monitored territories, which means that the quality of epidemiological surveillance of polio at the stage of infection elimination is satisfactory. Indicators close to 1.0 (0.95–0.96 per 100 000) were established in 2 regions, an insufficient number of diseases with AFP syndrome was detected in 3 territories, and 3 territories were classified as "silent" due to the complete absence of AFP registration (Fig. 37). In 2022, 11 territories had a satisfactory quality of supervision in terms of the level of registration of AFP, and an insufficient number of cases were detected in 3 regions; a high incidence of AFP in the Nenets Autonomous Okrug, 9.88 per 100 000,

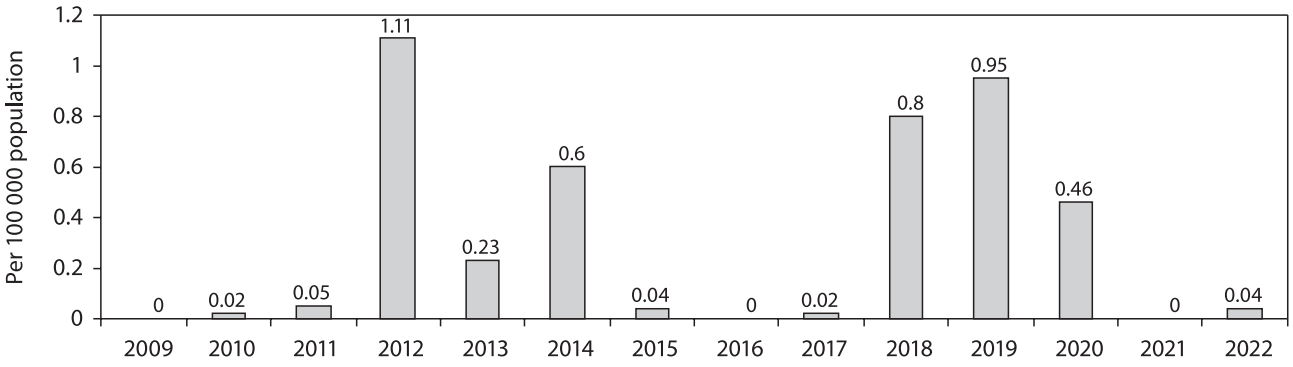

Figure 32. Measles incidence in the NWFD of Russia in 2009–2022

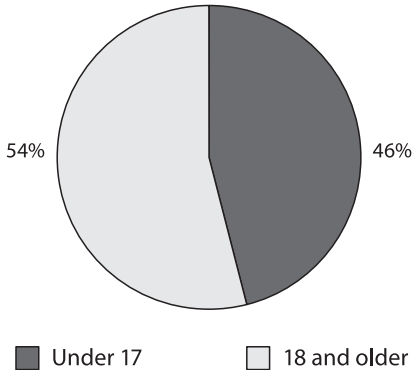

Figure 33. Distribution of measles patients by age in the territories of the NWFD in 2019–2022

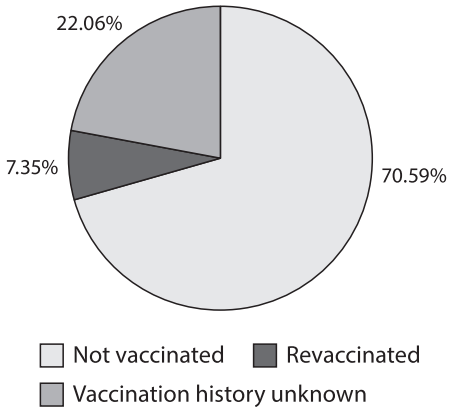

Figure 34. Distribution of measles patients depending on vaccination status in the territories of the St. Petersburg Regional Center in 2020–2022

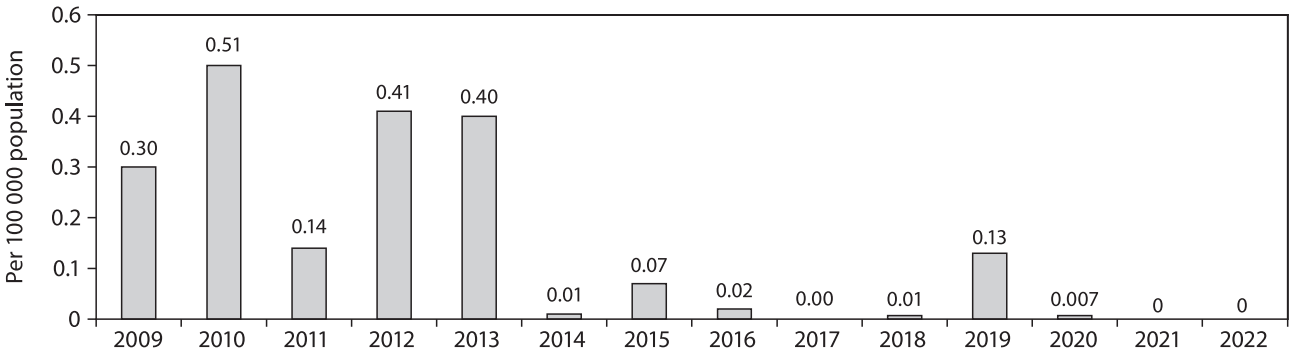

Figure 35. The incidence of rubella in the NWFD of Russia in 2009–2022

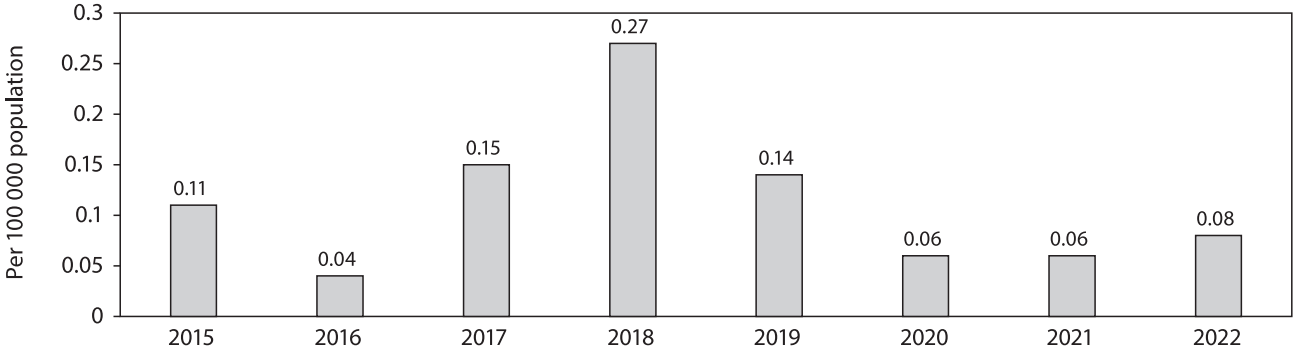

Figure 36. The incidence of epidemic parotitis in the NWFD of Russia in 2015–2022

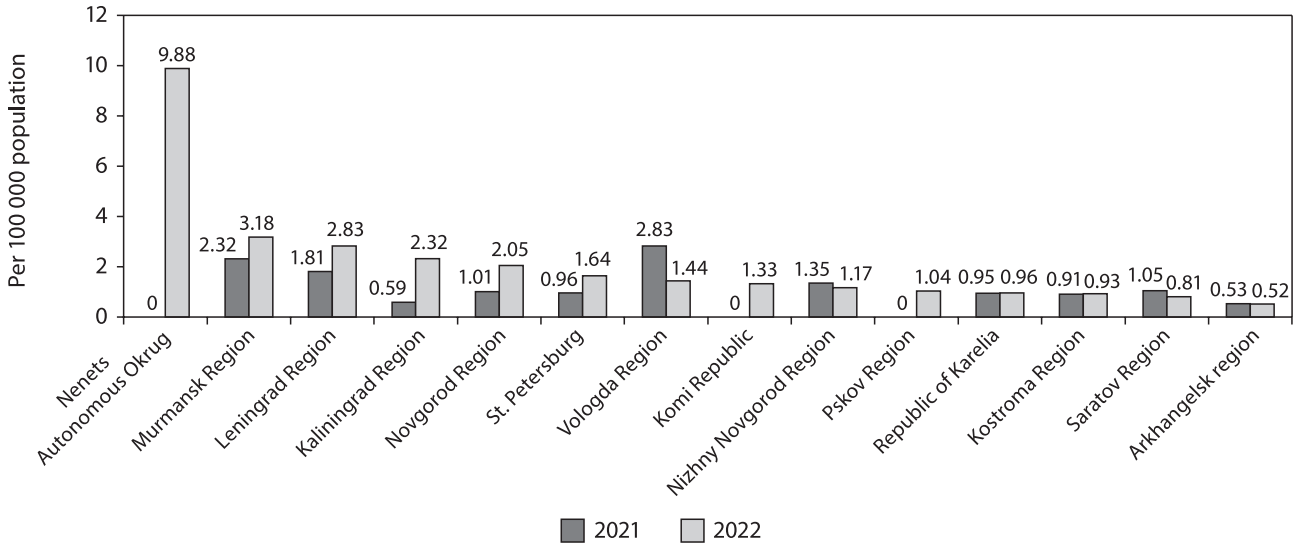

Figure 37. Registration of diseases with AFP syndrome among children under the age of 15 in the territories supervised by St. Petersburg RC in 2021–2022

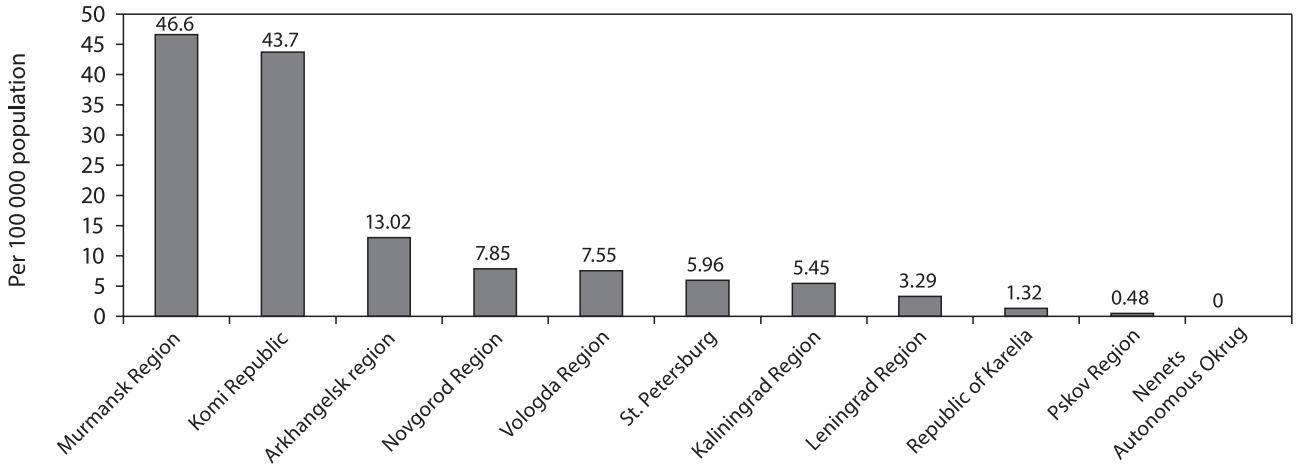

Figure 38. Ranking of territories by the incidence of enterovirus (non-polio) infection in the NWFD in 2022

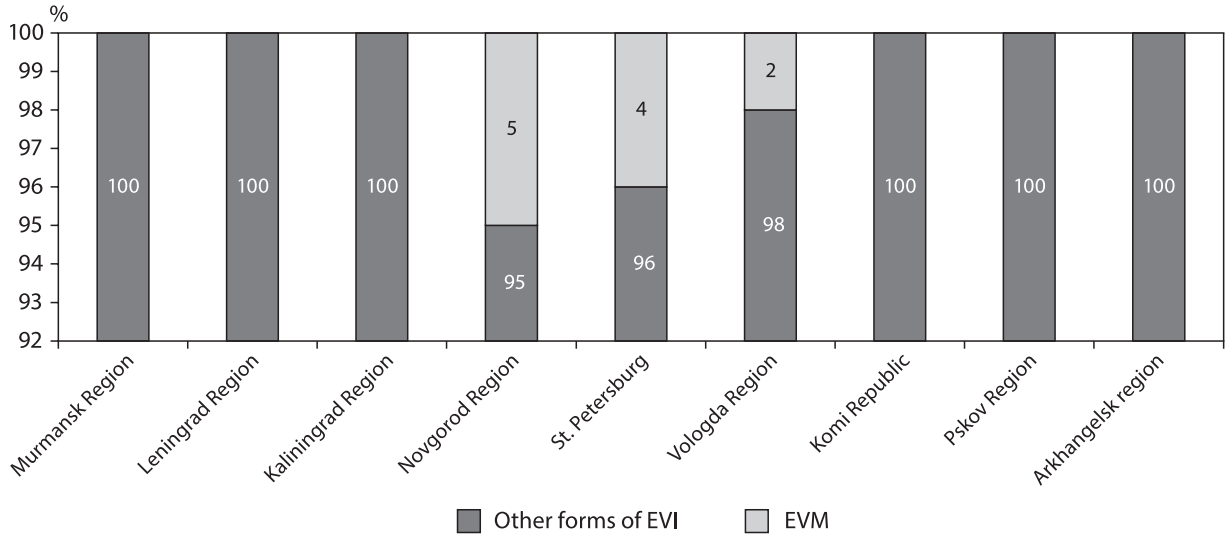

Figure 39. Registered cases of serous meningitis in the total number of EVI in the territories of the NWFD of Russia in 2021

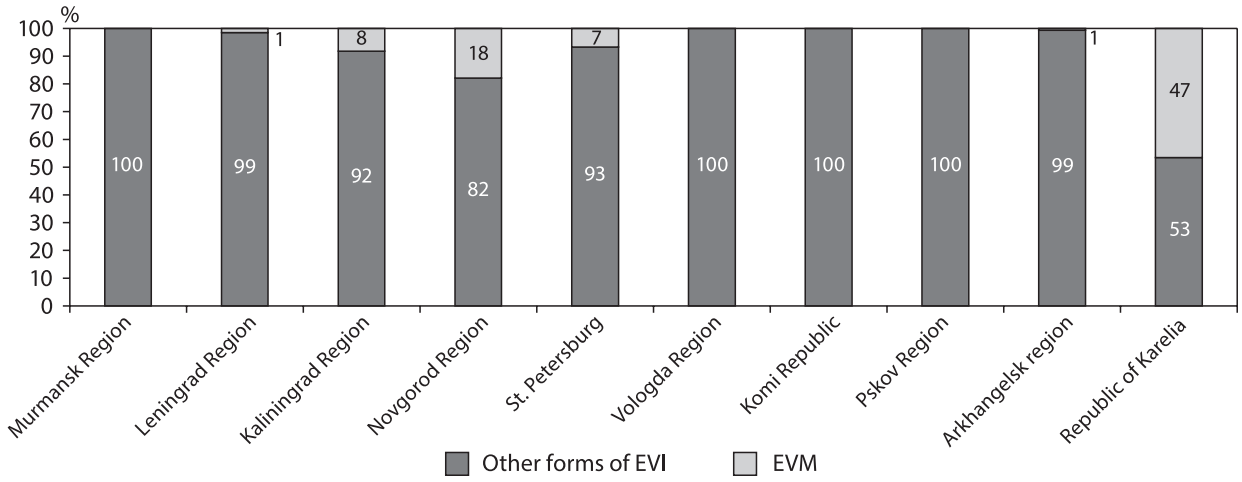

Figure 40. The share of serous meningitis among EVI in the territories of the NWFD in 2022

is due to a small number of children in the region (below 10 000). In 2021, 6 "hot" (suspected polio) cases were detected in the supervised territories, in 2022, there were 8 such cases, but polio was not confirmed.

The incidence of enterovirus (non-polio) infection in the territory of the NWFD of Russia in 2022 as a whole amounted to 9.99 per 100 000 population. The highest rates were in the Murmansk Region (46.6 per 100 000 (95% CI: 41.62–51.71), the Komi Republic (43.7 per 100 000 population (95% CI: 35.05–43.86)), and the Arkhangelsk Region (13.02 (95% CI: 10.89–15.19)). Fig. 38 shows the ranking of the territories of the NWFD by the incidence of enterovirus (non-polio) infection in 2022.

In 2021, enterovirus meningitis (EVM), a severe form of EVI, was registered in 3 territories of the NWFD, the share of enterovirus meningitis among all forms of EVI being within the range of 2–5% (Fig. 39).

In 2022, the incidence of EVM in the territories of the NWFD was 0.34 per 100 000 population. The highest rates were recorded in the Novgorod Region: 1.71 per 100 000 population (95% CI: 0.64–2.78), the Republic of Karelia: 1.15 per 100 000 (95% CI: 0.28–2.02) and the Kaliningrad Region: 0.49 per 100 000 (95% CI: 0.05–0.93). Enterovirus meningitis was registered in 6 territories of the NWFD, the proportion of this form among all forms of EVI varying from 1% to 47% (Fig. 40).

Differences in the registration of EVM in the regions may be related to the etiological structure of enteroviruses. In 2021–2022, no cases of enterovirus infection were detected in the Nenets Autonomous Okrug. The majority of patients with enterovirus infection in the territories supervised by the regional center in 2021 were children under the age of 17 (97%). The results of the study showed the need to continue epidemiological surveillance and prevention of polio and enterovirus (non-polio) infection.

Implementation of the acute viral hepatitis B elimination program in the NWFD of the Russian Federation

The program for the elimination of acute viral hepatitis B was initiated by St. Petersburg Pasteur Institute under the leadership of its director, Professor A. Zhebrun, in 2010–2012, and was approved by the head of the Federal Service for Supervision of Consumer Rights Protection and Human Well-being, the Chief State Sanitary Doctor of the Russian Federation, on August 14, 2013. The work was divided into stages, each of which included the solution of specific tasks, the achievement of which helped to determine the main tasks for the next period. In 2021–2022, the implementation of this program continued in the North-West of Russia (the population of the NWFD is about 14 million people).

The objectives of the III stage of the program included the implementation of organizational and advisory work, achieving the incidence of acute viral hepatitis B below 1.0 per 100 000 population in the regions or the absence of registration of cases of the disease, reducing the incidence of chronic hepatitis B, achieving 90% vaccination coverage of the adult population (up to the age of 55) against this infection. Meetings of specialists involved in the implementation of the program were held annually to summarize the work done and determine the tasks for the next period.

All the tasks were successfully solved. Vaccination of the population against hepatitis B helped achieve significant progress in reducing the incidence of acute viral hepatitis B in the Russian Federation and the NWFD. In 2021 and 2022, in the NWFD as a whole, the incidence rates were 0.28 and 0.18 per 100 000 population, respectively (in 2020, 0.29 per 100 000). No cases of acute hepatitis B were registered in 5 territories of the NWFD in 2022 (Table 18).

At the same time, the incidence of chronic viral hepatitis B among the population is decreasing much more slowly, and in some territories it does not show a significant downward trend. Analysis of cases with an established source of infection showed that the sourse for all patients with acute hepatitis B was patients with chronic hepatitis B.

Figure 41 shows the changes in HBV incidence in the Russian Federation, the NWFD and St. Petersburg in 2010–2021. The incidence is decreasing in all the studied territories. In 2020, amid the COVID-19 pandemic, the lowest level of morbidity was registered; with the removal of restrictive measures, an increase in indicators was observed, but the incidence does not exceed the level of the pre-epidemic period. In 2019 and 2022, in Russia as a whole, the incidence rates were 8.7 and 6.3 per 100 000 population, respectively, in the NWFD, 21.0 and 18.8 per 100 000, in St. Petersburg, 43.5 and 38.4 per 100 000 population.

The results of the study indicate the need to continue the implementation of the program for the elimination of viral hepatitis B in the territory of the NWFD of Russia with increased efforts for the control and prevention of chronic forms of the infection.

Table 18. The incidence of acute hepatitis B in the territories of the NWFD in 2018–2022

Territory	2018		2019		2020		2021		2022	
	abs.	per 100 000	abs.	per 100 000	abs.	per 100 000	abs.	per 100 000	abs.	per 100 000
NWFD	52	0.37	64	0.46	40	0.29	39	0.28	40	0.18
Republic of Karelia	1	0.16	1	0.16	2	0.32	2	0.33	1	0.20
Komi Republic	3	0.35	4	0.48	0	0	1	0.12	2	0.27
Arkhangelsk Region	4	0.36	0	0	1	0.09	0	0	0	0
Nenets Autonomous Okrug	1	2.27	0	0	0	0	0	0	0	0
Vologda Region	1	0.08	10	0.85	2	0.17	1	0.09	2	0.18
Kaliningrad Region	2	0.2	5	0.5	4	0.4	0	0	5	0.48
Leningrad Region	0	0	0	0	0	0	0	0	2	0.25
Murmansk Region	2	0.26	3	0.41	0	0	1	0.14	0	0
Novgorod Region	2	0.33	3	0.5	1	0.17	1	0.17	0	0
Pskov Region	0	0	0	0	0	0	0	0	0	0
St. Petersburg	36	0.68	38	0.71	30	0.56	33	0.61	28	0.50

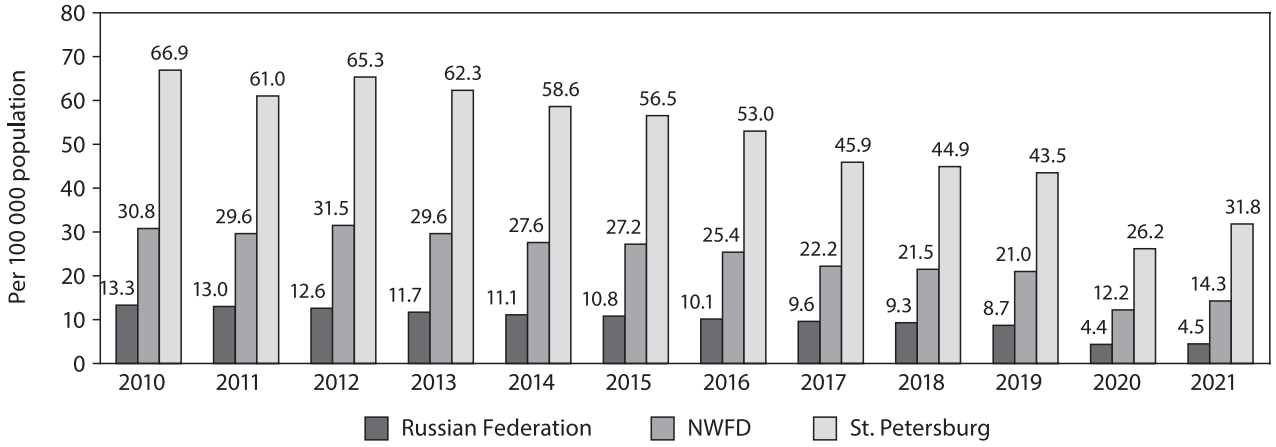

Figure 41. The incidence of chronic hepatitis B in the Russian Federation, the NWFD and St. Petersburg in 2010–2021

Publications

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LABORATORY OF VIRAL HEPATITIS

Head of the Laboratory: Elena Esaoulenko
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Acute viral hepatitides

The incidence of acute viral hepatitis (AVH) in the Russian Federation demonstrates a steady downward trend. In 2022, the largest share in the etiological structure of AVH in the Russian Federation was hepatitis A (58%), the proportion of which has decreased by 3.6 times (65% in 2013). Over the past ten years (2013–2022), the proportion of acute hepatitis B has decreased by 4.6 times and amounted to 11% of the total registered AVH cases in 2022 (15% in 2013). The share of acute hepatitis C increased by 1.9 times, to 27% in 2022 (16% in 2013). The proportion of acute hepatitis E in 2022 was 2% (1% in 2013), while other AVHs accounted for the remaining 2% (5% in 2013).

Currently, a significant amount of knowledge has been accumulated about A and E enteral hepatitides. The prognosis of the diseases is usually favorable, but the socio-economic consequences are negative and are becoming a burden on the health system. Globalization and improvement of sanitary conditions have led to significant changes in the epidemiology of hepatitis A. The most effective means of combating enteral hepatitis are improving sanitary conditions, in particular, the quality of water supply, food safety, and, for hepatitis A, increasing vaccination coverage.

Hepatitis A is transmitted through water, food, or objects on which microparticles of the patient's feces are present. Case-control studies have confirmed that infection with the hepatitis A is associated with a lack of hygiene, such as not washing hands after going to the toilet or before cooking, or sharing syringes. A retrospective analysis of hepatitis A transmission routes in patients hospitalized from 2018 to 2022 (n = 420) in St. Petersburg found that in 32.7% of cases the routes are not known. Among the known routes, water and food transmission routes were common, their share in the total structure being 27.4% and 24.2%, respectively. 15.7% of patients stated household contacts as the pathway.

In the long-term, the incidence of hepatitis A demonstrates a downward trend. In 2022, 2310 cases of hepatitis A were registered in the Russian Federation, the incidence rate being 1.6 per 100 000 population. Most patients were adults, and the proportion of children under the age of 17 was 43% (3.3 per 100 000 population). The highest incidence rate in 2022 was registered in the Ryazan region (18.1 in 2022, 18.4 in 2021). A high incidence was observed in the Kaluga Region and the Khanty-Mansi Autonomous Okrug. In 2022, no cases of hepatitis A were registered in 12 regions of the Russian Federation (an increase from 9 in 2021): the Republics of Kalmykia, Adygea, Ingushetia, Altai, Tyva, Kabardino-Balkaria, Karachay-Cherkessia, North Ossetia; in the Nenets and Chukotka Autonomous Okrugs; in Sevastopol and Astrakhan region. A similar trend has been maintained for many years in the Republics of Kalmykia, Tyva, Kabardino-Balkaria, the Jewish Autonomous Region and the Nenets Autonomous Okrug. In 2022, 5 foci of group morbidity were registered (in the Ryazan and Chelyabinsk regions, St. Petersburg, Khanty-Mansi Autonomous Okrug and Altai Krai), with a total of 63 cases. There is a significant decrease in the proportion of children in the age structure of the patients, to 20.6% from 76.9% in 2021. 431 283 people were vaccinated in the Russian Federation in 2022, including 165 932 children under the age of 17. The regions with the highest numbers of vaccinated people are Moscow (155 295), Sverdlovsk Region (39 937), Novosibirsk Region (18 537), Omsk Region (17 591), and the Republic of Crimea (15 799).

In 2022, 76 cases of hepatitis E were registered in 27 regions of the Russian Federation (in 2021, 57 cases in 19 regions), the incidence rate being 0.05 per 100 000 population. As it was in the past, no cases of hepatitis E are registered in the North Caucasus Federal Okrug. It should be noted that the quality and accessibility of laboratory diagnostics, as well as the healthcare alertness regarding this infection, play a significant role in the frequency of hepatitis E registration. In 2021–2022, the diagnosis of hepatitis E with the detection of anti-HEV IgM antibodies accounted for 74.5% and 86.2%, respectively.

The implementation of the program of mass immunization of the population of the Russian Federation against hepatitis B has resulted in significant progress in the fight against acute hepatitis B. Over the last decade (2013–2022), the incidence of acute hepatitis B has decreased by 4.6 times, from 1.3 to 0.3 per 100 000 population. In 2022, 15 cases of acute hepatitis B were registered in children under 17 years of age (0.1 per 100 000) in 13 subjects of the Russian Federation: Moscow, Tula, Samara, Novosibirsk regions; the Republics of Karelia, Dagestan, Ingushetia, Chechen, Udmurt, Chuvash; St. Petersburg, Krasnodar Krai, and Khanty-Mansi Autonomous Okrug. Compared with 2021, the incidence among the child population (0.04 per 100 000 children) increased slightly. In 2022, in 59 subjects of the Russian Federation (compared to 53 in 2021), no cases of acute hepatitis B were registered. In 7 regions, the incidence rate of acute hepatitis B is twice as high as the national average. The main measure of hepatitis B prevention is vaccination. In 2022, 2.1 million people were vaccinated against hepatitis B in the Russian Federation, including 1.4 million children (in 2021, a total of 2.3 million people). Vaccination coverage against hepatitis B in children aged 12 months in 2022 was 96.92% (in 2021, 96.9%). Vaccination coverage of the adult population is increasing every year. Vaccination coverage of the population in 2022 was 97.5% in the 18–35 age group, 91.1% in the 36–59 age group, and 39.7% in the 60 plus group.

The incidence of acute hepatitis C (AGS) in the Russian Federation decreased 35-fold from 2000 to 2022. The incidence rates in 2021–2022 were 0.6 and 0.8 per 100 000 population, respectively. The implementation of a set of anti-epidemic and preventive measures and the efforts of a number of government agencies and medical workers have definitely contributed to a decrease in the number of new infections. The sharp decline in basic epidemiological indicators for 2021–2022 is most likely associated with a decrease in the number of patients seeking outpatient

care and less active screening in the period of COVID-19 restrictions. The incidence of acute hepatitis C in children under 17 years of age was 0.17 per 100 000 children, and in 2021, 0.10 per 100 000 children. The proportion of children in the total number of acute hepatitis C cases was 4.9% (3.5% in 2021). The share of children under 1 year of age among all children with acute hepatitis C in 2022 was 45.3%, which is lower than last year (53.3% in 2021). In five regions, the incidence of acute hepatitis C exceeded the national average 2.0 to 2.7 times: St. Petersburg (2.03 per 100 000 population), Yamalo-Nenets Autonomous Okrug (1.64), Moscow (1.62), Ivanovo Region (1.53), and Chelyabinsk Region (1.52).

According to the results of the analysis of hepatitis C virus samples, there were no significant changes in 2021–2022. The prevailing genotype in the 44 196 samples is still genotype 1 (22 877 samples, 51.8%), with the dominating subtype being 1b (10 037 samples, 43.9%); genotype 3 was detected in 16 558 samples (37.5%), genotype 2 in 4436 samples (10.2%).

Chronic viral hepatitides

Despite the decrease in the incidence of acute forms of hepatitis B and C in the Russian Federation, high levels of new cases of chronic forms of viral hepatitis continue to be registered in the country. The incidence of chronic viral hepatitis (first diagnosed) in 2022 was 29.72 per 100 000 population, which is 1.6 times lower than the long-time average annual (48.47 per 100 000 population), and in children under 17 years of age, the incidence was 1.3 per 100 000 children. Chronic hepatitis C prevails in the etiological structure of newly reported cases of chronic viral hepatitis, with a share of 78.1%. The incidence of chronic hepatitis C in 2022 exceeded the incidence of chronic hepatitis B 3.6 times.

Over the last decade (2013–2022), the incidence of HCV has decreased by 1.7 times (from 39.2 to 23.2 per 100 000 population). In 2022, the incidence of chronic hepatitis C was 23.2 per 100 000 population, which is 1.6 times lower than the long-time average annual (37.03 in 2010–2019). In 2022, high morbidity rates, twice as high as the national average, were registered in St. Petersburg and Moscow.

The incidence of chronic hepatitis B in 2022 was 6.4 per 100 000 population, which is 1.7 times lower than the average long-term indicator (11.01 per 100 000 population). Over the past decade, the incidence of chronic hepatitis B has decreased 1.8 times (in 2013, 11.7 per 100 000 population), compared with 2021, it increased 42.5% (in 2021, 4.5 per 100 000 population).

