Saint Petersburg Pasteur Institute

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

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

At the Institute work:

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- North-West District Center for AIDS Prevention and Control;
- Regional Center for Epidemiological Surveillance of Poliomyelitis;
- Regional Center for Epidemiological Surveillance of Measles and Rubella.

The Institute has two reference centers of the Russian Federation:

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

Collaboration with WHO:

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

Contents

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Analysis of the results of antimicrobial susceptibility monitoring of *Salmonella* strains of "non-typhoid" serovars isolated in St. Petersburg in 2014–2019

***Salmonella* isolates:** 746 strains of non-typhoid *Salmonella* serovars, isolated from human; 482 strains of non-typhoid *Salmonella*, isolated from farm animals and foods of animal origin in St. Petersburg and the Leningrad region.

Methods (according to EUCAST): disc diffusion method (Oxoid), E-test (bioMerieux), MICE-strips (Oxoid).

A wide prevalence of antimicrobial resistant strains of *Salmonella* that cause diarrhea in humans, as well as those isolated from farm animals and from foods of animal origin, has been revealed. The susceptibility to all tested antibiotics was observed in 34.5% of strains isolated from humans, resistance to 1 or more class was found in 65.5%, including 10.1% of strains with multiple resistance to 3 or more classes (multi drug resistance, MDR) and 0.5% (4 strains) were found to be extremely resistant (extreme drug resistance, XDR) (to 5–6 classes of antibiotics). Of the *Salmonella* strains isolated from animals and food, 39.0% were sensitive, and 61.0% were resistant to 1 or more classes of antibiotics, including 32.4% of MDR strains and 0.6% of XDR strains.

60.9% of the strains isolated from humans, 26.2% from animals and 37.4% from livestock products were resistant to quinolones. Fluoroquinolone high-level resistance (MIC of ciprofloxacin 4–32.0 mg/l) was observed in 0.3% of strains isolated from humans, 12.7% of strains from animals and 13.9% from food. 0.9% of strains isolated from humans, 33.0% of those from animals and 17.9% from animal products were resistant to aminoglycosides. 12 strains isolated from humans (1.6%), 8 strains isolated from animals (1.9%) and from livestock products (2.4%) were resistant to the third- and fourth-generation cephalosporins.

We revealed the growth of antimicrobial resistance of *Salmonella* strains isolated from humans in 2014–2019 compared with 2002–2005: the general 4-fold increase in the proportion of resistant strains from 16.7% (95% confidence interval, 13.9–20.0) to 65.5% (95% CI 62.1–68.9) was accompanied by a 10-fold increase in resistance to quinolones from 5.9% (95% CI, 4.2–8.1) to 60.9% (95% CI 57.3–64.3) and 6-time to trimethoprim/sulfamethoxazole, from 1.1% (95% CI 0,5–2,3) to 6.0% (95% CI of 4.5 to 8.0).

Antimicrobial susceptibility was analyzed for the most significant *Salmonella* serovars: *S.* Infantis, *S.* Typhimurium, *S.* Enteritidis. Antimicrobial resistancewas detected in 69.9% of strains isolated from humans, 88.6% of those from farm animals, and 78.5% from food products. The proportion of resistant strains varied depending on the serovar and the source of isolation. The study revealed differences in the resistance indicators of *Salmonella* strains characteristic for serovars, regardless of the source of isolation (human, animal or food products) (Tabl. 1).

Serovar *S.* Enteritidis was characterized by the highest antimicrobial susceptibility (about 30% of the strains). The lowest proportion of MDR strains among serovars was noted for it (2.9% of strains from food products, 6.7% from humans, 13.1% from animals), most of the *S.* Enteritidis strains were isolated from humans and had an identical htpbcntynyjcnb profile (quinolones, chloramphenicol, tetracycline). *S.* Enteritidis strains isolated from animals were characterized by a higher proportion of resistance to "old" antibiotics (tetracycline, aminoglycosides, chloramphenicol, trimethoprim/sulfamethoxazole).

Strains of *S.* Typhimurium serovar were found to have marked differences in resistance, depending on the source of isolation. In general, the proportion of resistant strains was significantly higher than in *S.* Enteritidis (from 42.9% of strains from food to 97.4% of strains from animals). The proportion of MDR strains was 7–8 times higher than that in *S.* Enteritidis (21.6–88.2%, depending on the source of isolation), the majority of XDR strains belonged to this serovar. Unlike in *S.* Enteritidis, resistance to a larger number of classes of antibiotics was noted: aminopenicillins (21.4–82.9%), tetracyclines (28.5–77.6%), chloramphenicol (7.8–63.2%), trimethoprim/sulfamethoxazole (15.7–84.2%), cephalosporins of the 3rd and 4th generations (7.8%). Unlike other serovars, *S.* Typhimurium is not characterized by resistance to quinolones (7.1% among strains from food products, about 13.0% from animals and humans), but the indicators of resistance to beta-lactams reached maximum values (from 21.4 to 82.9% to aminopenicillins; 7.8% to cephalosporins). The strains isolated from animals had more pronounced indicators of resistance: almost all the strains were resistant to antibiotics, and 88.2% had multidrug resistance, generally, to "old" antibiotics: ampicillin (82,9%), trimethoprim/sulfamethoxazole (84,2%), tetracycline (77,6%), aminoglycosides (73,7%), chloramphenicol (63,2%).

Strains of serovar *S.* Infantis, regardless of the source of isolation, were characterized by extremely high rates of resistance to antibiotics (89.3–97.8% of strains): quinolones (88.9–94.4%), tetracyclines (82.1–97.8%), trimethoprim/sulfamethoxazole (35.6–64.3%). Most of the resistant strains (67.9–89.0%) were MDR strains with an identical phenotype (quinolones, trimethoprim/sulfamethoxazole and tetracycline).

When comparing strains isolated from different sources, no significant differences in resistance indicators were found, only serovar *S.* Infantis. *S.* Enteritidis strains differed significantly in their sensitivity to quinolones: the proportion of resistant strains from humans and from food was three times higher (61.4–68.7%) than among strains isolated from animals (21.1%). The differences depending on the source of excretion were most pronounced in *S.* Typhimurium: the indicators of resistance to almost all classes of antibiotics, as well as the proportion of MDR strains, in strains isolated from animals exceeded those among strains from

humans and food products. It was noted that resistance indicators of strains isolated from humans and from food products were similar. In animal strains, compared with those isolated from humans and foods, a significant proportion of strains resistant to "old" antibiotics (ampicillin, tetracycline, trimethoprim/sulfamethoxazole, aminoglycosides) was noted, there were also differences in resistance profiles.

Most MDR strains of *Salmonella* belonged to *S.* Typhimurium and *S.* Infantis serovars, and extremely resistant strains were of *S.* Typhimurium, *S.* London and *S.* Bredeney serovars. In the strains isolated from animals and from animal products, the most common was the MDR profile including five drugs: chloramphenicol, ampicillin, tetracycline, streptomycin and sulfanilamides. 93.7% of the strains with this profile belonged to Serovar *S.* Typhimurium, the rest to *S.* Agona and *S.* Dublin. *Salmonella* strains with such profiles were isolated from pigs and pig products produced mainly in the Leningrad region, several strains were isolated from pig products imported from Belarus, Brazil and Canada. Another common resistance profile is resistance to three drugs: tetracycline, nalidixic acid and nitrofurantoin, which is noted only in *S.* Infantis strains isolated from poultry or poultry products, both domestically produced and imported from EU countries (Germany, Lithuania, Portugal, France) and Brazil.

Beta-lactam resistance mechanisms

Salmonella strains resistant to aminopenicillins were represented by serovars *S.* Typhimurium (43.1% of the strains of this serovar), *S.* Enteritidis (2,2%), *S.* Infantis (7.1%), *S.* Kentucky (2 strains), *S.* Bredeney (1 strain) and *S.* London (1 strain). The broad-spectrum beta-lactamase gene bla_{TEM-1} was detected in 92.7% of aminopenicillin-resistant strains (Tabl. 2).

Table 1. Percent of antimicrobial resistant *Salmonella* isolated from different sources

	Humans (n = 594)			Animals (n = 38)			Products (n = 34)		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
S. Enteritidis									
Susceptible	179	30.1	26.6–33.9	12	31.6	19.1–47.5	11	32.4	19.1–49.2
Resistant	415	69.9	66.1–73.4	26	68.4	52.5–80.9	23	67.6	50.8–80.9
MDR (3 and more classes)	40	6.7	5.0–9.0	5	13.1	5.8–27.3	1	2.9	0.5–14.9
aminopenicillins	13	2.2	1.3–3.7	1	2.6	0.5–13.5	1	2.9	0.5–14.9
cephalosporins 3rd and 4th generations	7	1.2	0.6–2.4	0	0	0–9.2	0	0	0–10.2
carbapenems	0	0	0–0.6	0	0	0–9.2	0	0	0–10.2
quinolones	408	68.7	64.8–72.3	8	21.1	11.1–36.3	21	61.4	45.0–76.1
aminoglycosides	1	0.2	0–0.9	2	5.2	1.5–17.3	0	0	0–10.2
trimethoprim/sulfamethoxazole	15	2.5	1.5–4.1	3	7.9	2.7–20.8	0	0	0–10.2
chloramphenicol	26	4.4	3.0–6.3	3	7.9	2.7–20.8	1	2.9	0.5–14.9
tetracycline	38	6.4	4.7–8.7	12	31.6	19.1–47.5	3	8.8	3.0–23.0
S. Typhimurium									
Susceptible	20	39.2	27.0–52.9	2	2.6	0.7–9.1	8	57.1	32.5–78.6
Resistant	31	60.8	47.1–73.0	74	97.4	90.9–99.3	6	42.9	16.3–61.2
MDR (3 and more classes)	11	21.6	12.5–34.6	67	88.2	79.0–93.6	4	28.6	11.7–54.6
aminopenicillins	22	43.1	30.5–56.7	63	82.9	72.9–89.7	3	21.4	7.6–47.6
cephalosporins 3rd and 4th generations	4	7.8	3.1–18.5	0	0	0–4.8	0	0	0–21.5
carbapenems	0	0	0–7.0	0	0	0–4.8	0	0	0–21.5
quinolones	7	13.7	6.8–25.7	10	13.2	7.3–22.6	1	7.1	1.3–31.5
aminoglycosides	3	5.9	2.0–15.9	56	73.7	62.8–82.3	4	28.5	11.7–54.6
trimethoprim/sulfamethoxazole	8	15.7	8.2–28.0	64	84.2	74.4–90.7	5	35.7	16.3–61.2
chloramphenicol	4	7.8	3.1–18.5	48	63.2	51.9–73.1	2	14.2	4.0–39.9
tetracycline	21	41.2	28.8–54.8	59	77.6	67.1–85.5	4	28.5	11.7–54.6
S. Infantis									
Susceptible	3	10.7	3.7–27.2	1	5.5	1.0–25.8	1	2.2	0.4–11.6
Resistant	25	89.3	72.8–96.3	17	94.5	74.2–99.0	44	97.8	88.4–99.6
MDR (3 and more classes)	19	67.9	49.3–82.1	16	89.0	67.2–96.9	36	80.0	66.2–89.1
aminopenicillins	2	7.1	2.0–22.6	0	0	0–17.6	0	0	0–7.9
cephalosporins 3rd and 4th generations	0	0	0–12.1	0	0	0–17.6	0	0	0–7.9
carbapenems	0	0	0–12.1	0	0	0–17.6	0	0	0–7.9
quinolones	25	89.3	72.8–96.3	17	94.4	74.2–99.0	40	88.9	76.5–95.2
aminoglycosides	1	3.6	0.6–17.7	3	16.7	5.8–39.2	8	17.8	9.3–31.3
trimethoprim/sulfamethoxazole	18	64.3	45.8–79.3	11	61.1	38.6–79.7	16	35.6	23.2–50.2
chloramphenicol	0	0	0–12.1	0	0	0–17.6	0	0	0–7.9
tetracycline	23	82.1	64.4–92.1	16	89.0	67.2–96.9	44	97.8	88.4–99.6

Strains resistant to ESC were represented by serovars *S.* Enteritidis (7 strains), *S.* Typhimurium (7 strains), *S.* Abony (1 strain), *S.* Coeln (1 strain), *S.* Virchow (1 strain), *S.* Newport (1 strain). The strains produced "classic" extended-spectrum beta-lactamases (ESBL) of molecular class A of CTX-M genetic family (16 of 18 strains) and cephalosporinase of molecular class C (AmpC) of the CMY-2 genetic group (2 strains). Among the strains producing ESBL of the CTX-M family, ten strains produced ESBL of the CTX-M1 genetic group, three strains CTX-M9, two strains CTX-M2, and one strain, both CTX-M1 and CTX-M2.

Almost all *Salmonella* strainsproducing ESBL or AmpC (17 out of 18 strains) were resistant to 1–7 non-lactam classes of antibiotics, most often to quinolones (16 out of 18 strains). The beta-lactam preparations ceftazidime/avibactam and carbapenems were highly active against such strains; among non-lactam drugs, it was colistin and tigecycline to which all strains remained sensitive. Resistant strains isolated from animals also produced CMY-2 cephalosporinases (2 strains of *S.* Kentucky and one strain of *S.* Dublin) and ESBL of the CTX-M genetic family (*S.* Haifa, *S.* Derby).

Quinolone resistance mechanisms

Fluoroquinolones are the drugs of choice for the treatment of complicated and severe forms of *Salmonella* infection, including typhoid fever; nevertheless, the assessment of sensitivity of *Salmonella* strains to this class of antimicrobial agents, unlike of other *Enterobacterales* order bacteria, has its specific features in terms of methodology and interpretation.

Resistance to quinolones was detected in 69.1% of strains isolated from humans, and for *S.* Typhi strains this indicator was 1.5 times higher than for *Salmonella* strains of "non-typhoid" serovars (89.6% and 60.9%, respectively) (Tabl. 3). Most strains (66.8%) had low resistance to fluoroquinolones, which is regarded as clinically significant for *Salmonella* strains isolated in cases with generalized infections. High-level resistance was detected mainly in *S.* Typhi strains (7.3%), while among "non-typhoid" *Salmonella* serovars, two strains of *S.* Kentucky (0.3%) were found to have MIC of ciprofloxacin above 32.0 mg/l.

The level of resistance of *Salmonella* strains to quinolones depends on the molecular mechanism of acquired resistance. The low-level resistance is due to modification of DNA gyrase (one of the two enzymes involved in DNA replication) due to a single mutation (single nucleotide substitution) in the QRDR-region (quinolone resistance determining region) of *gyrA* or *gyrB* chromosomal genes, and much less often by plasmid-mediated mechanisms. High-level resistance occurs, as a rule, as a result of modification of both enzymes involved in DNA replication — DNA gyrase and topoisomerase IV (*parC* and *parE* genes) due to numerous single-nucleotide substitutions in these genes. We conducted a search for chromosomal mutations in resistant strains of *S.* Typhi (105 strains) and other serovars (39 strains) by analyzing nucleotide sequences of *gyrA*, *gyrB*, *parC* and *parE* genes using the ResFinder service of CGE bioinformatics platform (https://cge.cbs.dtu.dk/services/ResFinder), as well as in the BLAST program with reference sequences of quinolone-sensitive strains (*S.* Typhimurium LT2, GenBank CP014051.2 and *S.* Typhi Ty2, GenBank AE014613).

In strains with low-level resistance, one single nucleotide substitution was detected in 83 and 87 codons of the *gyrA* gene (Fig. 1).

In total, five variants of single nucleotide substitutions (corresponding amino acid substitutions) were detected, with various frequency of detection. All five substitutions were detected in *Salmonella* strains of "non-typhoid" serovars: G259T (Asp87Tyr) in 33.3% of strains; C248T (Ser83Phe) in 20.5%; G259A (Asp87Asn) in 23.1%; C248A (Ser83Tyr) in 10.3% and A260G (Asp87Gly) in 7.7%. *S.* Typhi strains were found to have three variants of substitutions: Asp87Asn (71.4% of resistant strains of *S.* Typhi), Ser83Tyr (6.7%), Ser83Phe (0.95%). The serovar specificity of some mutation variants is noteworthy: e.g., Asp87Gly was detected only in *S.* Enteritidis strains, Asp87Tyr in *S.* Infantis strains (the dominant mutation in this serovar). Serovar *S.* Enteritidis was characterized by the greatest variety of mutations: 4 out of 5 variants of substitutions were found, the most characteristic being Ser83Phe, detected in half of the studied strains of this serovar. Serovar *S.* Infantis was represented mainly by strains with the Asp87Tyr replacement.

Table 2. Beta-lactamase genes of various molecular classes identified in *Salmonella* strains isolated from humans

Genetic family of beta-lactamases	n	% of the number of resistant	Serovars (number of strains)
Strains resistant to aminopenicillins			
Total strains	41	100	
TEM	38	92.7	*S.* Enteritidis (12), *S.* Typhimurium (21), *S.* Kentucky (2), *S.* Bredeney (1), *S.* Infantis (1), *S.* London (1)
SHV, OXA, PSE	0	0	
Beta-lactamase not identified	3	7.3	*S.* Enteritidis, *S.* Typhimurium, *S.* Infantis
Strains resistant to aminopenicillins and ESC*			
Total strains	18	100	
AmpC, the CMY-2 genetic group	2	11.1	*S.* Newport, *S.* Enteritidis
ESBL: TEM, SHV	0	0	–
ESBL: CTX-M	16	88.9	
genetic groups: – CTX-M1	10	55.6	*S.* Typhimurium (4), *S.* Enteritidis (3), *S.* Abony (1), *S.* Virchow (1), *S.* Coeln (1)
– CTX-M2	2	11.1	*S.* Typhimurium (2)
– CTX-M9	3	16.7	*S.* Enteritidis (3)
– CTX-M1 + CTX-M2	1	5.5	*S.* Typhimurium (1)

*ESC refers to extended spectrum cephalosporins.

Table 3. The proportion of *Salmonella* strains with different levels of resistance to fluoroquinolones

The level of resistance to ciprofloxacin	Total (n = 1045)			Salmonella among "non-typhoid" serovars (n = 746)			*S.* Typhi (n = 299)		
	abs.	%	95% CI	abs.	%	95% CI	abs.	%	95% CI
Total resistant	722	69.1	66.2–71.8	454	60.9	57.3–64.3	268	89.6	85.7–92.6
Low level: MIC 0.12–0.5 mg/l	698	66.8	63.9–69.6	452	60.6	57.2–64.2	246	82.3	77.5–86.2
High-level MPC 4.0–32 mg/l	24	2.3	1.5–3.4	2	0.3	0.1–1.0	22	7.3	4.9–10.9

Multiple mutations in the *gyrA* and *parC* genes were detected in 24 strains with high-level resistance. Twenty-three strains (91.7%; *S.* Typhi and *S.* Kentucky) had an identical profile of three substitutions: *gyrA* Ser83Phe, *gyrA* Asp87Asn, and *parC* G239T (Ser80Ile), one strain of *S.* Typhi had two replacements: *gyrA* Ser83Phe and *parC* A251G (Glu84Gly).

Thus, identical resistance phenotypes in *Salmonella* strains were caused by different single nucleotide substitutions (in different genes, different codons, single or in combination with each other). Further evaluation of the phylogenetic proximity of the strains showed that single nucleotide substitutions in chromosomal genes can serve as an epidemiological label of the pathogen.

Detection of low-level plasmid-mediated resistance in 1045 strains revealed the production of the QnrS protein (*qnrS* gene) in three strains (*S.* Typhi, S. Corvallis, *S.* Typhimurium).

Our study showed that the development of antibiotic resistance in *Salmonella* strains in the Russian Federation match the global trends in both the growth of the proportion of resistant strains and the mechanisms of development of clinically significant resistance.

Evaluation of the prevalence and sensitivity to antibiotics of *Campylobacter* strains isolated from humans and chickens in St. Petersburg and the Leningrad Region

In 2019, 124 samples of chicken intestinal contents were examined for the presence of the main pathogens of Campylobacteriosis in humans (*Campylobacter jejuni* and *Campylobacter coli*). Due to the fact that poultry products these days are supplied to consumers from various types of poultry farms, a study was conducted of samples taken at a large poultry farm with a full production cycle, in small farms that use two types of chicken management methods (floor management without changing the litter and cage management), as well as in personal subsistence farms.

Significant contamination of the intestinal contents of chickens was revealed: Campylobacteria were found in 60.5% of the studied samples (*C. jejuni* — 47.6%; *C. coli* — 23.4%; both species — 10.5%). The highest level of infection of poultry was noted in a farm that practices floor management of poultry without changing the litter: Campylobacteria were found in 97.0%, almost all, of the examined birds (*C. jejuni* — 72.4%, *C. coli* — 44.8%, both species simultaneously — 24.1%). At a large poultry farm, Campylobacteria were found in 62.1% of samples (*C. jejuni* — 51.7%; *C. coli* — 17.2%; both species — 6.9%). In chickens kept on farms with cage management method, the level of infection with Campylobacteria was significantly lower — 37.5% (*C. jejuni* — 25,0%; *C. coli* — 12.5%). In private farms, Campylobacteria were detected in 52.9% of samples (*C. jejuni* — 44.1%; *C. coli* — 20.6%; both species — 11.8%).