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2. *Lyalina L.V., Esaulenko E.V., Khorkova E.V., Plavinskii S.R., Rishnyak O.Yu., Novak K.E., Totolian A.A. Implementation of the viral Hepatitis B elimination program in the North-West of Russia: results and prospects // Proceedings of the XII Congress of the All-Russian Scientific and Practical Society of Epidemiologists, Microbiologists and Parasitologists, Moscow, October 26–28, 2022 / Eds A.Yu. Popova, V.G. Akimkin. Moscow: Federal Budgetary Institution of Science "Central Research Institute of Epidemiology" of the Federal Service for Supervision of Consumer Rights Protection and Human Well-Being, 2022: 423. (In Russ.)*

Patents

1. *Patent No. 2759672 C2, Russian Federation, IPC G01N 33/53. Method for differentiating causes of development of initial stage of hepatic fibrosis in chronic viral hepatitides B and C. No. 2019141709; application: 2019.12.12: date of publication 16.11.2021 / Batsunov O.K., Semenov A.V., Arsenteva N.A., Lyubimova N.E., Ostankova Y.V., Esaulenko E.V., Totolian A.A. Proprietors: Federalnoe byudzhetnoe uchrezhdenie nauki "Sankt-Peterburgskij nauchno-issledovatelskij institut epidemiologii i mikrobiologii im. Pastera Federalnoj sluzhby po nadzoru v sfere zashchity prav potrebitelej i blagopoluchiya cheloveka" (FBUN NII epidemiologii i mikrobiologii imeni Pastera)*

LABORATORY OF MOLECULAR EPIDEMIOLOGY AND EVOLUTIONARY GENETICS

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Tuberculosis and mycobacteria

Projects and collaborations

- Joint project with National Institute for Public Health and Environment (RIVM, Bilthoven, Netherlands) on drug resistant tuberculosis, 2018–2020 (co-PI: I. Mokrousov and R. Anthony). 2018–2021.
- Russian Science Foundation, Project 19-14-00013 ("Uneven evolutionary and epidemic trajectory of the paradoxical ancient subtype of the East Asian lineage of *Mycobacterium tuberculosis*: stochastic fluctuations or causative correlations?" PI — Igor Mokrousov), 2019–2021. Funding continued for 2022–2023.
- Russian Science Foundation, Project 19-15-00028 ("Development of new efficient compounds against drug resistant *Mycobacterium tuberculosis* taking into account the population structure of the pathogen" PI — Anna Vyazovaya), 2019–2021.
- Project supported by PTR program of Institut Pasteur Paris "Transcriptional Response for AntimiCrobial Resistance detection in TB" (Coordinator — An van den Bossche, Belgium; Russian PI — Igor Mokrousov), 2019–2022.
- Russian Foundation for Basic Research, project 20-04-00686 "Deep machine learning methods in *Mycobacterium tuberculosis* genomics for the building of an open platform for the analysis of the pathogen's evolutionary signatures" (PI — E. Shitikov, Center of Physico-Chemical Medicine, Moscow), 2020–2022.
- Russian Foundation for Basic Research, project 19-515-55009 (joint project co-funded by National Natural Science Foundation China) "Integral insight into development of drug resistant tuberculosis in adults versus children: impact of bacterial strain and surrounding microbiome" (PI — Dr Zhdanova, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia), 2020–2022.
- Russian Foundation for Basic Research project 20-515-80006 under BRICS STI Framework Programme Response to COVID-19 global pandemic (PI — Prof. Y. Schwartz, Novosibirsk Research Institute of Tuberculosis), 2021–2022.
- Russian Science Foundation, project 22-15-00432 "The development of comprehensive mycobacterial diagnostics methods" (PI — Danila Zimenkov, Engelgardt's Institute of Molecular Biology RAS), 2022–2024.

International collaborations

National Institute for Public Health and the Environment, RIVM (2018–2021), Beijing Children's Hospital, China (2017–2021), Stephan Angeloff Institute of Microbiology and Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences (Sofia, Bulgaria), National TB Reference Laboratory, University Hospital Shefqet Ndroqi (Tirana, Albania), Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw (Poland), Instituto de Investigação do Medicamento, Faculdade de Farmácia, Universidade de Lisboa (Lisbon, Portugal).

National collaborations

Omsk State Medical University, Scientific Center of Family Health and Reproductive Problems (Irkutsk), Buryat State University (Ulan-Ude), Ural Research Institute of Phthisiopulmonology (Ekaterinburg), Northern Medical University (Archangelsk), Anti-tuberculosis dispensaries in Kaliningrad, Petrozavodsk (Karelia), Syktyvkar (Komi), Murmansk, Pskov, Omsk.

Major research results

Molecular monitoring of the *Mycobacterium tuberculosis* population in Murmansk region, Russia

In Murmansk oblast, the first molecular-genetic studies of circulating strains of *Mycobacterium tuberculosis* were carried out during the years of an increase in the incidence of tuberculosis (TB) (2003–2006). The study's aim was to carry out a genotypic characterization of the *M. tuberculosis* population in Murmansk oblast and analysis of changes in its structure over 15 years. Sixty-seven *M. tuberculosis* strains from patients with TB newly diagnosed in 2017 were studied. The strains were assigned to the Beijing genotype and its major clusters based on analysis of specific markers. The Beijing strains were typed by 24 MIRU–VNTR loci. All non-Beijing strains were subjected to spoligotyping. Genotypes of *M. tuberculosis* were identified: Beijing (52.2%), Ural (19.4%), T (9.0%), LAM (7.5%), Haarlem (3.0%), and X (1.5%). Among Beijing strains, the Central Asian/Russian cluster with heterogeneous MIRU–VNTR profiles was predominant — 34.3% (23/67). Multiple drug resistance (MDR) — resistance to rifampicin and isoniazid caused by mutations *rpoB* Ser-531Leu (TCG → TTG) and *katG* Ser315Thr (AGC → ACC) — was detected in 26.9% of the strains; the largest proportion of MDR strains were found in the Beijing B0/W148 cluster (85.7%), represented mainly by the MIRU–VNTR profile 100-32. High levels of clustering of Beijing Central Asian/Russian (CR = 0.68) and B0/W148 (CR = 0.71) strains reflect their current dissemination. In 2003–2017, a steady dominance of the Beijing genotype with a tendency to an increase from 44.0 to 52.2% was observed. The proportion of MDR strains in the cluster B0/W148 Beijing increased by 3.5 times, which indicates the selection and accumulation of this epidemiologically and clinically unfavorable variant of the tuberculosis pathogen in Murmansk oblast (Table 19).

Molecular Insight into *Mycobacterium tuberculosis* resistance to nitrofuranyl amides gained through metagenomics-like analysis of spontaneous mutants (collaborative project with Institute of Organic Chemistry with Centre of Phytochemistry, and The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences)

The information on the nitrofuran mode of action on mycobacteria and the molecular mechanism of mycobacterial resistance to nitrofurans is limited. Different spontaneous

Table 19. Genotypes and drug resistance of *M. tuberculosis* strains from Murmansk region in Russia

Genotype	Sensitive (n = 41)	Mono/ polyresistant (n = 8)	MDR (n = 18)	Total (n = 67)
Beijing	17	3	15	35
Beijing B0/W148	0	1	6	7
Central-Asian/Russian	13	2	8	23
Beijing, other	4	0	1	5
Non-Beijing	24	5	3	32
Ural	9	4	0	13
T	6	0	0	6
LAM	5	0	0	5
Haarlem	1	1	0	2
X	1	0	0	1
Unknown	2	0	3	5

mutations emerge in the *M. tuberculosis* population and may be selected and fixed if they are sufficiently beneficial for bacterial survival, adaptation, and fitness. In this study, we describe the synthesis of the new nitrofuranyl amides and investigate their anti-TB activity and possible mechanism of action/resistance through whole-genome sequencing of *M. tuberculosis* spontaneous mutants. We focused on nitrofuranyl amides since they possess strong antitubercular and antibacterial activity. However especially in case of antitubercular activity, their mechanism of action is still largely unknown.

Methods. A series of six new nitrofuranyl amides was synthesized by reaction of 5-nitrofuran-2-carbonyl chloride with different amines. The *in vitro* activity was assessed on the reference strain *Mycobacterium tuberculosis* H37Rv. The most active compound 11 was further used for *in vitro* selection of the spontaneous resistant mutants. Strain

Figure 42. Example of mutant position (genome position 411895 A>T, *iniA* gene) with mixed wild type and mutant alleles, with increasing percent of mutant alleles in *M. tuberculosis* H37Rv subcultures grown under higher (2x and 8x MIC) concentrations of compound 11 — 1.0 μM (A) and 4.0 μM (B). Only part of the reads is shown that fit the Geneious window. The reads were aligned to the reference genome H37Rv (NC_000962.3). The red arrow indicates the position

H37Rv was cultured at the elevated concentrations of 11, and DNA from pooled colonies was subjected to WGS followed by bioinformatics analysis.

Results. The same mutations in six genes were detected in bacterial cultures grown under increased concentrations of 11 (2x, 4x, 8x MIC). The mutant positions were presented as mixed wild type and mutant alleles while increasing the concentration of the compound led to a semi-proportional and significant (in 4 cases) increase of mutant alleles (see Fig. 42 as an example). The identified genes belong to different categories and pathways. Some of them were previously reported as mediating drug resistance or drug tolerance, and counteracting oxidative and nitrosative stress, in particular: *Rv0224c* (oxidative stress response), *fbiC* (F420 biosynthesis pathway that includes nitrofuran activating F420-dependent nitroreductase Ddn), *iniA* and lipase/esterase gene *Rv1592c* (efflux pump, maintenance of the plasma membrane integrity). Other genes were coding for PhiRv1 phage protein Rv1580c, and conserved membrane protein Rv1639c. Five of six mutations were non-synonymous and some likely led to significant changes in protein structure. Gene-set interaction analysis revealed a certain weak interaction for gene pairs *Rv1592–Rv1639c* and *Rv1592–Rv0224c*.

Conclusions. In conclusion, this study experimentally demonstrated a complex multifaceted genetic response of *M. tuberculosis* to the action of the nitrofuranyl amide that concerned multiple genes and different pathways. Six genes contained mutations that emerged in bacterial cultures grown under increased concentrations of nitrofuran, furthermore, the increasing concentrations led to a higher proportion of the mutant alleles. The identified genes belong to different gene categories and pathways. The same mutations were detected in different independent experiments that confirms a correlation between compound action and possible resistance mechanism. Furthermore, increasing the compound concentration of the compound led to a semi-proportional and significant (in 4 cases) increase of mutant alleles. Five of six mutations were non-synonymous and some could likely lead to significant changes in protein structure, especially mutations in *iniA* and *fbiC* with concordantly significant PAM1 and SIFT scores.

Further study should focus on experimental analysis of the role of the identified mutations, in particular by the transcriptomics and proteomics methods. Experiments focused on the interaction between nitrofurans and *M. tuberculosis* essential proteins in the presence and absence of a nitroreductase may shed light on the nitrofuran targets.

Extremely lethal and hypervirulent *Mycobacterium tuberculosis* strain cluster emerging in Far East, Russia (collaborative project with St. Petersburg Research Institute of Phthisiopulmonology)

Previously, the C57BL/6 resistant mice infected with highly virulent *M. tuberculosis* strain were shown as a TB model reproducing an exacerbated inflammatory response in a resistant host to hypervirulent mycobacteria, leading to irreversible necrotic lung lesions. Properties of the Beijing strains were previously studied in this model, including ancient and modern sublineages from different countries. However, only modern Beijing sublineage strains from Russia were analyzed in those studies. In this study, we aimed to investigate the virulence properties of the *M. tuberculosis* strains of the recently described and MDR-associated ancient Beijing clusters from Russia (Fig. 43) in the C57BL/6 mouse model.

In the murine model, strains 396 (14717-15-cluster, from Buryatia, Far East) and 6691 (1071-32-cluster, from Omsk, Siberia) demonstrated contrasting properties. The 396-infected group had significantly higher mortality, more weight loss, higher bacterial burden, and more severe lung pathology (Fig. 44 and 45). Furthermore, compared to the previously published data on other Russian epidemic Beijing strains (B0/W148, CAO, Central Asian Russian), strain 396 demonstrated the highest mortality (Fig. 45).

Strain 6691 belongs to the Beijing 1071-32-cluster widespread across different FSU countries but at low prevalence and is relatively visible only in Omsk, Western Siberia (7%). This situation follows a traditional assumption that multiple drug resistance mutations reduce fitness and virulence. In contrast, highly lethal and hypervirulent strain 396 represents an intriguing Beijing 14717-15-cluster predominant only in Buryatia, Far East (16%), sporadically found beyond it, but not forming clusters of transmission. This specific case does not fit a theory of the highly virulent and highly transmitted strain. We term this cluster 14717-15 conditionally transmissible as it is endemically prevalent only in one location. The reasons may lie in the particular interplay of the human immune system and the genetic background of this strain, and further in-depth study is warranted.

Impact of pathobiological diversity of *Mycobacterium tuberculosis* on clinical features and lethal outcome of tuberculosis (collaborative project with Omsk State Medical University and Clinical Tuberculosis Dispensary, Omsk)

Background. Recognition of the clinical significance of *Mycobacterium tuberculosis* population diversity is the key issue in molecular epidemiology and personalized medicine of tuberculosis (TB). Strains of different genetic lineages of *M. tuberculosis* demonstrate variability in some biological properties such as, in vitro growth rate, virulence in animal models, capacity to acquire drug resistance. The outcome of the disease, favorable or adverse, depends on a number of factors that act independently or synergistically and include not only the strain virulence, but also human genetics, HIV coinfection, immunosuppression, duration of tuberculosis disease, the timeliness of diagnosis, treatment efficacy, and social and environmental factors. *Mycobacterium tuberculosis* population in Russia is dominated by the notorious Beijing genotype which major variants are characterized by contrasting resistance and virulence properties. The simplified evolutionary pathway of the Beijing genotype with information on the above subtypes is shown in Fig. 43.

Here we studied how these strain features could impact the progression of pulmonary tuberculosis (TB) concerning clinical manifestation and lethal outcome.

Results. Study collection included 548 *M. tuberculosis* isolates from 548 patients with newly diagnosed pulmona-

ry TB in Omsk, West Siberia, Russia. Strains were subjected to drug susceptibility testing and genotyping to detect lineages, sublineages, and subtypes (within Beijing genotype). The Beijing genotype was detected in 370 (67.5%) of the studied strains. The strongest association with multidrug resistance (MDR) was found for epidemic cluster Beijing B0/W148 (modern sublineage) and two recently discovered MDR clusters 1071-32 and 14717-15 of the ancient Beijing sublineage. The group of patients infected with hypervirulent and highly lethal (in a mouse model) Beijing 14717-15 showed the highest rate of lethal outcome (58.3%) compared to Beijing B0/W148 (31.4%; p = 0.06), Beijing Central Asian/Russian (29.7%, p = 0.037), and non-Beijing (15.2%, p = 0.001). The 14717-15 cluster mostly included isolates from patients with infiltrative but not with fibrous-cavernous and disseminated TB. In contrast, a group infected with low virulent 1071-32-cluster had the highest rate of fibrous-cavernous TB, possibly reflecting the capacity of these strains of prolonged survival and chronicity of the TB process.

Conclusions. This study indirectly shows that the traditional approach to assessing virulence and lethality in murine models does remain useful. The group infected with hypervirulent and highly lethal in murine model 14717-15 cluster had the highest rate of the lethal outcome (58.3%) compared to Beijing B0/W148 (31.4%), and non-Beijing (15.2%) groups.

In Russia, a country with a very high rate of primary MDR-TB, treatment is empirical and takes into account a high probability of primary MDR-TB for some genotypes. Now it is time to attempt considering other features of the infecting strains as well. Not only drug resistance but also strain virulence should be taken into consideration in personalized medicine and TB treatment.

Practical approach to detection and surveillance of emerging highly resistant *Mycobacterium tuberculosis* Beijing 1071-32-cluster

The recently discovered genetic variant *Mycobacterium tuberculosis* Beijing 1071-32 is characterized by multiple or extensive drug resistance. Beijing 1071-32 isolates were identified in Siberia (the most likely region of its origin), but also in European Russia, Central Asia, Transcaucasia, and unexpectedly, in the Balkan countries. We developed a molecular method for rapid detection of strains of this genotype and applied to the large collection of DNA.

Based on the phylogenetic analysis of the genomic data, authors identified three cluster-specific synonymous SNPs in the genes *Rv0144, Rv0373c*, and *Rv0334* and developed and validated the real-time PCR assay for their detection. Analysis of the genetically and geographically diverse collection of ~2400 *M. tuberculosis* isolates sampled in 1996 to 2020 (European and Asian parts of Russia, former Soviet Union countries, Albania, Greece, China, Vietnam, Japan and Brazil), confirmed 100% specificity and sensitivity of this assay.

All Beijing 1071-32 isolates carried a characteristic signature of six mutations that confer resistance to the four first-line antibiotics. Intriguingly, this combination includes the most frequent and efficient mutations *(rpoB450, katG315, rpsL43)*, rare mutation *(embB497)*, and compensatory mutations *(rpoC485, katG335)*. The latter are supposed to restore the reduced strain fitness caused by previously acquired resistance mutations. The epistatic interaction of all these mutations could have influenced the spread of this genetic cluster.

In addition to the expected presence of the Beijing 1071-32 in Siberia and the European part of Russia, these strains were found in Central Asia, Transcaucasia, and also, quite unexpectedly, in the Balkan countries — Albania, Greece, Serbia. It is unknown whether the latter reflects already the local Balkan circulation of this strain or independent events of its introduction from the countries of the former USSR (Fig. 46).

Enigmatically, all geographically distant isolates of this Beijing 1071-32 cluster have the same set of six resistance mutations. No intermediate strains with some, but not all these mutations were found to date. Speculatively, the acquisition of new mutations made the strain more adaptable compared to intermediate variants that subsequently disappeared from the population.

Spatiotemporal dynamics of drug-resistant *Mycobacterium tuberculosis* in Estonia (collaborative project with North Estonian Medical Centre Foundation and Estonian Tuberculosis Registry, Tallinn, Estonia)

Different and contrasting trends related to human migration and the implementation of health control programs influence the spread of drug-resistant tuberculosis (TB). We analyzed the *Mycobacterium tuberculosis* population structure in Estonia, a high-priority EU country for TB control, to detect the dynamic changes and underlying factors. The study collection included 278 *M. tuberculosis* isolates recovered in 1999 and 2014–2015. The isolates were subjected to drug susceptibility testing, genotyping, and analysis of sublineage/cluster-specific markers and drug resistance mutations. The Beijing genotype was the most prevalent and its rate increased from 28.6% in 1999 to 38.5% in 2015 (p = 0.09). The non-Beijing strains represented Euro-American lineage (Latin American Mediterranean [LAM], Ural, Haarlem, T, X genotypes) and Indo-Oceanic lineage (one EAI-IND isolate) (Fig. 47). The proportion of isolates resistant to two or more drugs increased from 22.4% to 29.1% (p = 0.1). The pre-XDR/XDR isolates were identified only within the Beijing genotype. In contrast, the drug resistance rate decreased in the LAM genotype from 42.1% to 11.8% (p = 0.05). The Beijing B0/W148-cluster ("successful Russian strain") included only MDR, pre-XDR, or XDR isolates. All B0/W148-cluster isolates were resistant to two or more drugs compared to 28% of the Beijing 94-32-cluster (p = 0.0002) (Fig. 48). The Beijing genotype was not identified in the isolates from patients born in Estonia before 1940 compared to its 35.2% rate among other patients. In summary, the circulation of the highly drug-resistant isolates of the Beijing B0/W148 subtype, the increased prevalence of the Beijing genotype among HIV-coinfected patients, and the increased number of patients with alcohol abuse (47.5%) present major challenges of the current TB control in Estonia. The Beijing genotype was likely brought to Estonia after 1945 due to the massive human influx from the Soviet Union. In contrast, the main genotypes of the Euro-American lineage were likely endemic in Estonia during all 20th century.

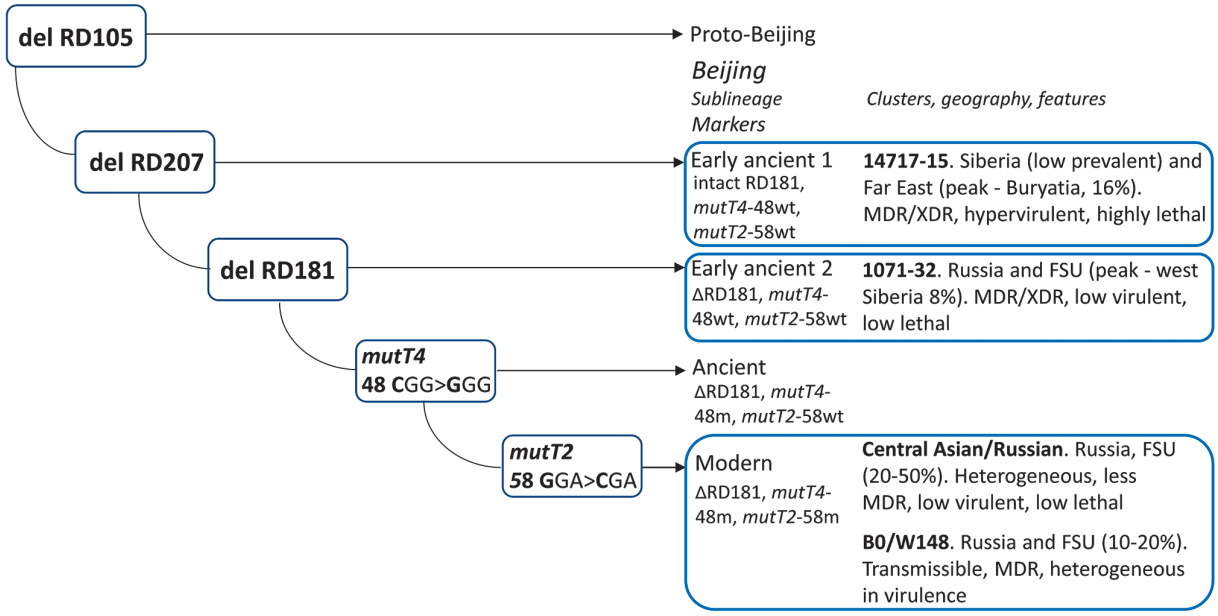

Figure 43. Simplified evolutionary pathway of the Beijing genotype with information on the above subtypes

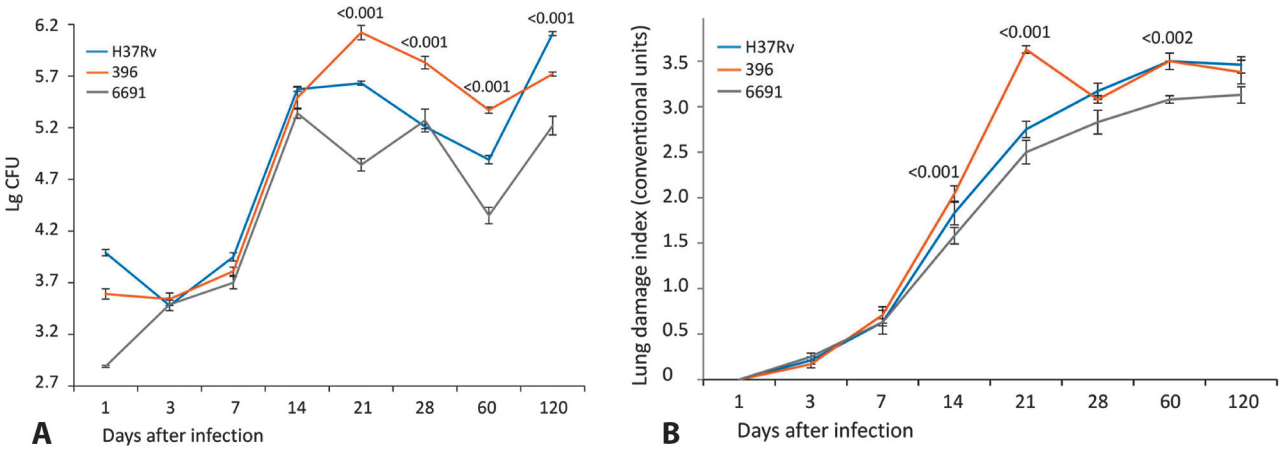

Figure 44. (A) Bacterial load in the lungs of mice infected with *M. tuberculosis* strains determined at different time points. (B) Lung pathology scores of mice infected with *M. tuberculosis* strains determined at different time points

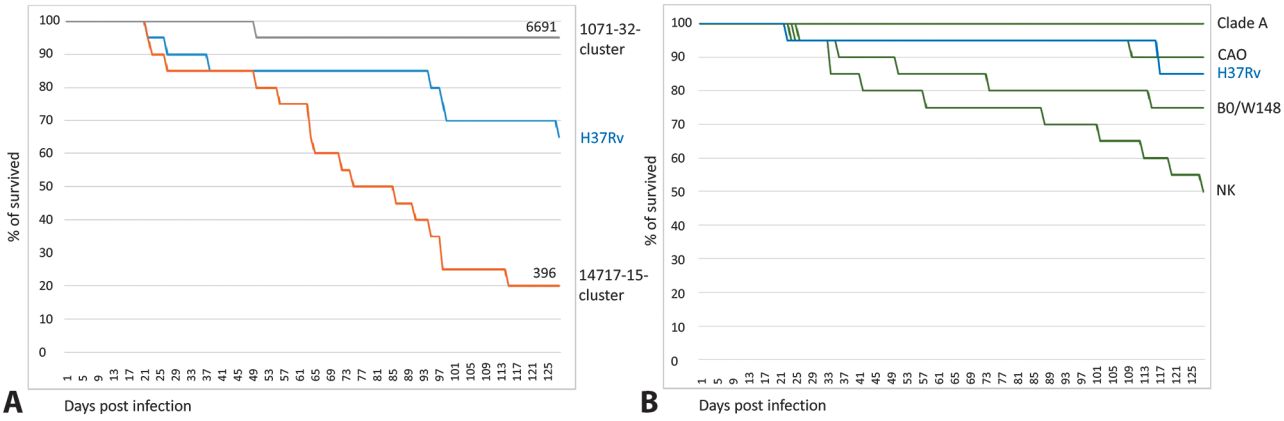

Figure 45. Comparison of survival of mice after infection with *M. tuberculosis* strains within 125 days p.i. in the similarly designed studies. The same strain H37Rv was used as reference. (A) Ancient Beijing sublineage (this study). Strain 6691 belongs to 1071-32- cluster (RD181 deleted), strain 396 belongs to 14717-15-cluster (RD181 intact). (B) Modern Beijing sublineage (adapted from Bespyatykh et al. [14]). B0/W148 strain is also named Russian epidemic or successful cluster. CladeA, CAO, NK strains belong to Central Asian Russian clade. The green color is used to show that all clinical isolates in this panel belong to the modern sublineage of the Beijing genotype

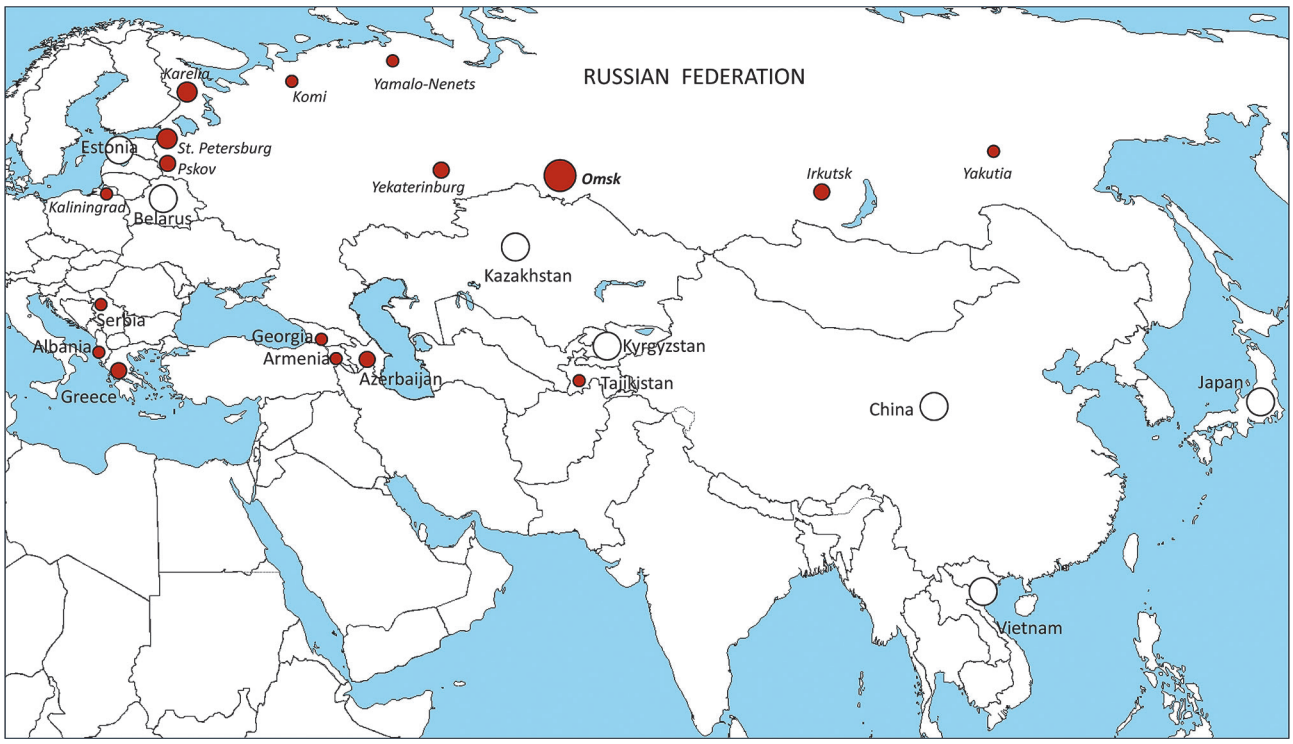

Figure 46. Geographic distribution of the Beijing 1071-32-cluster isolates identified in this study. Circle size roughly correlates with the proportion of identified isolates of this cluster (the smallest dots depict single isolates). Absence of these isolates in the local populations is shown by white circle

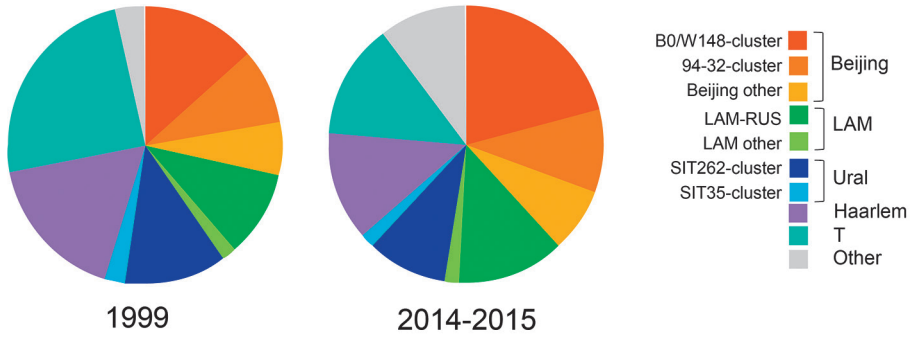

Figure 47. Prevalence of the main *M. tuberculosis* genotypes and subtypes in Estonia in 1999 and 2015

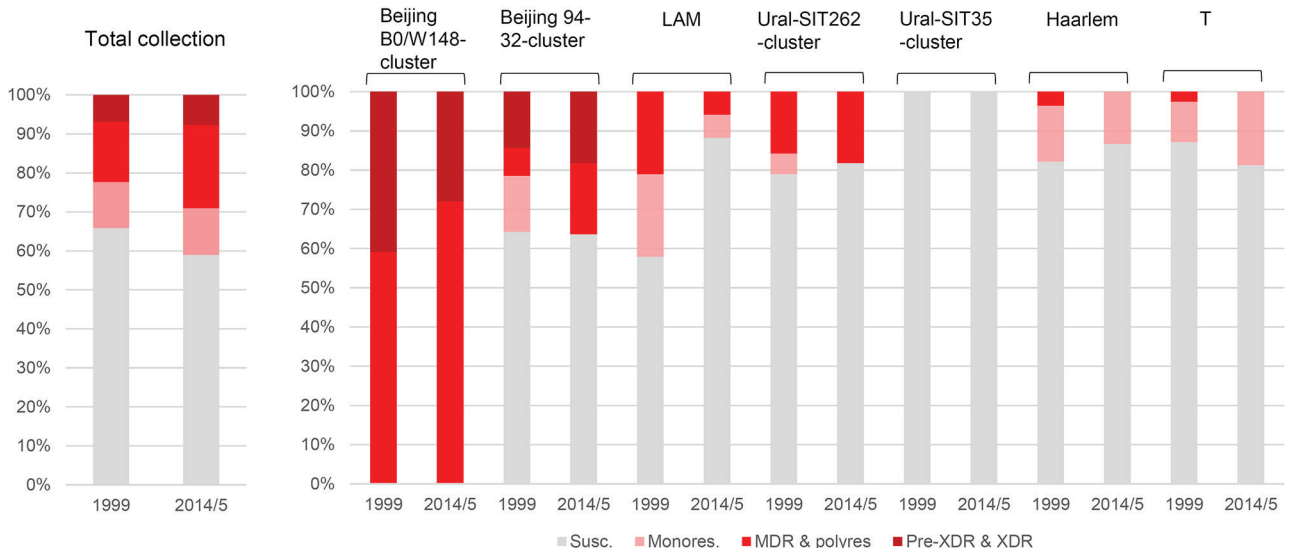

Figure 48. Drug resistance in the main *M. tuberculosis* genotypes in Estonia in 1999 and 2015

Drug resistance of non-tuberculous mycobacteria in Northwestern Russia

Among a large group of nontuberculous mycobacteria (NTM) (more than 150 species), slow-growing bacteria of the MAC complex (*Mycobacterium avium* complex) — *M. avium* and *M. intracellulare* have been recognized as clinically most important pathogens. Being the causative agents of pulmonary mycobacteriosis, MAC can cause lung destruction in immunocompetent individuals and a disseminated form of infection in HIV-infected people. The aim of the study was to study the drug resistance of *M. avium* and *M. intracellulare* isolates isolated from patients with mycobacteriosis in the Northwestern Russia. is the most frequent etiology of disease

Materials and methods. For the period from 2014 to 2020, 192 slow-growing MAC isolates (164 — *M. avium*, 28 — *M. intracellulare*) from HIV-negative patients with pulmonary disease were studied. Of the 164 *M. avium* isolates, 116 were isolated from newly diagnosed patients, 48 from previously treated patients (with an unknown treatment regimen). All *M. intracellulare* isolates were obtained from newly diagnosed patients. Drug susceptibility testing was performed using Sensititre SLOMYCO panels (Thermo Fisher Scientific). For clarithromycin, amikacin, moxifloxacin, and linezolid, the Clinical and Laboratory Standards Institute (CLSI) breakpoints have been used to interpret MIC values (CLA: S ≤ 8 mcg/ml, I = 16 mcg/ml, R ≥ 32 mcg/ml; MXF: S ≤ 1 mcg/ml, I = 2 mcg/ml, R ≥ 4, mcg/ml; LZD: S ≤ 8 mcg/ml, I = 16 mcg/ml, R ≥ 32 mcg/ml; AMI: S ≤ 16 mcg/ml, I = 32 mcg/ml, R ≥ 64 mcg/ml).