Thus, the main factor influencing the spread of *Campylobacter* in chickens is the method of their keeping: the highest level of infection of poultry was noted with floor management without changing the litter, and the lowest with cage management without litter.

In 2020, the sensitivity to antibiotics of 71 *Campylobacter* strains isolated in St. Petersburg from hospitalized patients (19 strains), as well as from the intestinal contents of chickens on poultry farms in the Leningrad Region (52 strains) was studied (Tabl. 4). 49 strains (69.0%) were of *C. jejuni*

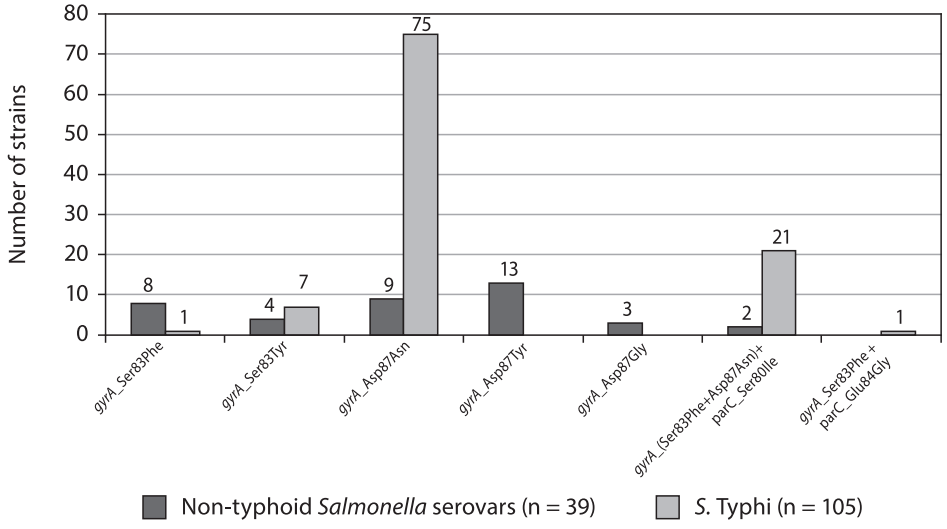

Figure 1. Single-nucleotide substitutions in the QRDR region of the *gyrA* and *parC* chromosomal genes detected in *Salmonella* strains resistant to quinolones

species, and 22 strains (31.0%) of *C. coli* species. The sensitivity of *Campylobacter*s to fluoroquinolones (ciprofloxacin), macrolides (erythromycin) and tetracycline was determined by the concentration gradient method.

Among the studied *Campylobacter*, no strains sensitive to antimicrobial agents were identified. All strains, regardless of the source of isolation (human, bird) and the species (*C. jejuni*, *C. coli*) were resistant to ciprofloxacin. Tetracycline resistance was observed in 66.2% of the strains and is most pronounced in *C. jejuni* (83.7%) compared to *C. coli* (27.3%). In general, the proportion of *Campylobacter* strains resistant to erythromycin did not exceed 7.0%, such a resistance phenotype was more characteristic of *C. coli* strains (18.2%) compared to *C. jejuni* (2.0%). More than 70.0% of *Campylobacter* strains were resistant to several antimicrobial agents simultaneously, the most common was combined resistance to ciprofloxacin and tetracycline (62.0%). Multiple resistance to ciprofloxacin, tetracycline and erythromycin was detected in three strains (4.2%) and did not depend on the source of isolation and species affiliation.

Campylobacter strains of two species have marked differences in the leading resistance phenotypes: the most characteristic resistance for *C. jejuni* is combined resistance to ciprofloxacin and tetracycline (in 64.7% of the strains from humans and 90.6% from chickens), whereas for *C. coli*, the characteristic resistance is to ciprofloxacin only (63.6%).

International high-risk clones

The assessment of sensitivity to antimicrobial agents and the detection of molecular mechanisms of resistance in *Salmonella* and *E. coli*strains isolated from patients with acute intestinal infections made it possible to identify strains of international high-risk polyresistant clones in St. Petersburg that have a global distribution and have caused food poisoning outbreaks in Europe and the United States.

Two strains of *S.* Kentucky were found to have multiple resistance to antimicrobial agents (ampicillin, streptomycin, gentamicin, chloramphenicol, sulfonamides, fluoroquinolones). Resistance to fluoroquinolones reached a high level (MIC of ciprofloxacin more than 32.0 mg/L) and was caused by multiple mutations in *gyrA* (Ser83Phe and Asp87Asn) and *parC* (Ser80Ile) chromosomal genes. According to the data of the salmonellosis reference center, occasional strains of *S.* Kentucky are isolated in the Russian Federation every year (from 2 to 8 strains annually over the last 10 years), and there are no data on the isolation of ST198 clone strains in the country. The strains that we isolated in St. Petersburg were, in terms of their phenotype of multiple resistance and the molecular mechanism of resistance to quinolones (the profile of multiple chromosomal mutations), identical to the international polyresistant high-risk clone *S.* Kentucky ST198. One of the Russian strains of *S.* Kentucky ST198 is deposited in GKPM-Obolensk under No. B-9045.

In Saint Petersburg, we isolated the *S.* Newport strain with multiple resistance to antimicrobial agents (aminopenicillins, ESC, chloramphenicol, tetracycline, streptomycin and sulfonamides). The resistance of the strain to ESC was due to the production of AmpC-cephalosporinase of the CMY-2 molecular family. The bla_{CMY-2} gene was located on a plasmid about 150 kb in size, which had a *Pst1* restriction profile, the most common in strains of the international high-risk polyresistant clone *S.* Newport MDR-AmpC/CMY-2. According to the Russian Reference center for salmonellosis monitoring, serovar *S.* Newport has been ranked fifth over the past 10 years in the *Salmonella* serovars "rating" (0.5–0.9%). Every year, from 100 to 200 strains of this serovar are isolated from people; there are no data on the isolation of strains of the international clone in our country. The *S.* Newport strain, which we isolated in the course of our study, in its multiple resistance phenotype, the molecular mechanism of resistance to ESC (AmpC-cephalosporinase CMY-2), and the restriction profile of plasmid resistance, matched the international high-risk polyresistant clone *S.* Newport MDR-AmpC/CMY-2. The strain was deposited in "GKPM-Obolensk" under No. B-9044.

In the Russian Federation, *E. coli* O26 is included in the list of causative agents of diarrheal diseases, which in the routine practice of clinical diagnostic laboratories are registered as EPEC without determining the H-antigen and the production of *shigA*-like toxin (STX). We studied 24 strains of *E. coli*

Table 4. Antibiotic resistance of *Campylobacter* spp. strains isolated from humans and poultry (number and % of resistant strains)

Antimicrobial drug	Total						Humans						Chickens					
	Total (n = 71)		*C. jejuni* (n = 49)		*C. coli* (n = 22)		Total (n = 19)		*C. jejuni* (n = 17)		*C. coli* (n = 2)		Total (n = 52)		*C. jejuni* (n = 32)		*C. coli* (n = 20)	
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Sensitive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Resistant	71	100	49	100	22	100	19	100	17	100	2	100	52	100	32	100	20	100
ciprofloxacin	71	100	49	100	22	100	19	100	17	100	2	100	52	100	32	100	20	100
tetracycline	47	66.2	41	83.7	6	27.3	13	68.4	11	64.7	2	100	34	65.4	30	93.8	4	20.0
erythromycin	5	7.0	1	2.0	4	18.2	1	5.3	0	0	1	50.0	4	7.7	1	3.1	3	15.0
Resistance phenotypes																		
ciprofloxacin	22	31.0	8	16.3	14	63.6	6	31.6	6	35.3	0	0	16	30.8	2	6.3	14	70.0
ciprofloxacin + tetracycline	44	62.0	40	81.6	4	18.2	12	63.2	11	64.7	1	50.0	32	61.6	29	90.6	3	15.0
ciprofloxacin + erythromycin	2	2.8	0	0	2	9.1	0	0	0	0	0	0	2	3.8	0	0	2	10.0
ciprofloxacin + tetracycline + erythromycin	3	4.2	1	2.1	2	9.1	1	5.2	0	0	1	50.0	2	3.8	1	3.1	1	5.0

of O26:H11 serovar classified as STEC, isolated from patients with diarrheal syndrome (hemocolitis) in St. Petersburg in 2014–2019. All strains were identical: they belonged to one enzymatic biovar 1, were characterized by the production of enterohemolysin and s*higA*-like toxin STX1. The analysis of the genomes revealed the presence of determinants encoding the major virulence factors of STEC: s*higA*-like toxin STX1 (*stx1a* gene) and additional genes encoded by plasmid pVF — *ehxA* (enterohemolysin), *katP* (peroxidase, catalase), *espP* (serine protease), as well as *cba* (colicin B), *gad* (glutamate decarboxylase), *cif* (secretion type III effector), *iss* (resistance to bactericidal action of blood serum). The strains belonged to the phylogenetic group B1 and sequence type 21 (ST21). 13 strains (54.2%) were sensitive to antimicrobial agents, the remaining 11 strains were characterized by ampicillin resistance in combination with resistance to three or more antimicrobial agent classes. Six strains had the MDR phenotype, two of them had the XDR phenotype and produced an extended-spectrum beta-lactamase of the CTX-M molecular family. All strains remained sensitive to carbapenems (Meropenem).

Thus, molecular methods made it possible to identify among the Russian clinical isolates of *Salmonella enterica* and *Escherichia coli* strains belonging to successful international high-risk clones: *Salmonella* Kentucky ST198, *Salmonella* Newport AmpC-MDR/CMY-2, *Salmonella* Typhi subclades 4.3.1 (haplotype H58) and *E. coli* O26:H11 ST21.

Conclusion

1. More than 60.0% of *Salmonella* strains isolated in St. Petersburg and the Leningrad region from humans, farm animals and food products of animal origin are resistant to antibiotics. The proportion of strains resistant to drugs used for the treatment of salmonellosis was: to quinolones, 26.2% (from animals) to 60.9% (from humans), to ESC — 1.6–2.4%. Strains of serovar *S.* Infantis was distinguished by extremely high levels of resistance to antimicrobial agents (89.3–97.8%), of which 67.9–89.0% were characterized by multiple resistance to quinolones, trimethoprim/sulfamethoxazole and tetracycline.
2. *Campylobacter* strains isolated from humans and poultry are resistant to antimicrobial agents used for the treatment of campylobacteriosis. Resistance to fluoroquinolones was noted in all the studied strains, resistance to tetracyclines reached 70%, to macrolides in *C. coli* strains: 18.2%, *C. jejuni*: 2.0%. More than 60.0% of *Campylobacter* strains were resistant simultaneously to fluoroquinolones and tetracycline; multiple resistance to fluoroquinolones, tetracycline and macrolides was found in 4.2%.
3. Resistance of *Salmonella* strains to beta-lactams is due to a mechanism of resistance characteristic of bacteria of the *Enterobacteriacae* family, namely, the production of β-lactamases of different molecular classes and genetic families: TEM-1 broad spectrum β-lactamases (90.5% of aminopenicillin-resistant strains), as well as of CTX-M (-1,-9, -2) (88.9%) and CMY-2 (11.1%) cephalosporinases. The production of CTX-M and CMY-2 was detected in strains of the following serovars: *S.* Enteritidis, *S.* Typhimurium, *S.* Virchow, *S.* Abony, *S.* Coeln, *S.* Newport, *S.* Haifa, S. Derby, *S.* Kentucky, *S.* Dublin.
4. Resistance to quinolones in *Salmonella* strains is mainly due to chromosomal mechanisms. We identified five variants of single-nucleotide substitutions in the *gyr*A gene, causing a low level of resistance to quinolones: Asp87Asn (*S.* Typhi — 71.4% of resistant strains; other serovars — 23.1%); Ser83Tyr (6.7% and 10.3%); Ser83Phe (0.9% and 20.5%); Asp87Tyr (33.3% — only in strains of *S.* Infantis); Asp87Gly (7.7% — only strains of *S.* Enteritidis). The high level of resistance to quinolones in almost all strains was due to the identical profile of single nucleotide substitutions: *gyrA* (Ser83Phe + Asp87Asn) and *parC* Ser80Ile. The plasmid resistance mechanism (*qnrS* gene) was detected in three strains (0.3%, *S.* Typhi, *S.* Corvallis, *S.* Typhimurium).
5. The research identified, among clinical isolates of *Salmonella enterica* and *Escherichia coli* in the Russian Federation, strains belonging to the successful international multidrug resistant clones at high risk of pandemic spreading, with global distribution in the world: *Salmonella Kentucky* ST198, *Salmonella Newport* AmpC-MDR/CMY-2, *Salmonella Typhi* subclade 4.3.1 (haplotype H58), *Escherichia coli* O26:H11 ST21 and *Escherichia coli* O25:H4-B2-ST131. Full genome sequencing of strains, the use of standardized methods of analysis and international databases containing detailed information on the genetic characteristics of pathogens have made it possible to detect high-risk international clones, as well as to assess their evolution and geographical distribution due to international tourism and trade in food, farm animals and feeds, and to identify specific transmission factors.

Publications

1. *Alieva E.V., Kaftyreva L.A., Makarova M.A., Tartakovsky I.S. Practical recommendations for the preanalytical stage of microbiological studies. Part I. Bacteriological studies // Laboratory Service. 2020; 9 (2): 45–66. (In Russ.)*
2. *Bilozor A., Balode A., Chakhunashvili G., Chumachenko T., Egorova S., Ivanova M., Kaftyreva L., Kõljalg S., Kõressaar T., Lysenko O., Miciuleviciene J., Mändar R., Lis D.O., Wesolowska M.P., Ratnik K., Remm M., Rudzko J., Rööp T., Saule M., Sepp E., Shyshporonok J., Titov L., Tsereteli D., Naaber P. Application of molecular methods for carbapenemase detection // Front. Microbiol. 2019; 10: 1755. doi: 10.3389/fmicb.2019.01755* **WoS**
3. *Egorova S.A., Kaftyreva L.A., Kazanovskaya N.S. Phenotypic and molecular characteristics of Salmonella strains resistant to extended-spectrum cephalosporins // Problems of Medical Mycology. 2020; 22 (4): 54–59. (In Russ.)*
4. *Egorova S.A., Kaftyreva L.A., Pomazanov V.V. Modern trends in the development of resistance of Salmonella bacteria to clinically significant antimicrobial drugs (literature review) // Russian Clinical Laboratory Diagnostics. 2020; 65 (5): 308–315. (In Russ.) doi: 10.18821/0869-2084-2020-65-5-308-315* **Scopus**
5. *Egorova S.A., Kaftyreva L.A., Suzhaeva L.V., Zabrovskaya A.V., Voitenkova E.V., Matveeva Z.N., Ostankova Yu.V., Likhachev I.V., Satosova N.V., Kitsbabashvili R.V., Smirnova E.V., Semchenkova L.I., Bystraya T.E., Sokolnik S.E., Utkina N.P., Sikhando L.Yu. Antimicrobial resistance and clinically significant mechanisms of resistance of Salmonella strains isolated in 2014-2018 in St. Petersburg, Russia // Russian Clinical Laboratory Diagnostics. 2019; 64 (10): 620–626. (In Russ.) doi: 10.18821/0869-2084-2019-64-10-620-626* **Scopus**

6. *Egorova S.A., Kaftyreva L.A. Methodological approaches to determining the sensitivity of Salmonella strains to fluoroquinolones // CMAC. 2020; 22 (4): 314–320. (In Russ.)*
7. *Egorova S.A., Kaftyreva L.A. Methodological specifics of determining the sensitivity of Salmonella strains to antimicrobial drugs // Russian Clinical Laboratory Diagnostics. 2019; 64 (6): 368–375. (In Russ.) doi: 10.18821/0869-2084-2019-64-6-368-375* ***Scopus***
8. *Egorova S.A., Kuleshov K.V., Kaftyreva L.A., Matveeva Z.N. The antimicrobial susceptibility, resistance mechanisms and phylogenetic structure of S. Typhi isolated in 2005-2018 in the Russian Federation // Russian Journal of Infection and Immunity. 2020; 10 (1): 99–110. doi: 10.15789/2220-7619-ASM-1171* ***WoS***
9. *Egorova S.A., Kuleshov K.V., Kaftyreva L.A. Modern methods of Salmonella subtyping in the investigation of Salmonella outbreaks // Immunopathology, Allergology, Infectology. 2019; 3: 36–42. (In Russ.) doi: 10.14427/jipai.2019.3.33* ***RSCI***
10. *Kaftyreva L.A., Egorova S.A., Makarova M.A. Detection of international high-risk clones of Salmonella and Escherichia coli, pathogens of food-borne diseases, in the Russian Federation // Russian Journal of Infection and Immunity. 2020; 10 (3): 565–569. (In Russ.) doi: 10.15789/2220-7619-DOI-1506* ***WoS***
11. *Kaftyreva L.A., Porin A.A., Ryzhman N.N., Kolosovskaya E.N. Screening studies in the diagnosis of chronic carriership of typhoid fever pathogen among residents of various countries // Bulletin of the Russian Military Medical Academy. 2020; 1 (69): 91–94. (In Russ.)*
12. *Likhachev I.V., Kraeva L.A., Samoylova A.A., Rogacheva E.V., Kaftyreva L.A., Egorova S.A., Mikhailov N.V. Practical evaluation of domestically produced test strips for determining the sensitivity of microorganisms to antimicrobial drugs by gradient diffusion method // Russian Clinical Laboratory Diagnostics. 2020; 65 (9): 557–561. (In Russ.) doi: 10.18821/0869-2084-2020-65-9-557-561* ***Scopus***
13. *Makarova M.A., Kaftyreva L.A. Genetic diversity of enteroaggregative Escherichia coli strains // Russian Clinical Laboratory Diagnostics. 2020; 65 (11): 699–703. (In Russ.) doi: 10.18821/0869-2084-2020-65-11-699-703* ***Scopus***
14. *Makarova M.A., Kruglov E.E., Matveeva Z.N., Zveryakina N.N., Kaftyreva L.A. The characteristics of Escherichia coli strains isolated in acute appendicitis and chronic ulcerative colitis // Problems of Medical Mycology. 2020; 22 (4): 66–71. (In Russ.)*
15. *Makarova M.A., Matveeva Z.N., Kaftyreva L.A., Mikhailova E.A., Kuzakova M.A., Laushkina O.I., Kuzmina T.M., Samsonova O.E., Alekseenko L.I., Postnova I.A., Fedorova L.M. Characteristics of antibiotic sensitivity of Shigella sonnei strains isolated from patients from foci of shigellosis that occurred in the North-Western Federal District in 2018 // Bacteriology. 2020; 5 (2): 18–23. (In Russ.) doi: 10.20953/2500-1027-2020-2-18-23* ***RSCI***
16. *Makarova M.A., Matveeva Z.N., Smirnova E.V., Semchenkova L.I., Derevyanchenko I.A., Sokolnik S.E., Zhirnova L.Yu., Kotova N.K., Pelenko T.F., Dudnikov D.S., Vasilyeva N.V., Kaftyreva L.A. Laboratory errors in the identification of Escherichia coli strains of serological groups O6 and O25 as causative agents of acute enteric infections // Russian Clinical Laboratory Diagnostics. 2020; 65 (6): 368–374. (In Russ.) doi: 10.18821/0869-2084-2020-65-6-368-374* ***Scopus***
17. *Sepp E., Andreson R., Balode A., Bilozor A., Brauer A., Egorova S., Huik K., Ivanova M., Kaftyreva L., Kõljalg S., Kõressaar T., Makarova M., Miciuleviciene J., Pai K., Remm M., Rööp T., Naaber P. Phenotypic and molecular epidemiology of ESBL-, AmpC-, and carbapenemase-producing Escherichia coli in Northern and Eastern Europe // Front. Microbiol. 2019; 10: 2465. doi: 10.3389/fmicb.2019.02465* ***WoS***
18. *Suzhaeva L.V., Makarova M.A., Kaftyreva L.A. Phylogenetic groups and virulence genes of Escherichia coli strains isolated from the intestinal microbiota of children // Russian Clinical Laboratory Diagnostics. 2020; 65 (4): 251–257. (In Russ.) doi: 10.18821/0869-2084-2020-65-4-251-257* ***Scopus***
19. *Suzhaeva L.V., Yegorova S.A. Resistance to antimicrobial drugs of Escherichia coli strains isolated from the intestinal microbiota of children // Russian Clinical Laboratory Diagnostics. 2020; 65 (10): 638–644. (In Russ.) doi: 10.18821/0869-2084-2020-65-10-638-644* ***Scopus***

Patents

1. *Patent RU2707548: Egorova S.A., Kaftyreva L.A. The bacterial strain Salmonella enterica subsp. enterica serovar Typhi B-8453 with low-level resistance to fluoroquinolones, used as a control strain for phenotypic and molecular studies for typhoid fever diagnostics. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
2. *Patent RU2707640 C1: Makarova M.A., Kaftyreva L.A. An Escherichia coli strain as a control test strain for phenotypic and molecular studies of Escherichia of serological group O144. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
3. *Patent RU2707925: Egorova S.A., Kaftyreva L.A. Salmonella enterica subsp. enterica serovar Typhi bacterial strain as a control strain for phenotypic and molecular studies in the diagnostics of typhoid fever. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
4. *Patent RU2744203: Egorova S.A., Kaftyreva L.A. The bacterial strain Salmonella enterica subsp. enterica serovar Kentucky B-9045 of the international polyresistant Salmonella clone Kentucky ST 198, used as a control strain for phenotypic and molecular studies in salmonellosis diagnostics. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
5. *Patent RU2744205: Egorova S.A., Kaftyreva L.A. The bacterial strain Salmonella enterica subsp. enterica serovar Newport B-9044 of the international polyresistant Salmonella clone Newport MDR-AmpC/CMY-2, used as a control strain for phenotypic and molecular studies in salmonellosis diagnostics. Applicant and copyright holder: St. Petersburg Pasteur Institute.*

Certificates of registration for databases

1. *Certificate of registration for database RUS 2019621507: Egorova S.A., Kaftyreva L.A., Porin A.A. S.Typhi-Museum: biological properties of the causative agent of typhoid fever. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
2. *Certificate of registration for database RUS 2019622278: Egorova S.A., Kaftyreva L.A. Salmonella-Museum: antibiotic sensitivity and mechanisms of resistance. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
3. *Certificate of registration for database RUS 2019621937: Makarova M.A. Molecular and genetic characteristics of Escherichia coli strains isolated in cases of extraenteric diseases. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
4. *Certificate of registration for database RUS 2020620406: Egorova S.A., Kaftyreva L.A. S.* Typhi*-Museum: molecular determinants of resistance. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
5. *Certificate of registration for database RUS 2020620407: Suzhaeva L.V., Kaftyreva L.A. Escherichia coli in the intestinal microbiota of children: antibiotic sensitivity and resistance mechanisms. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
6. *Certificate of registration for database RUS 2020620513: Suzhaeva L.V., Makarova M.A., Kaftyreva L.A. Escherichia coli in the intestinal microbiota of children: phenotypic characteristics, virulence genes, phylogenetic groups. Applicant and copyright holder: St. Petersburg Pasteur Institute.*

7. *Certificate of registration for database RUS 2020621588: Zabrovskaya A.V., Kaftyreva L.A., Makarova M.A., Egorova S.A. Opportunistic pathogens isolated from animals and from animal products, their resistance to antimicrobial drugs and virulence factors. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
8. *Certificate of registration for database RUS 2020620404: Zabrovskaya A.V., Kaftyreva L.A., Kuzmin V.A., Khakhaev I.A., Chunin S.A., Antipova N.A., Redina V.E. Salmonella isolated from animals, animal products and feeds. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
9. *Certificate of registration for database RUS 2020620646: Makarova M.A., Kaftyreva L.A. Molecular and genetic characteristics of commensal Escherichia coli strains isolated from healthy (without signs of acute intestinal infection) adult residents of St. Petersburg. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
10. *Certificate of registration for database RUS 2020621032: Makarova M.A., Kaftyreva L.A. Biological properties of enteropathogenic Escherichia coli (EPEC) strains isolated from stool samples of patients with diarrheal syndrome. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
11. *Certificate of registration for database RUS 2020621033: Makarova M.A., Kaftyreva L.A. Biological properties of enterotoxigenic Escherichia coli (ETEC) strains isolated from stool samples of patients with diarrheal syndrome. Applicant and copyright holder: St. Petersburg Pasteur Institute.*
12. *Certificate of registration for database RUS 2020621031: Makarova M.A., Kaftyreva L.A. Biological properties of shigAtoxin-producing Escherichia coli (STEC) strains isolated from stool samples of patients with diarrheal syndrome. Applicant and copyright holder: St. Petersburg Pasteur Institute.*

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

Relevance of the study. Pertussis remains an urgent healthcare problem in many countries of different regions of the world, including the Russian Federation. Neither vaccination nor a previous illness provide lifelong immunity. Pertussis is most dangerous for children of the first year of life; their older siblings, as well as adult family members, are most often the source of infection for infants. Since full protection against pertussis requires a full cycle of primary vaccination, which in the Russian Federation, according to the national vaccination schedule, is completed in children by the age of 6 months, in the first six months of life they can be protected only through anti-epidemic measures in their environment. National schedules of many countries include revaccination of children aged 5–7 years; revaccination of adolescents aged 14–16 years and medical officers working with children of the first year of life is carried out in a number of countries, and revaccination of adults every 10 years is recommended together with diphtheria and tetanus revaccination; the effectiveness of vaccination of pregnant women has been shown. Individual regional revaccination programs for preschool children (6–7 years old) are being implemented in the Russian Federation, but to date none of the options of additional revaccination has been widely implemented, including due to the lack of appropriate amendments to the national vaccination schedule. Therefore, establishing a scientific rationale for the introduction of an additional pertussis revaccination in the Russian Federation is relevant for determining the strategy of vaccinal prevention of this infection.

To understand the epidemiological situation regarding diphtheria and pertussis, systematic monitoring of circulating strains that cause the infections is necessary. Due to ongoing vaccination, the incidence of diphtheria has significantly decreased in recent years. For example, in 2017, no cases of diphtheria were registered in the Russian Federation and only 2 cases of bacterial carrier state were detected. In 2018, 4 cases of diphtheria and 3 cases of carrier state were reported. Mild localized forms of the infection prevail. However, diphtheria remains a relevant issue, since lack of alertness to it and likely import from other regions can contribute to new cases of diphtheria, especially among unvaccinated. Moreover, in recent years, there have been cases of atypical forms of infection caused by non-toxic strains of *C. diphtheriae*. It is especially important to study the patterns of spread of various bacterial clones, taking into account their pathogenicity factors and resistance to antibiotics prescribed as etiological therapy for diphtheria and pertussis.

Study objective. Research into seroprevalence to the causative agent of pertussis among the population of St. Petersburg; the study of antibiotic susceptibility of circulating strains of Bordetella; the study of genetic and phenotypic markers of virulence and antibiotic resistance in *C. diphtheriae* strains isolated in the Northwestern Region over the past 25 years.

Materials and methods. To assess seroprevalence to the causative agent of pertussis, ELISA kits were used to determine antibodies to pertussis toxin (IgG, IgA). The following levels of antibodies were considered positive: IgG ≥ 40 IU/ml, IgA ≥ 12 IU/ml. A positive level of IgG was regarded as evidence of contact with the causative agent (disease or latent immunization) in the last 1–2 years, whereas a high IgG level (≥ 100 IU/ml) or a combination of positive IgG and IgA were regarded as evidence of pertussis disease in the previous 6–12 months before the examination. For the screening of macrolide-resistant *Bordetella* strains, disk diffusion test and determination of MIC using E-tests were carried out, casein-charcoal agar being used as a medium.

Genetic and phenotypic methods were used to study the toxigenic properties of *C. diphtheriae* strains. To detect the toxigenic gene, a recently developed LAMP kit (Innova-plus, St. Petersburg) was tested using a certified kit for detecting the toxigenic gene by the Real-time PCR as a control. Phenotypic toxin production was detected in the Elek test. The ability to biofilm formation was studied using a fluorescent microscope. Antibiotic susceptibility was tested using the disk diffusion test according to the procedural guidelines MUK 4.12.1890–04 (Moscow, 2004) and the procedural guidelines "Antimicrobial susceptibility testing of microorganisms" (EUCAST, 2018).

Key results. To assess seroprevalence to the causative agent of pertussis, 200 adults were examined in 2019. IgG to pertussis toxin was detected in 32 persons (16%), including in 10 persons (5%), serological markers of recent pertussis were detected. Medical staff of a maternity hospital (78 persons) was examined: antibodies were detected in 15.4% of doctors, including in 5.1% there were serological markers of recent pertussis. A survey of doctors (51 person) in an outpatient facilities chain revealed 27.5% of seropositives, including 5.9% with signs of recent pertussis. The disk diffusion testing was used to examine 100 strains of *Bordetella* isolated from patients and contacts in St. Petersburg in 2000–2010. All strains were sensitive to erythromycin, amoxicillin, and cefotaxime. In 20 strains, the MIC of erythromycin was found; it averaged 0.06 μg/ml.

It was found that the strains of *C. diphtheriae* isolated in the Northwestern Region over the last 5 years did not show toxigenic properties in phenotypic tests. However, 40% of these strains carry a toxigenic gene detected by LAMP and confirmed by classical PCR. Thus, in contrast to the strains isolated during the diphtheria epidemic in 1993–1995, the *C. diphtheriae* strains currently circulating carry the "silent" toxogenicity gene much more often (in 13% and 40%, respectively). The ability to produce biofilms was found in 30% of *C. diphtheriae* strains isolated over the last 5 years. In addition, an increasing resistance of *C. diphtheriae* strains to the antibiotics of choice for diphtheria treatment has been noted over the years. For example, the resistance of *C. diphtheriae* strains isolated in 1993–1995 was found in 0.12% of cases, whereas the strains isolated in 2015–2019 were found to be resistant in 59% of cases. The same is true of tetracycline (0.06% of resistant strains in 1993–1995 and 83% — in 2015–2019).

Practical relevance. The conducted research revealed wide involvement of medical staff, including those working with infants, in the epidemic process of pertussis, suggesting that revaccination against pertussis of this professional group is needed. An unfavourable trend was revealed, i.e. an increasing proportion of *C. diphtheriae* strains carrying a "silent" toxigenic gene and resistant to the antibacterial drugs that are prescribed for diphtheria infection. A novel LAMP method was tested, which allows screening of strains and biomaterial from patients for the presence of toxigenic strains of *C. diphtheriae*, even in a poorly equipped bacteriological laboratory.

Prospects for further research into the topic. Seromonitoring of pertussis infection in children, adolescents and adults will continue in order to justify recommendations for pertussis revaccination in risk group. The study of antimicrobial susceptibility of the *Bordetella* strains circulating in St. Petersburg will continue.

It is necessary to further study various pathogenicity factors of *C. diphtheriae* strains that have been circulating in the Northwestern Federal District over the past 25 years. Testing of a number of promising methods for express diagnosis of pathogenicity of diphtheria pathogens will also continue.

Improvement of microbiological monitoring of pseudotuberculosis based on the use of new genotyping methods

Relevance of the study. Pseudotuberculosis is registered across much of the Russian Federation. At the same time, the nosoarea of the disease includes regions in the Far East, Siberia, the Urals and the Northwest of the country with a consistently high incidence rate exceeding the federal level by 2–5 times or more. Currently, in Russia, pseudotuberculosis occurs mainly as sporadic cases, whereas epidemic outbreaks are rare.

CRISPR-Cas systems have good prospects as a conclusive molecular marker for microbiological monitoring of circulating strains of *Y. pseudotuberculosis*. High resolution of genotyping based on spacer sequences of CRISPR loci has beed shown in the study of *Y. pestis* strains.

Study objective. Genotypic characterization of *Y. pseudotuberculosis* strains of various geographical origin based on the identification of pathogenicity factors and analysis of the locus composition of CRISPR-Cas systems.

Materials and methods. 167 strains of *Y. pseudotuberculosis* were examined, isolated from people and animals, including birds, with pseudotuberculosis, and from wipe samples of vegetables in the Russian Federation (127 strains), a number of neighbouring countries (Ukraine, Belarus, Abkhazia, Turkmenistan), as well as some other countries (UK, Sweden, Mongolia) (40 strains) over the last 80 years (1935–2014). Major pathogenicity factors were determined in the strains: chromosomal genes of superantigen YPM, pathogenicity islands YAPI and HPI, and plasmid genes pYV 46 MDa and pVM82. Primers for the amplification of CRISPR loci were developed earlier using the NCBI Primer-BLAST online application based on 15 full-genome sequences of *Y. pseudotuberculosis* strains deposited in the GenBank and RefSeq NCBI databases. Identification of CRISPR systems in the genomes of *Y. pseudotuberculosis* strains was carried out using the CRISPRone and CRISPRFinder online programs.

Key results. The study showed that all the examined strains have a CRISPR-Cas system with one to three loci. The size of the YP1 locus is 400–3500 bp, the YP2 locus is 250–500 bp, the YP3 locus is 400–2000 bp.

According to the results of the major pathogenicity factors identification, the studied strains of *Y. pseudotuberculosis* belong to one of four genogroups: G1 (HPI+YPM+) — 4 strains, G2 (HPI+YPM–) — 42 strains, G3 (HPI–YPM+) — 111 strains and G6 (HPI–YPM–) — 10 strains.

The majority of strains (144 strains, 86.2%) have a CRISPR-Cas system consisting of three loci. At the same time, the strains of the G3 genogroup are the most homogeneous ones, as 95.5% of these strains have three CRISPR loci. The strains of G2 genogroup are the most variable in terms of the locus composition of CRISPR systems: they have five combinations of CRISPR loci types.

It was shown that the length of the YP3 locus statistically significantly correlates with the presence of the major pathogenicity factors in the strains: chromosomal genes of superantigen YPM, pathogenicity islands YAPI and HPI and pVM82 plasmid genes (Fig. 2).

The majority of the studied strains of various origins (n = 91) were isolated within the Siberian Federal District (SFD) — one of the regions of the Russian Federation with the highest incidence of pseudotuberculosis. For comparison, we studied strains from the Far Eastern Federal District (FEFD) (n = 17) and the Northwestern Federal District (NWFD) (n = 12), where high incidence rates are also reported.

It was shown that the serotype O:1b strains of genogroup G3 (88 strains, 97%) predominate in the SFD; the genogroup is divided into subgroups depending on the presence of the pVM82 plasmid and the adhesion island of pathogenicity YAPI (including the *tcp* gene). Strains of the G3 genogroup cause pseudotuberculosis with symptoms of the Far East scarlet-like fever (FESLF) such as exanthema, peeling skin, red tongue, and joint lesions. The strains of the 3c genetic subgroup (HPI–YPM+YAPI+pVM82+) are predominant (43 strains, 47%). They have shown a variant of the CRISPR-Cas system including the CRISPR loci YP1 2000 bp/YP2 300 bp/YP3 2000 bp (Fig. 3, cluster I). The SFO also has strains of the genetic subgroup 3a (HPI–YPM+YAPI–pVM82–) (28 strains, 31%), with the sizes of CRISPR loci in the following ranges: YP1 1500–2000 bp/YP2 300 bp/YP3 800–1000 bp (Fig. 3, cluster II).

Y. pseudotuberculosis strains of genogroup G3 are also found in the territories of the FEFD and the NWFD, while some strains in these regions belong to subgroup 2a of genogroup G2 (HPI+YPM–YAPI–pVM82–), typical of pathogens mainly distributed in Europe. At the same time, in the Far Eastern Federal District, all strains of subgroup 2a belong are of O:1a serotype. These pathogens cause a disease without signs typical for Russia, manifesting mainly with gastrointestinal symptoms, without clinical manifestations of the FESLF. The strains of genogroup G2 isolated within the FEFD and the NWFD in the length of the loci of CRISPR-Cas systems differ from the strains of genogroup G3 characteristic of the SFD.

Among the Russian strains, four strains were also identified of a rare genogroup G1 (HPI+YPM+) and one strain of genogroup G6 (HPI–YPM–) (clinical symptoms require clarification).

The studied foreign strains differ from the Russian ones in a set of pathogenicity factors: they are mainly assigned to genogroup G2, subgroup 2a (HPI+YPM–YAPI–pVM82–)

(24 strains, 60%), as well as G6 (HPI+YPM+) (9 strains, 23%). Foreign strains have a variable locus composition of CRISPR-Cas systems and the length of CRISPR loci differs from that of Russian strains.

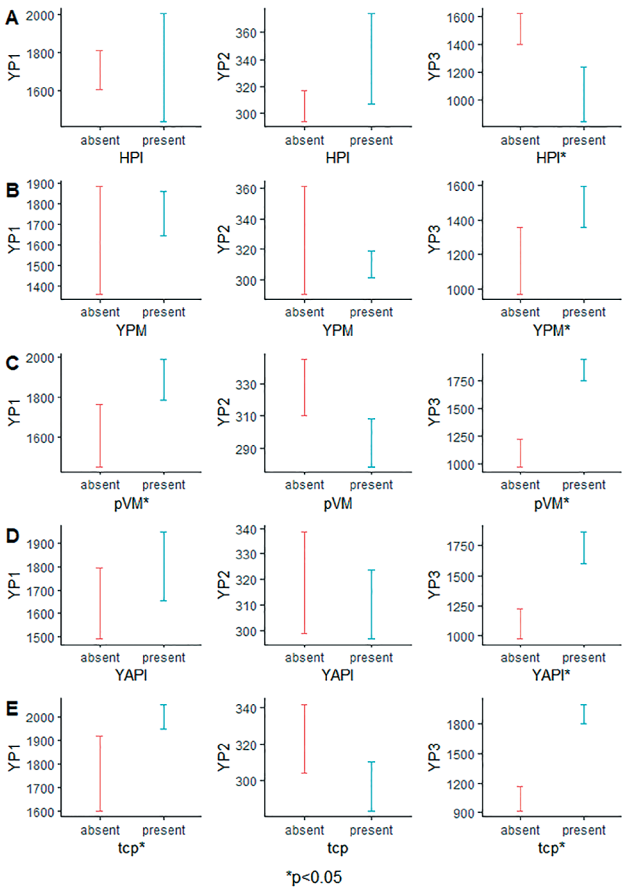

Figure 2. Average lengths of fragments of the YP1, YP2 and YP3 loci, depending on the presence of the major pathogenicity factors: A — HPI; B — YPM; C — pVM82; D — YAPI, including E — *tcp* gene. Absent — negative, present — positive

Earlier, no correlation was found between the spacer composition of loci and the sequence type of strains, but a clear geographical division of strains into "Asian" and "European" clades was revealed, depending on the type of CRISPR cluster (T. Seecharran et al., 2017). The study revealed a relationship between the lengths of the YP3 locus and the presence of the major pathogenicity determinants of the strains: chromosomal genes of superantigen YPM, pathogenicity islands YAPI and HPI, and pVM82 plasmid genes.

Besides, the coexistence of different phylogenetic groups in one territory suggests there are mechanisms in the population that restrict the transfer of genetic material (restriction-modification enzymes, the CRISPR-Cas system) (Seecharran et al., 2017). The CRISPR-Cas system of *Y. pseudotuberculosis*, which is regulated by multiple mechanisms, can contribute to the formation of a certain genotype of the strain, which, in turn, determines clinical manifestations of pseudotuberculosis.

Conclusion. New data on the structure of *Y. pseudotuberculosis* CRISPR-Cas systems isolated within the Russian Federation and some foreign countries were obtained. The locus composition of CRISPR-Cas systems of *Y. pseudotuberculosis* strains of various geographical origin and isolation sources was analyzed.

The variants of CRISPR-Cas systems (locus composition, length of CRISPR loci) were compared with the data on the geographical origin of the strains, the results of serotyping and attributing to genetic groups (subgroups) according to the presence of the major pathogenicity factors. As a result, genetic variants that dominate in certain territories were identified.

It was shown that the length of the CRISPR locus YP3 statistically significantly correlates with the presence of the major pathogenicity factors in the strains: the chromosomal genes of superantigen YPM, the pathogenicity islands YAPI and HPI and the pVM82 plasmid genes.

The obtained genotypic characteristics of *Y. pseudotuberculosis* strains from various regions of Russia and other countries, including data on the set of major pathogenicity factors, the locus composition of CRISPR-Cas systems and the length of CRISPR loci, can be used in phylogenetic stud-

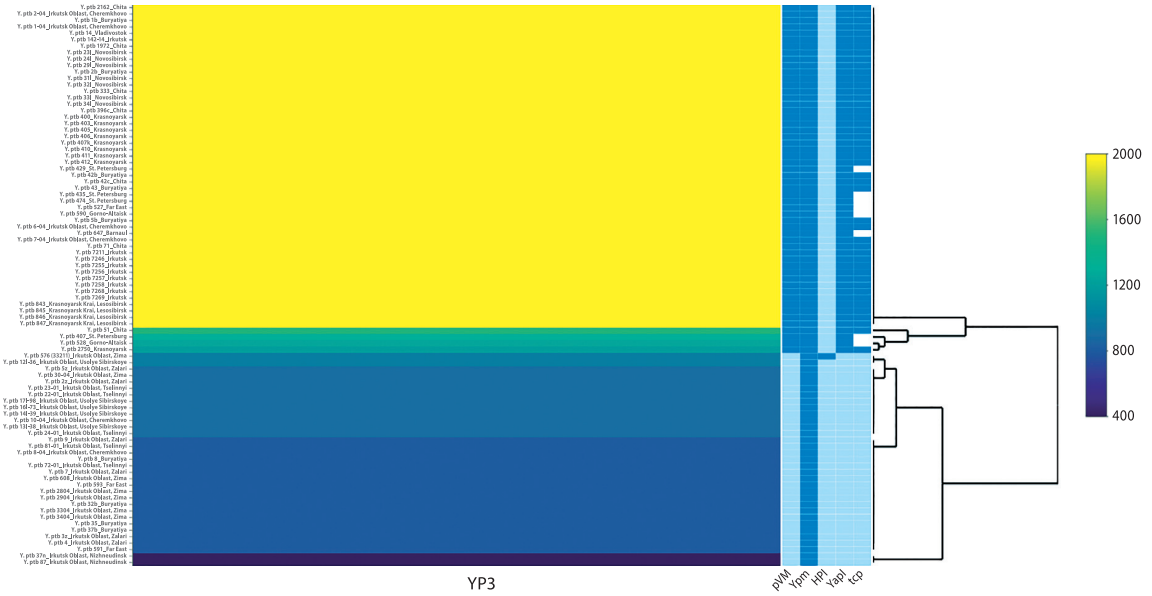

Figure 3. Distribution of the YP3 locus lengths depending on the presence of the major determinants of pathogenicity: pVM82, YPM, HPI and YAPI, including the *tcp* gene

ies and monitoring of circulating strains to identify pathogens of known genetic variants and novel ones that are not typical of the territory of interest.