Results. The main results are shown in Figures 49 and 50. Of the four antibiotics (clarithromycin, moxifloxacin, linezolid, amikacin), clarithromycin was the most effective against both *M. avium* (67.1%; 110/164) and *M. intracellulare* (60.7%; 17/28) without significant difference in susceptible rate between the species ($p > 0.05$). Overall, 57.3% *M. avium* and 53.5% *M. intracellulare* isolates were susceptible to linezolid. For moxifloxacin, 26.8% *M. avium* and 14.3% *M. intracellulare* isolates were susceptible; for amikacin — 57.3% *M. avium* and 53.5% *M. intracellulare* isolates were susceptible, respectively. Resistance rates to all antibiotics was higher in *M. intracellulare* compared to *M. avium*, but non-significantly ($p > 0.05$). The rate of CLA-susceptible *M. avium* isolates was 20% higher in newly diagnosed patients compared to previously treated patients ($\chi^2 = 6.296$; $p = 0.013$). The rates of *M. avium* first isolates group susceptible to moxifloxacin, linezolid, and amikacin was also higher than in group of follow-up isolates.

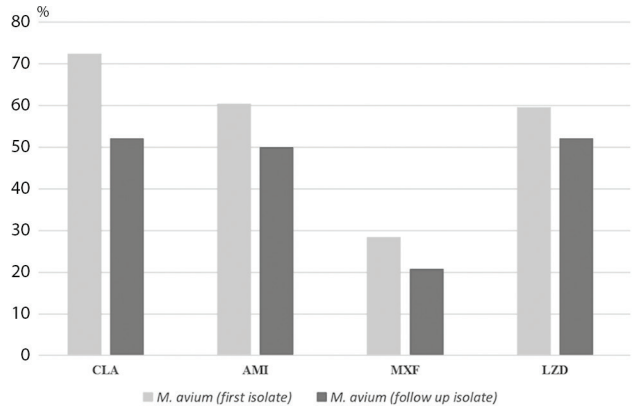

Figure 49. The distribution of proportions of *M. avium* strains sensitive to CLA (clarithromycin), AMI (amikacin), MXF (moxifloxacin) and LZD (linezolid) isolated from newly diagnosed (n = 116) and previously treated patients (n = 48)

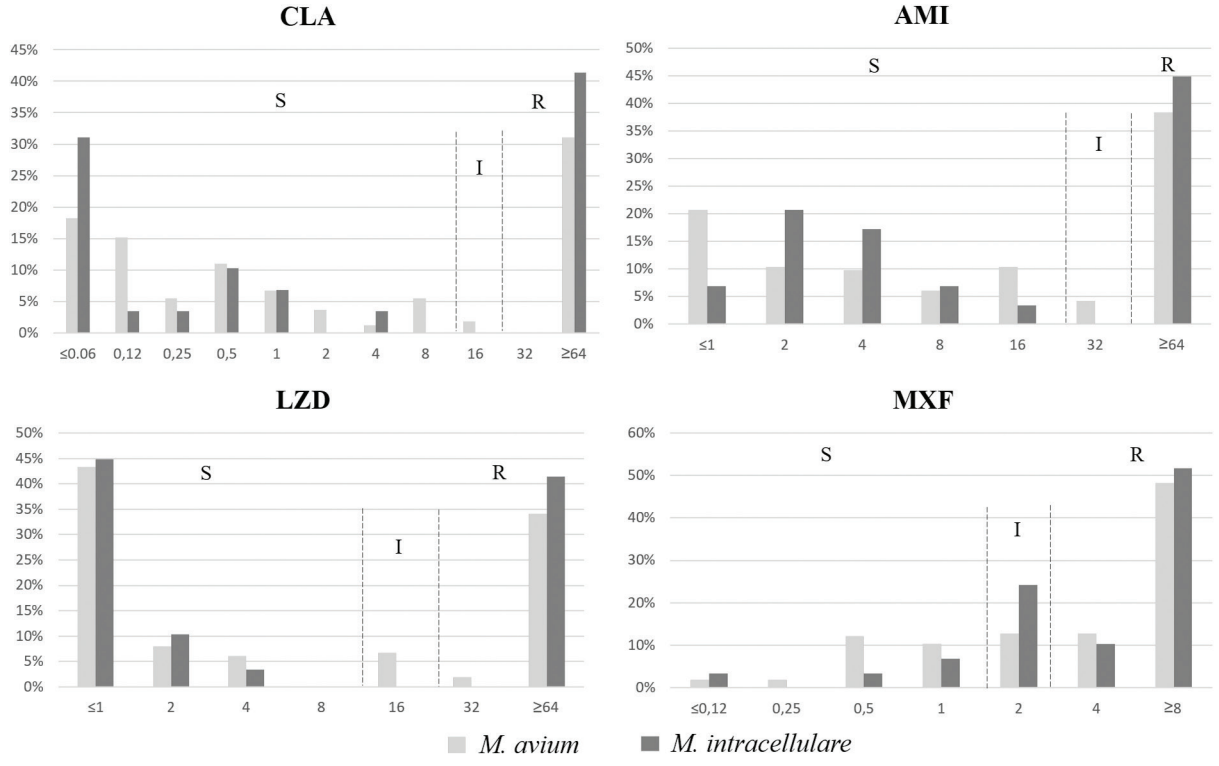

Figure 50. MIC distribution for CLA (clarithromycin), AMI (amikacin), MXF (moxifloxacin) and LZD (linezolid) in *M. avium* (n = 164) and *M. intracellulare* (n = 28) clinical isolates. The vertical dotted lines represent the MIC breakpoints for susceptible (S), intermediate (I), and resistant (R) MAC strains

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Book chapter

1. *Mokrousov I. Major Impact of Massive Migration on Spread of Mycobacterium tuberculosis Strains. In: Human Migration (Eds M. de Lourdes Moreno and M.H. Crawford) // Oxford University Press, 2021: 255–267. doi: 10.1093/oso/9780190945961.003.0020*

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2. *Bakuła Z., Wuyep V.B., Bartocha Ł., Vyazovaya A., Ikeh E.I., Bielecki J., Mokrousov I., Jagielski T. Molecular snapshot of drug-resistant Mycobacterium tuberculosis strains from the Plateau State, Nigeria // PLoS One. 2022; 17 (5): e0266837. doi: 10.1371/journal.pone.0266837*
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7. *Mokrousov I., Slavchev I., Solovieva N., Dogonadze M., Vyazovaya A., Valcheva V., Masharsky A., Belopolskaya O., Dimitrov S., Zhuravlev V., Portugal I., Perdigão J., Dobrikov G.M. Molecular Insight into Mycobacterium tuberculosis Resistance to Nitrofuranyl Amides Gained through Metagenomics-like Analysis of Spontaneous Mutants // Pharmaceuticals (Basel). 2022; 15 (9): 1136. doi: 10.3390/ph15091136.* ***Q1***
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9. *Mokrousov I., Vyazovaya A., Sinkov V., Gerasimova A., Ioannidis P., Jiao W., Khromova P., Papaventsis D., Pasechnik O., Perdigão J., Rastogi N., Shen A., Skiba Y., Solovieva N., Suffys P., Tafaj S., Umpeleva T., Vakhrusheva D., Yarusova I., Zhdanova S., Zhuravlev V., Ogarkov O. Practical approach to detection and surveillance of emerging highly resistant Mycobacterium tuberculosis Beijing 1071-32-cluster // Sci. Rep. 2021; 11 (1): 21392. doi: 10.1038/s41598-021-00890-7.* ***Q1***
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13. *Vinogradova T., Dogonadze M., Zabolotnykh N., Badleeva M., Yarusova I., Vyazovaya A., Gerasimova A., Zhdanova S., Vitovskaya M., Solovieva N., Pasechnik O., Ogarkov O., Mokrousov I. Extremely lethal and hypervirulent Mycobacterium tuberculosis strain cluster emerging in Far East, Russia // Emerg. Microbes Infect. 2021; 10 (1): 1691–1701. doi: 10.1080/22221751.2021.1967704.* ***Q1***
14. *Vyazovaya A., Felker I., Schwartz Y., Mokrousov I. Population structure of Mycobacterium tuberculosis from referral clinics in Western Siberia, Russia: Before and during the Covid-19 pandemic // Infect. Genet. Evol. 2022; 103: 105343. doi: 10.1016/j.meegid.2022.105343*
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4. *Patent pending. Mokrousov I., Vyazovaya A., Badleeva M et al. Method for detecting the genotype of Mycobacterium tuberculosis Beijing 14717-15-cluster. Application 2022122577, priority 23.08.2022.*
5. *Database "MIRU-VNTR profiles of strains of Mycobacterium tuberculosis isolated from patients with HIV-associated tuberculosis in St. Petersburg and the Leningrad region" Gerasimova A.A., Zhuravlev V.Y., Solovieva N.S., Mokrousov I.V., Narvskaya O.V., Vyazovaya A.A. Registration number 2021622630, Registration date 11/24/2021, Publication date 11/24/2021.*

LABORATORY OF MOLECULAR GENETIC MONITORING

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Genetic monitoring of SARS-CoV-2 in the Northwestern Federal District of Russia

Relevance of the study. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease (COVID-19). The COVID-19 pandemic is ongoing global pandemic that has been causing social and health complications since December 2019. These constituted major challenges to healthcare facilities and infrastructure that continue at present. In Russia, at the end of 2022, in total 21 717 748 COVID-19 cases and 393 712 fatalities have been identified. Systematic monitoring of key epidemiological indicators and genetic variants of SARS-CoV-2 is an integral part of anti-epidemiological measures, both at the regional and national levels. SARS-CoV-2 appears to be an actively evolving virus. As a result of intense and widespread circulation, SARS-CoV-2 genetic variants which pose an increased risk to global health began to appear in the second half of 2020. Some variants, due to their high transmissibility and epidemic potential, were classified by the World Health Organization (WHO) as variants of concern (VOC) and require increased vigilance by health authorities. At certain stages of the pandemic, strains of various lineages were given VOC status: Alpha (B.1.1.7); Beta (B.1.351); Gamma (P.1); Delta (B.1.617.2); and Omicron (B.1.1.529).

In 2021–2022 the team of laboratory, within the framework of the order of the Rospotrebnadzor, monitored SARS-CoV-2 strains on the territory Northwestern Federal District (NWFD) of Russia, the region with almost 14 million people population, which includes St. Petersburg, the second most populated city of Russia.

Study objective. The main study objective was to estimate key epidemiological indicators of COVID-19 epidemic in NWFD as well as estimate dynamics of genetic variants in the region.

Material and methods. In our study, we used statistical data from Rospotrebnadzor, obtained from an internal database based on daily regional reports.

In frame of SARS-CoV-2 genetic monitoring in Russia 10% of all positive samples from the clinics and hospitals in NWFD were sent to St. Petersburg Pasteur Institute for investigation. Nasopharyngeal swabs of patients with diagnosed COVID-19 were collected in 500 µL of special transport medium and stored at –20°C until further analysis. Molecular genetic methods include amino acid isolation, amplification of SARS-CoV-2 genome fragments. For genetic variants identification Sanger sequencing of S-gene was performed on ABI-3500 XL bioanalyzer. Belonging to the genetic variants was determined based on the presence of characteristic mutations in the S-gene, published on the WHO website.

Key results. In 2021, COVID-19 incidence in northwest Russia varied in waves, ranging from 258.8 per 100 K in April to 906.1 in December. At the same time, increases in incidence with the achievement of local maxima were recorded in January (1113.5 per 100 K), June (822.3 per 100 K), and November (1185.8 per 100 K) (Fig. 51).

In 2022 the COVID-19 cases dynamics was different, number of incidence dramatically varied during the year with highest level of COVID-19 cases in February (5544.3 per 100 K) to 105.4 per 100 K in June and 286.2 per 100 K in December, with a significantly fewer number of cases compared to Autumn of 2021 (Fig. 52).

SARS-CoV-2 genetic diversity in NWFD in the study period also varied and was characterized by the presence of variants of concern (VOC). During the first quarter of 2021, the 20B variant SARS-CoV-2 variant dominated in northwest Russia. However, in January 2021 the AT.1 SARS-CoV-2 variant was detected, which was admitted as a variant under monitoring (VUM). Its prevalence in January reached 1.1%, but reached 3.6% in March. A significant change in the genetic landscape began in May 2021. Dis-

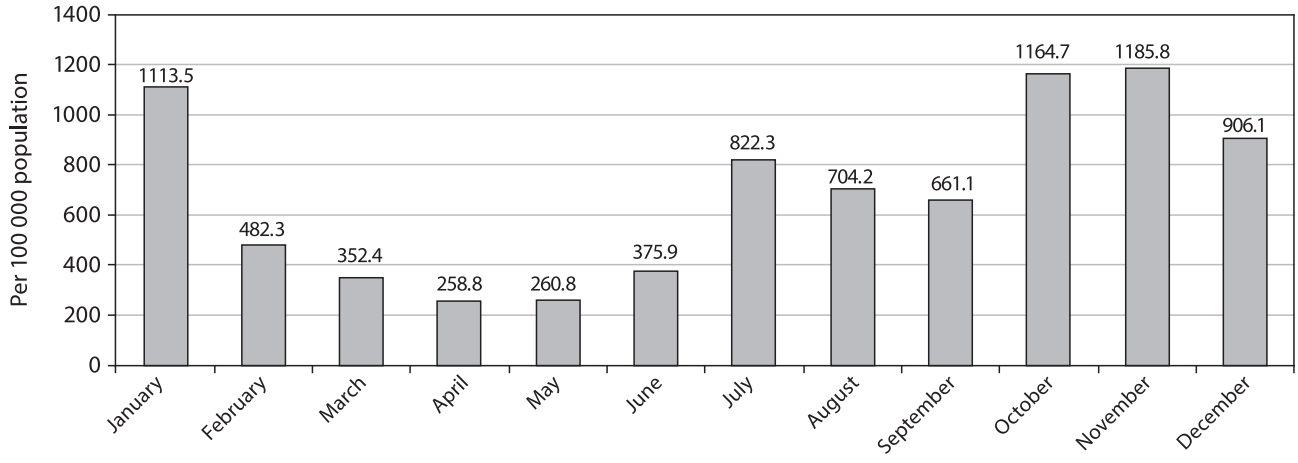

Figure 51. COVID-19 incidence in Northwest Russia in 2021. Periodic increases and decreases in incidence can be noted. Figure based on Rospotrebnadzor data

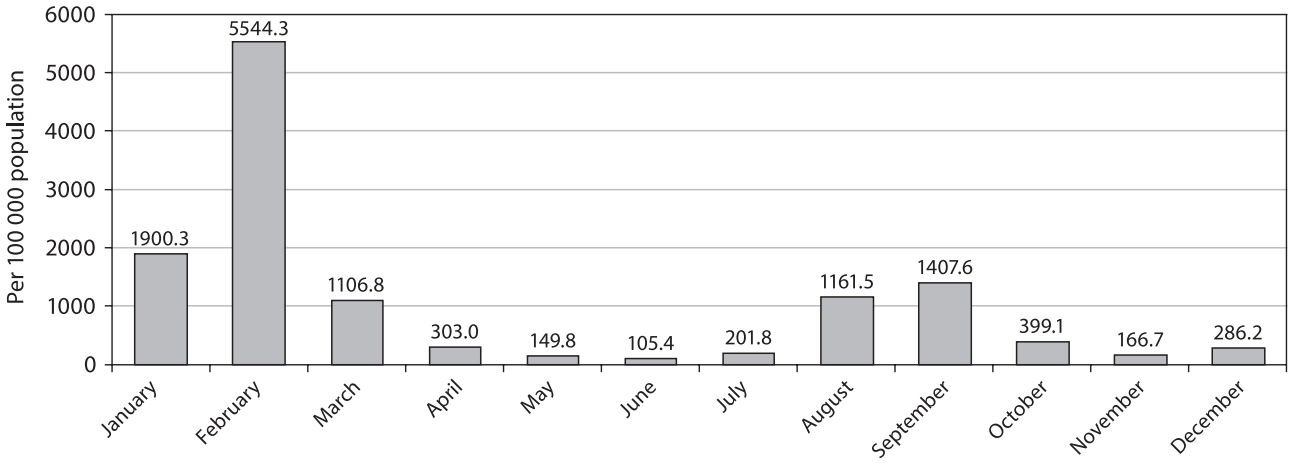

Figure 52. COVID-19 incidence in Northwest Russia in 2022. COVID-19 cases dynamics in 2022 was dramatically varied during the year, with highest level of COVID-19 cases in February. Figure based on Rospotrebnadzor data

placement of the wild-type virus continued, and its prevalence decreased to 42.4%. At the same time, AT.1 prevalence reached 28.2%, while Delta variant prevalence was 17.8%. The Alpha variant constituted 10.4% of all genetic variants in May. Such Delta variant dynamics led to the fact that, by the end of June, there was almost a complete displacement of all other SARS-CoV-2 variants. From the beginning of July to the middle of December, a complete dominance of the Delta variant was noted. Other VOCs, as well as wild type SARS-CoV-2, were eliminated (Fig. 53).

In 2022 Omicron was the dominant globally circulating variant of SARS-CoV-2. The first Omicron BA.1 variant case in St. Petersburg was registered in a woman arriving at Pulkovo Airport from the United Arab Emirates on 10 December 2021. In 2022, there was a further increase in the share of imported cases associated with Omicron strains. In January 2022 the share of Delta variant was 43%. On January 15, 2022 first case of BA.2 was identified in St. Petersburg.

The maximum percentage of BA.1 strains (69.8%) was registered on February, but to April the variant BA.2 almost completely substituted other variants. Next switch of genetic variants was in July with appearance and further expansion of new lineage BA.5. At the end of the year up to 20% consisted recombinants an subvariants of Omicron lineage BA.5 (Fig. 54).

Arrival and dissemination of Omicron Lineage SARS-CoV-2 in St. Petersburg, Russia

Relevance of the study. First isolated on November 11, 2021 the Omicron strain caused an explosive increase in incidence and rapidly spread globally, displacing the previously dominant Delta variant, despite the fact that 70% of the population in the developing/developed world were fully vaccinated. Currently, Omicron is the dominant glo-

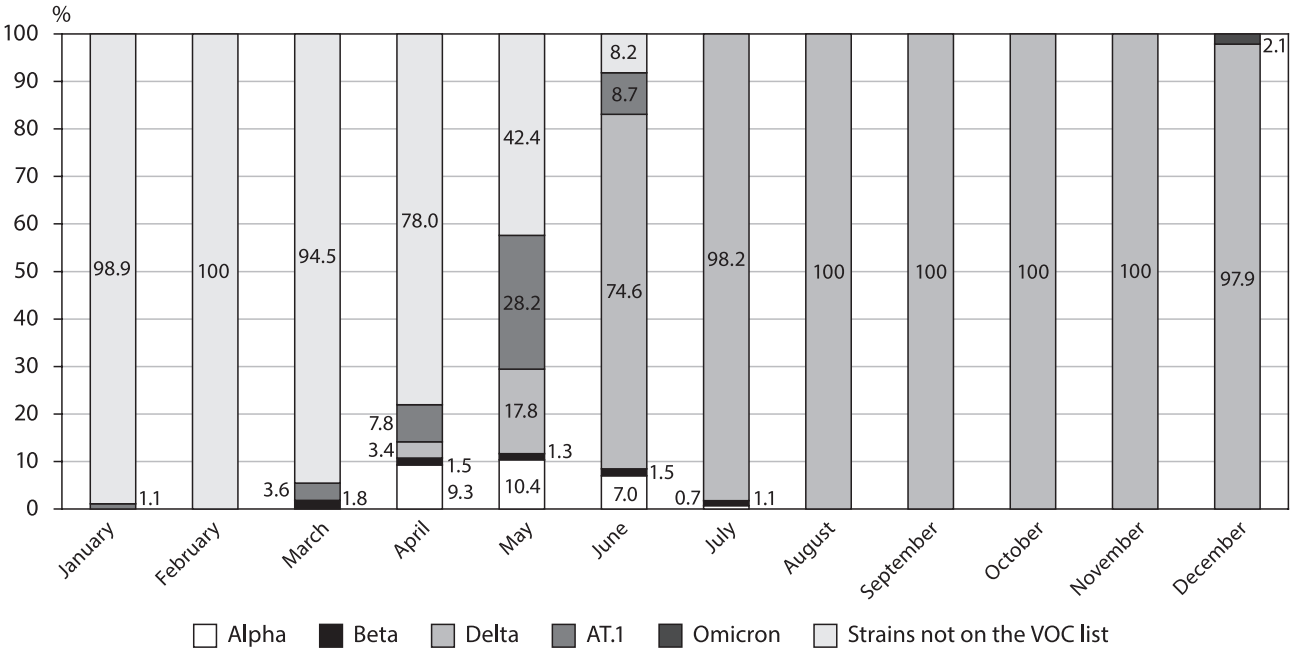

Figure 53. SARS-CoV-2 genetic diversity in Northwest Russia. From January to May 2021, various SARS-CoV-2 genetic variants, including VOCs, circulated in northwest Russia. However, from the beginning of June, they were completely displaced by the delta variant

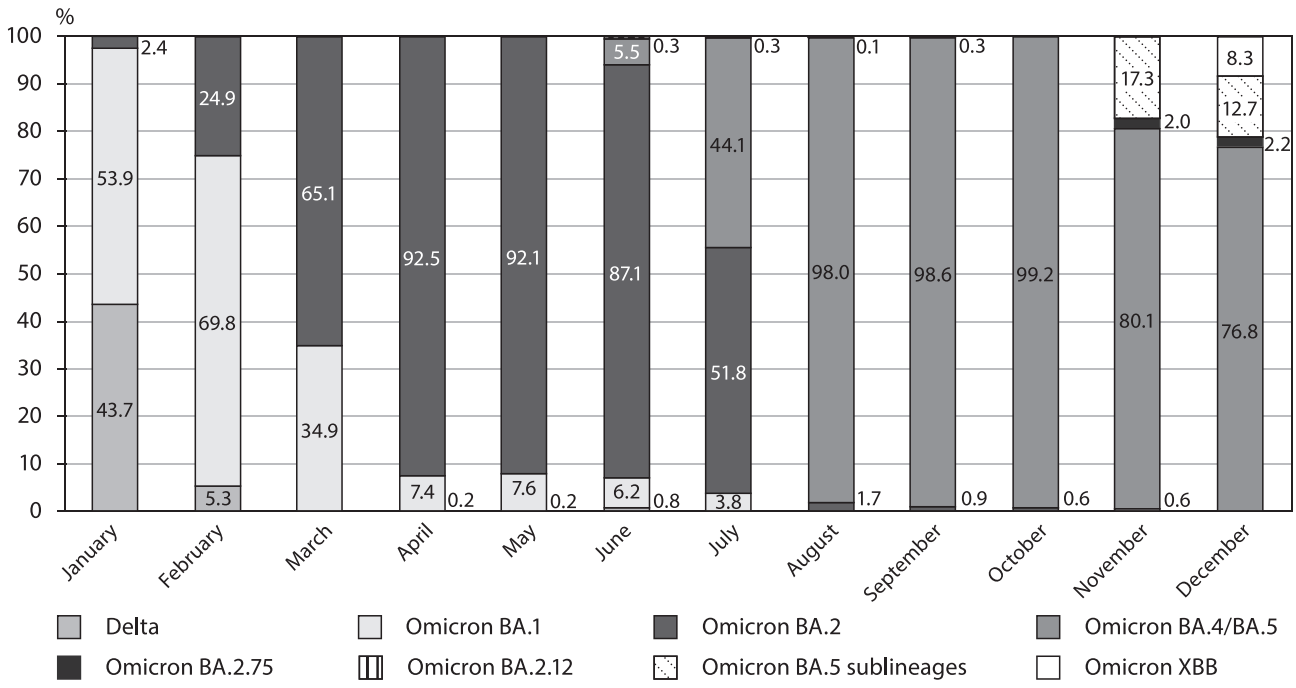

Figure 54. Genetic diversity of SARS-CoV-2 in Northwest Russia. Statistic analysis from January to December 2022 showed a rapid increase in the share from the Omicron BA.1 lineage and a decrease in the share of Delta in the beginning of the year and dominate of Omicron BA.5 variant at the end

bally circulating variant, accounting for > 98% of viral sequences shared on GISAID after February 2022.

St. Petersburg, Russia's second largest metropolis featuring a high population density, is home to Pulkovo International Airport. Without the strictest of quarantine measures, Pulkovo is most likely a gateway for the importation of new genetic variants into the city and their further distribution.

Study objective. Here, we analyze the dissemination dynamics of Omicron strains in St. Petersburg, Russia's second largest city. Our study is devoted to describing the distribution dynamics and variability of the Omicron BA.1 variant in St. Petersburg, as well as to trace the origin of the first imported strain, while assessing the effectiveness of the applied quarantine measures in preventing the spread of this kind of infection.

Material and methods. Nasopharyngeal swabs of patients with diagnosed COVID-19 during the period November 29, 2021 to May 01, 2022 were collected from hospitals, clinics, and at Pulkovo Airport (St. Petersburg) from arrivals and delivered to the St. Petersburg Pasteur Institute for sequencing and further genetic study. From hospitals and clinics, 10% of all positive samples were sent to the Institute for investigation. All COVID-19 positive samples from arrivals at Pulkovo airport with clinical manifestations, as well as without (voluntarily agreeing to examination), were sent to the Institute. Swabs were collected in 500 µL of special transport medium or phosphate buffered saline (pH 7.0) and stored at –20°C until further analysis. A total of 25 470 samples were examined, of which: 684 samples were obtained from persons arriving at Pulkovo Airport; 16 425 samples were from outpatients; and 8361 samples were from hospitalized patients in St. Petersburg.

Total nucleic acid samples were obtained by extraction and purification using the QIAamp Viral RNA Extraction Kit (QIAGEN, Germany) with the QIAcube Connect automatic station (QIAGEN, Germany). For screening of Omicron variants in the St. Petersburg population, an RT-PCR assay was developed with primers and probes to detect the BA.1 and BA.2 lineages. Initially, primers and probes were developed to detect the BA.1 lineage using a specific region (deletion 211 and insertion 214 in the S protein gene). Later primers and probes were also developed to detect the BA.2 lineage (using deletion 24–26 in the S gene).

In order to obtain near-complete SARS-CoV-2 genomic sequences (excluding 5′ and 3′ ends), a total of 138 primer pairs were designed using the Primal Scheme web-based design tool. The pairs produce 300–320 nt products with 50 nt overlaps. All SARS-CoV-2 sequence variants present in GISAID were considered at the moment of primer design. Reverse transcription used random hexamers and the Reverta-L Kit (AmpliSens®, Moscow, Russia).

Hot-start multiplex PCR amplification reactions were performed in a 25 µL total volume containing 2 µL of cDNA, 0.1 µM of each primer, and 12.5 µL of 2× BioMaster HS-Taq PCR mix (Biolabmix, Novosibirsk, Russia).The following thermal cycling parameters were used: 95°C for 3 min; 35 cycles (93°C for 10 s, 57°C for 30 s, 72°C for 30 s); and a final extension (72°C for 5 min). Reactions were performed in a C1000 Touch thermocycler (Bio-Rad, Hercules, CA, USA). Products were analyzed by 2.0% agarose gel electrophoresis in the presence of ethidium bromide. Amplified fragments cleaned by the AMPure XP Purification Kit (Beckman Coulter, UK), concentrations of the fragment mixes were measured with a Qubit 4.0 fluorimeter (Invitrogen, Waltham, MA, USA) using the dsDNA HS Assay Kit (Invitrogen, Waltham, MA, USA) and then used for library preparation.

Library preparation was performed according to the Illumina TruSeq Nano DNA Kit protocol with the TruSeq DNA CD Indexes Kit (Illumina Inc., San Diego, CA, USA). Amplicons were subjected to a series of enzymatic reactions: end repair; adenylation; and ligation of adapter sequences at the 5′ and 3′ ends. Products were then amplified by 8 cycles of PCR according to the protocol. The resulting libraries were purified using Illumina Sample Purification Beads and

eluted in 50 μL of resuspension buffer. Quality assessment of final libraries was carried out on the QIAxcel Advanced capillary system (QIAGEN, Hilden, Germany); fragment sizes (amplicon insert plus sequencing adapters) were about 420–450 bp. All libraries were quantified using the Qubit 4.0 fluorimeter and the Qubit dsDNA HS Assay Kit (Invitrogen) prior to sequencing. Sequencing was performed on the MiSeq instrument using MiSeq V3 chemistry.

The quality of Illumina reads was assessed using the FastQC program. Raw reads were filtered with Trimmomatic to remove adapters, low-quality nucleotides, and biased sequences at the ends of reads (parameters ILLUMINACLIP:TruSeq3-PE.fa:2:30:10:2SLIDINGWINDOW:4:20 HEADCROP:30 MINLEN:50). Genome assembly was carried out by mapping to the SARS-CoV-2 reference genome (Wuhan-Hu-1 strain, NCBI accession number NC_045512.2) using Bowtie 2. For variant calling and consensus generation, samtools and bcftools software were used. The Nextclade tool was used to assess the quality of assembled sequences and to assign genomes to lineages. Sequences were uploaded to GISAID.

Genomes were aligned with MAFFT v7.453. Ends (5′, 3′) were trimmed, and the phylogenetic tree was constructed with IQ-TREE. The workflow was tree reconstruction with ultrafast bootstrap (1000 replicates); the JC substitution model was used. The dendrogram was visualized with iTOL v.6. A global phylogenetic tree of SARS-CoV-2 variants was constructed using the tools implemented in Nextclade.

Phylogenetic network analyses were performed with Network v.10.2.0.0 using the median-joining algorithm.