The discovery in Russia of *Y. pseudotuberculosis* strains of the genetic subgroup 2a, which cause a disease manifesting mainly as gastroenteritis with fever and/or mesenteric lymphadenitis, acute appendicitis, and terminal ileitis, justifies the need for examination for pseudotuberculosis in patients with signs of an acute intestinal infection of unknown etiology.

Publications

1. *Belov A.B., Panin A.L. Theory of sapronoses: History of development and ways of improvement in the system of medical and biological sciences // Journal of Microbiology, Epidemiology and Immunobiology. 2020; 97 (1): 91–101.*
2. *Chuprun S., Dar'in D., Rogacheva E., Kraeva L., Levin O., Manicheva O., Dogonadze M., Vinogradova T., Bakulina O., Krasavin M. Mutually isomeric 2- and 4-(3-nitro-1,2,4-triazol-1-yl) pyrimidines inspired by an antimycobacterial screening hit: synthesis and biological activity against the ESKAPE panel of pathogens // J. Antibiotics. 2020; 10 (9): 666.*
3. *Dmitriev K.A., KraevaL.A., Lyalina L.V. The prevalence of antibiotic-resistant gram-negative pathogens of nosocomial pneumonia in St.- Petersburg, Russia // International Conference "Process Management and Scientific Developments". 2019: 78–84.*
4. *Erofeev V.T., Svetlov D.A., Smirnov V.F., Fedortsov A.P., Kaznacheev S.V., Bogatov A.D., Rodin A.I., Kraeva L.A. Production of Teflex biocidal preparations: from the synthesis of a new polymer to the product line. Part 2. Study of the effect of Teflex preparations on the properties of cement composites // Academia: Architecture and Biulding. 2020; 3: 106–115. (In Russ.)*
5. *Erofeev V.T., Svetlov D.A., Smirnov V.F., Kraeva L.A., Lubchenkov M.A., Svetlov D.D. Production of Teflex biocidal preparations: from the synthesis of a new polymer to the product line. Part 1. Development of manufacturing process for Teflex biocidal preparations // Academia: Architecture and Biulding. 2020; 2: 135–142. (In Russ.)*
6. *Gorbunov I.S., Gubal' A.R., Ganeev A.A., Rodinkov O.V., Kartsova L.A., Bessonova E.A., Arsen'ev A.I., Nefedov A.O., Kraeva L.A. Optimization of the conditions of analysis of exhaled air by gas chromatography-mass spectrometry for the noninvasive diagnostics of lung cancer // J. Anal. Chem. 2019; 74 (11): 1148–1158.*
7. *Il'in M.V., Sysoeva A.A., Bolotin D.S., Novikov A.S., Suslonov V.V., Rogacheva E.V., Kraeva L.A., Kukushkin V.Yu. Aminonitrones as highly reactive bifunctional synthons. An expedient one-pot route to 5-amino-1,2,4-triazoles and 5-amino-1,2,4-oxadiazoles — potential antimicrobials targeting multi-drug resistant bacteria // New J. Chem. 2019; 43 (44): 17358–17366.*
8. *Krasavin M., Lukin A., Vedekhina T., Manicheva O., Dogonadze M., Vinogradova T., Zabolotnykh N., Rogacheva E., Kraeva L., Sharoyko V., Tennikova T.B., Dar'in D., Sokolovich E. Attachment of a 5-nitrofuroyl moiety to spirocyclic piperidines produces non-toxic nitrofurans that are efficacious in vitro against multidrug-resistant Mycobacterium tuberculosis // Eur. J. Med. Chem. 2019; 166: 125–135.*
9. *Kunilova E.S., Kraeva L.A., Panin A.L. The importance of pathogenicity factors of some types of streptococci and klebsiella in determining their causative role in the development of inflammatory processes of the respiratory tract // Russian Journal of Infection and Immunity. 2020; 10 (1): 121–128.*
10. *Likhachev I.V., Kraeva L.A., Samoilova A.A., Rogacheva E.V., Kaftyreva L.A., Egorova S.A., Mikhailov N.V. Evaluation of domestic test strips designed for antimicrobial susceptibility testing by gradient diffusion (E-test) // Klin. Lab. Diagn. 2020; 65 (9): 557–562. (In Russ.)*
11. *Peretolchina N.P., Dzhioev Yu.P., Borisenko A.Yu., Stepanenko L.A., Voskresenskaya E.A., Klimov V.T., Reva O.N., Zlobin V.I. In silico comparative analysis of Crispr-Cas system structures of Yersinia pseudotuberculosis causing different clinical manifestations of pseudotuberculosis // J. Infectol. 2019; 11 (2): 80–87.*
12. *Peretolchina N.P., Klimov V.T., Voskresenskaya E.A., Kokorina G.I., Bogumilchik E.A., Trukhachev A.L., Igumnova S.V., Dzhioev Yu.P., Zlobin V.I. CRISPR-Cas loci of Yersinia pseudotuberculosis strains with different genetic determinants // Epidemiol. Vaccinal Prevent. 2020; 19 (2): 31–39.*
13. *Rostovskii N.V., Koronatov A.N., Sakharov P.A., Agafonova A.V., Novikov M.S., Khlebnikov A.F., Rogacheva E.V., Kraeva L.A. Azirine-containing dipeptides and depsipeptides: synthesis, transformations and antibacterial activity // Org. Biomol. Chem. 2020; 18 (46): 9448–9460.*
14. *Sakharov P.A. Koronatov A.N., Khlebnikov A.F., Novikov M.S., Glukharev A.G., Rogacheva E.V., Kraeva L.A., Sharoyko V.V., Tennikova T.B., Rostovskii N.V. Non-natural 2 H -azirine-2-carboxylic acids: an expedient synthesis and antimicrobial activity // RSC Advances. 2019; 9 (65): 37901–37905.*
15. *Samoilova A.A., Kraeva L.A., Likhachev I.V., Rogacheva E.V., Verbov V.N., Mikhailov N.V., Zueva E.V. Validation of the MPK-MICRO domestic kit designed for the serial microdilution antibiotic susceptibility testing // Clin. Microbiol. Antimicrob. Chemother. 2020; 22 (3): 231–236.*
16. *Sarycheva K.A., Kameneva S.V., Kushchenko N.V., Kraeva L.A., Usikova D.S. The relationship of the adhesion of the oral cavity microorganisms with the finishing method of dental material restoration // Int. J. Pharm. Res. 2020; 12 (4): 693–701.*
17. *Soultanov V., Kraeva L. Antibacterial activity of conifer green needle complex against Corynebacteria // Nat. Prod. Comm. 2020; 1 (15): 1934578X1990061.*
18. *Vedekhina T., Lukin A., Rogacheva E., Kraeva L., Krasavin M. ZN(OTF)2-catalyzed arenehydrazination of protected propargylamines leading to 3-amidoindoles // Tetragedron Letters. 2020; 61 (5): 151430.*
19. *Zimina T., Karasev V., Luchinin V., Kolobov A., Mandrik I., Sitkov N., Kraeva L., Shevchenko N., Orekhov Y. Peptide-based biosensor for express diagnostics of coronavirus respiratory infections // Proceedings. 2020; 60 (1): 52. doi: 10.3390/IECB2020-07059*

Database deposition

1. *Bogumilchik E., Voskresenskaya E., Kokorina G., Goncharov A. Yersinia pseudotuberculosis 379 StPb PI, whole genome shotgun sequencing project. NCBI Reference Sequence: SDWX01000001.1.*
2. *Peretolchina N.P., Dzhioev Yu.P., Klimov V.T., Voskresenskaya E.A., Kokorina G.I., Stepanenko L.A., Bogumilchik E.A., Borisenko A.Yu., Zlobin V.I. Database "Spacer sequences of CRISPR-Cas systems of Yersinia pseudotuberculosis strains" / State Register of databases, 25.12.2020, No. 2020622813.*

LABORATORY OF ZOOANTHROPONOSES

Head of the Laboratory: Nikolay Tokarevich
Researchers: N. Stoyanova, O. Freylikhman, Yu. Panfyorova, E. Syuzyumova, R. Baimova, O. Blinova

In 2019–2020, work was continued to improve the methods of epidemiological surveillance of relevant zoonoses based on molecular-genetic and immunological approaches. As part of this work, the following tasks were solved.

Optimization of laboratory diagnosis methods in order to improve the epidemiological surveillance of zooanthroponoses

Development of a PCR test system for the detection of *C. burnetii* in biological material

The need to increase the sensitivity and specificity of PCR test systems for the detection of *C. burnetii* in biological material is the reason why continuing the work we started earlier on the design of said systems is relevant. In the course of this study, a test system (a set of primers and a fluorescent probe) was developed for the detection of *C. burnetii* in biological material based on real-time PCR amplification of the *higA* gene fragment encoding a protein associated with the virulence of *C. burnetii* using the TaqMan method. The *higA* gene meets the requirements for genetic targets for real-time PCR in terms of conservativeness, variability, antigenic activity of the encoded protein and its relationship with the virulence features of the pathogen.

Real-time PCR with the developed test system was subjected to optimization of components and conditions for the detection of the Q fever causative agent, as well as to the assessment of its specificity and sensitivity. The obtained results suggest that the developed test system is more effective than the currently used commercial test system (based on the amplification of a *groEL* gene fragment). The conducted clinical and technical trials of this test system showed its potential for wide application and registration as a medical device by Roszdravnadzor. Positive results of testing of the developed test system were obtained by the Federal state budgetary healthcare institution of the Head Centre for Hygiene and Epidemiology of the Federal Medical and Biological Agency of Russia (Test Report No. 01.A-007/03.20 dated March 16, 2020).

Improvement of the real-time PCR for the detection of *Leptospira* in biological material

The main disadvantage of the existing kits for the detection of *Leptospira* DNA by real-time PCR is their insufficient sensitivity due to high intraspecific and intrageneric genetic diversity of *Leptospira*. High variability of the *Leptospira* genome is often the reason for the mismatch of the nucleotide sequences of primers and probes of the desired nucleotide sequence used as a target.

This study is aimed at creating a set of oligonucleotide primers and a fluorescent-labelled probe for the real-time PCR detection of a specific (with 100% degree of relatedness) genome area of the genome types of *Leptospira* belonging to the species *Leptospira interrogans* s.l. and *L. biflexa* s.l., genus *Leptospira* belonging to the group of pathogenic *(L. santarosai*, *L. kirschneri*, *L. noguchii*, *L. alexendari*, *L. interrogans*, *L. alstoni*, *L. weilii*, *L. borgpetersenii)* and intermediate *(L. kmetyi*, *L. mayottensis*, *L. inadai*, *L. fainei*, *L. licerasiae*, *L. wolffii*, *L. broomii)* species.

The 16S rRNA gene, which encodes the synthesis of the 16S ribosomal RNA subunit of *Leptospira*, was selected as a target.

When selecting primers, a number of established empirical requirements were met, making it possible to achieve high specificity and efficiency of amplification. To assess the specificity and sensitivity of the primers, DNA of 48 strains of *Leptospira interrogans*, including the genome type *Leptospira interrogans*, serogroups Pomona (7 strains), Canicola (10 strains) and Icterohaemorrhagiae (21 strain), genome type *Leptospira kirschneri*, serogroup Grippotyphosa (6 strains), genome type *Leptospira borgpetersenii*, serogroup Tarassovi (4 strains) kept in the collection of strains of the Pasteur Institute were used, as well as samples of total DNA from biological material: organs (kidneys, liver, spleen) of small wild mammals, blood of healthy donors, blood of people with laboratory-confirmed leptospirosis, and blood of intact guinea pigs. DNA of heterologous microorganisms (*Escherichia coli*, *Borrelia burgdorferi* s.l., *Rickettsia prowazekii*, *Rickettsia conorii*, *Brucella abortus*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Ureaplasma urealiticum*, *Ureaplasma parvum*, *Gardnerella vaginalis*, *Candida albicans*, *Klebsiella oxytoca*, and *Coxiella burnetii*) was also used. Efficacy of the set was assessed using the fluorescence signal accumulation curves resulting from amplification, as well as standard curves. The analysis showed high specificity and sensitivity of real-time PCR when using the developed set of primers and a probe, allowing to detect up to 10^2 GE/ml of biological material Fig. 4. The designed universal set of primers and probe as well as the real-time PCR conditions adapted for its use help to increase identification efficiency and accuracy of the leptospirosis causative agent by finding the genetic material of *L. interrogans* in samples of biological origin using real-time PCR.

On March 03, 2021, the Patent for Invention No. 2744186 for a designed set of reagents for detecting the causative agent of leptospirosis in biological material using real-time PCR was registered in the State Register of Inventions of the Russian Federation.

Development of an enzyme-linked immunoassay system for the detection of IgG antibodies to *C. burnetii* in human sera

To develop this diagnostic system, an inactivated *C. burnetii* antigen purified from tissue impurities was obtained, the optimal concentration of this antigen for plate sorption was determined, optimal dilutions of conjugates from various manufacturers were determined, and the optimal type of plates for the assay was selected. Higher sensitivity of the developed enzyme immunoassay system was established, as compared to the complement fixation test and the indirect immunofluorescence assay. When examining the sera of apparently healthy donors, satisfactory specificity of the enzyme immunoassay system was revealed. Regulatory doc-

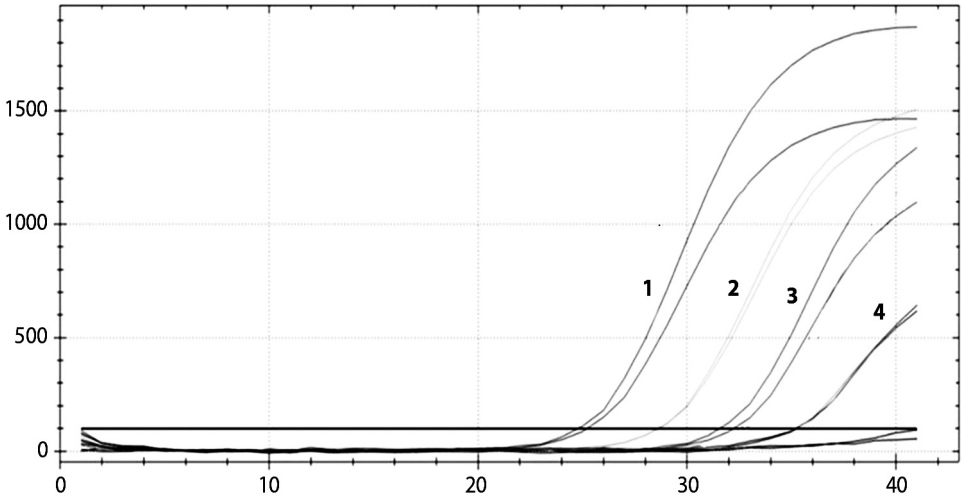

Figure 4. Fluorescence curves of tenfold dilutions of *Leptospira interrogans* DNA, strain Hond Utrecht IV
1 — 10^5 genome equivalents, 2 — 10^4 genome equivalents, 3 — 10^3 genome equivalents, 4 — 10^2 genome equivalents. Image courtesy V.G. Dedkov.

umentation was prepared for the manufacturing and use of an enzyme immunoassay system for the detection of IgG antibodies to *C. burnetii*. Registration certificate (No. P3H 2019/8718 dated 06.08.2019) for the first-ever enzyme immunoassay system developed in Russia for the detection of antibodies to *C. burnetii* in human blood serum was obtained.

Serological monitoring of infections transmitted by ticks and mosquitoes in the Northwestern region of Russia

Infection of residents of the Northwestern Federal District with vector-borne pathogens, including those that cause practically undiagnosed diseases in the studied territories, was established. These include human monocytic ehrlichiosis (in the Arkhangelsk and Pskov Oblast), human granulocytic anaplasmosis (in the Leningrad Oblast), Q fever (in the Arkhangelsk and Leningrad Oblast, in St. Petersburg and the Republic of Karelia) and fevers caused by arbaviruses of the California serogroup (in the Komi and Karelia Republics).

New data on the ecology of *Ixodes ricinus* in the Northwest of Russia and their role in the incidence surge of tick-borne encephalitis (TBE) and ixodes tick-borne borreliosis (TBB)

For many years, it has been believed that in the European territory of Russia, there are spring and autumn peaks in the number of adult ticks whereas in the middle of the summer they are almost completely absent. Our research shows that in the Northwestern region of Russia, the season of adult ticks activity lasts from April to October. The maximum number is recorded in July and August. During the entire season of activity, ticks of the older generation, born from August to early September of the previous year, predominate. At the beginning of the activity season (from April to June), ticks of the previous generation that were active last year may still be present in the populations (up to 17%). At the end of the activity season, from 30 to 60% of new generation individuals may be present in collections. Just like in adult ticks, in nymphs, two generations are present during the activity season. The results of ticks study using real-time PCR (based on the amplification of the 16SrRNA and Hbb gene fragments) indicate that the infection of imago ticks with *Borrelia burgdorferi* s.l. decreases as the population ages.

First cases of tick attacks on humans have been recorded in Arctic regions, with an average annual air temperature close to zero. An exponential increase in the tick-bite incidence rate (TBIR)* is observed in areas with temperatures from +0.5°C to +4°C. "Saturation", when the TBIR stops to increase with an increase in air temperature, are observed at average annual air temperatures exceeding +4°C.

The value of TBIR of about 1000 per 100 000 can be considered as the "saturation" value. This means that, as the air temperature in the region increases due to climate changes, the TBIR increases up to 1000, sometimes more, and then tends to be maintained at the level of 1000 and does not grow further. Hence we can draw a preliminary conclusion that the population of ticks also remains stable and does not grow with increasing temperature.

It was shown that in the Komi Republic, as well as in the neighbouring territories of the European North of Russia, ixodic ticks spread to the northern territories. As we found earlier, the main reason for the expansion of tick habitat is an increase in air temperature, especially during their activity period. Circulation of pathogens transmitted by ixodic ticks, and, consequently, the incidence of tick-borne infections, largely depend on environmental factors that act through the body of vectors. This paper shows that along with climatic changes, the transformation of vegetation cover in certain regions of the Komi Republic due to deforestation can cause a local increase in the number of *Ixodes persulcatus* in certain territories.

A surge in the incidence of TBE and TBB is caused by a whole set of reasons: the expansion of ticks into "new" territories, their increasing number and prevalence in terms of tick-borne encephalitis virus and *Borrelia burgdorferi*, both collected from grass on a flag and removed from people and animals, and the prolonged period of tick

*Tick-bite incidence rate (TBIR) is the number of tick victims per 100 000 of population.

activity. Social factors, such as professional activity of residents of the Komi Republic, age-specific activity of adolescents, deforestation etc., probably also contribute to the increased incidence of the TBE.

New data collected during this study on the ecological and epidemiological characteristics of TBE and TBB in the Komi Republic demand for targeted prevention of tick-borne infections, taking into account their local features. In our opinion, the northern territories of the Komi Republic, where cases of tick attacks on residents have been registered recently, should be considered as potentially endemic.