Key results. After the first case of Omicron lineage BA.1 was registered in St. Petersburg rapid expansion of the variant and increased incidence followed. A weekly analysis of COVID-19 morbidity in St. Petersburg showed a rapid increase starting from 145.9 per 100 K population in early January and reaching 2409.5 in February. The peak incidence was reached in February 2022, followed by an observed decline coinciding with the beginning of spread of the BA.2 variant. SARS-CoV-2 lineage change dynamics were shown in three categories: airport arrivals; clinical outpatients; and clinical inpatients and showed similar patterns: a rapid increase in the share from the Omicron BA.1 lineage in January 2022 and a decrease in the share of Delta; later in January, sporadic detections of the BA.2 lineage changed by increase of BA.2 variants up to 10% to the middle of February, following rapid expansion of BA.2 variant (more then 90% to May 2022).

Variability within genomes of the BA.1 and BA.2 lineages in St. Petersburg was also revealed. In addition to 30 known S gene SNPs, solitary mutations were identified in positions E132Q, S162I, M177I, P230Q, I231L, T284S, N354H, A672V, S686R, I1081V, D1163Y, and D1260G. On the basis of phylogenetic analysis, an attempt was made to trace the origin of the first imported strain, the first Omicron strain detected in St. Petersburg (brought from the UAE) clustered together with two strains from Austria and is included in one cluster with a strain from Israel.

The dramatic increase in COVID-19 incidence observed in St. Petersburg in the beginning of 2022 was due to the rapid expansion of Omicron lineage BA.1. Moreover, its dissemination in the city has a multiple-import signature. An analysis of BA.1 diversity showed the presence of several clonal complexes (Fig. 55). The analysis identified 20 clusters, and the rest formed distinct nodes. Nodes differed from each other by one or two nucleotide substitutions. Five main clusters (100, 165, 175, 145, SARS-CoV-2) included both imported strains and strains from those without a history of international travel. This suggests that the spread of the Omicron lineage in St. Petersburg occurred due to multiple imports.

A decline in COVID-19 incidence coincided with the emergence and spread of lineage BA.2. Regional restrictions in the fight against highly contagious SARS-CoV-2 strains showed low efficiency, and they were unable to prevent the rapid spread of Omicron in St. Petersburg. Continued monitoring of SARS-CoV-2 variant succession, including genomic analysis to identify or track mutations, remains critically important. Such data are needed to follow viral evolution and to develop appropriate measures to prevent pandemic burdens and complications.

Several factors likely indicate that it is impossible to stop the spread of this kind of respiratory viral infection in the conditions of a modern metropolis in the absence of strict quarantine measures. These factors are high transmissivity, high viral variability, and the existence of multiple contacts. After confirming the discovery of a new viral variant, named Omicron, Russia and a number of countries globally suspended air traffic or introduced quarantine measures for citizens arriving from African countries. Monitoring of Pulkovo Airport arrivals by country revealed that, in the absence of direct flights from African countries, arrivals with Omicron strains were imported from Europe and Asia.

Comparative Analysis of Library Preparation Approaches for SARS-CoV-2 Genome Sequencing on the Illumina MiSeq Platform

Relevance of the study. Conducting genomic research on the virus in the context of the ongoing pandemic is an important tool for serving public health needs. Mass sequencing permits the analysis of: viral spread and variability; the emergence of new, potentially dangerous variants; the ability to evade vaccines and acquire immune escape; new ways to treat and prevent disease; nucleotide changes in the genome that can affect virus detection using clinical diagnostic tools, such as real-time PCR; specific antiviral strategies or designs, including vaccine candidates.

The WHO also highlights the importance of whole genome sequencing for public health needs, including monitoring for changes in SARS-CoV-2 genetic structure along with associated metadata, such as viral spread and activity, and the analysis of circulating strain diversity, with the tracking of SARS-CoV-2 geographic distribution over time.

SARS-CoV-2 genomic sequence data are an integral part of the effort to counter the COVID-19 pandemic. Phylogenetic data analysis makes it possible to effectively assess ongoing processes, observe changes in the virus, respond to them in a timely manner, and make objective forecasts regarding the development of the epidemiological process. Multiple high-throughput sequencing technologies have been used for SARS-CoV-2 sequencing. Participated in the comparison commercial kits from different manufacturers in terms of data quality, genomic coverage, SNP determination, number of reads, and sequencing depth is important purpose for SARS-CoV-2 surveillance.

Study objective. In this study, we evaluated and compared several approaches for library preparation for SARS-CoV-2 whole genome sequencing using the Illumina MiSeq platform.

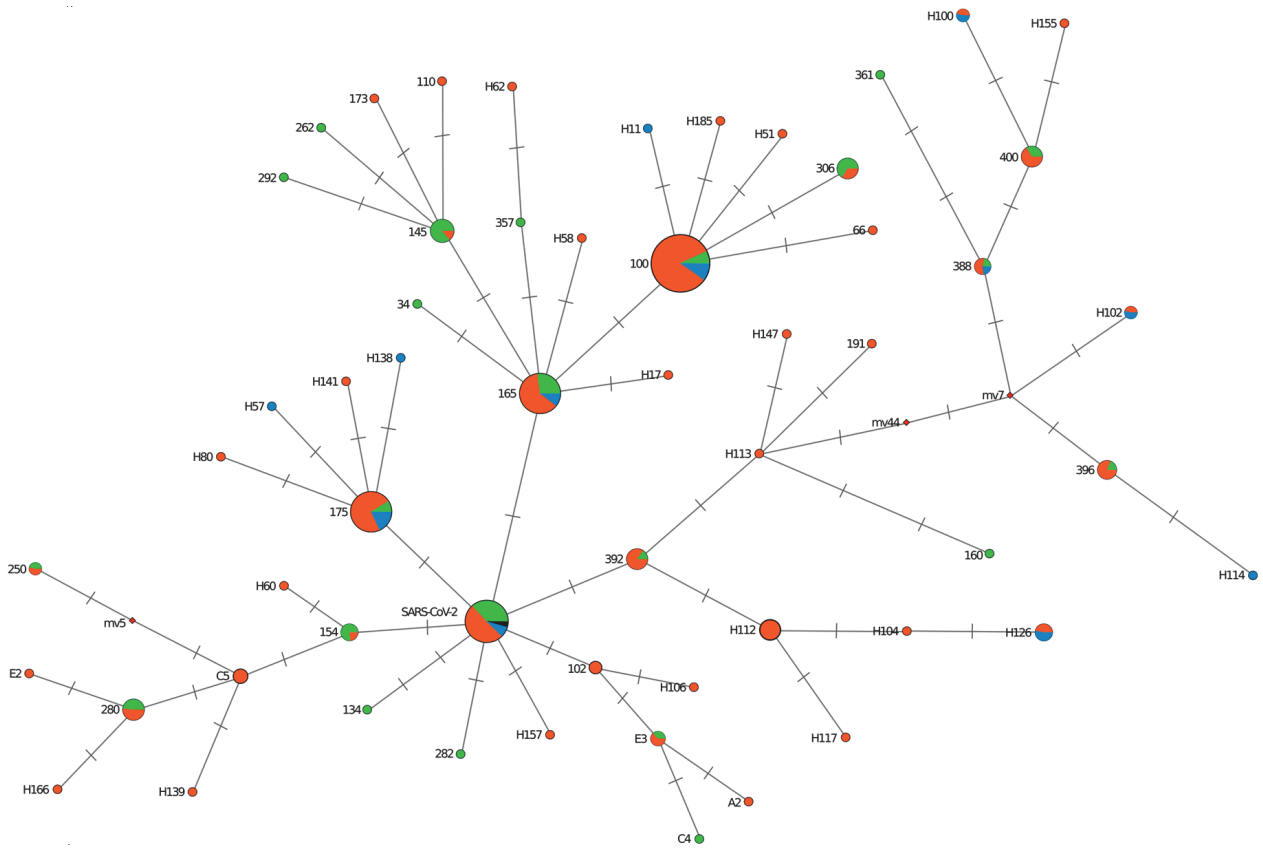

Figure 55. Phylogenetic network of SARS-CoV-2 Omicron BA.1 S gene sequences

Circle areas are proportional to the number of taxa, whereas each tick on the links represents a mutated nucleotide position. The median-joining network algorithm (epsilon parameter set to 10) was employed. Clusters are named by one of the sequences forming it. The node pie chart coloring illustrates the proportion of each group in the node. Key: strains introduced to St. Petersburg by Pulkovo Airport arrivals — green; strains from outpatients — orange; strains from inpatients — blue; and South African reference strain (EPI_ISL_6913991) — black.

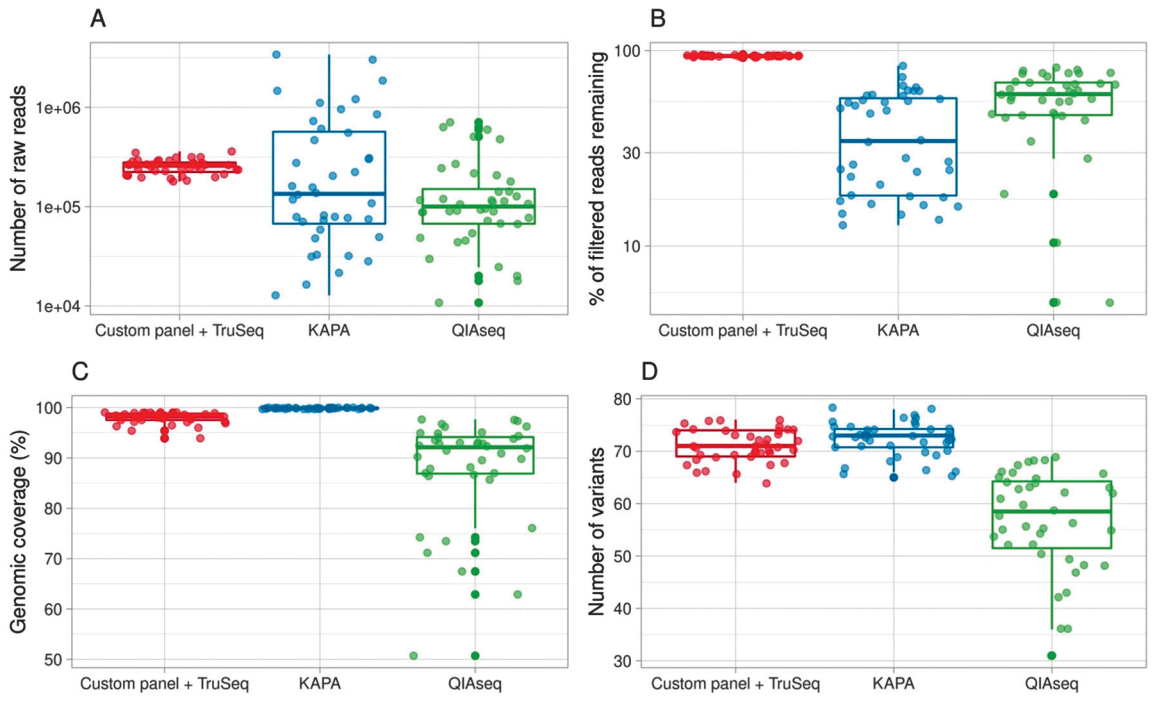

Figure 56. Comparison of the different library preparation kits

The x-axis shows the method. Boxplots show: (A) — number of raw reads achieved after Illumina MiSeq sequencing (y-axis is lg scaled); (B) — percent reads remaining after QC-trimming; (C) — percent SARS-CoV-2 genomic coverage; (D) — number of identified SNPs. Key: red — TruSeq DNA Nano Library Kit with custom primer panel; blue — KAPA HyperCap SARS-CoV-2; green — QIAseq DIRECT SARS-CoV-2.

Material and methods. The whole genome sequencing SARS-CoV-2 based on patient nasal swabs samples Northwestern Federal District of Russia (viral RNA load up to 23 cycles). For SARS-CoV-2 detection and to assess viral load, swabs were thoroughly analyzed using the COVID-19 Amp RT-PCR Kit (St. Petersburg Pasteur Institute, St. Petersburg, Russia). Total nucleic acid samples were obtained by extraction and purification using the QIAamp® Viral RNA Extraction Kit® (QIAGEN, Hilden, Germany) with the QIAcube Connect automatic station (QIAGEN, Hilden, Germany), according to the manufacturer's recommendations.

We choose three different library approaches: the amplicon-based QIAseq DIRECT SARS-CoV-2 Kit (Qiagen, Hilden, Germany), the target capture-based KAPA HyperCap SARS-CoV-2 Kit (Roche, Mannheim, Germany), and a whole genome sequencing custom primer panel (developed by the authors) adapted to the TruSeq Nano DNA Library Preparation Kit (Illumina, San Diego, CA, USA).

QIAseq DIRECT SARS-CoV-2 Library and KAPA HyperCap SARS-CoV-2 Library were prepared according to the protocol provided by the manufacturer. Custom Primer Panel with the TruSeq Nano DNA Library Preparation Kit consist of 138 primer pairs were designed in previous work. The target enrichment of the resulting cDNA was then performed with 6 primer pools. Sequencing was performed on the Illumina MiSeq platform. Assembly and SNP analysis was performed as described before. Statistical analysis was performed with R statistical language (R Core Team, 2022).

Key results. After the exclusion of samples with fewer than 10 000 reads in at least one method (one TruSeq, one QIAseq, and four KAPA) forty samples were taken for analysis. The number of obtained reads varied with the KAPA and QIAseq methods; it was quite uniform with TruSeq (Fig. 56A). The parameter "percent reads remaining after trimming" is presented in Fig. 56B. The quality of data obtained with "custom primer panel + TruSeq DNA Nano Library Kit" is high, whereas libraries prepared with QIAseq DIRECT SARS-CoV-2 or KAPA HyperCap SARS-CoV-2 kits yield numerous short reads.

All three approaches used in the study produced SNPs in the same positions subject to genomic coverage in the area. Two methods (KAPA HyperCap SARS-CoV-2 and TruSeq DNA Nano with custom panel) allowed the identification of the same number of SNPs, while less SNPs were identified with QIAseq DIRECT SARS-CoV-2 Kit.

Median coverage per position is shown in Fig. 57. Sequencing depth from libraries prepared with KAPA HyperCap SARS-CoV-2 was the most uniform along the entire genome. The "custom primer panel + TruSeq DNA Nano" and QIAseq DIRECT SARS-CoV-2 Library Kit approaches offered uneven genomic coverage in different genomic regions. The "custom primer panel + TruSeq DNA Nano" approach produced quite satisfactory coverage except for several regions.

When evaluating the results obtained, it can be concluded that all three approaches to creating libraries for SARS-CoV-2 sequencing can be used for research purposes. Each approach has its own advantages. In particular, with QIAseq DIRECT SARS-CoV-2, this is the speed of library preparation and a simple workflow. The capture-based KAPA HyperCap SARS-CoV-2 panel demonstrates the most accurate and complete coverage of the genome; however, in our experiments, we did not achieve a uniform distribution of reads across the samples. The developed custom panel has reliably proven itself in the genetic monitoring of various variants. There were no significant differences in genomic coverage or sequencing depth between the Delta and Omicron variants. Thus, at the moment, the custom panel is quite versatile for different variants of SARS-CoV-2, it presents results comparable to other platforms, but with a rather simple workflow. Its predictable distribution of reads per sample is well suited for monitoring genetic variants as part of COVID-19 surveillance. In addition, the user panel remains compatible with new variants. Despite the introduction of a new set of Omicron mutations, the panel produces results of predictable quality. Our results shows, that amplicon approach is suitable for routine monitoring, while the hybridization capture approach is more valuable for scientific research and the discovery of new mutations and variants.

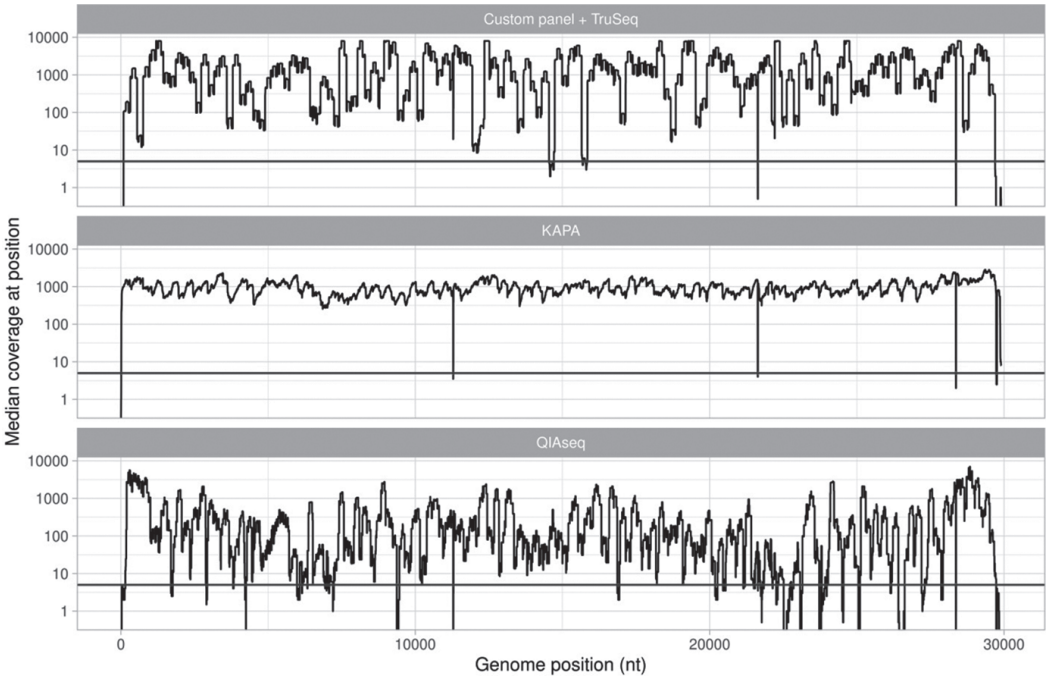

Figure 57. Median sequencing depth across the genome, depending on sample preparation method

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LABORATORY OF MOLECULAR GENETICS OF PATHOGENS

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I. Molecular diagnostics tools development

1. Real-time PCR diagnostic systems

A number of systems have been developed for the diagnosis of natural focal and other infections: SARS-CoV-2, Monkeypox virus (MPXV), Crimean–Congo hemorrhagic fever virus (CCHFV), Rabies virus (RABV), Measles virus (MV), Poliovirus type 2 (nOPV2), Nipah virus (NiV), Hendra virus (HeV), Bandia virus (BDAV), Kemerovo virus (KEMV). A system for differential diagnosis of Bunyamwera (BUNV)/Batai (BATV)/Ngari (NRIV) viruses has been developed. All systems have sensitivity of 10^2–10^3 copies per ml of the test sample.

2. Diagnosis of *Salmonella* Typhi in LAMP format

To date, several sets of primers have been described for the diagnosis of *S.* Typhi in LAMP format. These sets of primers were tested on strains specific to Malaysia and China. We evaluated these three LAMP variants to identify *S.* Typhi strains characteristic of the Russian Federation, and compared them with each other. The primer sets were taken from three publications. SALTYP1 is a fragment of the STY1607 gene from the article by Fan et al. (2015), SalTyp2 is from an article by the same authors in PLOS One (Fan et al., 2015), SalTyp3 is from a publication by Abdulla et al. (2014). Plasmids based on the target sequences were made to be used as positive controls. Amplicon was cloned into pGEM®-T Easy plasmid (Promega, Madison, USA).

Sensitivity

Sensitivity was measured on a series of dilutions of specific plasmids starting from 1×10^7 to 5 copies per reaction. For the SalTyp2 variant, dilutions higher than 1×10^6 were not detected. For SalTyp1 and SalTyp3, the sensitivity was 20 copies per reaction. This is comparable to the results in (Fan et al., 2015), in which the sensitivity was measured in the same way, in reaction copies using recombinant plasmids, and amounted to 15 copies per reaction.

Bioinformatics analysis

For SALTYP 1, the target sequence has analogues in a number of *Escherichia coli* strains: strain 90-9133, strain F16EC0617, strain RIVM_C036569, strain M-17, isolate L4_E1441_ETEC, strain 18SC05VL02-EC, strain RHB30-C19, O78 strain 3. For SalTyp 2, the sequence coincides with that of *Escherichia coli* strain Z0117EC0133 and *Escherichia coli* strain JL05. For SalTyp 3, the BLAST application found no matches with known strains that are not *Salmonella* Typhi. The search for homologies for SalTyp 1 among Russian strains revealed *Escherichia coli* strain EI0304, which contains 202 identical nucleotide residues, but does not overlap with the F3 primer. For SalTyp 2, a strain of *Escherichia coli* EI0386 was found, in which there was an almost identical sequence, with the only difference being in one nucleotide at the ends. Obviously, in this case, a false positive reaction can be expected. The *Escherichia coli* strain EI0274 contains 175 identical nucleotides of the target sequence of the SalTyp 3 system of the 221, but it does not overlap with the B3 primer, so there will be no positive signal here. It can be seen from the analysis that only for SalTyp 3 the sequence to which the primers are directed is selected correctly and will not produce false positive results (Fig. 58).

As a result, it is the third variant that is recommended for the detection of *Salmonella* Typhi. Specificity testing on 20 strains of *S.* Typhi isolates and 90 strains of other bacteria of 27 different species showed 100% specificity of the method.

3. CRISPR/Cas Detection Systems

Currently, CRISPR/Cas systems are widely used not only in genome editing, but also in various diagnostic platforms, e.g., DETECTR with the use of Cas12a protein and SHERLOCK with Cas13. The principle of operation of these methods is the ability of Cas12a and Cas13 proteins to trans-activity.

In order to recognize the target DNA and carry out nuclease activity, the Cas12a protein needs: 1) an RNA sequence for binding directly to the Cas12a enzyme, having a pseudo-hairpin structure, 2) a complementary part of the target DNA. Most often, these two sequences are combined into a single guide RNA (hereinafter gRNA). In addition, there is a restriction on the choice of the detection target: there must be PAM (protospacer adjacent motif) 5′-TTTN-3′ on the chain opposite the target sequence, which is complementary to the gRNA. Presumably, the PAM sequence serves as a seed for the divergence of two chains of the target DNA and hybridization of one of them with gRNA to form an R-loop.

The formation of the R-loop causes conformational changes in Cas12a, leading to the activation of nuclease activity in the RuvC domain, in which both chains of the target DNA are alternately cleaved to form cohesive ends. After that, this domain is able to non-specifically cleave any other single-stranded DNA. Thus, if fluorescent probes (short oligonucleotides with a fluorophore at one end and a suppressor at the other) are added to the solution as ssDNA, then an optical signal appears during their cleavage, which can be used to judge the presence of the target dsDNA in the solution.

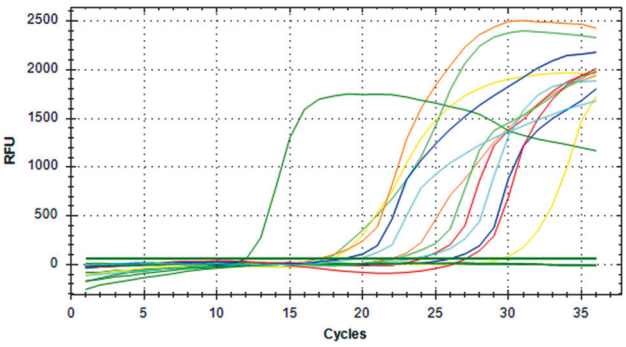

Figure 58. Real-time amplification on CFX96 Touch device to determine the sensitivity of detection reactions for SalTyp 3 variant

Dilutions in copies of the plasmid for the reaction: dark green — 1×10^7, red — 100, orange — 80, yellow — 60, light green — 40, blue — 30, blue — 20, black — 10, gray — 0.

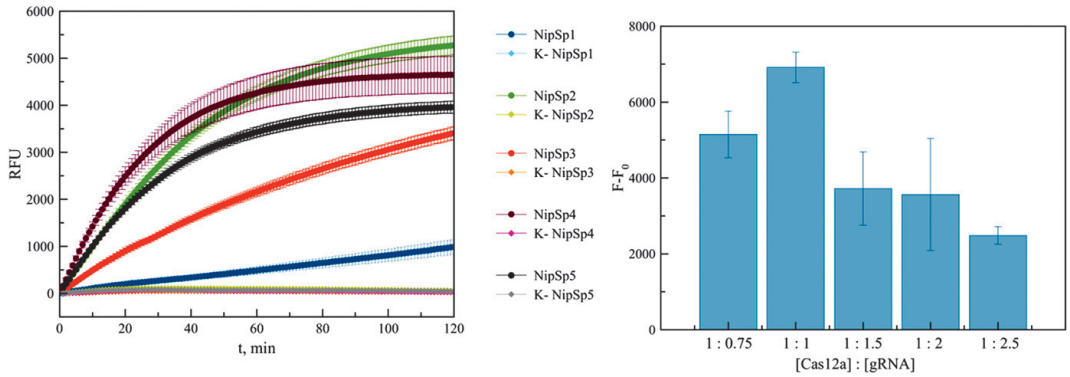

Figure 59. On the left: the averaged fluorescence signal via the FAM channel at CFX96 at Cas12a detection of Nipah virus with different guide RNAs. On the right: optimization of gRNA concentration relative to Cas12a

According to the assessment of the fundamental limitations of the kinetics of the stages of catalytic trans-activity of these Cas proteins, the sensitivity of such diagnostic platforms without amplification is in the order of picamol/l or 6×10^5 copies of the target DNA per ml. When combining these methods with amplification, it is possible to reach the detection limit of 10 copies/µl. The possibility of combining Cas detection with isothermal amplification (RPA, LAMP) in one test tube is particularly promising.

This paper describes the development of Nipah and Hendra virus detection systems based on the Cas12a DETECTR diagnostic platform.

Optimization of guide RNAs

Guide RNAs consist of two parts: a spacer complementary to the target DNA (23–24 nucleotides) and a loop interacting with Cas12a (21 nucleotides), which is the same for all considered variants of gRNA. The choice of spacer is limited for Cas12a due to the need for a PAM sequence (5′-TTTX-3′) on the chain of a complementary target sequence. The PAM sequence itself is not included in the guide RNA chain. For each target sequence of Nipah and Hendra, several variants of guide RNAs have been selected, which differ from each other by the spacer.

The gRNAs selected for the Nipah virus were NipSp1, NipSp2, NipSp3, NipSp4, and NipSp5. Similarly to the protocols from the articles [8, 10] concentrations initially selected for detection were c(Cas12a) = 100 nM, c(gRNA) = 200 nM, s(probe) = 1000 nM with the addition of 1 µl of the purified PCR product of the target DNA. Figure 59 shows the results of Nipah detection with different gRNAs. The highest value of the fluorescence signal was found when using gRNA NipSp2 (green) and NipSp4 (brown).

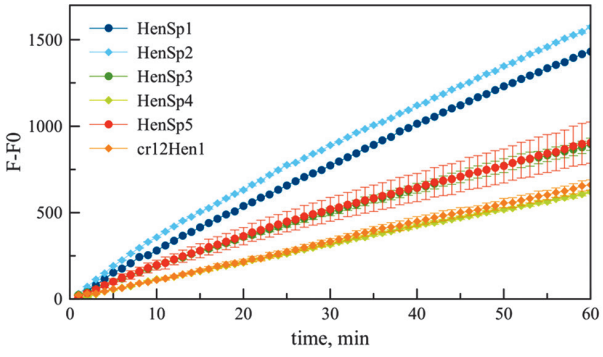

Figure 60. The averaged fluorescence signal via the FAM channel at CFX96 at Cas12a detection of Hendra virus with different guide RNAs

The concentration of gRNA relative to the concentration of Cas12a protein was optimized on Cas12a/NipSp2 complexes (Fig. 59 on the right). The most preferable concentration ratio was found to be [Cas12a]:[gRNA] = 1:1, i.e. c(Cas12a) = c(gRNA) = 100 nM.

Similarly, the most optimal guide RNAs for the detection of the Hendra virus were selected: HenSp1, HenSp2, HenSp3, HenSp4, HenSp5, Cr12Hen. The highest values of fluorescence relative to the background signal are observed when using HenSp1 (Fig. 60, deep blue) and HenSp2 (Fig. 60, light blue).

Selection of Cas12a detection conditions

Two variants of Lba Cas12a proteins and corresponding buffers to them from different manufacturers (New England Biolabs and GenScript) were analyzed. The reaction temperature of +40...+42°C turned out to be optimal for NEB enzyme (Fig. 61 on the left), whereas lower temperature reduces the efficiency of the reaction, and higher temperature leads to an increase in the signal spread. The temperature of +37°C was preferable for the protein from GenScript (Fig. 61, on the right).

The effect of preincubation of Cas12a complexes with gRNA on the efficiency of the reaction was investigated. It was found that detection is equally effective for cases of pre-incubation for 10 minutes at +25°C and 30 minutes at +37°C after subtracting background fluorescence in K– samples. Presumably, this is due to the fact that binding between Cas12a with guide RNA and finding the target DNA occurs in very short periods of time over a wide temperature range. The study of reaction kinetics confirms that the rate of complexation significantly exceeds the rate of protein trans-activity (Huyke D.A. et al., 2022).

Fluorophore optimization

Short oligonucleotides modified from the 5′ end with a fluorophore (FAM or HEX) and from the 3′ end with a suppressor (BHQ1) were used as probes in this work. With complementary binding of Cas12a/gRNA complexes to the target site, nuclease trans-activity is activated in the enzyme, which means the ability to cleave not only double-stranded target DNA, but also all single-stranded DNA in solution. Thus, after probes splitting, the fluorophore becomes free from the quencher, which leads to the emergence of a signal. 3 variants of probes with different oligonucleotide lengths (5 and 8 nucleotides) and two types of fluorophores have been examined. Fig. 62 shows that with all the probes examined, there is an increase in the detection signal, which eventually reaches saturation. When a longer chain (FB-long) probe is used, the signal appears faster, but after 20 minu-

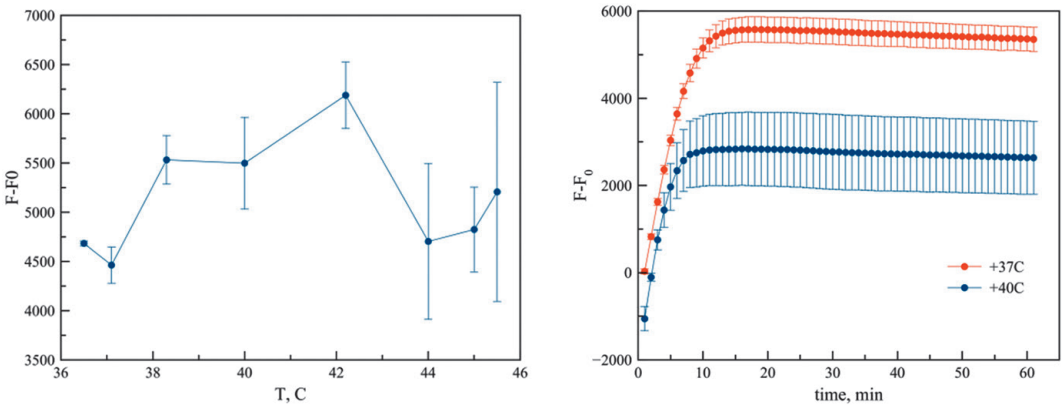

Figure 61. On the left: the temperature dependence of the magnitude of the averaged fluorescence signal for Cas12a detection when using an enzyme kit and a buffer from NEB. On the right: a comparison of the fluorescence signal at different detection temperatures for a set of enzyme and buffer from GenScript

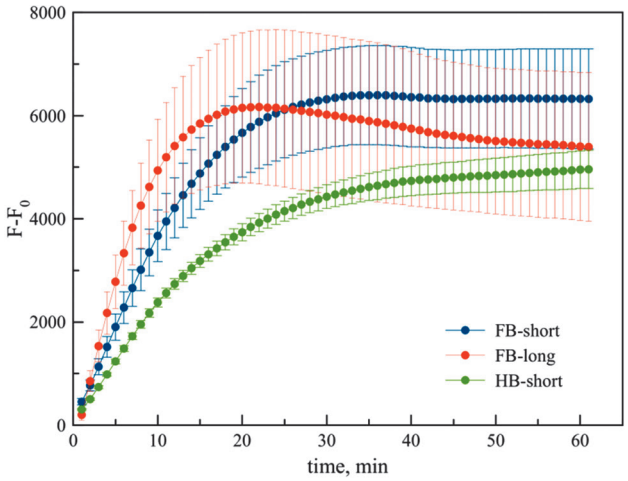

Figure 62. Comparison of probes in Cas12a detection

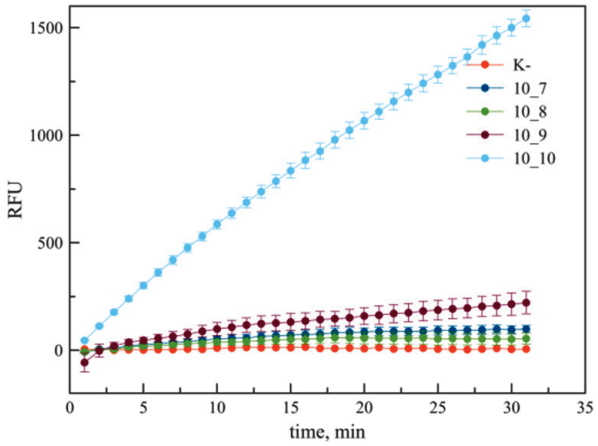

Figure 63. Testing the sensitivity of Cas12a detection for Nipah virus using NipSp2 gRNA

tes it begins to decrease. The probe with HEX fluorophore shows a smaller signal than with FAM. The choice of the probe concentration depends not only on the concentration of Cas12a/gRNA complexes, but also on the sensitivity of the fluorimeter. For Real-time CFX96 Touch BioRad, the best signal-to-noise ratio was with probe concentrations of 1000 and 2000 µM. In the case of the T16-ISO axxin fluorimeter, the optimal concentration of probes was 500 microns.

Sensitivity of the method

The target DNA site was preamplified and diluted with 10-fold dilutions to concentrations of 10^{10}–10^{4} copies/µl. The analytical sensitivity of the method was 10^{7} copies/µl (Fig. 63).

4. Detection systems based on deoxyribozymes

The Hendra and Nipah viruses (*Paramyxoviridae* family, *Henipavirus* genus) are highly pathogenic RNA-containing viruses, whose natural host is the fruit bat (genus *Pteropus*). These viruses cause outbreaks of serious diseases in humans and livestock in Australia, Malaysia, Singapore, and Bangladesh. The genetic similarity of these two viruses is about 80%, and the course of infection with them is also similar: from an asymptomatic or mild flu-like condition to a fatal respiratory or neurological disease.

Modern diagnostics uses real-time PCR to detect pathogens or ELISA to detect antibodies. Both technologies are time-consuming and require the use of expensive stationary equipment. Thus, there is a need to develop alternative simple, fast and accurate test systems for virus detection. The aim of this study is to develop a highly specific and easily standardized system for detecting Hendra and Nipah RNA viruses. We have developed a biosensor design based on the catalytic core of deoxyribozyme 10-23.

Deoxyribozymes are short, synthetic DNA oligonucleotides that have catalytic activity. Many of them catalyze the chemical reaction of cleavage of the phosphodiester linkage between nucleotides in the presence of divalent metal ions. For our test system, deoxyribozyme 10-23 was chosen as the catalytic core, since it is quite well studied, able to cleave the bond between any purine-pyrimidine pairs, uses magnesium cations as a cofactor and works at pH 7.4. To detect the target RNA, 2 sequences (binary Dz1 and Dz2) are synthesized, each consisting of a complementary portion of the target RNA, half of the catalytic core and a complementary portion of the fluorescent substrate. Thus, in the presence of the target RNA in the solution, due to Watson–Crick interactions, RNA binding occurs, as a result of which a catalytic core is assembled, which cleaves the substrate labeled with a fluorophore and a quencher (Fig. 64).

Unique target sequences were selected for both viruses (Fig. 65A and 65B): two for the Nipah virus and one for the Hendra virus; the biosensor design was common, the difference was only in the shoulder sequences that will bind the targeted RNA (Fig. 65C, 2D and 2E). In the biosensor de-

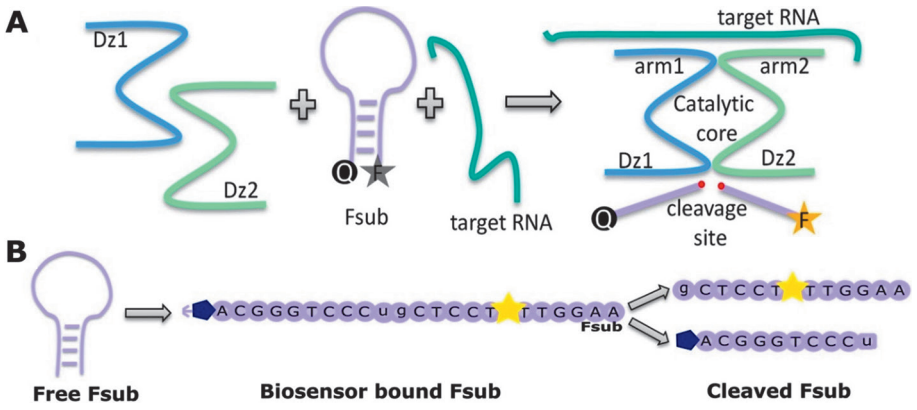

Figure 64. Scheme of target RNA detection using a binary probe

(A) Target RNA detection at the presence of binary probe and fluorescence substrate (Fsub). (B) The Fsub structure before and after its cleavage with Dz_NiV.

sign, the T1 chain acts as a platform or "core", the T2 and T3 threads are complementary to the T1 core, but, nevertheless, the T2 chain is connected to the Dz1 chain through a hexaethylene glycol linker. The Dz_2 chain is a free oligonucleotide that does not interact with T3, which is needed to exclude the possibility of assembling the catalytic core of DNAzyme 10-23 in the absence of the target RNA. T1, T2_Dz1 and T3 require pre-assembly in the buffer for assembly at 95°C for 5 minutes. We also found out that after cooling, the assembled structures can be stored at –20°C, and they do not lose their catalytic properties. We named these constructs Dz_NiV and Dz_HeV, in accordance with the viruses they define.

To test the specificity of target RNA recognition, each biosensor was incubated with RNA sites of five more different RNA viruses of similar length: Machupo, Sabia, Junin, Guanarito and SARS-CoV, and positive results were obtained only if target RNA was present in the reaction mixture (Fig. 66A, B and C).

To optimize the cleavage reaction, we determined the influence of such factors as pH and Mg^{2+} cations concentration. The acidity of the reaction buffer has an important effect on the physicochemical properties and biological activity of proteins and nucleic acids. To determine the effect of pH on our detection system, we tested the buffers for splitting with pH = 5; 6.5; 7; 7.5; 8; 8.5 and 9. The biosensor was most active at pH 7.5. At a more acidic pH (5–7), the fluorescent signal, both from the control and from the samples, was 4–6 times less than at pH 7.5. At a pH above 7.5, nonspecific denaturation of the fluorescent substrate was detected, leading to a significant decrease in the F1/F0 ratio.

The presence of magnesium cations is necessary for the formation of the correct structure of the DNAzyme and the cutting of the substrate, i.e., the presence of Mg^{2+} ultimately affects the magnitude of the fluorescence signal. For all biosensors, the presence of 50 mM Mg^{2+} was optimal, since with an increase in the magnesium concentration above 50 mM, the catalytic activity decreased, and the value of the fluorescent signal in samples containing targeted RNA decreased. With the addition of 10 mM Mg^{2+}, the situation was reversed: cleavage in the samples with the addition of RNA was active and the fluorescence value

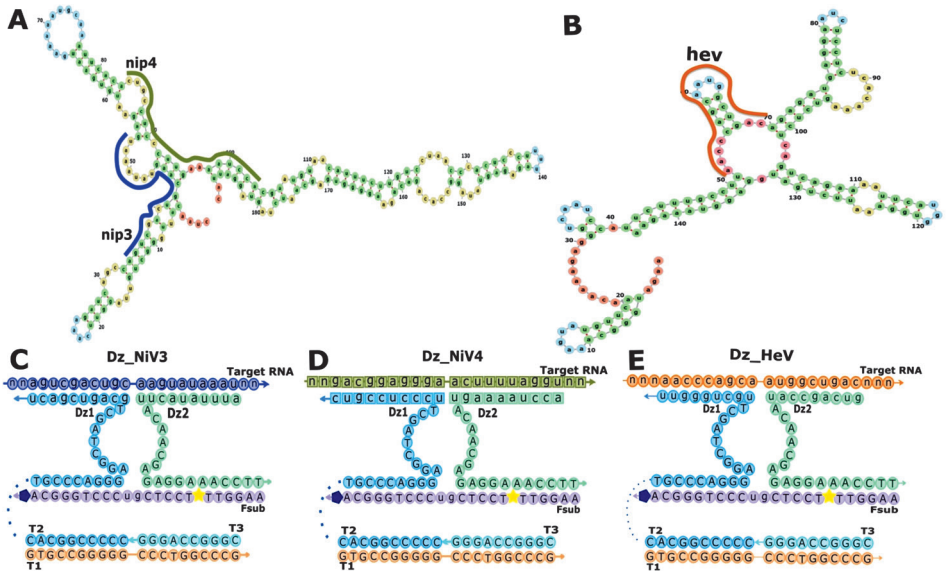

Figure 65. The principle of deoxyribozyme biosensor design and activity

(A) Secondary structure of the G gene of Nipah virus. Purple and green lines indicate RNA regions complementary to DNAzyme arms Dz_NiV3 and Dz_NiV4, respectively. (B) Secondary structure of the N gene of Hendra virus. Orange line indicates RNA region complementary to DNAzyme arms Dz_HeV. (C) Dz_NiV3 design consisting of T1, T3, nip_Dz1_T2_v3 and nip_Dz2_v3. (D) Dz_NiV4 design consisting of T1, T3, nip_Dz1_T2_v4 and nip_Dz2_v4. (E) Dz_HeV design consisting of T1, T3, hev_Dz1_T2 and hev_Dz2. The arrows indicate the 5'–3' direction of the sequence. The yellow stars indicate FAM fluorophore, the blue pentagons — BHQ1 quencher, dotted lines connecting oligos — hexaethylene glycol linker (HEG).

in them was at the level of samples containing 50 mM Mg^{2+}, however, the control fluorescence value also increased significantly, which is why the F1/F0 ratio decreased.

The next step was to estimate the limit of detection (LOD) for biosensors. For this experiment, samples containing 100 nM of already annealed biosensors and 100 nM Fsub were incubated with various amounts of targeted RNA (5 nM, 10 nM, 25 nM, 50 nM and 100 nM) for 20 minutes at 37°C. According to the data obtained, the sensitivity of biosensors reaches 10 nM (Fig. 67A, B and C).

During the work, the researchers designed inexpensive, high-precision, fast biosensors based on deoxyribosime 10-23 to detect Nipah and Hendra viruses. In the presence of targeted RNA and magnesium ions, the catalytic core of the DNAzyme is assembled, which cleaves the hairpin substrate labeled with a fluorophore and a quencher, due to which the fluorophore is released into the solution. 20 minutes is enough to obtain the detection result, and incubation takes place at 37°C. The biosensor requires pre-assembly, but it can be stored at –20°C, which is important when creating a test system with pre-amplification and detection stages and transferring it to users. In the future, we plan to optimize biosensors for detecting viral RNA in clinical samples. Biological samples may contain insufficient copies of the virus to be detected by a biosensor, so the test system being developed will include the following steps: reverse transcription, amplification, transcription and detection.

II. Development of an ELISA format diagnostic method for HPV16 papillomavirus infection

HPV16 papillomavirus infection causes cervical cancer, cancers of the anogenital tract and oral cavity. Protein L1 (56 kDa) is the main capsid protein of papillomaviruses (HPV) and is part of the preventive vaccines available in Russia, Cervarix and Gardasil. In natural human contact with the infection, only in 15% of cases are specific IgG antibodies produced, since HPV16 has various mechanisms of evasion from the immune system. This fact provides justification for vaccination. Currently, immunization against papillomavirus infection in the Russian Federation is not included in the national vaccination calendar. The substantiation of the medical, social and economic urgency of the problem has been developed. With the introduction of vaccination with a quadrivalent vaccine, provided a high vaccination coverage of the recommended age groups is maintained, it is possible to reduce the incidence and economic costs associated with the treatment of patients.

The developed ELISA-based test system will allow monitoring the vaccination campaign and evaluating the effectiveness of the vaccine.

A full-length sequence of the L1 gene with a length of 1515 bp was assembled de novo by SOE PCR (Splicing by overlap extension polymerase chain reaction). Mutations synthesized during gene assembly were corrected by PCR-based site-directed mutagenesis. The resulting gene encodes the amino acid sequence of L1 protein, which fully corresponds to the sequence of the natural virus.

Expression constructs based on the pGD plasmid were created for the expression of L1 in a state merged with additional tags. The conformational features of the tag contribute to improving the solubility of the target protein, its proper folding and simplification of purification. Different expression conditions were selected depending on the tag. The following expression constructs were collected: pGD–L1, pGD–GST–L1, pGD–MBP–L1, pGD–NusA–L1, pGD–SlyD–L1, pGD–SUMO–L1, pGD–Fh8–L1, pGD–TrX–L1, pGD–TF–L1. The induction of transformed *E. coli*–BL21(DE3) cells was carried out. The most effective protein synthesis was in the following expression constructs: pGD–GST–L1, pGD–MBP–L1, pGD–SUMO–L1, pGD–TrX–L1.

MBP-L1 protein containing *E. coli* maltose binding protein (MBP), and GST-L1 protein containing glutathione-S-transferase (GST), were obtained in a soluble fraction. The SUMO-L1 protein containing a ubiquitin-like modifier protein (SUMO) and the TrX-L1 protein containing *E. coli* thioredoxin (TrX) were obtained in the form of inclusion bodies. Additionally, expression constructs contain a 6His sequence for purification. MBP-L1 and GST-L1 were purified by nickel-affinity chromatography in native conditions, and SUMO-L1 was purified in denaturing conditions. The SUMO-L1 and TrX-L1 proteins from the cell culture were refolded. The obtained proteins were tested by Western blotting and enzyme immunoassay (ELISA) in reaction with commercial Abcam antibodies specific to the L1 protein. The MBP-L1 protein was selected for ELISA testing with blood sera, since it is produced in a soluble fraction, it has a purification protocol selected for it, and there is no nonspecific binding of commercial antibodies during Western blotting.

The HPV16 550-116-PHG Alpha Diagnostic commercial ELISA test system was used as a comparison system. According to the results of the analysis on a commercial test system, positive and negative samples of blood sera were selected. The blood serum of a patient vaccinated with Gardasil-4 was also collected.

For the ELISA reaction, the MBP-L1 protein was titrated from 5 mcg/ml to 0.156 mcg/ml. Commercial protein L1 at a concentration of 1.25 mcg/ml was taken as a control. Protein planting on a solid substrate was carried out in a PBS buffer at +4°C during the night. In order to avoid nonspecific binding of antibodies to the ELISA substrate, blocking with 3% casein in the PBS + Tween buffer at 37°C was performed for 30 minutes. Blood serums were introduced into wells of 100 µl with a dilution of 1:100. A PBS-based buffer with the addition of Tween was also used for washing. A conjugate of goat antibodies to human IgG with horseradish peroxidase was used as secondary antibodies. The dilution of secondary antibodies was 1:10 000. The obtained OD_{450} values are presented in Table 20.

It is planned to select the conditions for the ELISA reaction and create a characterized panel of samples.

III. Development of methods for expression and purification of VP7 protein of Wad Medani and Kemerovo viruses for ELISA diagnostics of sheep

The Wad Medani (WMV) and Kemerovo (KEMV) viruses are classified as arboviruses transmitted by ticks. WMV and KEMV are 57% homologous for VP7 protein. The viruses cause benign fever, but the danger lies in cases of encephalitis that KEMV may cause.

The VP7 protein (40 kDa) participates in the interaction of the virion with the cell surface and, like the VP2 protein, is one of the virulence factors determining the infectivity of the core particle of the virion.

The main task is to develop a methodology for creating ELISA-based diagnostic kits where the recombinant VP7 protein for Wad Medani and Kemerovo viruses will be used as an antigen. The test system to be developed will allow assessing the presence of specific antibodies to WMV and KEMV in African sheep.

The gene encoding the Vp7 protein was assembled de novo by SOE PCR (Splicing by overlap extension polymerase chain reaction). To avoid nucleotide substitutions, the Vp7 sequence (1100 bp) was cloned in two parts with lengths of 560 and 540 bp. Clones containing the correct sequence were selected for subsequent reactions. After that, in order to combine fragments of 560 and 540 bp, PCR was performed from two plasmids using terminal primers. As a result, a full-length sequence of the Vp7 gene without mutations was assembled.

To create an expression construct, the Vp7 gene was cloned into a pGD plasmid vector. The appropriate induction conditions were determined. The Vp7 protein was obtained in the form of inclusion bodies. Protein purification was done with Ni-affinity chromatography in denaturing conditions. Since proteins in the soluble fraction are the most optimal for ELISA, it is necessary to clone the Vp7 gene into a plasmid vector with an additional fusion protein (tag). The tag affects the conformational features of the target protein, promotes solubility and simplifies its purification. Expression constructs containing the following tags were created: pGD–GST–Vp7, pGD–MBP–Vp7, pGD–NusA–Vp7, pGD–SlyD–Vp7, pGD–SlyD–Vp7.

At the moment, a soluble protein MBP-Vp7-WM has been obtained, which was purified on Ni-affine mini spin columns in native conditions. In the future, it is planned to develop Vp7 protein with different tags in order to determine the most suitable for conducting ELISA with mouse antibodies.

IV. Protected RNA based on MS2 bacteriophage

Protected RNA is a recombinant RNA packed into the protein envelope of MS2 bacteriophage. In this form, RNA is resistant to ribonucleases, and it is also stable during storage. Such protected RNAs can be used as quantitative RNA standards for determining the absolute number of copies of a specific RNA sequence, including for determining the quantity of RNA viruses in plasma or serum. In addition, they can be used as qualitative RNA standards in diagnostic methods for detecting nucleic acids, including as positive controls to verify the correct functioning of the diagnostic system.

Currently, the production of protected RNA is at its optimization stage. More suitable conditions are selected for the synthesis of pseudovirus particles, their purification, and the design of the original genetic structure used to develop the particles is modified. The created design is an expression vector that includes sequences of maturase genes and the MS2 phage envelope protein, sites necessary for the assembly of the viral particle, as well as the target sequence of the target virus. One of the optimization goals is to increase the number of pseudovirus particles produced.

It is known that the control of protein translation in MS2 bacteriophage is carried out by the secondary RNA structure (depends on the spatial stacking of RNA). For instance, before the maturase gene there is an untranslated section that is 130 bp long, which plays an important role in regulating the translation of this gene. In this area, three secondary structures emerge: they are hairpins forming a structure resembling a clover leaf. At the 5'-end of the RNA chain there is the Shine–Dalgarno sequence, and a complementary sequence to it is found in 30 nucleotides from the 3'-end. When these sites interact, the repression of maturase protein synthesis occurs. The formation of a secondary RNA structure for a short period of time allows ribosomes to bind to the Shine–Dalgarno sequence and begin translation. Removal of the complementary site leads to a 5- to 10-fold increase in the gene expression. Given the available information, a part of this untranslated sequence was added to the vector before the maturase gene. In the original version, 40 bps was added, and then 80 bps was added. According to the results of real-time PCR, protected RNA controls with an elongated (80 bp) untranslated sequence demonstrate an increase in the number of phage particles compared to the previous version. Thus, there is no complementary sequence in our genetic design that interacts with the Shine–Dalgarno sequence, but epy RNA can form a secondary structure that controls the synthesis of pseudovirus particles.

V. Recombinant depolymerases of *Klebsiella pneumoniae* bacteriophages

The growing number of bacteria with multiple drug resistance is becoming one of the main problems for the health sector. According to WHO, the most critical situation is observed with the following bacteria: *Acinetobacter*, *Pseudomonas*, and *Enterobacteriaceae* family, including *Klebsiella pneumoniae*.

The high rate of bacterial evolution and the limited number of antimicrobial drugs lead to the search for alternative treatments for many infectious diseases. Phage therapy was considered one of the alternatives for some time, but is currently allowed only in a few countries. Now the interest in bacteriophages is growing again. Modified bacteriophages and their components are used not only in medicine, but also in agriculture and industry. Since bacteriophages are quite successful in bacteria lysis, close attention is paid to their various proteins, e.g., endoliases and depolymerases. It is believed that these proteins can be used in antimicrobial therapy as direct and auxiliary agents.

Another advantage of bacteriophages is their high specificity, which is due to the presence of receptor-binding proteins that recognize primary receptors on the surface of the bacterial cell. By their structure, receptor-binding proteins have a domain responsible for attachment to the tail of the phage, and an enzymatic domain. If necessary, it is possible to combine these domains from proteins of different bacteriophages and create chimeric proteins. In bacteriophages that infect encapsulated bacteria, receptor-binding proteins have depolymerase activity, and can destroy capsule exopolysaccharides, lipopolysaccharides, and biofilm matrix. In the case of *K. pneumoniae*, in which many variants of the capsule structure are described, the bacteriophage depolymerase is able to cleave only a certain type of capsule. Thus, the actual tasks are the creation of a synthetic bacteriophage having a set of different receptor-binding proteins, as well as the production of recombinant proteins of bacteriophages.

At the moment, we have managed to obtain recombinant depolymerases of *K. pneumoniae* bacteriophages, which can destroy types K2 and K20 capsules. The effectiveness of the recombinant depolymerases obtained was

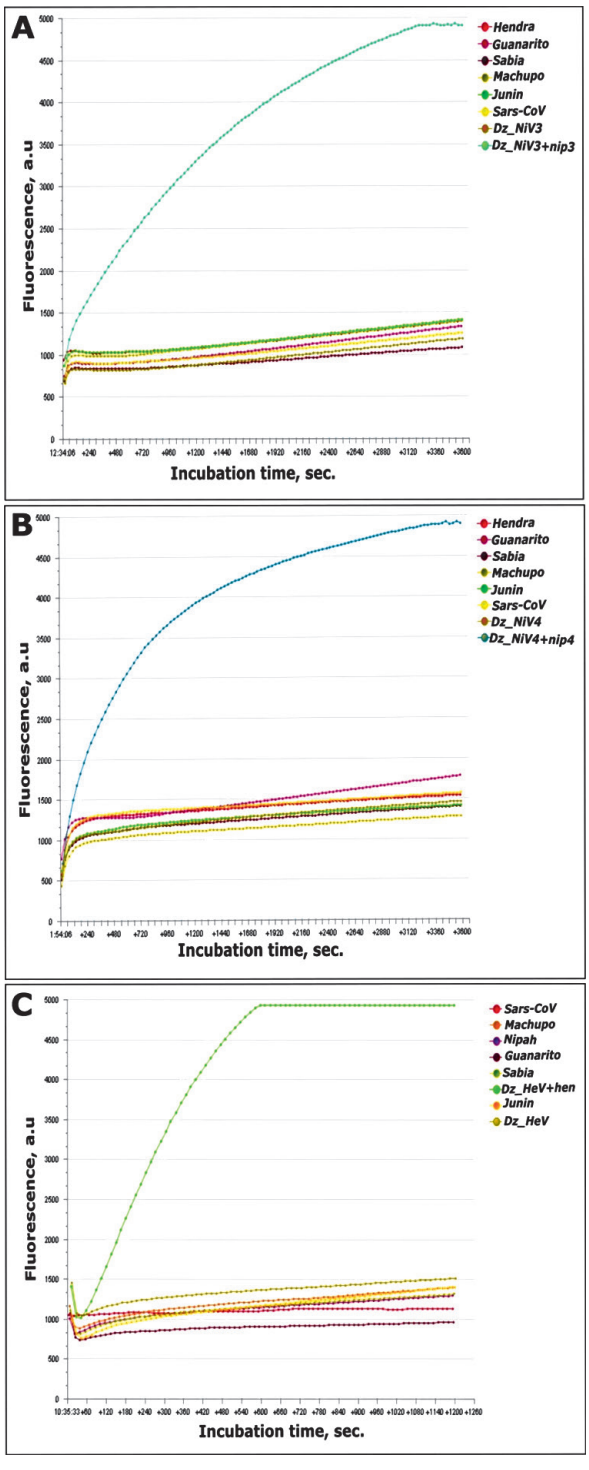

Figure 66. Selectivity of Dz_NiV3 (A), Dz_NiV4 (B) and Dz_HeV (C)
Images were directly obtained from the AxxinT16-ISO Isothermal Fluorescence Reader.

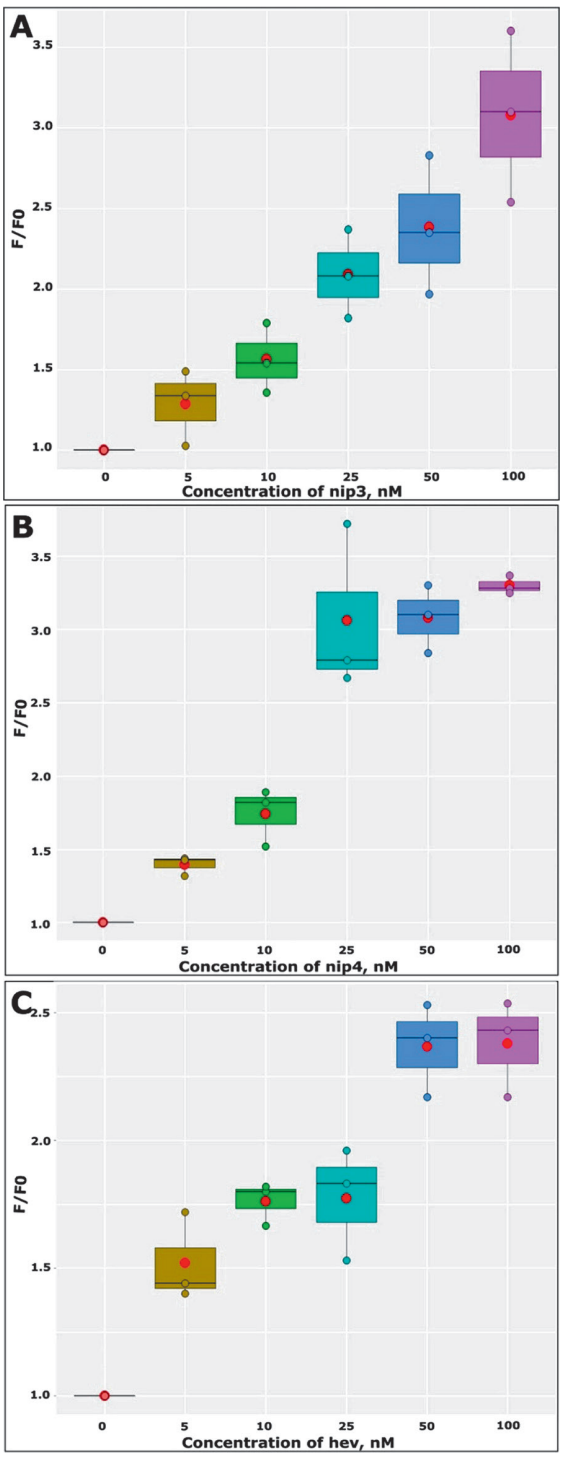

Figure 67. Sensitivity of Dz_NiV3 (A), Dz_NiV4 (B) and Dz_HeV (C)
Images demonstrate a fluorescent response from samples containing the different synthetic RNA. Red dot on the graphs indicates the mean F1/F0 ratio.

Table 20. Verification of binding of soluble MBP-L1 protein with antibodies from blood sera, OD_{450} values

MBP-L1	Vacc.	(–)	(+)	(–)	(–)
5 mcg/ml	0.940	0.331	1.00	0.588	0.472
2.5 mcg/ml	0.782	0.164	0.696	0.352	0.284
1.25 mcg/ml	0.596	0.125	0.634	0.262	0.210
0.625 mcg/ml	0.448	0.097	0.585	0.246	0.155
0.3125 mcg/ml	0.509	0.124	0.699	0.290	0.169
0.156 mcg/ml	0.327	0.110	0.841	0.309	0.205
Commercial L1 1.25 mcg/ml	0.480	0.198	0.655	0.461	0.257
0 mcg/ml	0.050	0.054	0.058	0.051	0.049

tested on different strains of *K. pneumoniae* with a spot test. Translucent lysis zones were observed on cups with *K. pneumoniae* strains of the corresponding capsule type after application of purified recombinant protein, and they were not present on cups with strains with different capsule types. It follows from this that recombinant depolymerases have the same high specificity as native phages. In addition, purified recomibinant depolymerases affect a wider range of bacteria with one capsule type than native bacteriophages can. This can be explained by the fact that the interaction of a bacterium with a bacteriophage is a complex process that can be affected by various factors, e.g., the recognition of a secondary receptor on the surface of the bacteria, the protective mechanisms of bacteria from virus penetration, low lytic activity of the phage, etc.

The use of recombinant depolymerases can enhance the effectiveness of antimicrobial drugs, since a bacterium deprived of a capsule becomes more susceptible to the effects of various substances. In the future, it is planned to obtain recombinant depolymerases that destroy the capsule types of those *K. pneumoniae* strains that are most often the most virulent and have multidrug resistance.