Publications

1. *Gnativ B.R., Tokarevich N.K. Results of long-term monitoring of tick-borne viral encephalitis and tick-borne borreliosis in the Komi Republic // Russian Journal of Infection and Immunity. 2021; 11 (4): 707–722. (In Russ.)*
2. *Grigoryeva L.A., Tokarevich N.K., Freilikhman O.A., Samoylova E.P., Lunina G.A. Seasonal changes in of sheep tick, Ixodes ricinus (L., 1758) (Acari: Ixodinae) in natural biotopes of St. Petersburg and Leningrad province, Russian Federation // Syst. Appl. Acarol. 2019; 24 (4): 701–710. doi: 10.11158/saa.24.4.14*
3. *Panferova Yu.A., Freilikhman O.A., Tokarevich N.K., Naidenova E.V., Senichkina A.M., Agafonov D.A., Konstantinov O.K. Detection of Coxiella burnetii in ticks collected from cattle on the territory of some provinces of the Republic of Guinea // Epidemiology and Infectious Diseases. 2019; 4 (5–6): 234–2020. (In Russ).*
4. *Samoilov A.E., Stoyanova N.A., Tokarevich N.K., Evengard B., Zueva E.V., Panferova Y.A., Ostankova Y.V., Zueva E.B., Valutite D.E., Kovalev E.V., Litovko A.R., Goncharov A.U., Semenov A.V., Khafizov K., Dedkov V.G. Lethal outcome of Leptospirosis in Southern Russia: characterization of Leptospira interrogators isolated from a deceased teenager // Int. J. Environ. Res.Public Health. 2020; 17 (12): 4238. doi: 10.3390/ijerph17124238*
5. *Syuzyumova E.A., Telnova N.V., Shapar A.O., Stoyanova N.A., Tokarevich N.K. Ecological and epidemiological characteristics of tick-borne encephalitis in St. Petersburg // Russian Journal of Infection and Immunity. 2020; 10 (3): 533–542. (In Russ.) doi: 10.15789/2220-7619-EAE-924*
6. *Tronin A., Tokarevich N., Blinova O., Gnativ B., Buzinov R., Sokolova O., Evengard B., Pahomova T., Bubnova L., Safonova O. Study of the relationship between the average annual temperature of atmospheric air and the number of tick-bitten humans in the North of European Russia // Int. J. Environ. Res. Public Health. 2020; 17 (21): 8006. doi: 10.3390/ijerph17218006*
7. *Tronin A.A., Tokarevich N.K., Gnativ B.R. Abundance of Ixodes persulcatus ticks in Komi Republic as a function of an air temperature // Russian Journal of Infection and Immunity. 2019; 9 (5–6): 811–816. doi: 10.15789/22207619201956-811-816*

Department of Virology

LABORATORY OF ETIOLOGY AND VIRAL INFECTIONS CONTROL

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Surveillance of poliomyelitis and enterovirus infection

St. Petersburg subnational laboratory for the diagnosis of polio on the basis of the Pasteur Institute is part of the Polio Laboratory Network of the World Health Organization (WHO). As part of the Global Polio Eradication Initiative, it oversees polio and acute flaccid paralysis cases in 14 regions of the Russian Federation. The laboratory receives samples of primary material from children under 15 years of age with the acute flaccid paralysis syndrome, from people exposed to them, from patients diagnosed with enterovirus infection and from children from risk groups, including children from migrant families who arrived from polio-affected regions. The laboratory monitors the circulation of polioviruses and non-polio enteroviruses among various population groups, and also examines samples from the environment in order to detect polioviruses and non-polio enteroviruses.

All virological studies, which are the "gold standard" of the Global Polio Eradication Initiative, and molecular studies are carried out in accordance with the WHO protocols and in compliance with the containment requirements. Every year, the laboratory performs professional tests provided by the WHO, and undergoes the annual WHO accreditation. Every year, the Researchers produce summary reports on maintaining the polio-free status of 14 administrative territories of Russia. These reports are sent to the Federal Service of Rospotrebnadzor to draft a report from the Russian Federation to the WHO for the certification of the Russian Federation as a polio-free country.

In 2019–2020, 204 samples were examined from patients with the acute flaccid paralysis (AFP). The tests revealed 3 polioviruses type 3 (PV3), which, according to the results of intratypic differentiation, were vaccine-derived. 8 non-polio enteroviruses were also isolated from patients with AFP, including Coxsackievirus A2, Coxsackievirus B1–6, and Echovirus 11.

In 2019, one case of vaccine-associated paralytic polio was registered in a child aged 10 months. The child received the first dose of polio vaccine, which was, according to the local records, the Polimilex inactivated trivalent vaccine. The child A.D., together with his mother, was admitted to the Central Infectious Disease Hospital of the FMBA of the Russian Federation in St. Petersburg for treatment. In the laboratory, a vaccine-derived PV3 was isolated from two fecal specimens. The mother's fecal specimens were negative. In the first blood serum of the child, antibodies to polioviruses were detected in the following titres: PV1 — 1:256, PV2 < 1:8, PV3 — 1:64. The absence of antibodies to poliovirus type 2 could be evidence of the use of an oral polio vaccine not containing poliovirus type 2 for vaccination of the child, and erroneous data in the vaccination papers. In the second blood serum of the child, taken 21 days after the first one, an eight-fold increase in the titre of antibodies to PV3 was detected (1:512), which was the causative agent of the disease. On day 60, the patient had residual paralysis. The final diagnosis is "vaccine-associated paralytic polio in a recipient/contact".

Throughout 2019 and 2020, the laboratory examined samples of children from migrant families in order to search for polioviruses and non-polio enteroviruses. In 2019–2020, the virological laboratory of the St. Petersburg RC examined material from 375 healthy children from migrant families who arrived from Tajikistan, Uzbekistan, Kazakhstan and Ukraine, as well as from the polio-affected territories of the Russian Federation (the Chechen Republic and the Republic of Dagestan). During the examination of the samples, five vaccine-derived polioviruses (1.3%) were isolated from healthy children from migrant families: three polioviruses were classified as type 1 and two were classified as type 3. 16 (4.3%) non-polio enteroviruses were also isolated. Polioviruses were isolated from children who arrived from Tajikistan and the Chechen Republic. Non-polio enteroviruses of various serotypes were isolated from healthy children from migrant families who arrived from different territories.

An important step in the surveillance for the confirmation of the polio-free status of the Russian Federation is the assessment of the level of immunity to polioviruses in the population (in accordance with procedural guidelines MU 3.1.2943-11). Currently, in accordance with the National Immunisation Schedule, a sequential polio vaccination scheme is used in the Russian Federation, i.e. the first two vaccinations are given to children using an inactivated poliovirus vaccine, whereas for the next four vaccinations, an oral poliovirus vaccine is used.

Comparative analysis of the state of herd immunity to polioviruses among the pediatric population in one of the 14 territories was carried out. Until 2016, a trivalent oral polio vaccine was used for the immunization of children, while after the exclusion of type 2 poliovirus from the vaccine, starting from 2017, vaccination was carried out with a bivalent oral polio vaccine. 1300 blood sera of children from the indicator group of 3–4 years and from the indicator group of 15–17 years were examined (Tabl. 5).

The obtained results demonstrated a negative trend in the herd immunity to poliovirus type 2 during the use of a bivalent oral polio vaccine. In 2012–2014, no sera seronegative to poliovirus type 1 and 2 were detected. At the initial stage of use of the bivalent oral polio vaccine (2016–2017), there were still no seronegative blood sera for poliovirus type 2 in children. In 2018, 1% of seronegative sera was revealed in the indicator group of 3–4 years and 2% of seronegative sera was revealed in the indicator group of 15–17 years. In 2019, the proportion of such sera increased to 2% in the younger group and to 6% in the older group, respectively. The absence of antibodies to poliovirus type 2 in vaccinated children is a wake-up call and requires further research.

Table 5. Results of a study of children's blood sera for the presence of poliovirus antibodies in the territory in 2012–2014 and in 2017–2019

Type of vaccine	Years	Number of children	Percentage of sera seronegative to PV					
			Children aged 3–4 years			Children aged 14–15 or 16–17 years		
			PV 1	PV 2	PV 3	PV 1	PV 2	PV 3
t OPV	2012	203	0	0	1	0	0	12
	2013	222	0	0	2	0	0	7.4
	2014	269	0	0	5.3	0	0	6.0
b OPV	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

In 2019–2020, 8320 samples from patients with enterovirus infection were examined in 14 territories. The detection rate of polioviruses was low (0.1–0.2%) and in all cases polioviruses were isolated from children recently vaccinated with OPV. According to the results of intratypic differentiation, all poliovirus isolates were vaccine-derived. In 2020, the situation was different from what it was in previous years. Due to the coronavirus pandemic, the number of conducted studies significantly decreased.

The general picture of non-polio enteroviruses isolated from EVI patients in 2018 and 2019 is shown in Fig. 5. Coxsackie A enteroviruses of vaious serotypes were frequently isolated (40%) from EVI patients. Coxsackie B1–6 enteroviruses were isolated in 19–20% of cases. It should be noted that the Echovirus 30 has a rather high proportion in the structure of all enteroviruses (11–12%).

The proportion of Echovirus 6 was high (18.5%) in 2018 and decreased to 5.6% in 2019. The proportion of enterovirus 71 increased from 4.6% in 2018 to 8.9% in 2019. Non-polio enteroviruses of other serotypes were rarely isolated from EVI patients during these years.

In 2020, 702 samples from patients with EVI were examined. Vaccine-derived polioviruses were isolated in 0.6% of children recently vaccinated with an oral poliovirus vaccine. Among the isolated enteroviruses, as in previous years, Coxsackieviruses A of various serotypes prevailed (51.3%), most of them were represented by Coxsackieviruses A6 and A16. The proportion of Coxsackie B1–6 enteroviruses was approximately the same as in previous years (18.9%), and half of these were of Coxsackievirus B5 serotype. The proportion of Echovirus 30 was 11%.

ECHO 30 enteroviruses, isolated in 2008–2009 in the Northwest of Russia, belonged to the eC2 genotype; enteroviruses of this genotype were circulating in Western European countries at that time. From 2013 to 2017, in many territories of Russia ECHO 30 genotype h viruses were actively circulating, which were previously virtually not detected in the country and were imported to Russia from Southeast Asia. In 2018, ECHO 30 viruses of the eC2 genotype were again imported to Russia from Turkey, and they are currently still circulating in many territories of Russia, including in the Northwest (Fig. 6).

Throughout the entire observation period, the epidemic process of enterovirus infection in the territories of Russia developed independently, and the rises in the incidence of EVI were usually associated with a change of the leading enterovirus serotypes or genotypes. Clinical forms of the enterovirus infection vary significantly. In different territories, different clinical forms of infection prevailed. Severe clinical course of the disease was caused by the development of enterovirus meningitis (EVM), usually caused by type B enteroviruses. Exanthematic forms of the disease usually had a mild clinical course and were associated with type A enteroviruses.

Over the past five years, diseases with the clinical signs of enterovirus meningitis have prevailed in some territories. Their proportion in the total EVI significantly exceeded ($p < 0.05$) the proportion of exanthematic forms of EVI in St. Petersburg, the Republic of Karelia, Arkhangelsk and Saratov Oblast. The main causative agents of the EVM were type B enteroviruses ECHO 30, ECHO 6 and Coxsackievirus B1–6. In other territories — in the Komi Republic, Leningrad,

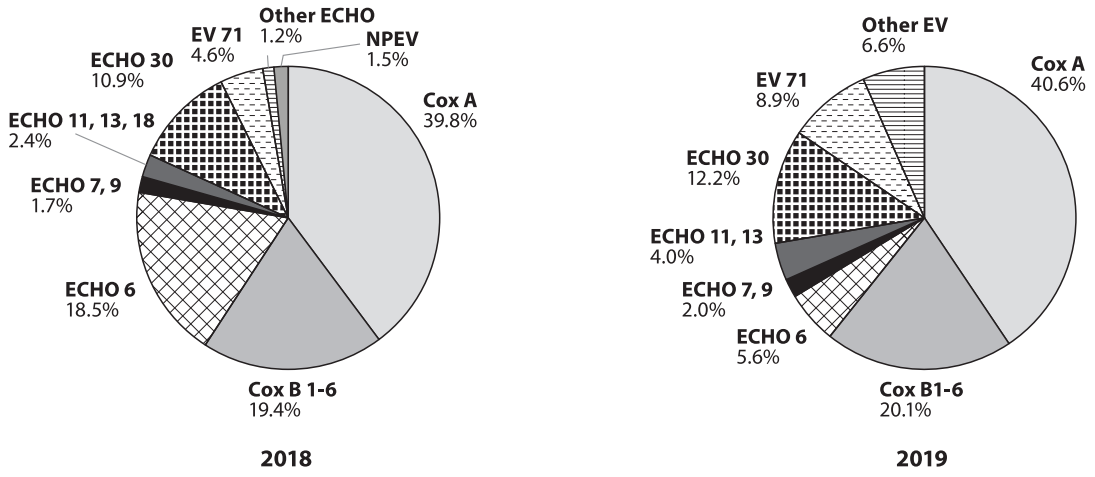

Figure 5. Structure of non-polio enteroviruses isolated from samples of EVI patients in 2018–2019

Murmansk and Vologda Oblast — EVI with the clinical signs of hand, foot and mouth viral exanthems (HFMD) or herpetic angina prevailed with a high degree of confidence ($p < 0.05$). The main causative agents of exanthematic forms of the disease were type A enteroviruses — Coxsackieviruses A6, A10, and A16.

Monitoring of environmental objects by examining wastewater samples for the detection of polioviruses and non-polio enteroviruses in environmental samples is one of the key elements of surveillance of the circulation of polioviruses and non-polio enteroviruses in the population during the final phase of the Global Polio Eradication Initiative.

In 2018–2019, 3590 samples of wastewater were examined virologically in the territories of the region. The structure of enteroviruses isolated from wastewater samples was dominated by polioviruses, which is due to the ongoing routine vaccination of children with oral polio vaccine. According to the results of intratypic differentiation, all polioviruses were vaccine-derived. Coxsackie B1–6 enteroviruses dominated among non-polio enteroviruses; their proportion was 27–29%. These viruses can be a causative agent of enterovirus meningitis, and they can present as an asymptomatic carrier state. The proportion of ECHO 30 and ECHO 6 enteroviruses, which can also cause enterovirus meningitis, was 8–9% and 3–11%, respectively (Fig. 7).

In 2020, during the coronavirus pandemic, the number of examined wastewater samples decreased by about three times: 663 wastewater samples were examined virologically. 50 polioviruses (7.5%) were isolated, which, according to the intratypic differentiation, were vaccine-derived; of these, 8 polioviruses were classified as type 1 and 42 polioviruses — as type 3.

At the post-certification stage of Global Polio Eradication Initiative, improving the surveillance of polio and acute flaccid paralysis is crucial. Increased virological surveillance of patients with AFPs, combined with the most important types of additional surveillance, such as surveillance of children from risk groups, enterovirus infection and the environment, will make it possible to maintain the polio-free status of individual territories of Russia and the country as a whole and ensure the implementation of the Global Polio Eradication Initiative. Information on of non-polio enteroviruses circulation patterns in the population of certain territories at different time periods and on the evolution of enteroviruses will make it possible to understand the laws and features of the epidemic process in this infection, as well as

Figure 6. Phylogenetic relationships of ECHO 30 enterovirus of genotype eC2, identified in regions of Russia in 2018–2019

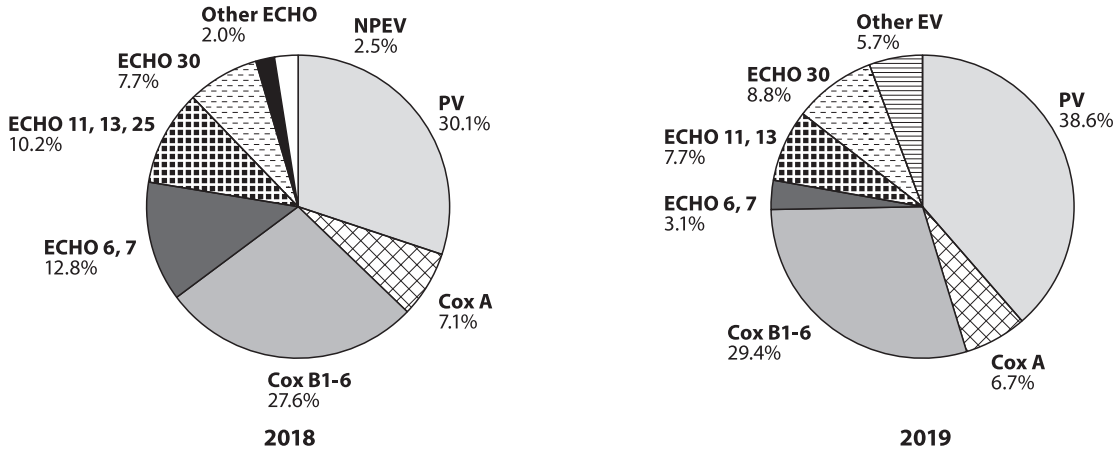

Figure 7. The structure of non-polio enteroviruses isolated from wastewater samples in 2018–2019

to contribute to improving of enterovirus infection prevention in order to reduce the incidence.

In 2020, nucleotide sequences of 58 enterovirus strains were deposited in GenBank, which can be used by other researchers working with the enterovirus infection.

Improvement of epidemiological surveillance of measles and rubella at the stage of infection elimination

In 2019, 332 samples of blood sera from measles patients, 72 samples of blood sera from rubella patients and 331 samples of blood sera from patients with exanthematic forms of diseases from 11 territories of the Northwestern Federal District (NWFD) were examined at the laboratory. The incidence of measles in 2019 was 0.96 per 100 000 population, showing an increase by 1.2 times as compared to 2018 (Fig. 8).

Measles was registered in 6 territories of the region, a total of 132 cases were detected (Fig. 9).

Of the patients, adults accounted for 65.9%, children under 18 years of age — for 34.1%, and the same structure was revealed in 2018.

As for vaccination status, the same percentage was made up of unvaccinated patients (41.7%) and patients with unknown history (43.9%), in 5.3% of cases the patients had been vaccinated, and in 9.1% of cases — revaccinated (Fig. 10). 99.2% of cases were confirmed in the laboratory (in one patient, the case was epidemiologically related to another patient).

In 2020, 112 blood serum samples from measles patients, 29 blood serum samples from rubella patients and 84 blood serum samples from patients with exanthematic forms of diseases were examined.

In 2020, 63 cases of measles were registered in two territories of the Northwestern Federal District, the incidence rate was 0.45 per 100 000 population. The measles incidence rate decreased by 1.7 times as compared to 2019. 49 cases were registered in St. Petersburg and 14 cases — in Leningrad Oblast.

The age composition of patients has changed. Whereas in previous years there was an almost equal proportion of adults and children, in 2020 adults accounted for only 28% of patients. The vaccination status of patients has also changed. Among measles patients, 97% were unvaccinated or people with unknown history. Only 3% had evidence of previous vaccination.

Etiology of 6 family and home foci of measles with the number of cases from two to four people in St. Petersburg and two family foci with two cases in the Leningrad Oblast has been specified. Only one outbreak in St. Petersburg in January 2020 was caused by the measles virus of D8 Gir Somnath genotype, whereas all other cases of measles in St. Petersburg and the Leningrad Oblast were caused by a single B3 Kabul genotype. The last case of measles in the region was registered in May 2020.

In 2020, there were no cases imported from abroad, 3 cases were brought from other subjects of the Russian Federation, and the remaining 60 cases were local.

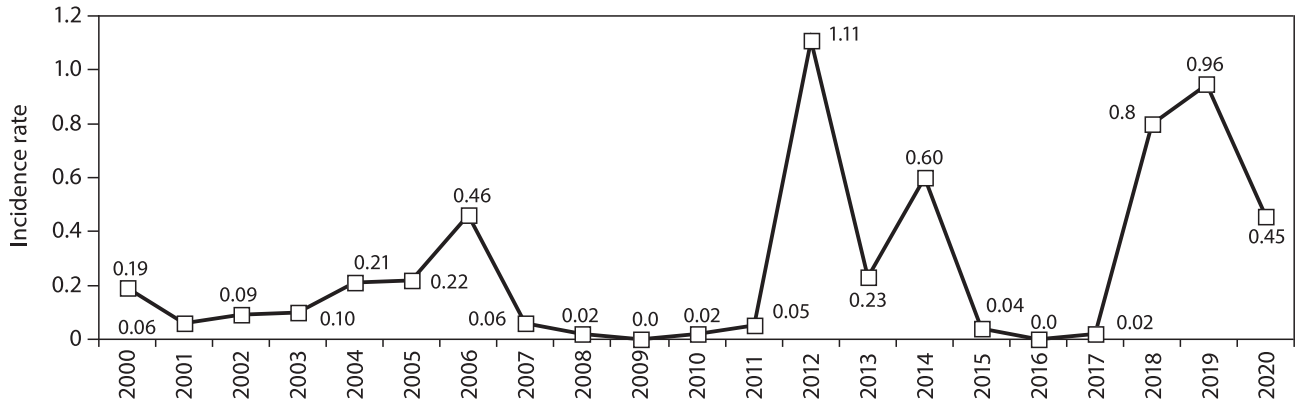

Figure 8. Incidence of measles in the territories of the Northwestern Federal District in 2000–2020 (per 100 000 population)

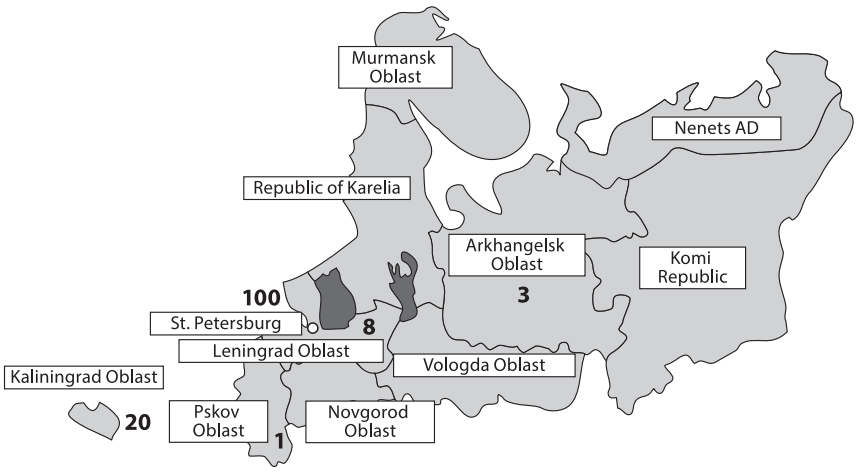

Figure 9. Distribution of measles cases in the territories of the Northwestern Federal District in 2019

The incidence of rubella in 2019 in the territories of the Northwestern Federal District increased. 18 cases were registered, i.e. 0.13 per 100 000 population (Fig. 11). All cases were registered in St. Petersburg.