VI. Study of the genetic diversity of orthobunyaviruses of Bunyamwera serological group

At the time of the beginning of the research, there was controversy in respect of the species of many viruses of this group, which led to some confusion in the published research papers and made it difficult to study them further and classify newly discovered representatives. Currently, GenBank NCBI database contains sixty-four genome-wide sequences of members of the Bunyamwera serogroup. In accordance with the current ICTV demarcation criteria, the species identity of many strains should be reviewed. In particular, of the sixteen sequences designated as Bunyamwera orthobunyavirus sp., three strains should be attributed to Fort Sherman

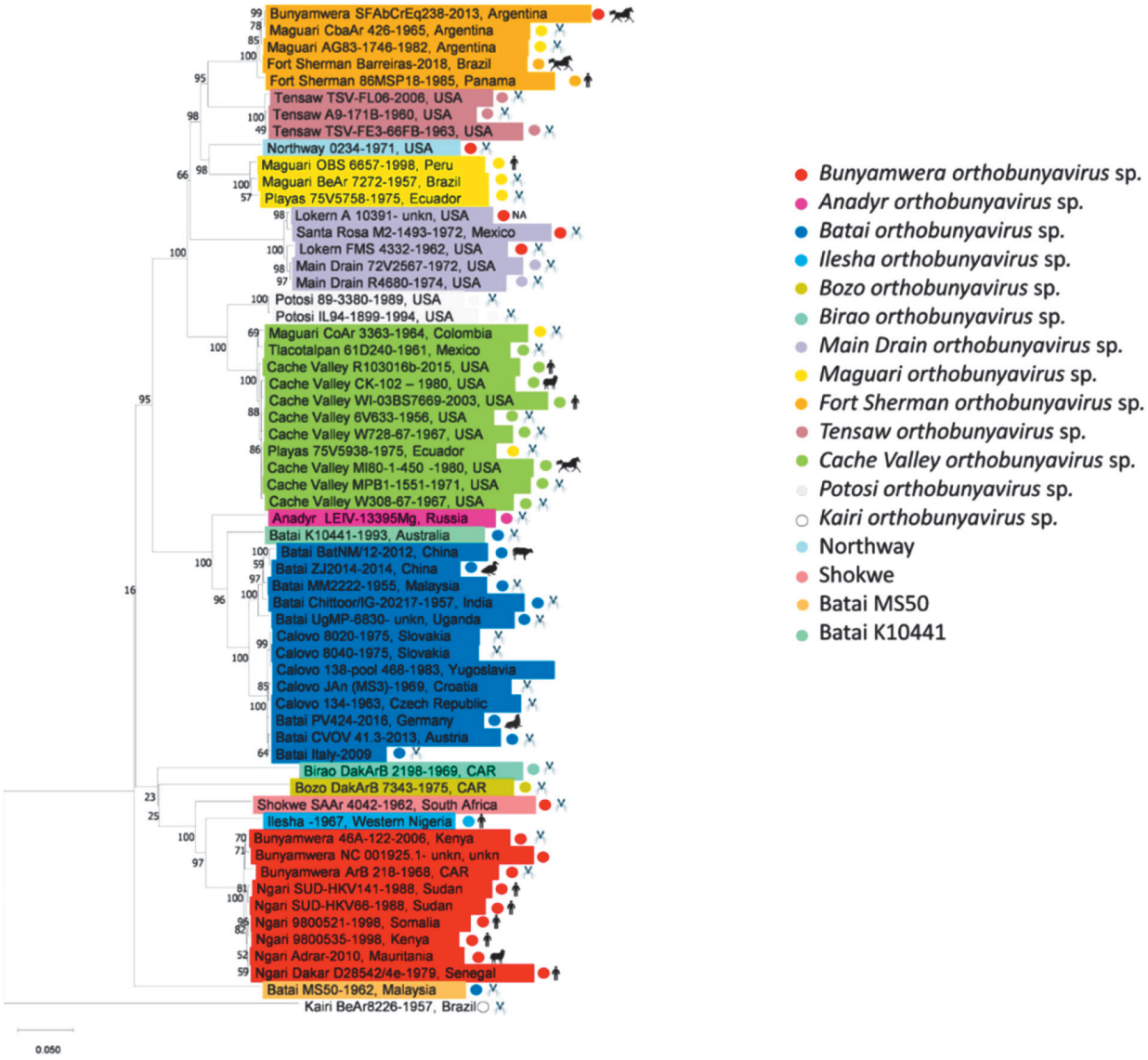

Figure 68. Phylogenetic tree of Bunyamwera serological group members based on L segment sequence (amino acid level)

The tree was rooted to the genome of the La Crosse virus (EF485032). Alignments of amino acid sequences were performed using MEGA v.11 software using the MUSCLE algorithm. Phylogenetic trees were reconstructed using maximum-likelihood estimation based on the general time-reversible (GTR) parametric model allowing gamma-distributed frequency variation between sites and a proportion of invariant sites in the sequence. The robustness of the tree was tested using 1000 bootstrap replicates. The GTR substitution model evaluated 24 models with various combinations of parameters of nucleotide substitution on the basis of maximum-likelihood fits and selected the best model among them. Attribution to the orthobunyavirus species according to ICTV is indicated by a colored dot. The real attribution to the orthobunyavirus species is indicated by colored highlighting. Isolate source listed as an animal picture.

orthobunyavirus sp.: SFCrEq231 (KP063892-KP063894), SFBzEq232 (KP063895-KP063897) and SFAbCrEq238 (KP063898-KP063900). Two isolates out of sixteen belong to different types of viruses: NORV (MH484312-MH484314) and SHOV (MH484330-MH484332). Moreover, three of them should be attributed to the Main Drain orthobunyavirus sp.: LOKV isolate A10391 (MH484303-MH484305), LOKV isolate FMS 4332 (MG820264, MG828823 and MG696865) and SARV isolate M2.-1493 (MH484324-MH484326).

Of the fifteen sequences classified as Batai orthobunyavirus sp., two should be considered separate virus species: BATV K10441 isolate (KU661980, KU661984 and KU661991) and BATV MS50 strain (NC_043579-NC_043581). BATV strain M150 is considered a reference strain of the species. However, in accordance with current ICTV criteria, it belongs to a different viral species. Since from a historical point of view, the M50 strain was not the first BATV strain to be detected, it would be logical to leave the Batai orthobunyavirus sp. name for the main group and give a new species name to the M50 strain. This assumption is supported by Groseth et al., who determined that strain MS50 is, in fact, unrelated to BATV and likely represents a novel genotype in the genus *Orthobunyavirus* (Groseth et al., 2012). BATV strain K10441 also could not be attributed to Batai orthobunyavirus sp. This strain was isolated in Western Australia near Willare village. Perhaps it should be designated as Willare orthobunyavirus sp. This name was approved jointly with the team of authors who discovered the virus (Briese et al., 2016). Among sequences classified as Maguari orthobunyavirus sp., three require reclassification: PLAV 75V5938 strain (KX100124-KX100126), MAGV CoAr 3363 strain (KX100109-KX100111), and MAGV AG83–1746 strain (KX100112-KX100114). The first two should be attributed to Cache Valley orthobunyavirus sp., the third should belong to Fort Sherman orthobunyavirus sp.

We also defined M segment reassortment events between FSV strain 86MSP18 (ac. no. MH484294-MH484296) and CVV. Reassortments in M were also identified between MDV and POTV: MDV strain 72V2567 (ac. no. MH484306-MH484308) or MDV strain R4680 (ac. no. MH484309-MH484311) with POTV strain 89–3380 (ac. no. MH484321-MH484323) or POTV strain IL94–1899 (ac. no. NC_043645-NC_043647). Moreover, we found signs of reassortment of LOKV and SARV or MDV with an unknown virus (Fig. 68).

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Northwestern District Centre for AIDS Prevention and Control

LABORATORY OF HIV IMMUNOLOGY AND VIROLOGY

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The tasks of the North-Western District Center for AIDS remain the study and generalization of data on the epidemic process of HIV infection in the Northwestern Federal District (NWFD) of the Russian Federation. The analysis of the epidemic process is based on official statistical information on cases of HIV infection detected in the administrative territories of the NWFD during serological screening and registered in the reporting forms of the Federal State Statistical Observations.

The various aspects of this work can be summarized as follows:

- analysis of the results of screening of the population for HIV infection in 2021–2022 in the regions of the NWFD to assess the intensity of the epidemic process and the degree of involvement of various population groups;
- characteristics based on the performed studies in the dynamics of the epidemic process evolution in terms of morbidity, prevalence and ways of HIV infection;
- definition the role of different population groups in the spread of the epidemic in different time periods;
- assessment of the nature of the course of the epidemic based on the results of the development of the AIDS stage in patients and fatal outcomes as an indicator of the adequacy of organizational, diagnostic and therapeutic measures;
- development of a three-level model for assessing the epidemic process from the level of districts and constituent entities of the Federation to the Federal District with the determination of the dominant causes of the increase in the incidence and prevalence of HIV infection, viral hepatitis and tuberculosis, including geographical, economic, social and demographic factors, as well as determining the optimal ways to direct organizational measures in accordance with the developed assessment model for the epidemic process;
- study of the course of viral and bacterial infections, including HIV infection, tuberculosis and hepatitis C, in the context of COVID-19 pandemic;
- evaluation the interacting role of these infections in cases of the severe course of each of them;
- systematization of the role of general organizational and epidemiological measures in countering the spread of HIV infection and comorbid conditions, as well as determining the significance of personalized factors that can affect the course and outcome of the disease.

The NWFD includes 11 constituent entities of the Russian Federation with a population of 13.9 million people (9.5% of the Russian population). The population of the NWFD decreased by 0.3% (by 40 859 people) in 2022 compared to 2021.

The total screening coverage of Russian citizens for HIV antibodies in 2022 increased by 27.7% compared to 2021, and by +23.7% in 2020 (Fig. 69). 4 207 140 citizens of the Russian Federation were examined for HIV infection in the NWFD in 2022, 30.2% of the total population of the District. The number of foreign citizens tested in 2022 increased by 8.9% compared to 2021 (253 442 people). The share of foreign citizens in the total number tested in 2022 was 6.0%, in 2021, 6.6%, compared to 12.4% in 2015. An increase in screening for HIV infection among citizens of the Russian Federation was noted in all territories of the NWFD, except the Republic of Karelia (–2.2%). The highest growth in the number of people covered by screening is observed in the Arkhangelsk (+47.4%) and Pskov (+42.3%) regions, St. Petersburg (+39.8%), Leningrad region (+30.1%). In the remaining territories, the increase in HIV infection screening did not exceed the average for the District.

The analysis of Form No. 4 "Information on the results of the HIV antibody testing" showed revealed that most of the HIV infection tests in the District in 2021 were in the following groups: tested due to clinical indications (code 113, Table 21), which was 31.8%, "other" (118) — 30.5%, donors (108) — 8.8%, pregnant women (109) — 7.8%, tested at the initiative of the patient (101) — 6.8%, foreign citizens (200) — 6.6%. The lowest share in the structure of testing was occupied by such groups as men who have sex with men (MSM) (103) — 0.02%, persons engaged in the provision of commercial sexual services (104) — 0.1%, contact persons identified during epidemiological investigation (except for children born to HIV-infected mothers) (121) — 0.4%, persons using psychoactive substances (102) — 0.5%, persons in places of deprivation of liberty (112) — 0.8%.

In 2021, testing in vulnerable groups, the so-called risk groups (people who use psychoactive substances, MSM, persons with a suspected or confirmed STD diagnosis, and persons in custodial settings), decreased in the NWFD as a whole by 3.4% compared to 2020 (Fig. 70). Compared to 2007, this indicator decreased 51.9%.

The average detection rate for the District (code 100) in 2021 was 160.1 per 100 000 tested persons (171.1 in 2020, 175.3 in 2019, 195.5 in 2018), a decrease of 6.4% (Fig. 69).

It should be noted in respect of the detection rate per 100 000 tested persons by the territories of the NWFD that the growth of this indicator in 2021 was observed in Novgorod (+3.3%) and Kaliningrad (+1.7%) regions,

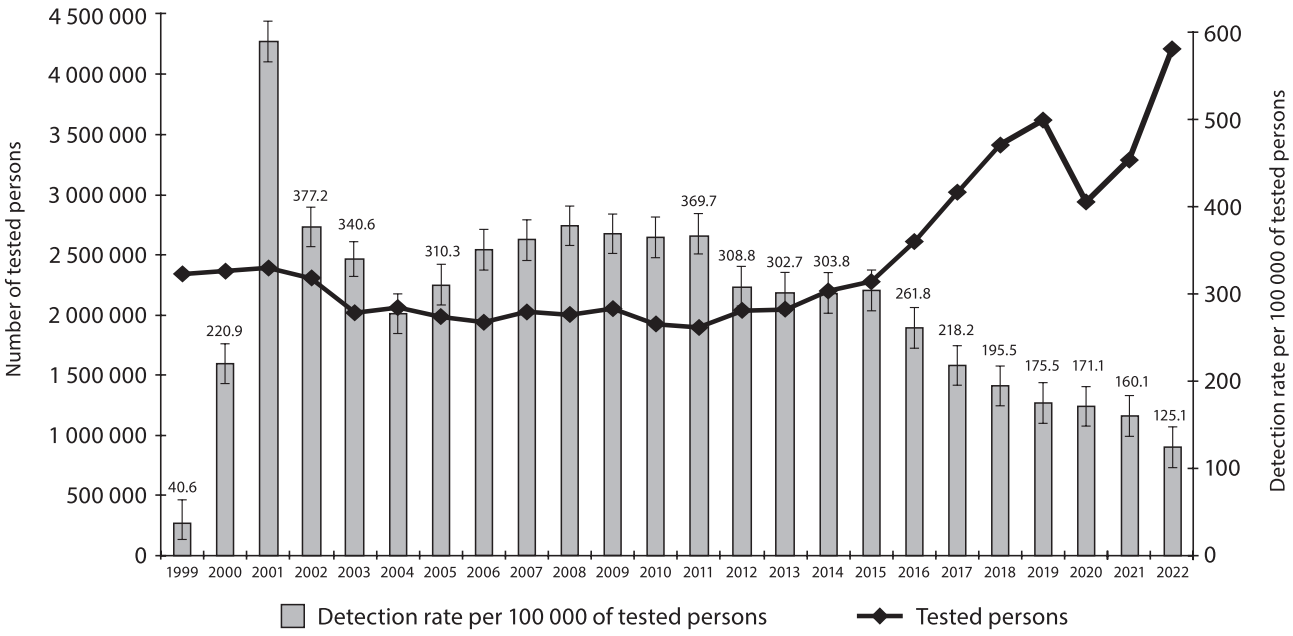


Figure 69. The number of HIV antibody tests and the detection rate per 100 000 of tested individuals in the NWFD, 1996–2020

and in St. Petersburg (+0.5%). In the remaining territories, the detectability index decreased.

The analysis of the effectiveness of screening by group (according to the codes of Form No. 4) in the NWFD as a whole in 2021 shows that the detection rate in the group of people using psychoactive substances (code 102) was 1.1% (1.2% in 2020, 2.0% in 2019, 2.0% in 2018, 2.6% in 2017, 3.0% in 2016) (Fig. 71). The detection rate in this group higher than the district average was achieved in Kaliningrad (4.2%) and Arkhangelsk (2.6%) regions, the Komi Republic (3.6%) and St. Petersburg (1.9%).

In the group of contact persons identified during the epidemiological investigation (except for children born to HIV-infected mothers) (code 121), the detection rate was 1990.8 per 100 000 tested individuals (2306.9 in 2020, 2855.8 in 2019, 2855.8 in 2018, 1921.6 in 2017, 4038.8 in 2016).

The detection rate in the group of persons in detention facilities (code 112) was 1.8% (1.7% in 2020, 1.6% in 2019, 1.6% in 2018, 1.8% in 2017, 2.9% in 2016)

In 2021, in the NWFD, the detection rate in the group of men who have sex with men (code 103) was 4.1% (6.1% in 2020, 3.7 in 2019, 3.7% in 2018, 3.5% in 2017, 3.5% in 2016).

Table 21. Explanation of codes of population testing for HIV infection

The cohort studies	Cohort code
Donors (of blood, biological fluids, organs and tissues)	108
Medical and other personnel working with patients with HIV or infected material	115
Persons conscripted for military service, starting contract military service (equivalent service), or entering military training institutions	111
Tested at the initiative of the patient (in the absence of other reasons for the test)	101
Persons who use psychoactive substances	102
Men having sex with men	103
Persons with suspected or confirmed diagnosis of sexually transmitted infections	104
Persons providing commercial sexual services	105
Pregnant women	109
Husbands/sexual partners of women registered for pregnancy care	110
Persons in custodial settings	112
Examined due to clinical manifestations (sum of codes 114+116+117)	113
Patients with clinical manifestations of HIV infection, AIDS-defining illnesses	114
Persons tested for HIV when seeking medical care (in accordance with the standards of medical care), except for patients with hepatitis B and C	116
Persons with a suspected or confirmed diagnosis of hepatitis B or hepatitis C	117
Others	118
Contact persons identified during epidemiological investigation (except for children born to HIV-infected mothers)	121
Children born to HIV-infected mothers	124
Participants in emergency situations that involve skin/mucous membranes exposure to blood and biological fluids	125
Citizens of the Russian Federation	100
Foreign citizens and stateless persons	200

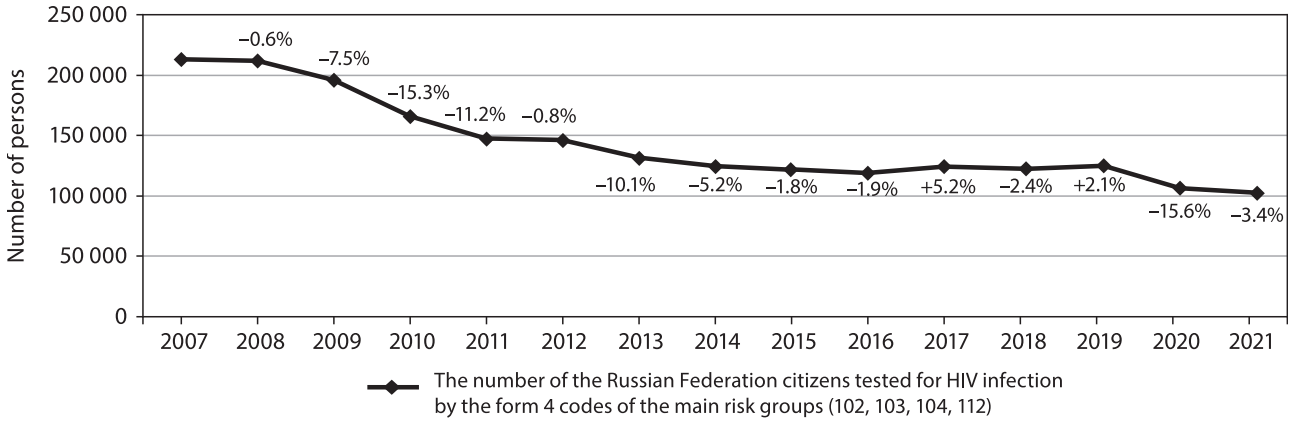


Figure 70. Time profile of HIV testing in the NWFD by year for the most numerous risk groups (codes 102, 103, 104, 112) in 2007–2021

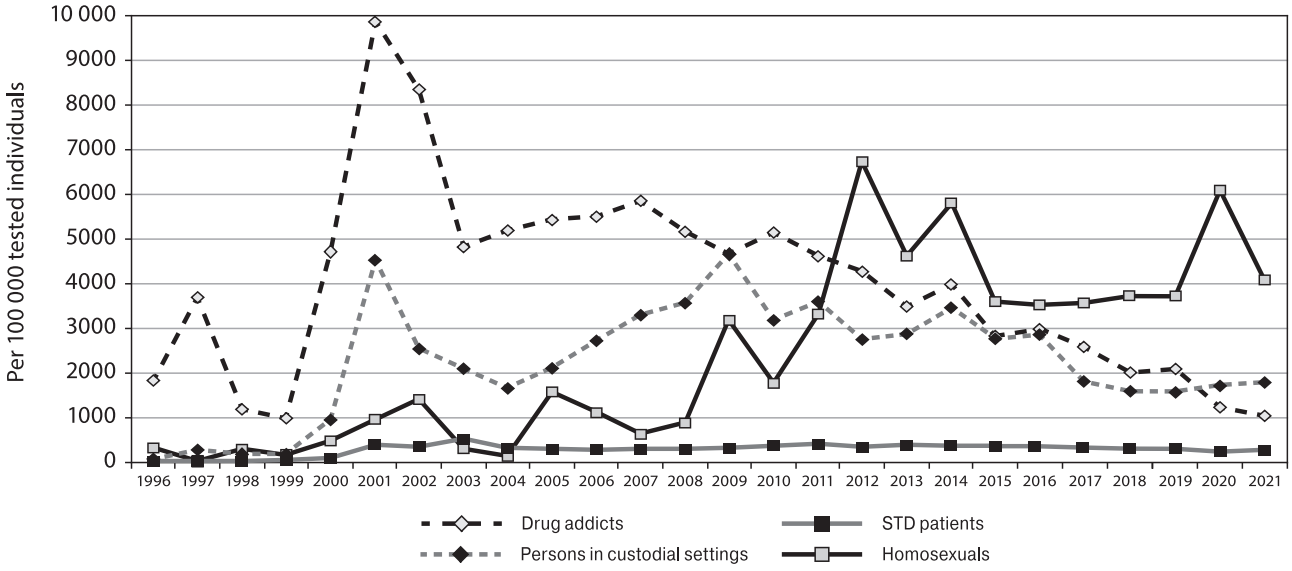


Figure 71. Detection of HIV infection in various population groups according to serological screening in the NWFD, 1996–2021

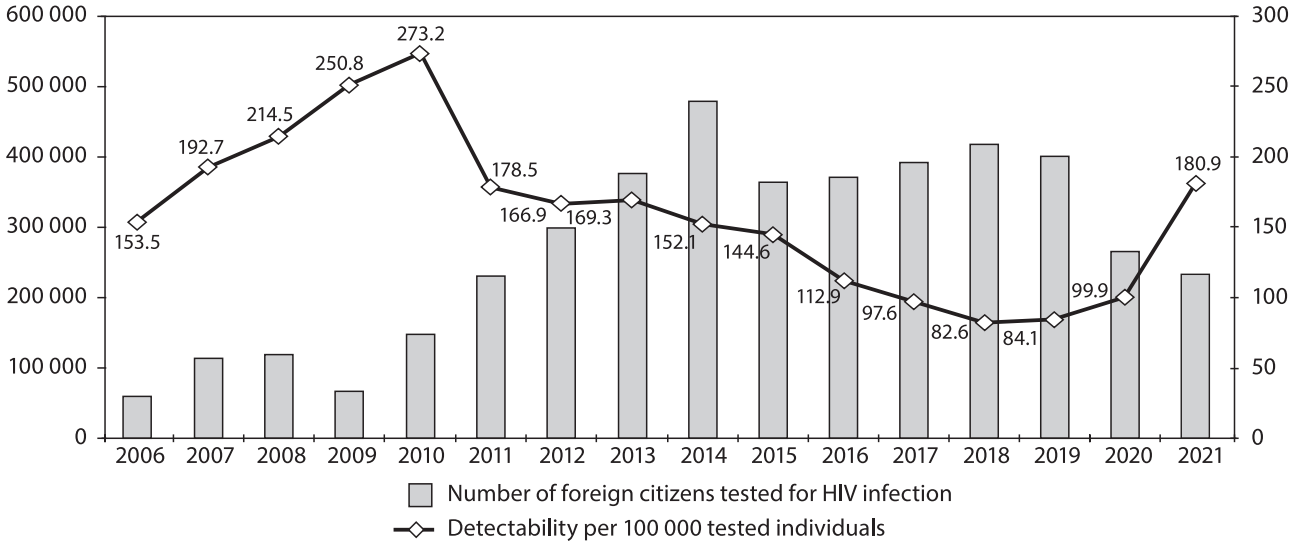


Figure 72. The number of HIV antibody tests and the number of foreign citizens identified as HIV-positive in the NWFD, 2006–2021

This indicator was above 5% and above the average for the District in Murmansk (33.3%), Arkhangelsk (25.0%), Vologda (14.3%), Kaliningrad (7.7%), Leningrad (4.8%) regions and St. Petersburg (4.2%).

The HIV detection rate among foreign citizens in 2021 increased by 81.1%, reaching 180.9 per 100 000 examined (99.9 in 2020, 84.1 in 2019, 82.6% in 2018, 97.6 in 2017, 112.9 in 2016). Of 232 750 foreigners tested for HIV in 2021, 421 had positive results (356 in 2020, 337 in 2019, 345 in 2018, 386 in 2017, 419 in 2016) (Fig. 72). St. Petersburg accounts for 69.1% of the tests and 80.7% of detections (160 721 and 340, respectively).

A total of 158 270 cases of HIV infection were registered in 11 territories of the NWFD over the entire registration period as of 31.12.2022. In general, this is 10% of the total number of HIV-infected persons registered in the Russian Federation by the specified date, which is 1 640 000 people. Excluding the deceased (39 150 people), by the end of 2022, 119 120 people infected with HIV lived in the NWFD.

In 2022, 5263 new cases of HIV infection were registered among citizens of the Russian Federation in 11 territories of the NWFD, which is 0.3% less than in 2021 (Fig. 73, Table 22)

A decrease in the number of new cases of HIV infection was noted in five of the 11 territories of the NWFD.

The decrease in new cases in the NWFD, which began in 2009, brought the incidence rate below the national average as early as in 2013, and in 2021, as in 2012, the indicators were almost at the same level, with a difference of 10 units: 37.9 in the NWFD and 48.7 in the Russian Federation as a whole (Fig. 74).

For many years, the highest prevalence of HIV infection in the district is found in St. Petersburg and the Leningrad Region, which outnumber both the NWFD (837.1 per 100 000 population) and the Russian Federation as a whole (778.1 per 100 000 population).

By the cumulative indicator of the number of seropositive people per 100 000 population (prevalence), excluding the deceased, the territories of the NWFD are ranked as follows: St. Petersburg: 1218.6, Leningrad Region: 1058.2, Murmansk Region: 826.3, Kaliningrad Region: 688.0, Komi Republic: 560.4, Novgorod Region: 530.1, Republic of Karelia: 382.4, Vologda Region: 335.0, Arkhangelsk Region (excluding cases identified in the Federal Penitentiary Service): 245.5, Pskov Region: 177.7, NAO: 210.8.

The NWFD has a greater involvement of older age groups in the epidemic process of HIV infection. Since 2011, the highest incidence rates have been recorded among 35–39 y.o. and 40–44 y.o. groups; in 2020, the levels were 101.5 and 96.5, respectively. This can be explained both by the identification of patients who were infected at a younger age and by a new stage in the development of the epidemic, characterized by mainly sexual transmission of HIV in all age groups.

In the distribution based on age and gender, it should be noted that the highest incidence rate in 2021 among men was observed in the age group of 40–44 y.o. and in the group of 35–39 y.o. among women, 107.2 and 62.3 per 100 000 populations, respectively (Fig. 75).

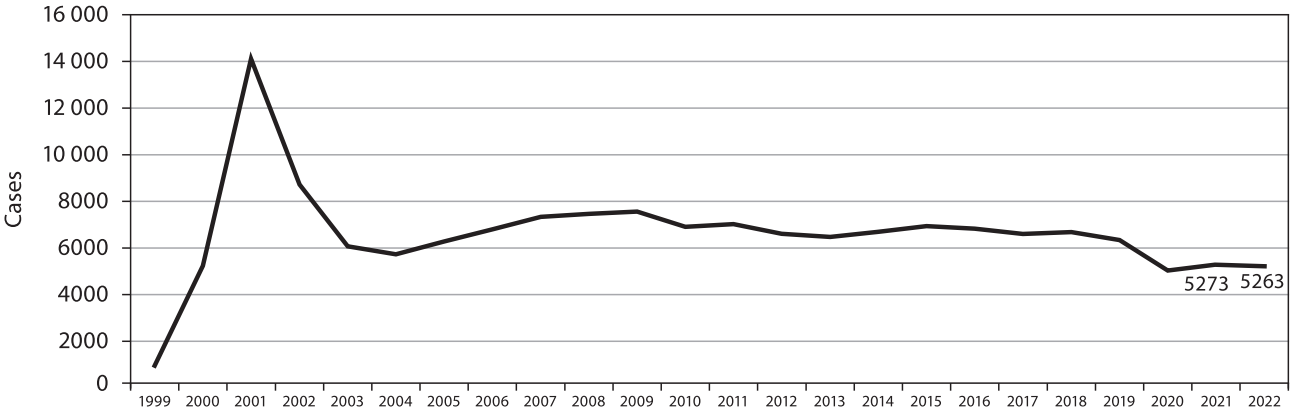
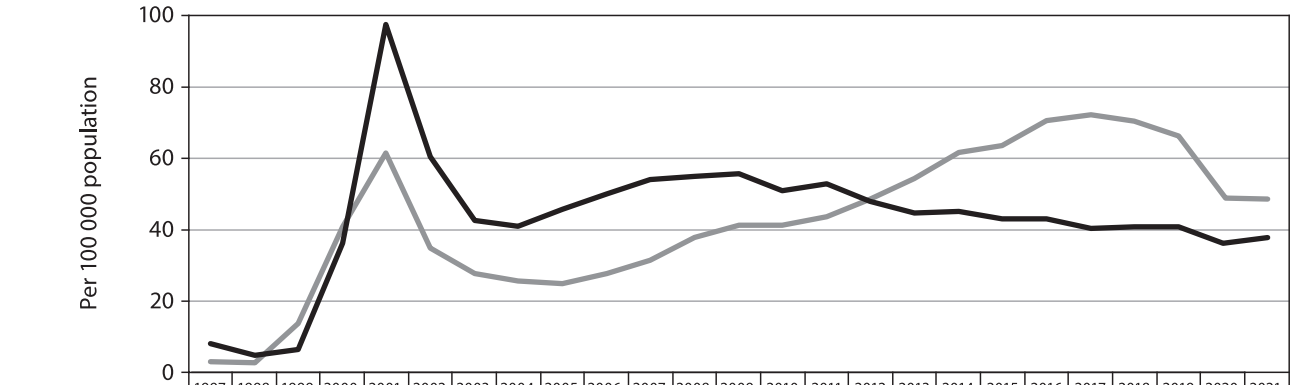


Figure 73. Annual changes in the number of newly detected cases of HIV infection in the NWFD (1996–2022)

Table 22. Registration of new cases of HIV infection in the territories of the NWFD

Territory	2019	2020	Increase/decrease (%)	2021	2022	Increase/decrease (%)
Arkhangelsk Region	363	301	–17.1	263	235	–10.6
Vologda Region	404	365	–9.7	358	343	–4.2
Kaliningrad Region	416	349	–16.1	394	429	8.9
Republic of Karelia	241	180	–25.3	177	169	–4.5
Republic of Komi	397	383	–3.5	365	368	0.8
Leningrad Region	1023	719	–29.7	763	715	–6.3
Murmansk Region	426	387	–9.2	367	337	–8.2
Novgorod Region	352	249	–29.3	259	275	6.2
Pskov Region	108	94	–13.0	88	92	4.5
NAO	7	7	0.0	15	17	13.3
St. Petersburg	2601	1997	–23.2	2224	2283	2.7
NWFD	6338	5031	–20.6	5277	5263	–0.3



	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Russian Federation	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	61.6	63.6	70.6	72.2	70.5	66.2	49.9	48.7
Northwest Federal District	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	45.2	43.1	43.1	40.4	40.8	40.8	36.3	37.9

Figure 74. The incidence of HIV infection in the Russian Federation and in the NWFD, 1997–2021

In 2021, the proportion of young people among newly diagnosed HIV-infected persons continues to decrease. In 2005, the age group of 15–19 y.o. accounted for 8.4%, and for 1.0% in 2021, for; the group of 20–24 y.o. in 2005 accounted for 30.6%, and for 4.6% in 2021 (Fig. 76).

There were more males (60.1%) than females among HIV-infected people in the NWFD in 2021. However, the share of women in the total structure of HIV-infected people is steadily growing, from 18.9% in 1995 and 26.2% in 2000 to 39.9% in 2021.

In 2021, transmission of the virus via heterosexual contacts was registered in 67.8% (68.4% in the Russian Federation), and via intravenous drug administration, in 26.4% of cases (27.8% in the Russian Federation). In 2021, the transmission of the virus with intravenous drug use did not exceed 50% in any of the territories of the NWFD (Fig. 77). If one examines data for Arkhangelsk or Vologda regions, it can be noted that the levels of HIV transmission through intravenous drug use and heterosexual contacts are at approximately the same level.

HIV prevalence among IDUs registered with drug abuse clinics in the territories of the NWFD in 2021 was 93.1 per 100 000 populations (109.5 in the Russian Federation). The share of HIV-infected drug users above the average for the District (36.9%), was detected in the Kaliningrad region (50.4%), St. Petersburg (45.4%), and Leningrad Region (40.0%).

Over the past five years of HIV monitoring, the share of the MSM group in the NWFD statistics remains high (as in the Russian Federation as a whole), at 4.5% in 2021 (5.0% in 2020, 4.1% in 2019, 3.8% in 2018, 1.2% in 2012). In some territories, the share of MSM group in 2021 was higher than the average for the district in St. Petersburg (13.1% in 2021, 12.1% in 2020, 7.8% in 2019) (Fig. 78).

The analysis of HIV risk factors distribution for men and women shows that heterosexual transmission of the pathogen remains the most prevalent for women (Fig. 79).

In 2021, in HIV-infected women, sexual transmission as the main risk factor for infection was registered in 85.3% (45.4% in 2005). In men in 2021, sexual transmission as a risk factor for infection was established in 56.5% of cases (13.5% in 2005), and intravenous drug use in 35.2% of cases.

Cases of HIV identified in 2021 were distributed by social status as follows: 25.8% were unemployed people, 29.8% workers, 10.4% office-based employees, 9.7% persons in detention facilities, 0.4% university and vocational schools and colleges students, and 0.2% military personnel.

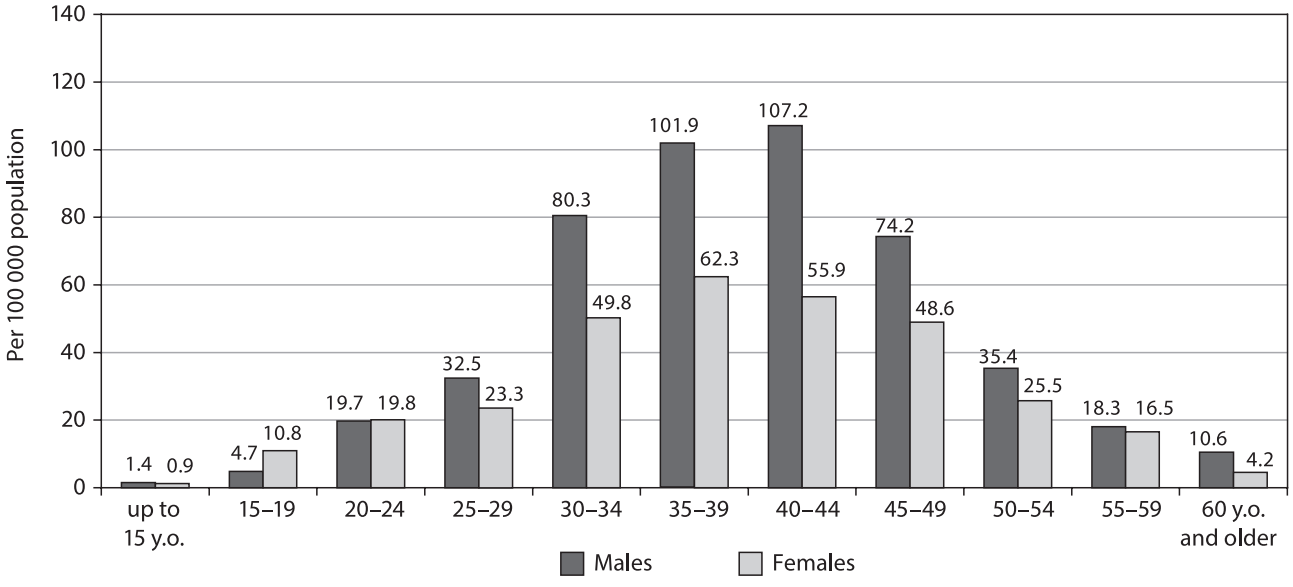


Figure 75. Distribution of HIV incidence by age group and by gender in the NWFD in 2021

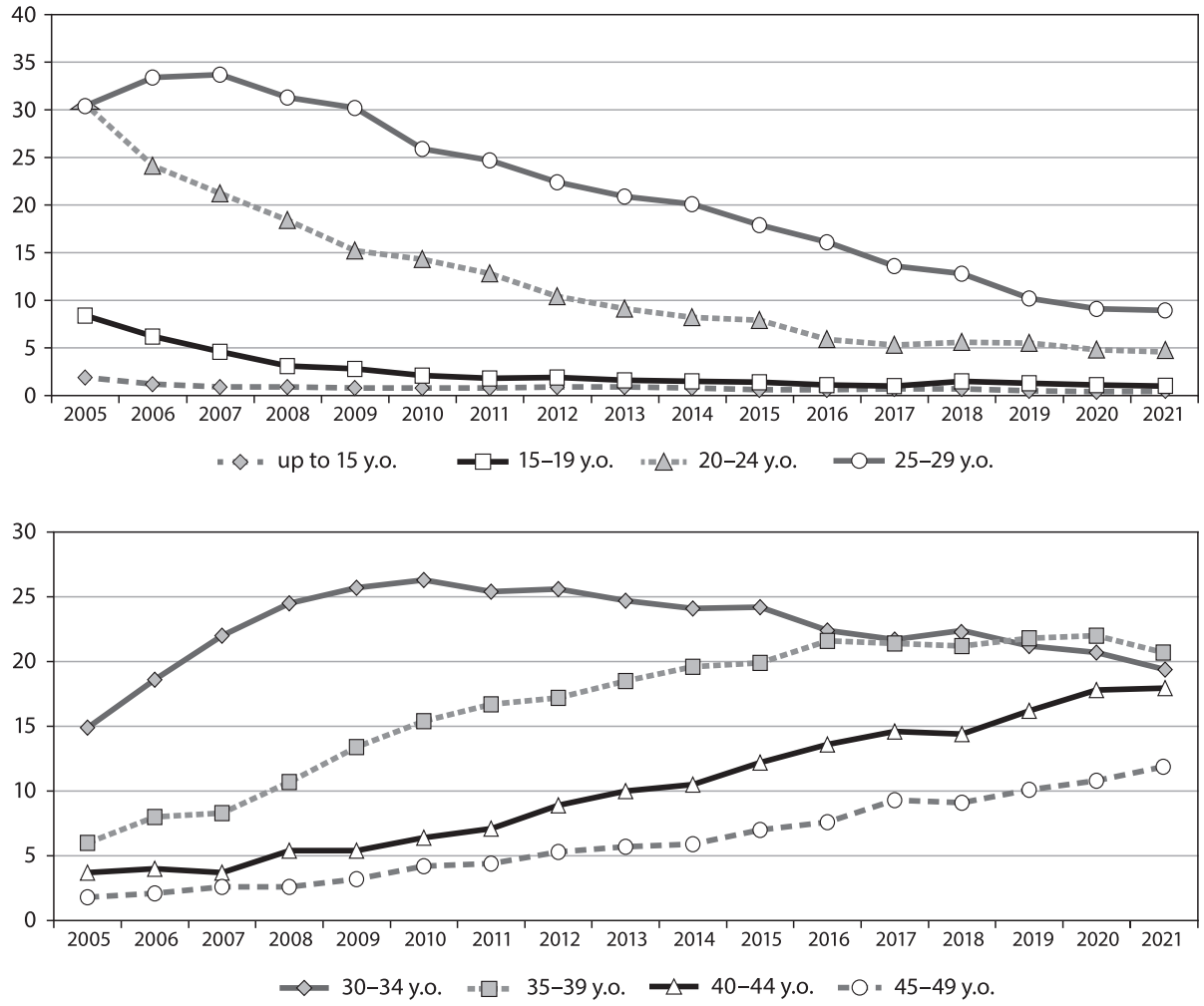


Figure 76. Time profile of the distribution of HIV-infected people in the NWFD by age, 2005–2021

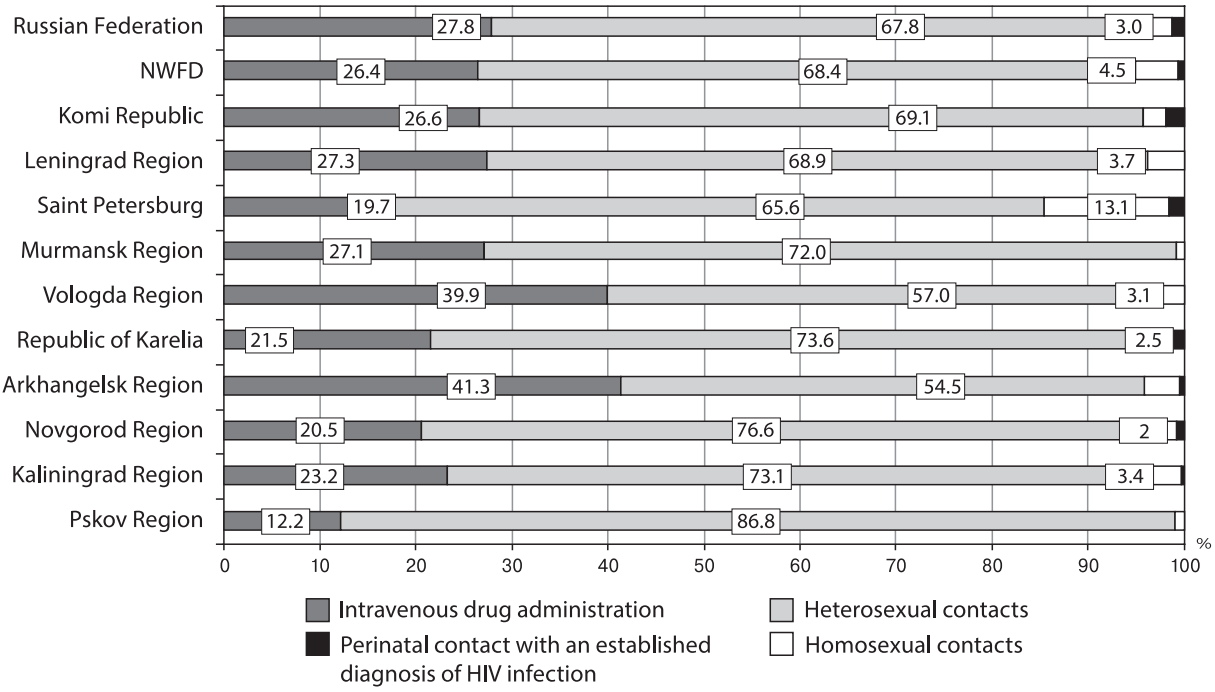


Figure 77. Distribution of HIV-infected people in the NWFD by infection risk factors (excluding cases where these factors were not known) by territories in 2021

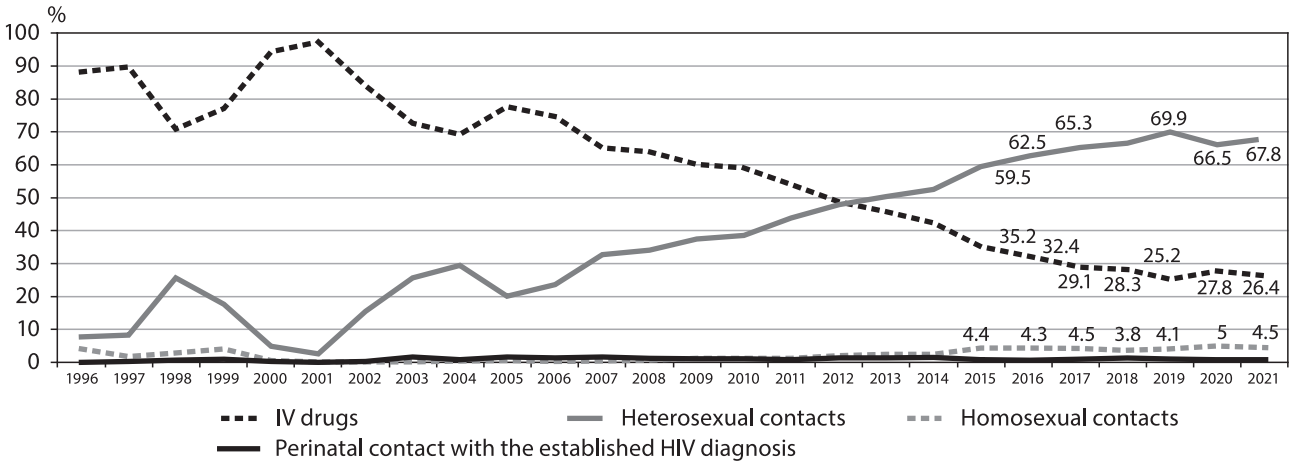


Figure 78. Distribution of HIV cases in the NWFD by infection risk factors from 1996 to 2021 (excluding cases where the factors were unknown)

In 23.2% of cases, the social status of HIV-infected people was not known (Fig. 80). Over the years, there is a growing trend of involvement of non-socially disadvantaged HIV-infected patients in the epidemic process.

Due to the late detection and delay in seeking medical help, as well as an increase in the number of patients with concomitant diseases in the District, the number of lethal outcomes among infected patients remains fairly high, including those diagnosed with AIDS (Fig. 81).

1752 HIV infected people died for various reasons unrelated to HIV in the NWFD in 2021 (1569 in 2020, 1554 in 2019, 1501 in 2018, 1490 in 2017, 1252 in 2016). 748 deaths with AIDS diagnosis were registered (755 in 2020, 908 in 2019, 919 in 2018, 1039 in 2017, 1328 in 2016). The causes of death in patients with AIDS were mainly tuberculosis (50%), pneumonia, lymphoma, and meningoencephalitis. Since the beginning of the registration of HIV infection, from 1987 to 2021, 36 722 HIV-infected people died, of which 13 049 were diagnosed with AIDS.

The growing number of women involved in the epidemic process has contributed to an increase in the number of children born to HIV-infected mothers; this year was marked by an increase in the number of children born to HIV-infected mothers and the number of children diagnosed with HIV infection (Fig. 82). Since the beginning of HIV infection registration in 1987, 21 803 children in the District had perinatal exposure to HIV infection. The cumulative number of children with a confirmed diagnosis of HIV infection due to perinatal transmission at the end of 2021 was 1023. It should be noted that more than half of the children who were diagnosed with HIV infection in 2021 were residents of the Republics of Karelia and Komi. Perinatal infection of children decreased in the NWFD from 25 to 1.1%, varying in individual territories from 0 to 8.9%.

The coverage with three-stage chemoprophylaxis of HIV transmission from mother to child remained at fairly high level. A full course of three-stage chemoprophylaxis of HIV transmission from mother to child in 2021 was received by 90.1% of mother-child couples, in 2018, by 88.9%, in 2006, by 72.5%. The lowest indicators in the District were observed in the Republic of Karelia (30.8%), the Vologda Oblast (81.8%), and in the Komi Republic (84.5%).

The number of infected persons registered at AIDS Centers in the NWFD increases every year. During the year, a total of 71 732 HIV-infected people underwent dispensary observation in 11 territorial AIDS centers, which is 85.6% of those to be monitored and 61.6% of those living with HIV (for the Russian Federation as a whole, this indicator is 68.6%). Every 10th or 11th of those found to have HIV is not followed up by a doctor. This indicator tends to decrease, but it is necessary to take into account that a significant part of infected people still do not know about the fact, and their number is commensurate with the number of people who have been registered for follow-up in the specialized centers.

HIV infection at subclinical (latent) stage 3 was observed in 28.6% of the observed individuals. The stage of secondary manifestations (4A, 4B, 4B) was diagnosed in 70.1% of patients (66.7% in 2020, 63.6% in 2019, 61.1% in 2018, 61.1% in 2017, 66.3% in 2016) (Fig. 83).

In 2021, specific ART was administered to 55 372 patients with HIV infection (51 177 in 2020, 48 719 in 2019, 41 081 in 2018, 34 180 in 2017, 29 816 in 2016), which is 77.2% of those registered at the Centers (82.2% in the Russian Federation) and 47.6% of those living with HIV (56.4% in the Russian Federation).

In the Federal Penitentiary Service of Russia system, 4139 HIV-infected prisoners were provided with ART therapy as of 31.12.2021 (3821 in 2020, 2944 in 2019, 2210 in 2018, 2900 in 2017, 2614 in 2016).

In 2021, the staff of the AIDS Center of the NWFD conducted a repeated survey of 54 infectious disease physicians (the first was held in 2018, with 74 participants) on the conditions of work in the territorial AIDS Centers and the role of specific causes of the development of HIV cases into severe and comorbid forms. The comparison of the data obtained shows that there were no significant changes in the rating of the reasons. The reasons ranked first were the same: late detection of HIV infection and delayed beginning of ART, low level of ART adherence, low coverage of patients with ART, lack of personnel for the entire cycle of diagnostics, medical examination and treatment, poor access to medical organizations, insufficient choice and quality of ART. The first three reasons lead to an increase in the number of patients with advanced stages of HIV infection.

The doctor's opinions on the priorities for funding have also changed little. The issues that remain most relevant are the expansion of ART coverage and screening organization. Others have lost their significance, e.g., the expansion of personnel in proportion with the loads, or new ART.

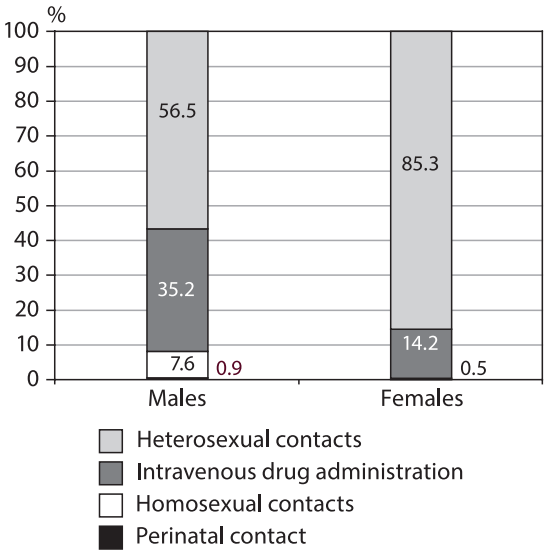


Figure 79. Main ways of HIV infection in men and women and in the NWFD in 2021

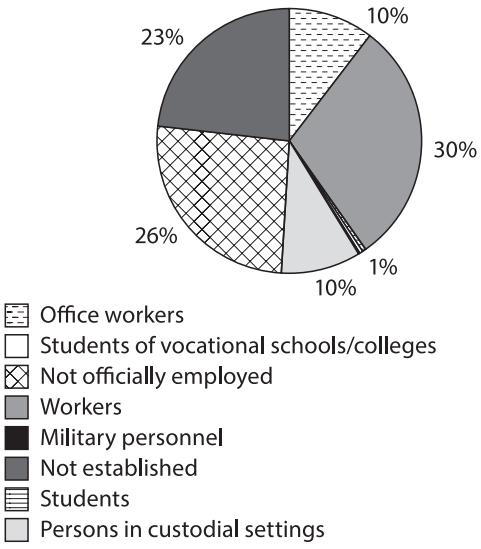


Figure 80. Social status of people at the moment of HIV detection in the NWFD (2021)

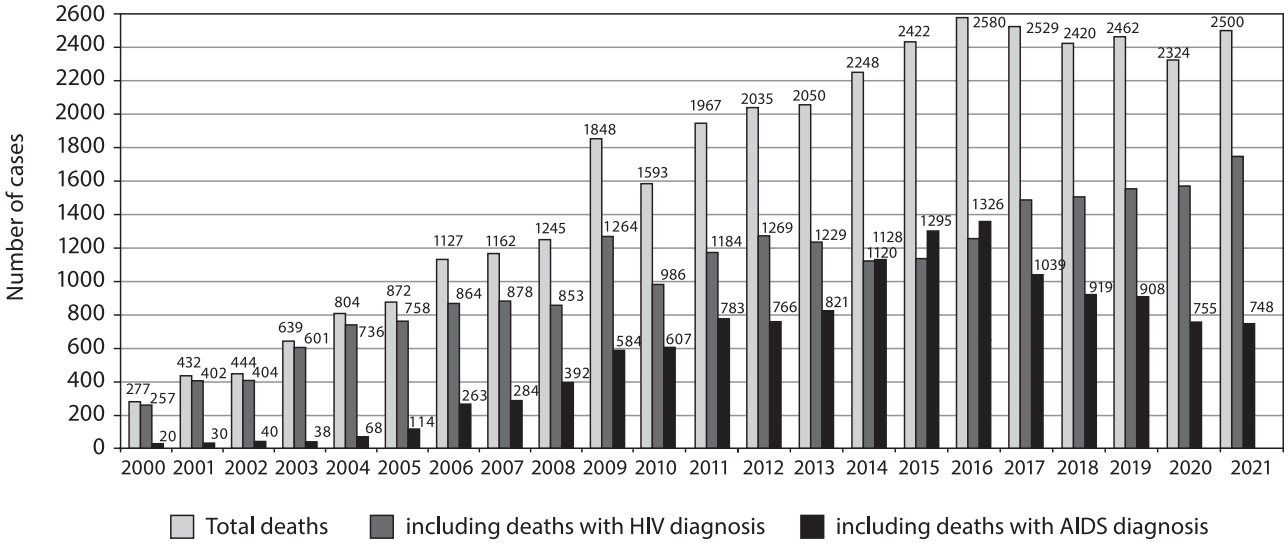


Figure 81. Registration of deaths of patients with HIV and AIDS in the territories of the NWFD, 2000–2021

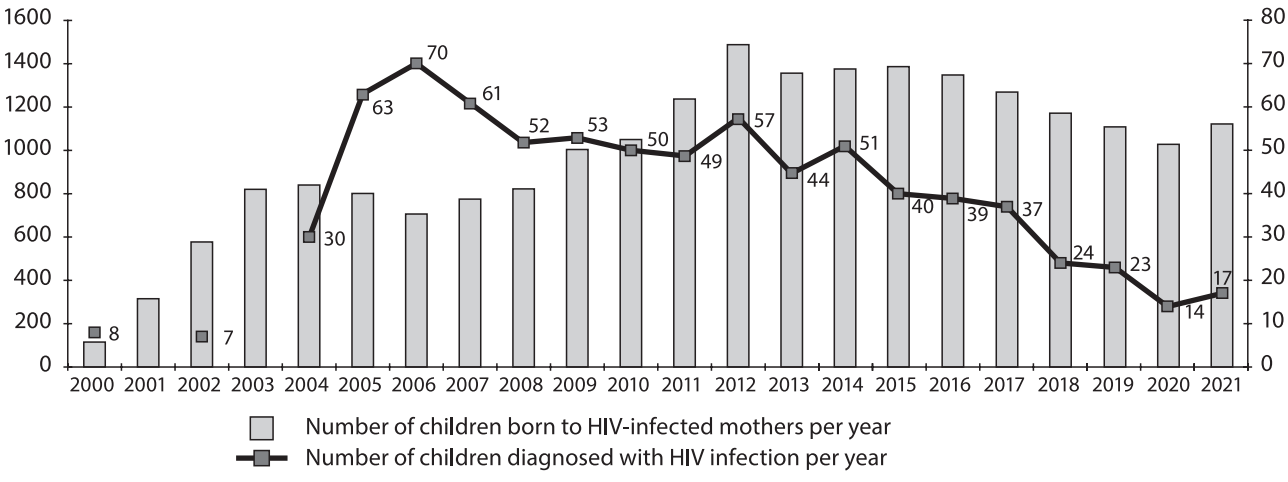


Figure 82. Children born to HIV-infected mothers in the territories of the NWFD, 2000–2021

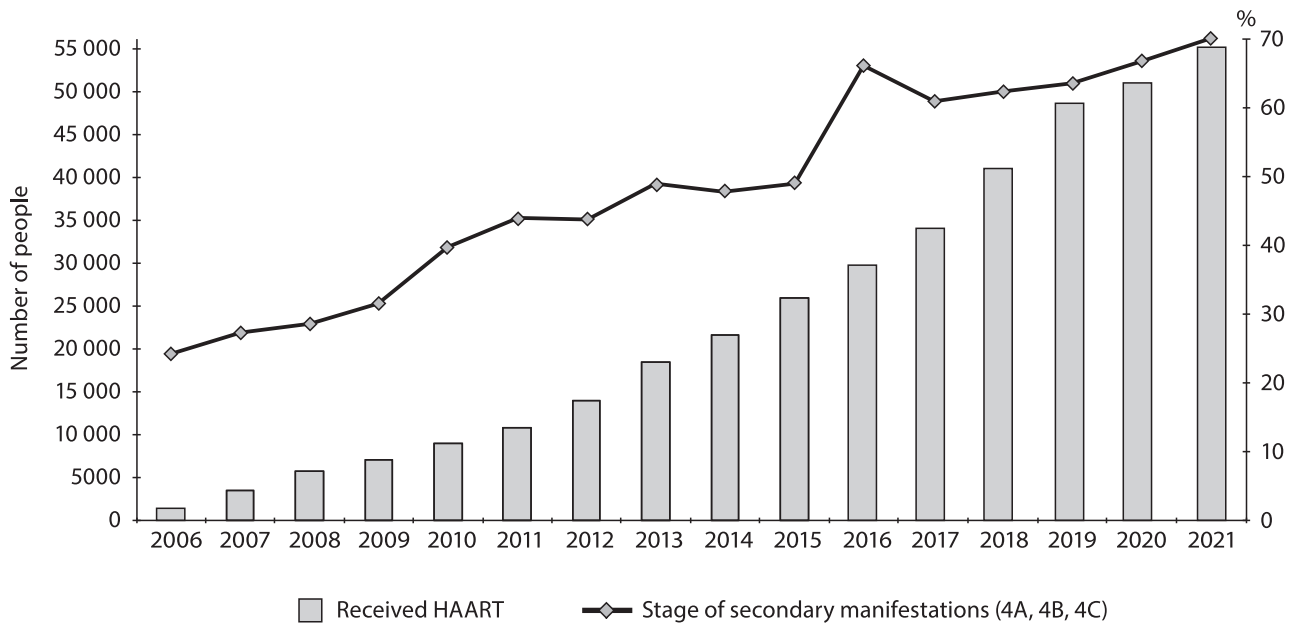


Figure 83. The number of patients with HIV receiving ART and the share of patients with severe stages of the disease in the period from 2006 to 2021

Having evaluated the results and some variation of the initial data on individual positions of the survey for doctors in comparison with the survey four years before that, we come to the following conclusion:

- in general, the opinion of the participants in the treatment process coincides with the findings concerning the most vulnerable places in ART organization for patients with HIV infection;
- there are regional differences in working conditions and supplies;
- opinions on the significance of the reasons vary depending on the perception of events, views and priorities.

Laboratory of Immunology and Virology for HIV Infection

The Laboratory of Immunology and Virology of HIV infection conducts research on HIV, as well as viral hepatitis, including in the framework of international projects. In addition, the researchers of the laboratory are studying the immune status of adults and newborns in the NWFD, as well as genetic features associated with the development of some primary immunodeficiency.

HIV isolates obtained from 643 individuals with antiretroviral therapy (ART) inefficiency in the NWFD were studied. The ART regimen usually included two nucleoside reverse transcriptase inhibitors (NRTI) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) (84.22%). Schemes with protease inhibitors (IP) in the composition were much less common (15.78%). Genotypes were determined on the basis of two typing methods — phylogenetic analysis and sequence analysis with the use of REGA HIV-1 Subtyping Tool v.3.0 application. The A6 HIV subtype characteristic for Russia prevailed (71.86%), and the circulating recombinant form CRF03_AB was also found (13.13%), while other HIV genotypes found mainly in the Kaliningrad Region were significantly less represented. According to the analysis, 72.05% of patients had at least one significant mutation associated with ART resistance of the corresponding virus subtype. In total, we found 140 different drug resistance mutations (75 NRTI, 65 NNRTI). In the vast majority of the studied isolates (60.98%), mutations of drug resistance to NRTI + NNRTI were found. In 3.94% of cases, multiple drug resistance (MDR) to three classes of drugs was detected. The analysis of stable mutation combinations in the studied isolates showed mutation patterns of resistance to thymidine analogues (TAM): TAM-1 (3.94%) and TAM-2 (2.44%). A stable combination of mutations other than TAM was also observed: L74V + Y115F (8.82%). In addition, stable combinations of mutations associated with resistance to NNRTI were identified: K101E + G190S (17.82%), K103N (18.20%), and K103N + V108I (8.26%).

We analyzed 138 blood samples obtained from HIV-infected individuals with antiretroviral therapy failure from the Leningrad region. The following ratio of HIV-1 subtypes was shown: A6 subsubtype characteristic of Russia prevailed (98%), while subtype B (1%) and a recombinant between the circulating recombinant form of CRF_03AB and the A1 subsubtype (1%) were found in isolated cases. According to the results of the analysis, 96% of patients had at least one significant mutation associated with drug resistance in the corresponding virus subsubtype. In comparison with the 2012 data for St. Petersburg, the incidence of HIV-1 drug resistance mutations increased by more than 3 times, from 30% to 95%. In total, we encountered 105 different drug resistance mutations in 35 positions *pol* gene of HIV-1. The mutations encountered in the majority (89%) of cases cause drug resistance to NRTI (43%) and NNRTI (47%). The smallest proportion among the mutations encountered (11%) is mutations that determine resistance to PIs. In the vast majority of detected cases (94%) of drug resistance, 2 or more mutations were found in the analyzed isolates. In such patients, the virus is most often resistant to 2 classes of ARP (73%), sometimes to 3 classes of ARP (8%). The M184V mutation was the most prevalent (77%), followed by the Q151M mutation (51%), associated with drug resistance to several NRTI at once, which was not detected in 2012. In 2012, mutations G190S (47%) and K103N (13%) associated with drug resistance of the virus to NNRTI were the third most frequent; in our study, muta-

tions in the 101st position (47%), also associated with drug resistance to NNRTI, were in the 3rd place. Among the mutations of drug resistance to PIs, all cases featured the major mutation M46I/L, and 3 cases had a minor mutation L89T.

The analysis of 162 blood samples obtained from HIV-infected individuals with ART failure from the Kaliningrad region showed the predominance of recombinant forms between subtypes A and B (74%), with the most common recombinant found between CRF03_AB and subtype A (33.95%), followed by the circulating recombinant form of CRF03_AB (25.31%), and with a significant proportion (13.58%) of a recombinant form similar to CRF03_AB (CRF03_AB-like), while the contribution of "pure" subtypes to the formation of this recombinant form is not completely clear. The A6 gene variant characteristic of Russia was detected in 16.67% of cases. A negligible quantity of HIV subtypes less characteristic and not typical for the country were found in insignificant quantities: A3, B, G, CRF02_AG, as well as a recombinant between subtypes K and J. In addition to genotypic analysis, a study was conducted on the occurrence of drug-resistance associated mutations in this region. Strains obtained from patients with ART inefficiency (n = 107) and those with newly diagnosed infection (n = 55) were studied. Primary drug resistance was detected in only two cases (3.64%), so further analysis covers all patients with identified drug resistance mutations. In total, 80 different mutations associated with drug resistance were found. Of these, the majority are mutations of drug resistance to RT inhibitors, including 31 substitutions with resistance to NRTI (38.75%) and 35 mutations with resistance to NNRTI (43.75%); a smaller proportion of the mutations (14, i. e., 17.50%) are substitutions associated with drug resistance to PIs. HIV-1 strains with at least one mutation associated with drug resistance to ARP were detected in 96 patients (59.26%). The most common drug resistance mutations were to RT inhibitors. In 13 cases, mutations of drug resistance to NRTI were found, in 4, to NNRTI, and in 66, to NRTI + NNRTI. In addition, 13 patients had mutations of drug resistance to PIs, including 10 to PIs + NRTI and 3 to PIs + NRTI + NNRTI. The study of drug-resistance mutation profiles in the obtained strains allowed us to find a connection between the K101E + G190A/S substitutions, and this combination mainly occurs in the absence of the most common mutation, K103N. We also revealed the dependence of the substitution in the 190th position for alanine (A) or serine (S) on the subtype of the virus. The substitution of 190A was found only in recombinants between subtypes A and B, and the mutation of 190S was mainly in subsubtype A6 strains (five out of six cases).

Based on the analysis of the nucleotide sequence of the V3 loop of the env gene, HIV tropism was determined in 76 patients with ART failure from the Arkhangelsk Region. In most cases (43.4%), 1 ART scheme was used, 3 schemes were administered in 22.4% of cases, 2 and 4 schemes in 11.8%, and 5 schemes in 10.5%. The period from HIV detection to the ART start varied from 0 to 12 years and averaged 3.48±3.47 years, and the total duration of therapy, including all regimens, ranged from 1 to 13 years, on average 3.64±2.36 years. The virus of subtype A subsubtype A6 was much more frequent in the studied group (89.5%) than subtype B (9.2%); in one case (1.3%), variant CRF03_AB was detected. The result of the analysis of the distribution of samples by tropicity in the tested group is presented in Table 23.

While at least 57% of subtype B strains were identified as R5X4/X4-tropic variants, the share of R5X4/X4-tropic samples among HIV subsubtype A6, even with a high FPR level, only slightly exceeded 22%. This shows that A6 subsubtype strains predominantly demonstrate R5 tropism even at a higher FPR. To determine X4-tropic viruses, an analysis with an FPR level of 5% was additionally performed, since, according to a number of studies, patients with FPR ≤ 5% were infected with X4 viruses, the presence of R5 virus could be predicted in patients with FPR ≥ 20%, and most patients with FPR > 5% and < 20% were infected with dual-tropic R5X4 virus. No samples with FPR < 5%, i.e., X4-tropic, were detected among the studied strains, regardless of the subtype of the virus. However, it should also be noted that two strains of subtype B (which accounted for 28.57% of HIV subtype B in the group) can still be classified as true X4-tropic, according to rule 11/25, i.e., by the presence of a positively charged amino acid at positions 11 and/or 25 of the V3 loop. The predominance of the R5-tropicity of the virus among patients with A6 subsubtype HIV also indicates the genotype-specificity of co-receptor tropism. It should also be noted that the predominance of CCR5-tropism was previously found in HIV subsubtype A1, and it could be assumed that the low incidence of non-R5-tropic viruses is characteristic of subtype A. The distribution of the occurrence of amino acid residues of the V3-region sequence in the study group was evaluated. Significant differences between R5-tropic and R5X4/X4-tropic strains of subsubtype A6 are shown for positions 18 (χ^2 = 7616, p = 0.0058), 21 (χ^2 = 7281, p = 0.007), 24 (χ^2 = 5587, p = 0.0181), 34 (χ^2 = 5144, p = 0.0233). Among the R5X4/X4-tropic strains of the A6 subsubtype, amino acid substitutions were found in positions 6, 19, 21, 26, 29, 30, and not found in R5-tropic A6 strains. Moreover, positively charged amino acids K and H appeared in positions 6 and 21, respectively. In addition, there was a tendency to an increased occurrence of substitutions for positively charged amino acids in positions 10, 18, 32 and 34. It should be noted that the most frequent substitutions among the maraviroc-resistant R5-tropic strains were shown to be in positions 10, 13, 14, 18 and 25.