One of the 18 cases of rubella was imported from Cambodia, one more — from the Republic of Mari El.

The proportion of patients who were not vaccinated against rubella and did not have information about vaccination was 94.4%. Only one of the patients had evidence of a single vaccination.

In 2019, there were 2 foci of infection spread, one of which was registered in a dormitory in St. Petersburg, where 2 students from Algeria fell ill. The second focus with four cases was registered among adult staff members at the place of employment. Rubella virus of genotype 1E was identified in patients in the latter focus.

In 2020, one case of rubella was registered in an unvaccinated adult in St. Petersburg. The incidence rate was 0.007 per 100 000 population. The low incidence of rubella in the NWFD during 2017–2020 and adequate data of a molecular examination of the material from patients confirm that the NWFD is in rubella elimination phase.

Work was carried out to identify rubella in pregnant women and cases of congenital rubella syndrome. In 2019–2020, seven pregnant women with suspected rubella or after a contact with a laboratory-confirmed case of rubella were examined. The diagnosis of rubella was ruled out in six women, in one woman with a pregnancy of 25 weeks, the diagnosis was confirmed by a three-time repeat testing for IgM antibodies (increased), IgG antibodies (increased) and low IgG antibodies avidity (6.9%).

Study of influenza etiology in St. Petersburg in 2019–2020

The long-term monitoring of the incidence of influenza and acute respiratory viral infections (ARVIs) in St. Petersburg was continued (Fig. 12).

In St. Petersburg, almost every year saw rises in the incidence of influenza and ARVIs. The incidence of influenza and ARVIs in 2019 was high, almost at the level of 2018. In 2020, the incidence significantly decreased and since March it has been at the inter-epidemic level. The recording of influenza and ARVI cases was affected by the COVID-19 pandemic that began in Russia.

In 2019, the epidemic threshold for the incidence of influenza and ARVIs in St. Petersburg (11 045 cases daily) was exceeded on 09.01.2019.

The maximum number of cases (14 245) was registered at week 8. In March, the threshold was exceeded several times with a maximum of 11 335 cases, with a further decrease by the end of the month to 8000–9000 cases per day, as in the inter-epidemic period.

Analysis of the age structure of patients revealed the highest rates in the age group of 3–6 years (a maximum of 7.03 per 100 children of this age group). In the age group of 0–2 years and 7–14 years, the incidence rate was lower and amounted to 4.76 and 3.87, respectively. The incidence in the adult group was at 0.56 insignificant (Fig. 13).

In order to study the etiological structure of infection, from week 46 of 2018 to week 18 of 2019, nasopharyngeal swabs were collected from patients admitted to the Botkin Clinical Infectious Disease Hospital for subsequent testing using real-time PCR and virological method.

A total of 569 samples were examined by PCR using test kits for type A and B influenza viruses, type I, II, and III parainfluenza viruses, adenoviruses, RS viruses, coronaviruses, rhinoviruses, and *Mycoplasma pneumoniae*. 312 positive samples were detected, which accounted for 52.0%. Influenza viruses prevailed (260 positive samples).

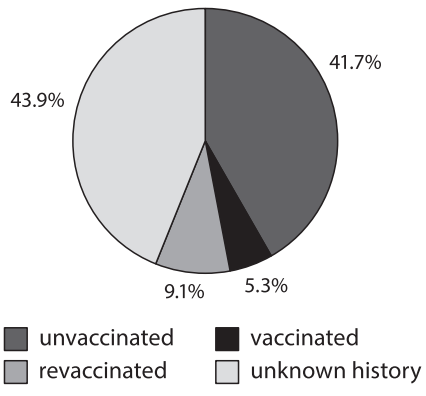

Figure 10. Breakdown of measles patients by vaccination status in the territories of the Northwestern Federal District in 2019

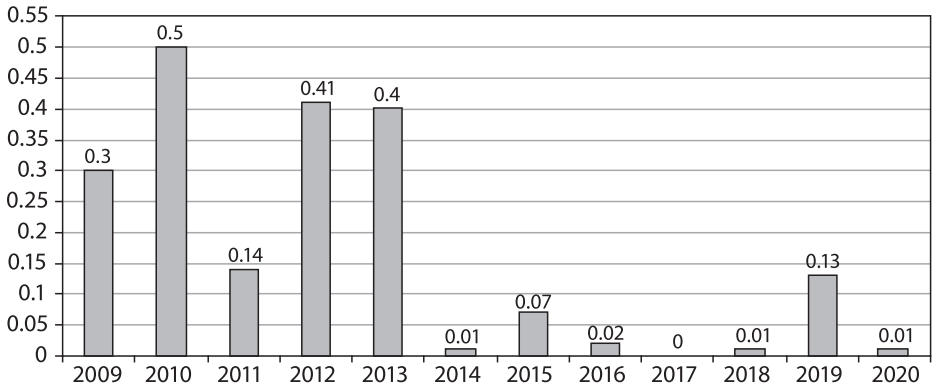

Figure 11. Incidence of rubella in the Northwestern Federal District in 2009–2020

As during the epidemic season of 2018, influenza A(H3N2) viruses prevailed, they were detected in 47.7% of cases. At 32.1%, the influenza A(H1N1) virus was the second most detectable. Occasionally, influenza B viruses were detected.

During the virological examination of nasopharyngeal swabs on MDCK cell culture from 36 patients with influenza and ARVIs, in whom the RNA of the influenza virus was detected, 24 strains of the influenza virus were isolated. In 50% of cases, influenza viruses of serotype A(H1N1) were identified, which were closely related to the pandemic variant of the influenza virus A(H1N1)pdm09 and the vaccine strain A/MichigAn/45/15. Some of the isolates (41.7%) were of the serotype A(H3N2) and were related to the strain A/Singapore/16-0019/16.

During the epidemic season, one influenza B virus of the Victorian Line was isolated from hospitalized patients, which, according to its antigenic properties, did not correspond to the vaccine strain B/Colorado/06/17. The strain of influenza virus B/SPb/22/19 isolated in the laboratory reacted to 1/16 of the homologous titre with the rat antiserum made for the vaccine strain of influenza virus B/Colorado/06/17. The strain of influenza B virus isolated in 2019, which had 3 deletions, belonged to another antigenic group that had not previously circulated in Russia. The strain of the influenza virus B/SPb/22/19 of the Victorian Line significantly differed in antigenic properties from the influenza virus strain that is part of the inactivated influenza vaccine.

In 2020, the epidemic threshold was exceeded three times in February with a maximum number of 12 533 cases per day.

When analyzing the age structure of influenza and ARVI cases, it was found that, as in 2019, the highest rates were in the age group from 3 to 6 years (with a maximum of 5.63 per 100 persons of this age group). In the age group from 0 to 2 years, the maximum value was 4.05 and in the age group from 7 to 14 years — 3.99. The incidence in the adult group was not more than 0.59.

In 2020, the study of the etiological structure of diseases with respiratory viral infections continued. From week 46 of 2019 to week 22 of 2020, nasopharyngeal swabs were taken from patients admitted to the Botkin Clinical Infectious Disease Hospital and then examined in order to detect genetic material of respiratory viruses. A total of 1580 samples were examined, of which 727 samples, i.e. 46.0%, turned out to be positive.

In the epidemic season of 2020, the share of influenza viruses among all respiratory viruses was 57.6%. Influenza B viruses prevailed: their detection rate was 29.7%, and influenza viruses B of the Yamagata Line were detected most often, influenza A(H1N1) viruses were in second place — 23.5%, whereas influenza A(H3N2) viruses and untyped influenza A viruses were detected in a small percentage of cases. In addition to influenza viruses, other respiratory viruses were identified.

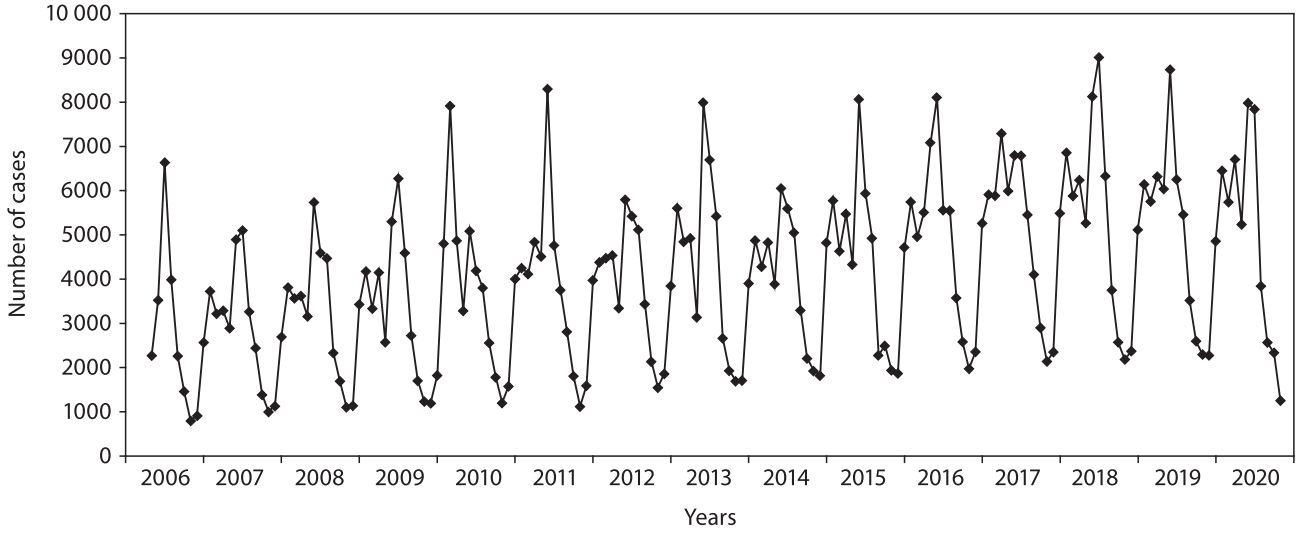

Figure 12. Average daily incidence of influenza and acute respiratory viral infections in St. Petersburg by months

In 2020, nasopharyngeal swabs from 40 patients in whom influenza viruses were detected by PCR were examined virologically on a cell culture. In 23 cases, influenza viruses were isolated and identified. They were distributed as follows: A(H3N2) — 1 strain, A(H1N1) — 13 strains, and 9 strains of the Victorian Line influenza B virus. No strains of the influenza B virus of the Yamagata Line were detected.

Antigenic features of isolated influenza virus strains: 1) influenza A(H3N2) virus SPb/16/20 was related to the reference strain A/Singapore/16-0019/16, reacting to 1/4 of the homologous titre with serum to the reference strain; 2) influenza A(H1N1)pdm09 viruses were antigenically related to the vaccine strain A/Brisbane/02/18, interacted to 1–1/2 of the homologous titre with serum to the vaccine strain; 3) influenza B viruses of the Victorian Line were antigenically related to the reference strain B/Victoria/02/19 and interacted to 1–1/2 of the homologous titre with serum to the reference strain.

Thus, in 2019–2020, the etiology of influenza epidemic rises in St. Petersburg was specified. Because there are two strain lines of influenza B virus circulating among the population, i.e. Yamagata (2019) and Victorian (2020) Lines and they appear unpredictably, a four-component vaccine containing influenza B viruses of both lines should be used more actively for vaccination.

The ethical component of the epidemic process control

In the reporting years, essential attention was given to further studying the ethical component of the control and management of the epidemic process, with an emphasis on emergency situations, which include research on the global elimination of infections and the formation of an ethical protocol for the COVID-19 pandemic. The extreme conditions of such situations are due to unknown and unpredictable circumstances, which require an up-to-date, prompt and balanced ethical approach to both an individual and civil society as a whole.

A key element of understanding the ethical essence of implementation of WHO programs for the elimination of vaccine-preventable diseases was the study of the role of an informed consent as a universal tool for protecting human rights and dignity. Of particular importance for this work was our direct participation in WHO programs for the global elimination of polio, elimination of measles and rubella within the framework of the activities of WHO Subnational Laboratories and the authors' membership at the UNESCO International Bioethics Committee. The implementation of programs for the eradication and elimination of infections requires not only consistency at the global level, but also the use of scientific potential and economic resources, which is impossible without the support of society. This should be supported by appropriate awareness raising, equal access to personnel training and public information, which, in turn, requires transparency, objectivity, integrity and accessibility, as well as the study of responses and rapid response to achieve a fair benefit/risks information balance, prevent misinformation and disorientation. When carrying out specific measures to eradicate infections, ethical integrity was achieved by studying the lessons of the program "Institutional memory and lessons learned", as well as taking into account the diversity of situational cases with the different cultural, social, religious, economic and psychological status of the cohorts involved. All this requires adherence to the ethical principles of recognizing human vulnerability, having respect for cultural diversity, equality, justice, equality and pluralism. Real achievements can be based on the ethics of transnational interaction practices, the observance of which contributes to the exchange of new technologies and educational, professional training and information in the field of global bioethics.

In the current period, special attention was given to finding an algorithm for ethically verified decisions and actions during the current COVID-19 pandemic, which was achieved through consistent monitoring and the formation of an ethical comment on the pandemic. At the beginning of the pandemic (March 2020), basic, guiding and procedural ethical principles were identified and analyzed, which are important for planning work during an infectious disease pandemic in order to make ethically verified decisions. During the dynamic assessment of the ethical component of the epidemic situation caused by COVID-19, the viability of checking the effectiveness of recommended standards and identifying unforeseen ethical conflicts, causes and consequences of a possible ethical crisis during COVID-19 pandemic and the possible options of its prevention was identified. The focus of public health ethics has identified three es-

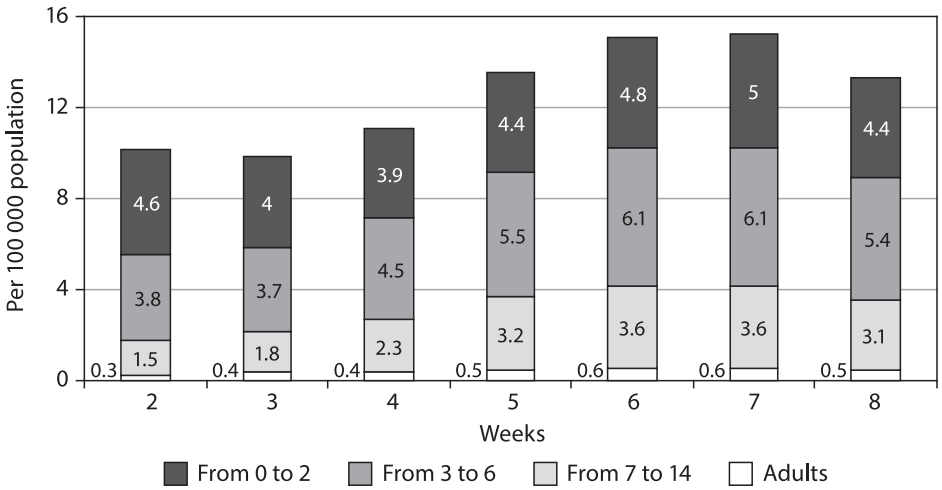

Figure 13. Incidence of influenza and ARVIs in St. Petersburg during the epidemic rise of 2019 in different age groups

sential duties of foreseeing and resolving ethical problems when organizing work with COVID-19, which include the duty to plan, protect and guide. Summing up the materials on research ethics presented in this report, it can be stated that, from the point of view of public health, any reasonable response in a crisis begins with science, morals and civil responsibility. The conceptual integrity of decisions and actions at all levels of management in an emergency situation is tested by the ability to create new and advanced conditions for the development of science and ethics in combination and unity with commitment to the ideals and needs of civil society.

Publications

1. *Bichurina M., Romanenkova N., Rozaeva N., Kanaeva O. Surveillance for Enterovirus Infection in Russian Federation's North-West // Conference of the polio laboratory network, national poliovirus containment coordinators, national authorities for containment. 24–26 September 2019, Copenhagen, Denmark.*
2. *Bichurina M.A., Voloshchuk L.V., Go A., Pisareva M.M., Guzhov D.A. Clinical features of rhinovirus infection in hospitalized adult patients during the epidemic season 2017–2018 // Journal Infectology. 2020; 12 (4): 19–22. doi: 10.22625/2072-6732-2020-12-4-19-22*
3. *Camara J., Antipova A.Yu., Bichurina M.A., Zarubaev V.V., Magassouba N., Lavrentieva I.N. Implementation of the Program of measles elimination in the WHO African Region // Russian Journal of Infection and Immunity. 2019; 9 (3–4): 449–456. doi: 10.15789/2220-7619-2019-3-4-449-456*
4. *Golitsyna L.N., Nguyen T.T., Romanenkova N.I., Luong M.T., Vu L.T., Kanaeva O.I., Bichurina M.A., Novikova N.A. Enterovirus infection in the Socialist Republic of Vietnam // Russian Journal of Infection and Immunity. 2019; 9 (3–4): 467–475. doi: 10.15789/2220-7619-2019-3-4-467-475*
5. *Kubar O.I., Bichurina M.A., Romanenkova N.I. Ethical consideration regarding COVID-19 // EC Microbiology SI.02. 2020: 14–15.*
6. *Kubar O.I., Bichurina M.A., Romanenkova N.I. Ethical Principles for Infectious Disease Eradication // EC Microbiology. 2019; 15 (8): 769–770.*
7. *Kubar O.I., Bichurina M.A., Romanenkova N.I. Second ethical comments towards COVID-19 (one year later) // Russian Journal of Infection and Immunity. 2021; 11 (1): 17–24. doi: 10.15789/2220-7619-SEC-1645*
8. *Kubar O.I., Klingmann I., de Balincourt C., Sampson H. Improving and bringing together approaches to education as a factor for the harmonious development of Good Clinical Practice (GCP) // Remedium. 2019; 5: 46–51. doi: 10.21518/1561-5936-2019-5-46-63*
9. *Kubar O.I. Ethical comments on COVID-19 // Russian Journal of Infection and Immunity. 2020; 10 (2): 287–294. doi: 10.15789/2220-7619-ECO-1447*
10. *Kubar O.I. Ethical interaction of three elements of medicine during COVID-19 // Bioethics. 2020; 2 (26): 9–14. doi: 10.19163/2070-1586-2020-2(26)-9-14*
11. *Kubar O.I. Health and dignity. value of UNESCO's universal ethical principles during pandemic of COVID-19 // Bulletin of the Commission of the Russian Federation for UNESCO. 2020; 42: 44–49.*
12. *Kubar O.I. Search for the ethical point of genetic dilemmas in medicine // Scientific conference Bioethical problems of genetic technologies development in the Russian Federation. Moscow, 10–11 November 2020: 24–27.*
13. *Kubar O.I. Social value of infectious diseases ethics // Medical Ethics. 2020; 10 (2): 35–41.*
14. *Lavrentieva I.L., Khamitova I.V., Camara J., Antipova A.Yu., Bichurina M.A., Magassouba N.F., Nikishov O.N., Kuzin A.A., Semenov A.V. The Status of humoral immunity to parvovirus B19 in population of certain geographical regions // Journal of Microbiology, Epidemiology and Immunobiology. 2020; 97 (3): 233–241. doi: 10.36233/0372-9311-2020-97-3-5*
15. *Lavrentieva I.N., Antipova A.Yu., Bichurina M.A., Khamitova I.V., Nikishov O.N., Kuzin A.A. Parvirus infection markers in persons with exantemic diseases and in risk groups // Journal Infectology. 2019; 11 (3): 110–117. doi: 10.22625/2072-6732-2019-11-3-110-117*
16. *Mamaeva T.A., Zheleznova N.V., Bichurina M.A., Naumova M.A., Govoruhina M.V., Toptygina A.P. Evaluation of age-related distribution of measles cases with primary and secondary immune response in Russian Federation, 2010–2016 // Russian Journal of Infection and Immunity. 2020; 10 (4): 717–728. doi: 10.15789/2220-7619-EOA-1407*
17. *Pelčić G., Karačić S., Mikirtichan G.L., Kubar O.I., Cheng-tek Tai M., Morishita N., Vuletić S., Tomašević L. Religious objections to vaccination or religious justifications to refuse vaccination: is it really true? // Medicine and Health Care Organization. 2020; 5 (1): 58–84.*
18. *Romanenkova N., Rozaeva N., Bichurina M. Diagnostics of poliomyelitis and acute flaccid paralysis in some territories of the Russian Federation // Conference of the polio laboratory network, national poliovirus containment coordinators, national authorities for containment. 24–26 September 2019, Copenhagen, Denmark.*
19. *Romanenkova N.I, Rozaeva N.R., Kanaeva O.I., Bichurina M.A. Peculiarities of enterovirus infection on 14 territories of Russia in 2018 // Morbidity, etiology and prevention of enterovirus infection. Information bulletin. 2019; 6: 30–32.*
20. *Romanenkova N.I., Rozaeva N.R., Bichurina M.A., Kanaeva O.I., Chkhyndzheriya I.G., Shishkina L.V., Madoyan A.G., Valdaitseva N.V. Epidemiological aspects of enterovirus infection in the Russian Federation during the period of 2018–2019 // Journal Infectology. 2021; 13 (1): 108–116. doi: 10.22625/2072-6732-2021-13-1-108-116*
21. *Romanenkova N.I., Rozaeva N.R., Bichurina M.A., Kanaeva O.I., Chkhyndzheriya I.G. Vaccine associated paralytic poliomyelitis and acute flaccid paralysis on some territories of Russia during 20 years // Journal Infectology. 2019; 11 (3): 102–109. doi: 10.22625/2072-6732-2019-11-3-102-109*
22. *Stoiljkovic V.D., Bichurina M.A., Lavrentieva I.N., Filipovic-Vignjevic S.B., Bancevic M.D., Zheleznova N.V., Antipova A.Y. Rise in 2017–2018 measles morbidity in Serbia and Northwest Russia // Russian Journal of Infection and Immunity. 2020; 10 (4): 729–734. doi: 10.15789/2220-7619-RIM-1342*
23. *Voloshchuk L.V., Go A.A., Pisareva M.M., Guzhov D.A., Bichurina M.A., Petrova P.A. Clinical and laboratory characteristics of influenza infection in hospitalized adult patients during the 2018–2019 epidemic season // Russian Journal of Infection and Immunity. 2021; 11 (1): 191–196. doi: 10.15789/2220-7619-CAL-1467*
24. *Yusupov R.M., Fedorchenko L.N., Naumov V.B., Kubar O.I. Interparliamentary session recommendations on issues of nanotechnology ethics for legislative support member States of the Commonwealth of Independent States // Scientific Dialogue: Issues of medicine. XXV International scientific conference. 15 Jul 2020: 6–15. doi: 10.18411/sciencepublic-15-07-2020-02*

LABORATORY OF EXPERIMENTAL VIROLOGY

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Improving virological surveillance of airborne viral infections during the implementation of the measles and rubella elimination program in the Russian Federation

During the implementation of the WHO measles and rubella elimination program in different regions of the world, including in the Russian Federation, it is necessary to carry out differential laboratory diagnosis of measles, rubella and other exanthematic diseases, among which parvovirus infection (PVI) is medically a very significant one.