Table 23. Distribution of HIV strains in the examined group by tropicity, determined with Geno2pheno[coreceptor] application, at different false-positive results cut-off levels

Subtype	FPR 10%		FPR 20%	
	R5	R5X4/X4	R5	R5X4/X4
A6	62 (91.18%)	6 (8.82%)	53 (77.94%)	15 (22.06%)
B	3 (42.85%)	4 (57.15%)	1 (14.28%)	6 (85.72%)
CRF03_AB	1 (100%)	0 (0%)	1 (100%)	0 (0%)
χ^2 p value	0.0014		0.0013	
Total	66 (86.84%)	10 (13.16%)	55 (72.37%)	21 (27.63%)

The most common substitutions in these positions were 10R, 13H/P, 14I/M, 18R and 25D, some of which are shown in our study. In the group we studied, isoleucine is present in position 14 in all the examined sequences, and variant 25D is present in both R5X4/X4-tropic strains and in most R5-tropic strains. No cases of 13H/P were identified, and no substitutions of 10R. However, 13.5% of R5-tropic strains and 26.67% of R5X4/X4-tropic strains were found to have 10K substitutions. 18R substitution was also present in 26.67% of R5X4/X4-tropic strains and only in 3.77% of R5-tropic strains. Our results confirm the predominant change in HIV tropicity from R5 to R5X4/X4 over time in subtype B viruses among patients with failed ART in the Arkhangelsk region. However, a significantly lower share of R5X4/X4-tropic variants of HIV in individuals with long-term infection with A6 subsubtype virus compared to B subtype has been shown. It can be assumed that the dynamics of changes in the tropicity of HIV depends on the subtype of the virus. The high frequency of occurrence of a number of mutations previously described as mainly associated with resistance to maraviroc and similar drugs may indicate a natural polymorphism characteristic of the A6 subsubtype that has no correlation with resistance to CCR5 coreceptor antagonists.

The priority areas of the laboratory's research work include the identification of markers of hepatitis B (HBV), C (HCV) and D (HDV) viruses in various groups, as well as molecular genetic analysis of isolates of these viruses.

A method has been developed for detecting hepatitis B virus DNA in biological material at low viral load based on two-stage real-time PCR with detection by three targets. HBV was detected by nested PCR. Analytical sensitivity was tested by gradual dilution method. At the first stage of analysis according to the method, hepatitis B DNA was amplified using oligonucleotides complementary to the regions of greatest similarity of the genomes of various hepatitis B isolates flanking the entire genome of the virus. At the second stage, the amplification product of the first stage was used as a matrix, and PCR was performed with three pairs of oligonucleotides and corresponding oligonucleotide probes with fluorescent labels to three regions (Core gene, S gene and X gene) of the virus genome, as well as one pair of primers and a corresponding probe complementary to the human HPRT gene site. The sensitivity of the method when extracting DNA from 100 ml of plasma was 5 IU/ml, obtaining a Ct threshold cycle for only one fluorophore may indicate the presence of hepatitis B DNA in a sample with a load of less than 5 IU/ml, and the detection of hepatitis B is possible with repeated PCR examination of the corresponding sample with extraction of hepatitis B DNA from an increased plasma volume (200–1000 μl). The developed method makes it possible to identify the disease in various subgenotypes of hepatitis B and can be used for laboratory diagnostics of chronic viral hepatitis B (HBV) in population as a whole and in risk groups, including HBsAg-negative form of the disease.

For the first time in the Russian Federation, hepatitis B isolates of D4 subgenotype were detected in blood plasma samples of three patients from the Republic of Dagestan. All three isolates belonged to ayw2 serotype. Drug-resistance mutations L80V and M204I were detected in an HIV-infected patient with HBsAg-negative latent hepatitis B. The high frequency of natural polymorphic variants of Pol gene was found in all three isolates. In the Precore/Core region, all three isolates were found to have Precore A1846T, C1858T mutations and Core E40D, N74T, N87S, I97F mutations. V5L and P38S mutations were detected in the X region in all three isolates. Phylogenetic analysis of the full-length hepatitis B genome showed that the isolates belong to the same group as the isolate of D4 subgenotype from South Africa. All three patients with the above isolates come from the same geographical region, and the phylogenetic proximity of the nucleotide sequences of entire virus genomes and a number of identical mutations and polymorphic variants in different genes in all three isolates indicate an independent single import of the virus with its subsequent spread. The ways of distribution, as well as the frequency of occurrence of HBV D4 in the Republic of Dagestan remain unknown and need further investigation.

A comparative analysis of the prevalence of viral hepatitis in patients of hemodialysis centers in St. Petersburg and Belgrade (Republic of Serbia) was performed. Antibodies to hepatitis C were detected in 7.5% and 11.1% of patients from St. Petersburg and Belgrade, respectively. Markers of hepatitis B were detected in the analyzed groups in 92.5% and 87.1% of cases. However, most of these cases are associated with vaccine antibodies to hepatitis B anti-HBs IgG. HBsAg was detected only in 1.1% of cases in the group from the Russian Federation and in 0.9% of cases in the group from Serbia. In the group of patients in St. Petersburg, HBV DNA was detected in HBsAg-positive individuals (1.1%), as well as in 3 HBsAg-negative patients (1.7%). Thus, HBV DNA was detected in 2.8% of individuals. In the group of Belgrade patients, hepatitis B DNA was also detected in an HBsAg-positive patient (0.9%), as well as in three HBsAg-negative individuals (2.8%). Thus, HBV DNA was detected in 3.7% of patients. In all cases of HBsAg-negative hepatitis B we identified, the viral load of hepatitis B was less than 50 IU/ml. Four of the samples obtained from patients in St. Petersburg belonged to D2 subgenotype, and one to D3 genotype. Four samples obtained from patients from Belgrade belonged to different subgenotypes (D1, D2, D3, A2, respectively). We analyzed the Major Hydrophilic Region (MHR) and the region of determinant "a", which is a cluster of major B-cell epitopes located between 124 and 147 (or 149) amino acid residues. Mutations in the MHR region were detected in all cases, but it is only in HBsAg-negative isolates that mutations in the region of 124–147 amino acids were detected. Mutations P120T, R122K, A128V, Q129R, M133I, G145R are known to affect the recognition of HBsAg by anti-HBs antibodies, while amino acid substitutions P120T, Q129R, M133I, G145R are associated with the vaccine resistance resistance of the virus. The results of this study show that the problem of transmission of pathogens of hemocontact viral hepatitis in the hemodialysis departments of the Russian Federation and the Serbian Republic persists.

A study of 1400 plasma samples of donated blood obtained from HBsAg-negative regular blood donors of the Ural Federal District showed the presence of hepatitis B DNA in 4.93% of blood donors. Among anti-HBcore IgG-positive DNA samples, hepatitis B was detected in 18.08% of cases, while in individuals with detected HBV DNA, anti-HBcore IgG was found in 46.38% of samples. 8.69% of isolates were identified in which anti-HBs IgG antibodies and virus DNA were detected simultaneously in the absence of anti-HBcore IgG. The phylogenetic analy-

sis of 69 isolates showed that hepatitis B of genotype D (94.2%) prevailed in the examined group, while genotype C (5.8%) was detected in four cases. At the same time, among patients with hepatitis B genotype D, the highest incidence was determined for subgenotype D3 (56.92%) compared with subgenotypes D2 (23.08%) and D1 (20%). Thus, among 69 samples from HBsAg-negative blood donors, hepatitis B subgenotypes are presented as follows: D3 — 53.62%, D2 — 21.74%, D1 — 18.84%, C2 — 5.8%. When determining the serological subtype of the detected isolates, the ayw2 serotype prevailed (73.91%), while serotypes ayw3 (14.49%), adw3 and adr (5.8% each) were less frequent. ayw2 serotype is determined for all D1 subgenotype isolates and for most of D2 and D3, ayw3 is also found for D2 and D3, wile adw3 is found only for hepatitis B subgenotype D2, and adr for C2. The presence of amino acid substitutions was found in 22 positions out of 144 (15.28%). The following polymorphic variants were identified: Y100S/L, Q101R/H, G102R, L109Q/I, I110L, G112E, T113S, T114S, T118V/A, P120S, K122R, T125M, T127P, A128V, N131T, S132Y, F134Y, A159G, Y161F, V168A. In all hepatitis B isolates of genotype C substitutions were identified in the specified region that were not found among isolates of genotype D: T126I, K160R. A number of polymorphic variants were also identified in the reverse transcriptase region of the P gene: A7V, E11G, H12L, I16T, A21S, A38G/E, R41G, Q48E, Y54H/N/D, V63I/C/G, N71K, L72R, N76T/G, S85C/L, F88S, Y89*/L, H90S, L91I, H94D, A97G, H100D, S105C, Y111N, S117Y, N118T, F122L, H124Y/N/H, H126R, T128I/N/T/A, M129L, Q130P, D134N, Y135S, N139D, L145M, K154Q, L164M, R167G, L180M, T184A, I187L, M204V, S213T, V214A, H216Q, L229F, A223S, N248H, C256S, Y257H, D263E/A, I266V/R, Q267H/L, E271D, V278I, I282V, L293H, F300L, M309K, S317A/F/S, K318R, A329T, N337T. At the same time, the majority of subjects (21.74%) were found to have seven mutations in the RT region, 15.94% had six mutations, 11.59% had eight mutations, three and four mutations were found in 10.14%, five mutations in 8.7%, nine mutations in 7.25%, and 10 or more mutations in 11.59% of donors. In three cases (4.35%), mutations were identified that determined drug resistance of the virus: M204V, L180M, T184A. The analyzis of the pre-Core region showed polymorphic variants W28L/S and G29D/A. W28L/S was found in 13.04% cases, G29D/A in 23.19%, and both mutations simultaneously, in 5.8%. Thirty-nine positions in which amino acid substitutions occurred were identified in the Core region: T12S, S21T/H/A/Q, F24Y, V27I, D29Q, A34T, E40D/Q, A41P, P45H, L55I, E64D, M66L/R, T67N/S, A69V/S, N74G/V, E77D, P79Q, A80I/T, N87S, N92H, I97F/L, I105V, T109S, E113Q/D, L116V/I, P130A, A131T, P135Q, N136D, T142L, L143I, T147A, V149I, R151P, D153*, R154*, S157T, Q179K, S183P. The high incidence of HBsAg-negative HBV among blood donors indicates not only the widespread prevalence of latent hepatitis B in the population, but also the insufficiency of generally accepted methods of testing and/or sensitivity of diagnostic tests to detect HBV, so attention must be paid to the issue and effective measures must be taken to ensure safe blood transfusion. The revealed hypervariability of the virus genome confirms the need to study the distinctive features of the pathogen and the immune response of the host in the latent course of viral hepatitis B.

As part of HCV drug resistance study, three patients with chronic viral hepatitis C (HCV) who did not respond to treatment with sofosbuvir + daclatasvir combination were examined. For all 3 samples, nucleotide sequences of the target genes NS3, NS5A, NS5B were obtained by direct sequencing, of satisfactory quality with a length of 800, 750 and 600 bps, respectively. The genotype of all isolates is 1b. The study revealed drug resistance mutations in all patients, including the Y93H mutation in the NS5A region, which causes the resistance of the virus against daclatasvir drugs included in the therapy regimen, as well as elbasvir, ledipasvir, ombitasvir, velpatasvir, which are not included in it; the L31V mutation in the NS5A region leads to decreased sensitivity to daclatasvir and ombitasvir. Along with that, L159F and Y56F mutations were detected in the NS5B and NS3 regions, respectively. The L159F nucleotide substitution causes a decrease in sensitivity to sofosbuvir. The Y56F nucleotide substitution in the NS3 region is the reason for the decreased sensitivity to grazoprevir. All patients were treated with the combination of glecaprevir (NS3 inhibitor) + pibrentasvir (NS5A inhibitor), as no mutations were found that would provide drug resistance against the therapy. Patients 2 and 3 achieved SVR12 and SVR24 due to this therapy regimen. Patient 1 decided not to follow the recommendations and refused further treatment.

Further, to determine the frequency of occurrence of primary HCV drug resistance mutations, we studied virus isolates from 42 HCV patients living in the NWFD who did not receive treatment with direct-acting antiviral (DAA) agents. The viral load in patients ranged from 100 to 1.8×10^8 IU/ml, and 33% had a viral load of less than 300 IU/ml, making further analysis impossible. As for hepatitis C genotype, 19 patients (68%) were infected with HCV genotype 1 (two people 1a and 17 people 1b) and 9 patients (32%) with genotype 3a virus. The nucleotide sequence of all three sites (NS3, NS5A, NS5B) was determined in 17 samples. Due to the low viral load (less than 2×10^3 IU/ml), two sites were identified in 4 samples and one genome site in 7 samples. Mutations associated with HCV resistance to DAA were found in 5 patients: 1 mutation in the NS5A region (A30K), 1 mutation in the NS5B region (L159F) and 3 mutations in the NS3 region (Q80K, Y56F, N174S). The analyzed regions of NS3, NS5A, NS5B genes demonstrate a high frequency of natural polymorphic variants. Several unusual mutations were recorded among the studied isolates, including in the positions characteristic of drug resistance mutations: NS5A (P58S/T — 14%, A62S/F — 25%); NS5B (C316T/N — 29%, L159P — 7%, S282R — 7%); NS3 (R117Q/C — 14%, V170I — 21%, S122N — 7%, Y56F — 7%). Unfortunately, there is no information about most of them in available sources, which determines the need to monitor and evaluate the significance of these nucleotide substitutions. In our study, 18% of patients were found to have virus mutations that could lead to a failure of treatment at the beginning of therapy or at later stages. It is very important to conduct an epidemiological analysis of an HCV patient before prescribing DAA and starting therapy, in order to exclude the possibility of transmission of a resistant strain of the virus.

Based on the agreement on scientific cooperation with the Republic of Guinea, a number of studies were conducted on the assessment of HIV and viral hepatitis in the country. Thus, 2616 blood plasma samples obtained from apparently healthy individuals living in the Republic of Guinea were tested. The incidence of serological markers of hepatitis B (HBV) was 80.77%, the incidence of HCV antibodies was 18%. Serological markers of hepatitis B and C in the

same person were found in 5.12% of individuals. However, HBsAg was detected only in 16.01% of individuals. 2.75% of the tested patients were found to have both HBsAg and anti-HCV. The assessment of the prevalence of serological markers depending on gender showed that serological markers are generally found in 80.93% of men and 80.42% of women, HBsAg was detected in 18.61% of men and 10.5% of women, and anti-HCV antibodies in 19.06% of men and 15.75% of women. The data of the prevalence of serological markers by age groups show that in the group of children under 18, the incidence of seropositive markers of hepatitis B was 70.43%, in the 18–22 y.o. group, 54.7%, in the 23–40 y.o. group, 79.59%, in the 41 y.o. and older age group, 89.46%. The incidence of anti-HCV was 11.96% among the tested persons aged 18–22 y.o., 22.76% among patients aged 23–40 y.o., 20.35% in over 41 y.o. age group. The examination of samples for the presence of HBV DNA found the virus in 585 people (22.36%), including 166 HBsAg-negative individuals. Hepatitis C RNA was detected in 58 samples (2.2%). 27 people were found to have RNA of both HCV and HBV, including 19 HBsAg-negative cases, which amounted to 1.03% in the examined group. In the examined group, hepatitis B of genotype E was more common (75.5%) than other genotypes (D1: 9.39%, D2: 4.02%, D3: 6.37%, A2: 4.7%). Amino acid variability among hepatitis B samples was higher in the PreCore/Core region than in the preS1/preS2/S region. SHB mutations were detected in 83.89% of cases, Core mutations in 94.29%, and PreCore amino acid substitutions in 16.77% of patients.

HIV Ag/At was detected in 239 people, and HIV RNA was detected in 58. The following HIV subtypes were identified: CRF02_AG (41.9%), A1 (29.1%), A3 (12.9%), URF A1_G (12.9%), and G (3.2%). 25% of patients had at least one significant mutation leading to HIV drug resistance. The mutations that occur cause resistance to NRTI and NNRTI, while resistance to protease inhibitor was not observed, and one case of multiple resistance was identified.

The assessment of HIV, hepatitis B and hepatitis C in 305 pregnant women from Conakry (Republic of Guinea) showed serological markers of hepatitis B in 76.06%. Hepatitis C antibodies were detected only in 1 case (0.32%). HIV markers were detected in 3 cases (0.98%). In the analyzed group, the prevalence of HBsAg significantly differed between the groups of pregnant women aged 13–19 years (17.33%) and 20–24 years (12.12%), $p < 0.0001$, RR = 5.107 at 95% CI: 2.458–10.612. When assessing the overall prevalence of molecular biological markers among patients, we did not detect HIV RNA, and HCV RNA was detected in one patient (0.32%), while the occurrence of HBV DNA was 20%. Among HBsAg-positive individuals, hepatitis B DNA was detected in 86.11%, accounting for 10.16% of the whole group. Among HBsAg-negative individuals, hepatitis B DNA was detected in 11.15%, accounting for 9.84% of the whole group. It should be noted that in nine cases, HBV DNA was detected in the absence of any serological markers, which amounted to 14.75%, i. e., 2.95% of the group as a whole. The assessment of the prevalence of hemocontact infections in pregnant women is significant for the subsequent identification of routes of transmission in order to control and/or prevent the spread of infection. Of particular interest are cases (n = 3) of detection of HBV DNA with a viral load of more than 200 IU/ml in the absence of HBsAg but in the presence of anti-HBs IgG antibodies. For these isolates, we sequenced the complete genomes. All three HBV samples belonged to genotype E, and in each case, escape mutations were found that result in the virus escaping from diagnosis during screening for HBsAg (mutations L216*, G145A, and C147T were detected).

250 blood samples obtained from donors living in Conakry (Republic of Guinea) were examined. The occurrence of serological markers of hepatitis B in the group was 83.2%, while HBsAg was detected in 16.4% of the subjects. The high occurrence of serological markers of hepatitis B in the studied group of patients, showing that most of them had contact with the virus, confirms the data on the prevalence of the pathogen in the African region. The high incidence of HBsAg in the examined group is probably due to the fact that a significant proportion of the studied individuals were primary donors (64.8%), who in African countries often donate blood to receive remuneration, being also interested in free testing for HIV, syphilis, and parenteral viral hepatitis. A significantly higher occurrence of this marker was demonstrated in men (19.55%) than in women (8.45%), while the relative risk (RR) of infection with the virus with the formation of HBsAg-positive form of hepatitis C in males was significantly higher compared to women (RR = 2.314; 95% CI: 1.018–5.251; $p = 0.0369$). The prevalence of hepatitis B DNA among blood donors was 30.4% (95% CI: 24.76–36.51), including 15.6% (95% CI: 11.33–20.7) of cases of HBsAg-negative hepatitis B. Based on the phylogenetic analysis of 76 hepatitis B isolates, it was shown that variants of the pathogen of genotype E (85.53%) predominate in the examined group compared with genotype A of subgenotype A3 (11.84%) and genotype D of subgenotype D2 (2.63%). Of particular interest are cases of detection of viral DNA in HBsAg-negative blood donors in the presence of anti-HBs IgG (n = 4) and the simultaneous presence of anti-HBs IgG and anti-HBc IgG (n = 7), which is characteristic of convalescents and indicates established protective immunity. In all these cases, the viral load exceeded 200 IU/ml. All these samples belonged to genotype E; in each case, mutations associated with HBsAg-negative HBV (Y100C, M103I) and/or escape mutations localized in the MHR region of the S gene were detected, and the mutations helped virus escape from diagnostics during screening for HBsAg (L115I/E, T127P, Q129H/R, M133I/A/F, C137Y, K141E, D144E, G145A/R, C147T, R149A/D).

Based on the agreement on scientific cooperation with the Socialist Republic of Vietnam, a number of studies were conducted on the assessment of HIV and viral hepatitis in the country. To assess the prevalence of serological and molecular biological markers of viral hepatitis B and C, blood plasma samples obtained from apparently healthy residents of South Vietnam were analyzed. The analysis of the overall prevalence of serological markers showed that among apparently healthy individuals, HBsAg and HCV antibodies were detected in 12.3% and 3.27% of individuals, respectively. The prevalence of HBsAg in men (19.1%) was significantly higher than that in women (5.9%), $\chi^2 = 14.688$ with $p = 0.0001$, $df = 1$, calculated odds ratio OR = 3.751 (95% CI: 1.892–7.439). Hepatitis B was detected in 26.95% of apparently healthy patients, taking into account HBsAg-positive and HBsAg-negative DNA samples. Phylogenetic analysis of HBV showed that the main subtype is B4 (64.49%), with other subtypes detected being C1 (14.95%), B2 (9.35%), C2 (6.54%), C3 (0.93%) and C5 (3.74%). HCV RNA was detected in 7 samples (1.76%). Phylogenetic analysis showed that all HCV isolates belong to genotype 6, subtype 6a.

To assess the prevalence of hepatitis B and hepatitis D among HIV-infected persons, samples were taken from 316 HIV-infected residents of the Socialist Republic of Vietnam undergoing ART. The shares of serological markers of hepatitis B and hepatitis D found were the following: HBsAg: 9.17%, anti-HBs IgG: 10.44%, anti-HBcore IgG: 42.08%, and general anti-HDV: 9.81%. Anti-HDV antibodies were distributed as follows: 2.53% were detected among HBsAg-positive samples (27.58% in the subgroup) and 7.27% among samples without HBsAg (8.01% in the subgroup). The prevalence of HBsAg among men in the group (12.28%) significantly exceeded that of women (5.52%), with the calculated odds ratio OR = 2.398, p = 0.04 (95%, CI: 1.028–5.592). A tendency to an increased incidence of HBsAg among HIV-infected individuals was revealed in the age group of 30–49 y.o. (10.7%) compared with 18–29 y.o. (5.9%). HBV DNA was detected in 32.58% of cases, including 23.41% in HBsAg-negative individuals. HBD RNA was detected in 7 (24.13%) HBsAg-positive individuals and 16 (21.62%) HBsAg-negative individuals, which amounted to 22.33% of HBD-positive individuals and 7.27% of the total group. For all 23 samples of HIV+HBV+HDV, nucleotide sequences of complete HBV and HBD genomes were obtained. Phylogenetic analysis of hepatitis B nucleotide sequences showed the predominance of hepatitis B genotype B (69.59%) compared to genotype C (30.41%). Among HIV-infected patients, hepatitis B of subgenotype B4 (60.89%) prevailed, followed by C1 (21.73%), B2 (8.7%), C2 (4.34%) and C5 (4.34%). The serological subtype of hepatitis B was determined based on HBsAg amino acid sequence. The ayw1 serotype was found in 60.89% of cases, adr in 26.07%, and adw2 in 13.04%. However, the ayw1 serotype is found only for hepatitis B subgenotype B4, adr is represented in all hepatitis B C1 and hepatitis B C2, adw2 in hepatitis B B2, as well as in a single sample of the hepatitis B C5 subgenotype. Phylogenetic analysis of hepatitis D nucleotide sequences showed that genotype 1 of hepatitis D was more widespread (78.26%) than genotype 2 (21.74%). Our work shows a high occurrence of parenteral viral hepatitis markers both among individuals healthy for the purposes of the study and among HIV-infected residents of South Vietnam. Particular attention should be paid to the prevalence of HBsAg-negative hepatitis B in the region, which indicates the insufficiency of currently used methods both for virus detection and for preventing infection. It is also obvious that regular screening of chronic hepatitis B patients for the presence of hepatitis D must be held, with using HDV RNA as a marker.

The prevalence of hepatitis A and hepatitis E in the Southern region of Vietnam was studied based on the analysis of the frequency of detection of antibodies to hepatitis A and E viruses in the local population. Serological markers of enteral viral hepatitis were determined in blood serum samples of adults aged 18 to 65 years of three groups: apparently healthy individuals (n = 397), HIV-infected persons (n = 316), and patients with chronic viral hepatitis (n = 268). The analysis of the prevalence of anti-HAV-IgG in samples obtained from conditionally healthy individuals, HIV-infected persons and patients with chronic viral hepatitis revealed no differences between the groups. The occurrence of anti-HAV-IgG in the general group (n = 981) was 80.1%, in the absence of anti-HAV-IgM. There were no gender/age differences in the frequency of anti-hepatitis A IgG in the examined groups. Anti-HEV-IgG in the groups of persons healthy for the purposes of the study, patients with chronic viral hepatitis, and HIV-infected persons were present in 36.2%, 33.2% and 39.8% of cases, respectively. The prevalence of anti-HEV IgM in these groups was 3.27%, 4.1% and 3.79%, respectively. In the general group (n = 981), anti-HEV IgG was detected in 36.6% of cases, anti-HEV IgM in 3.66%, which corresponds to the prevalence of antibodies to HEV in endemic regions. The markers of enteral viral hepatitis in residents of South Vietnam were found in a significant share of cases. There is an obvious need for further studies of the extent of the spread of hepatitis A and hepatitis E in the Socialist Republic of Vietnam with currently available highly sensitive diagnostic methods.

One of the directions of the laboratory's research is the study of immunity and primary immunodeficiencies (PID) in humans. As part of this research, a highly sensitive method was developed for laboratory assessment of the immune status of patients with real-time PCR, based on determination of the concentration of T-receptor excision rings (TREC) and B-cell excision rings (KREC) in children and adults. The study used samples of whole blood and dried blood spots obtained from newborns and adults, including apparently healthy individuals, and from patients with diagnosed PID and HIV-infected patients. The total sample size is 2577 people. Multiplex PCR was performed to analyze the number of target TREC and KREC molecules, as well as fragments of the reference genes HPRT and RPP30 with the use of the developed series of plasmid calibrators. The analytical range of TREC/KREC DNA measurements ranged from 10^3 to 10^9 copies/ml. The accuracy of measurements on a CFX tablet was 95.84%, and 95.11% on a rotary design device (Rotor-Gene 3000), which corresponds to the standard indicator. The results obtained with whole blood and dried blood spots were shown to be equivalent. The reference limits of the target TREC and KREC molecules for newborns, as well as for adults, by age categories, have been determined. The method makes it possible to diagnose a decrease in T- and/or B-cell immunity in children and adults and can be used to detect TREC and KREC molecules in whole peripheral blood samples and in a dried blood spot on Guthrie cards. It is also possible to apply uniform reference values, regardless of the analyzed clinical material. The test results indicate the possibility of effective use of multiplex PCR diagnostics both for complex primary testing/screening of newborns and for assessing the state of immunity in order to identify adult patients with PID and as part of the diagnosis of patients with secondary immunodeficiency, e.g., with HIV infection. Thus, the analysis of the quantity of TREC and KREC in whole blood samples obtained from HIV-infected patients showed a significantly lower levels of target analytes in patients with long-term infection with ART treatment failure compared with healthy people. The area under the curve for the TREC parameter was 0.9997±0.0003, with 95% CI: 0.9989–1.000, $p < 0.0001$. For the KREC parameter, the area under the curve was 0.9948±0.0024, with 95% CI: 0.9900–0.9996, $p < 0.0001$. No difference was found in TREC and KREC DNA levels between healthy people and people with newly diagnosed HIV with an infection period of less than one year. Timely personalized assessment of the state of immunity will help to preserve the life of patients and improve its quality.

Primary immunodeficiencies include hereditary angioedema (HAE), a rare genetic disorder characterized by recurrent swelling of soft and submucosal tissue that poses a threat to the patient's life. The diagnosis is made with due account for the clinical picture, family history, laboratory values of C1-esterase inhibitor, complement 4 component, complement 1q component, antibodies to C1 and genetic testing for a number of mutations in the SERPING1, F12, PLG, ANGPT1, KNG1, MYOF, and HS3ST6 genes. However, other genes may be involved in the pathogenesis, the negative effect of mutations in which has not yet been studied. Since an extensive range of genes can be involved in the development of non-monogenic diseases such as hereditary angioedema (HAE), it is especially important to identify groups of the most likely candidate genes presumably involved in the development of the pathology. The most likely genes were identified with bioinformatic analysis methods using a number of web resources (Humanetv3, GeneMania, FUMA GWAS in GENE2FUNC mode) to build genetic and protein-protein networks, and identify the biological context of selected candidate genes. We identified one hundred potential candidate genes, mutations in which may be associated with HAE. The biological context of the identified genes has been determined. The data of the biological context, genetic and protein-protein interactions made it possible to exclude a number of genes from the list of the most likely participants in the pathogenesis and classify the remaining ones into groups with greater or lesser involvement potential. The group of the most likely candidate genes for HAE includes the following: PLAT, HRG, SERPINA1, SERPINF2, MASP2, GRB14, C1QBP, DOK2, KLKB1, F11, TEK, KLK10, KRT1, APOH, CPB2, F2. The results obtained can contribute a lot to the study of the molecular mechanism of HAE, as well as in the diagnosis and prognosis of the course of the disease. The identified candidate genes are potentially capable of serving as diagnostic biomarkers for patients with idiopathic angioedema.

Conclusion

According to the results of the study and the data of many publications, prolonged ART did not lead to significant changes in the epidemic process of HIV infection, and it did not realize its clinical potential successfully at the population level, judging by the indicators of the spread of clinical stages of the disease, as well as the mortality of patients with HIV-related immune suppression.

The prevalence of HIV infection in the NWFD is increasing, and this is accompanied by the detection of cases at late stages with signs of secondary and comorbid diseases, mainly hepatitis C and tuberculosis. In recent years, with a decrease in the incidence of tuberculosis and chronic hepatitis C, there has been an increase in the proportion of patients in who have these diseases in combination with HIV. A comparison of the patterns of the course of the epidemic process of HIV and co-infections shows the general trends and features of the evolutionary patterns of the spread and manifestation of pathogens in the NWFD.

The obtained results determined the need for an integrated approach to patients with HIV infection, taking into account their personal and clinical needs. Medical personnel providing care to people living with HIV should take into account not only the clinical presentation of the disease, but also the psychological and social status of the patient, in order to improve the outcomes of HIV infection.

The current epidemic situation requires awareness and improvement of approaches to the organization of prevention of socially significant infections in the context of the ongoing HIV epidemic.

Publications

2021

Monographs

1. *Evolution of the COVID-19 pandemic / Belyakov N.A., Bagnenko S.F., Rassokhin V.V., Trofimova T.N., Kolbin A.S., Lukina O.V., Simbirtsev A.S., Trofimov V.I., Emelyanov O.V., Kabanov M.Y., Neznanov N.G., Rybakova M.G., Isaeva E.R., Didur M.D., Totolian A.A. // St. Petersburg: Baltic Medical Education Center, 2021: 410.*
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Analytical reviews

1. *HIV infection and comorbid conditions in the North-West Federal District of the Russian Federation in 2021: analytical review / Ed. by N.A. Belyakov. St. Petersburg: St. Petersburg Pasteur Institute, 2023: 48.*

Patents

1. *Patent No. RU 2 753 309 C2, CPC C12Q 1/68 (2021.02). Method for detecting hepatitis B virus DNA in biological material at low viral load based on two-stage PCR with real-time detection. No. 2019137626; application: 21.11.2019: date of publication 13.08.2021 / Ostankova Yu.V., Serikova E.N., Semenov A.V., Totolian A.A. Proprietors: Federalnoe byudzhetnoe uchrezhdenie nauki "Sankt-Peterburgskij nauchno-issledovatelskij institut epidemiologii i mikrobiologii im. Pastera Federalnoj sluzhby po nadzoru v sfere zashchity prav potrebitelej i blagopoluchiya cheloveka" (FBUN NII epidemiologii i mikrobiologii imeni Pastera) (RU)*
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