However, as there is no official recording of parvovirus infection cases in the Russian Federation, it is impossible to fully assess the extent of infection with parvovirus B19 (PVB19). High proportion (50% and more) of asymptomatic forms contributes to infection spread, including in risk groups, i.e. among recipients of blood, tissues and organs, patients with chronic infectious and oncological diseases. Data on the effect of PVB19 infection on the course of the underlying disease in these groups of patients are few and inconsistent. Data on PVB19 gene variants circulating in the region are limited.

In 2019–2020, new results were obtained and previous results were summarized in order to characterize the spread and molecular genetic features of the causative agent of parvovirus infection among healthy individuals and in risk groups in the Northwestern Federal District (NWFD) of the Russian Federation.

Research objectives:

1. describe the spread of parvovirus infection in the territories of the NWFD;
2. estimate the error rate of the primary diagnosis of PVI;
3. study the herd immunity to parvovirus B19 among residents of St. Petersburg, in an organized group (blood donors), and among migrant workers;
4. determine the effect of PVB19 infection on the course of the underlying disease in the risk group (patients with oncohematological diseases);
5. identify the PVB19 isolates circulating in the territories of the NWFD and to conduct their phylogenetic analysis.

Materials and methods

Research materials

A total of 2093 samples of blood serum and/or plasma of apparently healthy individuals, as well as patients with exanthematic or oncohematological diseases aged from 7 months to 70 years, living within the NWFD, were examined.

The samples were obtained from the collections of the virological laboratory of the Regional Centre for Measles and Rubella Surveillance in the NWFD, in the laboratory of virology of the Pasteur Institute, from the State healthcare institution City Clinical Hospital No. 30, as well as from the clinic of Gorbacheva Research Institute of Pediatric Oncology, Hematology and Transplantology of St. Petersburg. All the examined persons gave their written informed consent to participate in the study. Description of the groups of examined persons is given in Tabl. 6.

Diagnosis, clinical examination of patients, complete blood count, urinalysis, and blood chemistry were carried out by doctors of respective medical institutions.

A total of 3187 laboratory tests were performed. 1162 samples were examined for IgM, 931 samples for IgG antibodies, and 1087 samples for PVB19 DNA. 7 samples were sequenced.

Research methods

Enzyme immunoassay. Qualitative determination of IgM antibodies to PVB19 was carried out using the diagnostic kit Anti-Parvovirus B19 ELISA IgM (EUROIMMUN, Germany) (test systems) according to the manufacturer's instructions. The presence of IgM antibodies to PVB19 in blood sera was classified as acute PVI.

Quantitative and qualitative determination of IgG antibodies to PVB19 was carried out using the diagnostic kit Anti-Parvovirus B19 ELISA IgG (EUROIMMUN, Germany) according to the manufacturer's instructions.

Molecular genetic methods. The extraction of nucleic acids (DNA) from blood plasma was carried out using the commercial kit AmpliPrime Ribo-prep (Central Research Institute of Epidemiology, Moscow), according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR). Detection and/or quantitative determination of PVB19 DNA, assessment of the viral load of samples and pools when analyzing the sensitiv-

Table 6. Numerical characteristics of the laboratory-examined groups

Characteristics of the group	Number
Blood samples of apparently healthy persons living in St. Petersburg	817
Blood samples of migrant workers from the Republics of Tajikistan and Uzbekistan staying in the Russian Federation on a work visa	114
Blood samples of patients with exanthematic manifestations of the infection living in the territories of the NWFD	1044
Blood samples of PVI patients admitted to the City Clinical Hospital No. 30	64
Blood samples of patients with allo-HSCT from the clinic of the Gorbacheva Research Institute of Pediatric Oncology, Hematology and Transplantology" (St. Petersburg)	54
TOTAL	2093

Table 7. Nucleotide sequences of primers additionally used for sequencing of the NS1/VP1 region of PVB19

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

ity of the method being developed was carried out by qualitative or quantitative real-time PCR with hybridization-fluorescence detection based on the commercial set AmpliSens® Parvovirus B19-FL (Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow) according to the manufacturer's instructions.

Specific primers (Syntol, Russia) were used for amplification and sequencing of the product. The sequence of primers and fluorescent probes was chosen based on literature sources, and also selected using the NCBI/Primer-BLAST program according to generally accepted recommendations (Tabl. 7).

Purification of the amplification and sequencing products was carried out using two methods:

1. using a commercial reagent kit Qiaquick PCR Purification kit (Qiagen, Germany) according to the manufacturer's instructions;
2. using the method of ethanol precipitation in the presence of sodium acetate.

The quality of purification of amplification products was analyzed by dissolving the precipitate in 30 µl of TE buffer, and visualized in an agarose gel. DNA concentration was measured according to the standard method recommended by the manufacturer on a Qubit 2.0 fluorimeter.

PVB19 DNA Sequencing. Plasma samples with a viral load of at least 10^2 IU/ml were used for sequencing. A purified DNA fragment with a concentration of 50–100 ng was used to perform sequencing reactions from direct and reverse primers in three repetitions for each pair of primers of each sample. PVB19 primers were used for the reaction, which make it possible to analyze the NS1/VP1 region (NS1-VP1u locus), recommended for geno- and subgenotyping of PVB19, with a length of about 994 base pairs (b.p.), according to the isolate J35 (AY386330) present in the international GenBank database, as well as additional primers.

For additional control of the samples, two analytical systems were used with the corresponding reagents according to the manufacturer's instructions: in the GenomeLab GeXP genetic analyzer (Beckman Coulter Inc., USA) and the ABI PRISM 3500 genetic analyzer (Applied Biosystems, USA).

Phylogenetic analysis. To identify the genotypes of PVB19 isolates, a fragment of the genome was selected that included the conservative fragment of the areas of the NS1 and VP1 genes (locus NS1-VP1u), 994 b.p. Primary analysis of the fragments obtained during the sequencing was performed using the NCBI Blast program as compared to the nucleotide sequences of reference samples present in the GenBank international database.

The alignment of nucleotide sequences was performed in the MEGA 7.0 software using the ClustalW algorithm. For the creation of phylogenetic trees and subsequent phylogenetic analysis, the distances between sequences were examined using the Neighbour-joining method, which allows tree optimization in accordance with the criterion of "balanced minimal evolution" using the "Maximum Composite Likelihood" model, and bootstrap was performed for 1000 repetitions to assess the reliability of the constructed trees.

Statistical data processing was performed using the MS Excel and Prizm 5.0 software packages (GraphPad Software Inc). The Pearson χ^2 test was used. The value of the χ^2 test was compared to the critical values for the corresponding number of freedom degrees. If the obtained value of the χ^2 test exceeded the critical value, it was concluded that there was a statistical relationship between the studied risk factor and the outcome at the appropriate significance level.

The Pearson correlation coefficient r_{xy} was used as an indicator of strength of relationship between the quantitative indicators x and y with normal distribution. The statistical significance of the correlation was evaluated using the t-test. The values of the correlation coefficient r_{xy} were interpreted in accordance with the Cheddock scale. The probability value $p < 0.05$ was used as the threshold for the reliability of differences.

Table 8. Detection of IgM antibodies to PV B19 in the blood sera of patients with exanthematic diseases in the territories of the NWFD (2014–2017)

Year		2016		2017		2018		2019		Total	
Territory (republic, oblast)		n*	including IgM+ PV B19	n	including IgM+ PV B19	n	including IgM+ PV B19	n	including IgM+ PV B19	n	including IgM+ PV B19/% M±m
Republic of Karelia		1	0	13	2	19	6	15	5	48	13/27.1±6.4
Republic of Komi		2	0	36	4	19	4	21	4	78	12/15.4±4.1
Arkhangelsk Oblast		0	0	22	1	23	2	23	0	68	3/4.4±2.5
Vologda Oblast		12	7	30	3	32	5	25	2	99	17/17.2±3.8
Kaliningrad Oblast		6	3	23	4	20	2	19	4	68	13/19.1±4.8
Leningrad Oblast		10	1	36	8	29	7	32	10	107	26/24.3±4.2
Murmansk Oblast		1	1	13	2	17	4	16	0	47	7/14.9±5.2
Novgorod Oblast		0	0	13	1	16	3	29	7	58	11/18.9±5.1
Pskov Oblast		0	0	10	0	13	1	13	2	36	3/8.3±4.6
St. Petersburg		37	11	139	25	135	21	123	29	434	86/19.8±1.2
Nenets AO		0	0	1	0	0	0	0	0	1	0/–
TOTAL	abs.	69	23	336	50	323	55	316	63	1044	191
	% M±m	33.3±5.7		14.9±1.9		17.0±2.01		19.9±2.3		18.3±0.3	

Results

1. Identification of PVI cases within the territory of the NWFD, clinical manifestations of the infection and errors of clinical diagnosis

Identification of PVI cases within the territory of the North-Western Federal District (NWFD) of Russia in 2016–2019 was indicative of the extent of infection spread in the Northwest of the Russian Federation and made it possible to compare the results with those obtained earlier (2009–2012). The district consists of 11 entities of the Russian Federation.

In general, in 2016–2019, specific PVB19 IgM antibodies were detected in 18.9% of the examined samples. The frequency of detection of acute PVI cases in different years was different. The minimum proportion of positive cases (14.9%) was noted in 2017, whereas the maximum (33.3%) — in 2016. Positive samples were received from ten of the eleven territories of the NWFD, with the exception of the Nenets Autonomous Okrug (NAO) (Tabl. 8).

IgM antibodies to PVB19 were most commonly detected in the sera of patients from the Republic of Karelia and the Leningrad Oblast: 27.1% and 24.3%, respectively, whereas least often — in the patients from the Pskov (8.3%) and Arkhangelsk (4.4%) Oblast. These data are consistent with the results obtained in 2009–2012 and indicate a wide spread of B19 parvovirus infection in the NWFD.

Across the district, there was a higher level of positive findings in 2012 and 2016 (33.3% and 45.5%, respectively) as compared to 2009, 2010 and 2011 (5.4%, 14.5% and 14.0%, respectively), as well as to 2017, 2018 and 2019 (14.9%, 17.0%) and (19.9%). These values suggest an epidemic increase in the incidence of PVI in different territories of the NWFD in 2012 and 2016, which corresponds to a 3–6-year epidemic cycle typical of the natural spread of the infection.

The maximum proportion of IgM+ samples, out of the total number studied in 2016–2019, was revealed from December to May (24–39%), the minimum — from August to October (7.4–9.2%%), which confirms the data available in the special literature on the winter-spring seasonality of the disease.

Among the patients, children aged 3–6 and 7–14 years prevailed: 25.3% and 33.3%, respectively, of the number of examined in this age group. Among persons over 30 years of age, PVI is statistically significantly ($p < 0.05$) more common in females: 17.2% versus 8.7%.

To assess the severity and frequency of clinical manifestations of the disease, the main clinical symptoms and syndromes were evaluated in adult patients with PVI (average age was 35.2 years) who were treated in an infectious disease hospital in St. Petersburg. It was found that in 78.6% of cases, the disease was of moderate severity, in 14.3% of cases it was mild, and in one case (7.1%) — severe (Tabl. 9).

In all hospitalized patients, a syndrome of general infectious intoxication, mainly moderately pronounced, was noted. Fever was observed in 92.9% of patients; exanthema was detected in 85.7% of patients. The rash appeared at different times from day 1 to day 1 of the disease. Rashes on the skin were mainly macular-papular in nature, and in 14.3% of patients, hemorrhagic elements of the rash were also detected. Some patients had respiratory syndromes in the form of rhinitis (28.6%), pharyngitis (85.7%) and bronchitis (14.3%). Upon admission to the hospital, some patients were diagnosed with hepatomegaly (57.1%) and splenomegaly (42.9%) during examination and according to the results of abdomen ultrasound.

Table 9. Frequency and duration of the main clinical symptoms and syndromes in parvovirus infection

Main clinical symptoms and syndromes	Frequency (in %) of occurrence	Duration (days), $M \pm m_x$
Fever (duration)	92.9	6.8±1.08
Febrile fever	85.7	7.3±1.05
General infectious intoxication syndrome	100	7.1±0.99
Rhinitis	28.6	4.7±1.89
Pharyngitis	85.7	4.4±0.87
Bronchitis	14.3	8,0±1,0
Cough	14.3	8.5±1.5
Exanthema	85.7	10.3±2.02
Hepatomegaly	57.1	–
Splenomegaly	42.9	–

The overall incidence of complications in PVI was 28.6%. Among the complications, pneumonia was predominant (14.3%) as well as complications from the ENT organs: acute sinusitis (14.3% of patients) and acute tubootitis (7.1% of cases).

All the children we examined had a mild form of the disease. The clinical picture was similar: subfebrile fever, rash on the face ("spanked cheeks" syndrome), then on the limbs, which became brighter with physical exertion. No complications were detected.

In none of the cases the diagnosis of "parvovirus infection" or "infectious erythema" was established after the initial examination. Among the errors of clinical diagnosis, the diagnosis of "exanthema of unclear etiology" prevailed (in 46% of patients).

Analysis of the structure of primary diagnoses of 192 patients with parvovirus infection showed that diagnosing PVI is challenging for clinical specialists. During the follow-up period, the correct clinical diagnosis was established in only 4% of cases. The most common errors in the primary diagnosis were rubella (34%) and acute respiratory viral infections (20%), as well as viral infections of unknown etiology (14%). With a high frequency (22%), diagnoses were mistakenly made that characterize the disease as non-infectious and not requiring anti-epidemic measures, e.g. toxicoderma/allergic dermatitis/exanthema of unclear etiology.

2. Identification of PVI markers in blood samples of apparently healthy residents of St. Petersburg

Taking into account that no methods of specific prevention of parvovirus infection have been developed, the spread of PVI in a particular population can be judged by the level of humoral immunity. DNA of parvovirus B19 also stays in blood long enough (from a few weeks to several years). These two laboratory markers can be used both for assessing population immunity (IgG antibodies) and for detecting cases of a relatively recent disease (virus DNA).

Blood samples of apparently healthy residents of St. Petersburg (n = 317) were obtained from persons aged 18–87 years (average age 42.3±12.09, median 39.0 years) and divided into 4 age groups. Specific IgG antibodies to PVB19 were detected in all age groups (Tabl. 10).

Table 10. Detection of specific IgG to PVB19 in blood samples of apparently healthy residents of St. Petersburg in different age groups

Age (years)	Number of examined samples (abs.)	Including IgG + to PVB19	
		Number (abs.)	Proportion (%), M±m
18–20	18	6	33.3±11.11
21–30	68	40	58.8±5.97
31–40	78	40	51.3±5.66
41 and older	153	111	72.5±3.61
TOTAL	317	197	62.1±2.72

Table 11. Identification of specific IgG to PVB19 in blood samples of migrant workers from Central Asia in different age groups

Age (years)	Number of examined samples (abs.)	Including IgG + to PVB19	
		Number (abs.)	Proportion (%), M±m
18–30	50	19	38.0±6.86
31–40	33	18	54.5±8.67
41 and older	31	17	54.8±8.94
TOTAL	114	54	47.4±4.68

In general, about 62 % of people seropositive to PVB19 were identified, with a tendency to increased proportion in older age groups ($r = 0.164$, $p = 0.003$). It was found that DNA-containing samples dominated among people aged 18–30 years, in whom samples with the most copies were also registered, which indicates a more active virus circulation among young people under 30 years of age.

Another kind of herd immunity to PVB19 was formed in an organized group consisting of teachers and students of one of the universities of St. Petersburg. 500 blood samples of persons aged from 18 to 60 years were examined (average age 25.2 years, median 21 years). A high number of people seropositive to PVB19 was registered already among 18–20-year-old students — their proportion was 85.2±2.38% and remained without statistically significant changes in all the examined age groups ($df = 3$, $\chi^2 = 3.966$, $p = 0.266$).

The largest number of DNA of PVB19-positive samples was obtained in the age group of 18–20-year-olds (11.1±7.41%). In the same age group, the maximum values of the PVB19 DNA content were detected ($\geq 10^6$ IU/ml).

Thus, in the context of long-term exposure, the intensive formation of herd immunity to PVB19 occurred already in the persons of the first age group (18–20 years), which is associated with the latent pathogen circulation in this population. This assumption is confirmed by the fact that in a blood sample containing PVB19 DNA at a concentration of 1.1×10^8 IU/ml, virus-specific IgM and IgG antibodies were detected. And the total share of 18–20-year-old cadets seropositive to PVB19 (85.2%) significantly exceeded the seroprevalence index in the same aged group of apparently healthy residents of St. Petersburg (33.3%).

3. Identification of PVI markers in blood samples of migrant workers from Central Asia temporarily residing in St. Petersburg

This section presents the results of studying the level of humoral immunity to PVB19 in migrants from Central Asia who were in St. Petersburg on a work visa to determine the significance of this population in the spread of parvovirus infection.

To identify laboratory markers of PVI, 114 blood samples of migrant workers from the Republics of Uzbekistan and Tajikistan aged from 18 to 56 years (average age 33.4 years, median 33.5 years) were examined.

In general, the number and proportion of IgG-positive samples was 54 out of 114, or 47.4±4.68%. Blood samples were divided into 3 age groups. Specific IgG antibodies were detected in each of them (Tabl. 11).

Altogether, IgG-positive blood samples obtained from persons aged 18–30 years amounted to 38.0±6.8% of the number of examined samples. In the group of persons 40 years and older, significant differences ($p = 0.05$) in seroprevalence were revealed between migrant workers from Central Asian countries living in St. Petersburg (47.4%) and permanent residents of the city (72.5%).

4. Study of the effect of PVB19 infection on the course of the underlying disease in patients with oncohematological diseases

There are separate reports in the specialized literature that parvovirus infection can complicate the course of diseases accompanied by immunodeficiency (oncological, hematological, etc.).

The main task of this section is to determine the viral load with PVB19 by quantitative real-time PCR in patients before and in the early stages after the allogeneic hematopoietic stem cell transplantation (allo-HSCT), as well as to analyze the relationships with the levels of PVB19 antibodies in patients after allo-HSCT.

A total of 54 patients who underwent allo-HSCT were examined, the median age was 7.2 years (0.6–19 years). The majority of patients (51 out of 54) of this group were followed-up for 2 months after the allo-HSCT. The majority of the examined group consisted of patients with acute myeloblastic leukemia (n = 20; 37%) and acute lymphoblastic leukemia (n = 14; 26%). A third of the patients (33%) were in remission after previous treatment. Laboratory tests included complete blood count, urinalysis, serum chemistry, etc. The DNA of parvovirus B19, herpesviruses (CMV, EBV, and HSV), and polyomaviruses (BK, JC) was determined before allo-HSCT (0 days), as well as 30 and 60 days after the transplantation.

The average DNA content and levels of IgG antibodies to PVB19 at the time of transplantation did not correlate with the age of the examined patients, nor with the disease status, the general condition of patients or the presence of additional viral infections.

Comparison of the average levels of viral load with PVB19 DNA or the concentration of IgG antibodies to parvovirus within 30–60 days after intensive therapy and allo-HSCT showed no significant changes over time. But there was a correlation found between these laboratory values and clinical manifestations in patients. In particular, non-zero values of viral load were observed in 28% before allo-HSCT, 29% and 30.4% 30 and 60 days after allo-HSCT, respectively, i.e. about 70% of patients showed negative results during the period after allo-HSCT. Before allo-HSCT, IgG to PVB19 were detected in 37 (68.5%), at D+30 — in 41 (80.4%), at D+60 — in 38 (77.6%) patients. Detection of PVB19 DNA both before and after allo-HSCT was not accompanied by detection of IgM antibodies at all follow-up periods.

A significant positive correlation was found between the viral load and the levels of anti-PVB19 IgG in all accounting parameters (r = 0.351; p = 0.000008), and it is maximum 60 days after allo-HSCT. Thus, a highly reliable correlation was shown between the initial viral load (before allo-HSCT) and the levels of IgG antibodies to parvovirus at different follow-up periods (Fig. 14).

It is important to note that with elevated IgG levels to parvovirus B19, graft acceptance disorders were observed, which, in general, were detected more often 60 days after allo-HSCT (r = 0.315; p = 0.034; n = 46).

In addition, a significant correlation was shown between the low content of neutrophils and platelets (and, to a lesser extent, red blood cells) in the blood of patients and increased concentrations of virus-specific IgG antibodies. This may indicate a connection between the long-term persistence of parvovirus and the delayed recovery of hematopoiesis within 30–60 days after allo-HSCT (Fig. 15A, B, C).

Similar correlations (less pronounced) were also shown between the initial presence of parvovirus and the delayed recovery of red blood cells and platelets in the blood (r = –0.281; p = 0.02; r = –0.303, p = 0.01, respectively).

The presence of viral B19 DNA in the blood on day 30 after allo-HSCT was associated with febrile neutropenia at this time in 100% of cases (14/14), whereas in the absence of parvovirus DNA, febrile neutropenia was revealed in 68% of patients (23/34) (p = 0.016; RR = 1.478; 95% CI: 1.172–1.865).

Thus, the presence of parvovirus B19 DNA in the blood of children before hematopoietic cell transplantation was accompanied by an increase in the level of IgG antibodies in the blood at all follow-up periods after it and was associated with neutro- and thrombopenia occurring after allo-HSCT.

5. Molecular-genetic characteristics of PVB19 isolates isolated from blood samples of residents of St. Petersburg and Leningrad Oblast

To obtain isolates circulating in the territories of the Russian Federation, 9 blood samples of apparently healthy persons of different ages, regardless of gender, living in the city of St. Petersburg and Leningrad Oblast, were examined. The genetic sequences are deposited in the GenBank international database under the numbers: MH534950, MH166338, MG779501, MG779500, MG711455, MF481196, MF408298, MF405142, and MT543168. All the analyzed samples belonged to genotype 1 of subtype 1A. 7 PVB19 isolates were used to identify phylogenetic relationships. These nucleotide sequences can be divided into two subgroups: six isolates (85.7%) belonged to subgroup 1A2, one isolate (14.3%) belonged to subgroup 1A1. Phylogenetic relationships between the studied isolates and the reference sequences are shown in Fig. 16.

The sequences obtained in this study are closely related to those identified earlier in 2010–2011 on the territory of the NWFD and in 2005–2011 on the territory of the Russian Federation in the studies of other research groups. These data indicate ongoing viral circulation. However, a certain level of clustering of viruses by year may indicate the re-import of the virus.

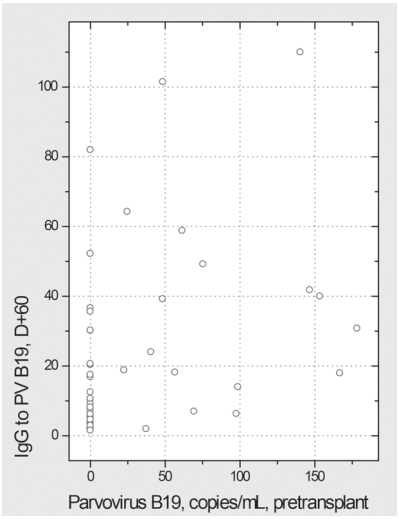

Figure 14. Relationship between the initial viral load with PVB19 (day 0) and the concentration of IgG antibodies to parvovirus B19 on day 60 (r = 0.461; p = 0.0004)

On the X axis is the concentration of PVB19 (copies/ml), on the Y axis are the levels of IgG antibodies 2 months after allo-HSCT.

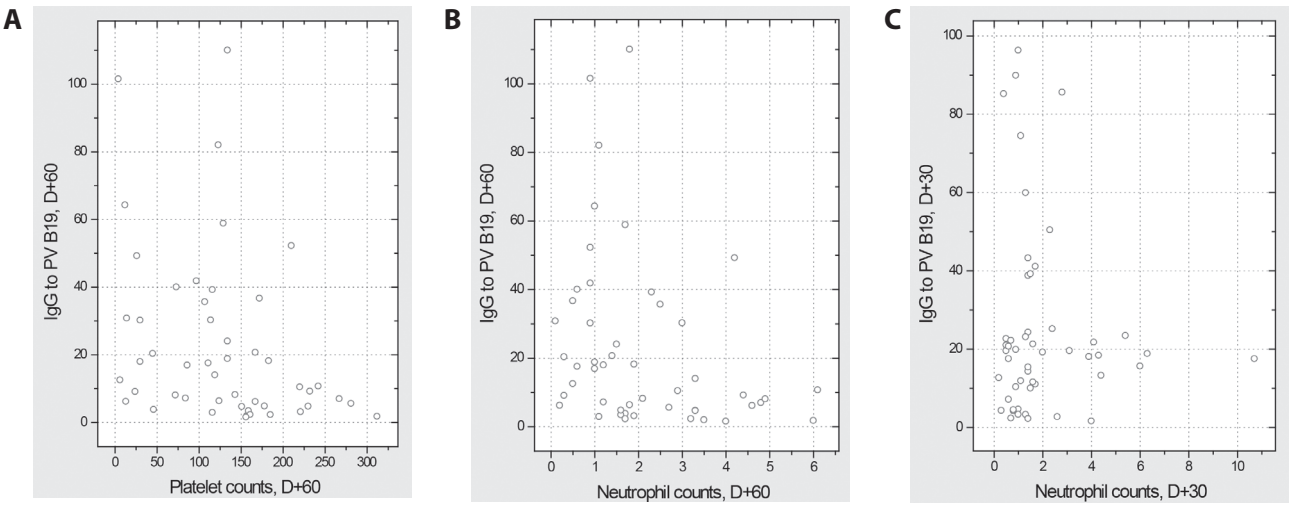

Figure 15. Correlation between hematological parameters and the level of IgG PVB19 in patients after allo-HSCT

A. Dependence of the number of platelets in the blood on day +60 after allo-HSCT and the levels of anti-PVB19 IgG on day +60 (r = –0.422; p = 0.001).
B. Negative correlation between the concentration of neutrophils in the blood on day +60 and the levels of anti-PVB19 IgG (day +60) (r = –0.422; p = 0.002).
C. Dependence of the level of neutrophils in the blood on day +30 on the levels of anti-PVB19 IgG (day +30) (r = –0.380; p = 0.003).

Comparative phylogenetic analysis of the studied samples. The isolates identified in this study from apparently healthy individuals from St. Petersburg and the Leningrad Oblast were compared to isolates from the Republic of Kazakhstan and the Republic of Serbia that were isolated earlier (outside the scope of this study). All isolates are classified as genotype 1A.

Isolates of both subgroup 1A1 and subgroup 1A2 with a predominance of one or another subgenotype in the population were found in each group. When comparing isolates of parvovirus B19, similarities of PVB19 strains in subgroups 1A1 and 1A2 were found.

At the same time, there are some geographical differences in the distribution of 1A subtypes, i.e. the predominance of 1A1

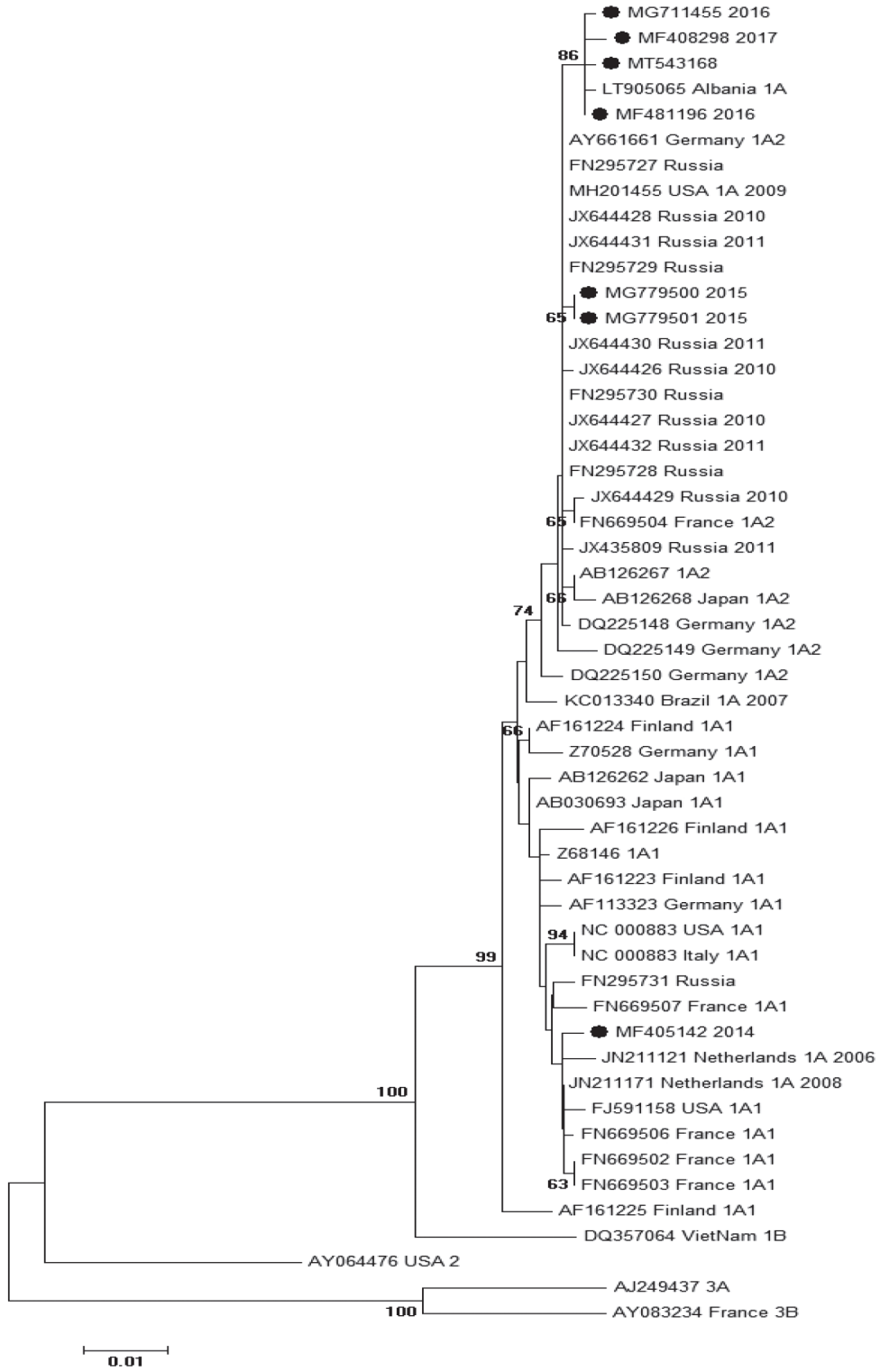

Figure 16. A dendrogram showing phylogenetic relationships between the studied isolates of parvovirus B19 isolated from blood samples of persons living in St. Petersburg and the Leningrad Oblast (●) as compared to the reference sequences presented in the international GenBank database. The reference sequences are labelled by the GenBank codes indicating the genotype, the region of origin and the date of material collection. Bootstrap values ≥ 60

subtype isolates isolated from blood samples of apparently healthy individuals of the Republic of Kazakhstan, and of 1A2 subtype isolates isolated from blood samples of apparently healthy individuals of the Russian Federation and the Republic of Serbia. The identification of isolates with similar nucleotide composition within one subtype in the territories of different geographical regions may be evidence of the common origin of the isolates, as well as the spread of parvovirus B19 strains associated with migration processes and globalization. Detection of nucleotide sequences of PVB19 DNA, subtype 1A2, clustering into a separate branch (Republic of Kazakhstan), may indicate the endemicity of these isolates for this territory.

The results obtained indicate an active circulation of parvovirus B19 in Europe and Asia.

Conclusion

Thus, using the example of such a vast territory as the NWFD of the Russian Federation, the widespread occurrence of PVI in Russia was confirmed.

It was shown that the formation of herd immunity to PVB19 depends on such factors as population density and the duration of social contacts.

In recent years, there has been a tendency for the spread of infectious diseases due to active migration. A large number of migrant workers from Central Asian countries arrive in the Russian Federation every year.

Migrants with a low level of population immunity are certainly a target for PVB19 infection. The crowding of these ethnic communities, which is characteristic of their stay in St. Petersburg, can contribute to the active spread of infection with the involvement into the infectious process of permanent residents of the city who are sensitive to infection, including people from risk groups.

It has been proven that infection with parvovirus B19 in patients with hematological diseases with a high degree of confidence correlates with the aggravation of the underlying disease.

Based on the phylogenetic analysis of PVB19 isolates, it was shown that under the conditions of natural distribution, PVB19 isolates identified both in the Russian Federation and in other geographical regions have a fairly high degree of homology.

In general, the results obtained indicate the high medical significance of the parvovirus infection and the need for further research into the geographical and social features of the spread of PVI, as well as the impact of PVB19 infection on the course of diseases such as chronic anemia, immunodeficiency conditions, etc., and the principles of diagnosing PVI in risk groups. Further molecular genetic studies are also needed to gain more knowledge about the biological features of parvovirus B19, the features of its distribution and evolution.

Publications

1. *Antipova A.Y., Bichurina M.A., Lavrentieva I.N. On the implementation of the WHO Western pacific regional plan of action for measles elimination // Russian Journal of Infection and Immunity. 2018; 8 (4): 465–472.*
2. *Bichurina M.A., Lavrentyeva I.N., Zheleznova N.V., Romanenkova N.I., Kubar O.I. Solidarity in the concept of vaccinal prevention of infections — a new aspect of ethics // Ethics of vaccination (criteria for scientific and humanitarian breakthrough) / Ed by. O.I. Kubar. St. Petersburg: St. Petersburg Pasteur Institute, 2018: 145–158.*
3. *Bichurina M.A., Zheleznova N.V., Lavrentieva I.N., Antipova A.Y., Kuliashova L.B., Totolian A.A. Study of ELISA test-systems of different formats for detection of measles virus specific IgM in different geographic zones // Russian Journal of Infection and Immunity. 2018; 8 (2): 230–234. (In Russ.)*
4. *Camara J., Antipova A.Yu, Bichurina M.A., Zarubaev V.V., Magassouba N., Lavrentieva I.N. Implementation of the program of measles eliminaton in the WHO African Region // Russian Journal of Infection and Immunity. 2019; 9 (3–4): 449–456.*
5. *Khamitova I.V., Lavrentyeva I.N., Averyanova M.Yu., Chukhlovin A.B., Zubarovskaya L.S., Afanasyev B.V. Parvovirus B19 incidence, specific antibody response, and delayed hematopoetic recovery after allogeneic hematopoetic stem cell transplantation // Cell. Ther. Transplant. 2018; 7 (1): 36–43.*
6. *Khamitova I.V., Ostankova Yu.V., Antipova A.Yu., Semenov A.V., Lavrentieva I.N. Molecular-genetic characteristics of Parvovirus B19 isolates circulating in The North-Western Federal District // Journal of Microbiology, Epidemiology and Immunobiology. 2018; 6: 55–66.*
7. *Khamitova I.V. Ostankova Y.V., Antipova A.Y., Lavrentieva I.N. Molecular genetic characteristics of parvovirus B19 isolates circulating in the Northwestern Federal District // Journal of Microbiology, Epidemiology and Immunobiology. 2018, 6: 55–61.*
8. *Kraeva L.A., Tokarevich N.K., Lavrentyeva I.N., Roshchina N.G., Kaftyreva L.A., Kunilova E.S., Kurova N.N., Stoyanova N.A., Antipova A.Yu., Svarval A.V., Zueva E.V., Porin A.A., Rogacheva E.V., Zheltakova I.R., Khamitova I.V., Timofeeva E.V., Bespalova G.I. Infection of labour migrants from Central Asia and residents of St. Petersburg and their susceptibility to various infectious diseases // Russian Journal of Infection and Immunity. 2018; 8 (1): 61–70. (In Russ.)*
9. *Lavrentieva I.N., Antipova A.Y., Bichurina M.A., Khamitova I.V., Nikishov O.N., Kuzin A.A. Markers of parvovirus infection in individuals with exanthemic diseases and in risk groups // Journal Infectology. 2019; 11 (3): 110–117. (In Russ.)*
10. *Lavrentieva I.N., Khamitova I.V., Camara J., Antipova A.Y., Bichurina M.A., Magassouba F.N., Nikishov O.N., Kuzin A.A., Semenov A.V. The status of humoral immunity to Parvovirus B19 in population of certain geographical regions // Journal of Microbiology, Epidemiology and Immunobiology. 2020; 97 (3): 233–241. (In Russ.)*
11. *Lavrentyeva I.N. , Khamitova I.V., Slita A.V. , Levkovski A.E., Diallo A.A., Diallo A.K., Sow T.C., Naydenova E.V., Agafonov D.A., Senichkina A.M. Impact of coinfection of PV B19 on the course and prognosis of malaria caused by plasmodium falciparum // Russian Journal of Infection and Immunity. 2018; 8 (3): 383–387.*
12. *Nikishov O.N., Kuzin A.A., Zobov A.E., Lavrentieva I.N., Antipova A.Y., Ostankova Y.V., Khamitova I.V., Nikishov S.N. Results of a study of parvovirus B19 (Parvoviridae, Parvovirinae, Erythroparvovirus, Primate erythroparvovirus 1) prevalence and circulation activity in socially significant categories of the population // Vopr. Virusol. 2020; 65 (3): 143–149.*

Development of means of prevention and treatment of socially significant viral infections

As the coronavirus infection has come to the fore, during the reporting period, the laboratory developed a model of infection caused by the coronavirus OC43 in cell culture. The most permissive culture was selected from the collection of cell lines, culture conditions were improved and the growth parameters of the virus determined.

The obtained model was used to analyse the properties of a chemical library of compounds based on the naturally occurring alkaloid cytisine (Fig. 17), and 9 compounds with high antiviral activity were identified. Selectivity index of the most promising of them was 89. Thus, the developed model allows screening for antiviral activity of chemical compounds against coronavirus infection.

Studies aimed at identifying and developing new synthetic low-molecular-weight inhibitors of influenza viruses were continued. Derivatives of the natural sesquiterpene alcohol ginsenol showed high potential (Fig. 18). The most active of them, referred as leader compound, or ginsamide, or compound 4981, demonstrated a directed inhibitory effect on the fusogenic activity of viral hemagglutinin, which explains its ability to block the reproduction of the influenza virus at the earliest stages of the viral cycle. It is important that the leader compound was active both against rimantadine-resistant (Fig. 19A) and oseltamivir-resistant (Fig. 19B) strains of the influenza virus, which is evidence of its ability to overcome the drug resistance of viruses, which is a serious issue when bringing new drugs into clinical practice.

The ability of the influenza virus to develop resistance to compound 4981 and the properties of the resulting resistant strain were also studied. A resistant variant was obtained by serial passages of the virus in the presence of increasing concentrations of the compound. It was shown that after 8 passages in a cell culture, the IC_{50} of the compound 4981 for the virus passaged in the presence of the drug was 15 times higher as compared to its IC_{50} for the control virus, which indicates the ability of this ginsamide derivative, as other anti-influenza drugs with a direct mechanism of action, to cause drug resistance.

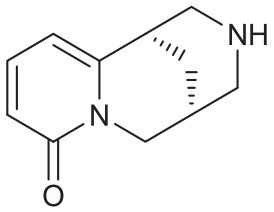

Figure 17. Cytisine structure

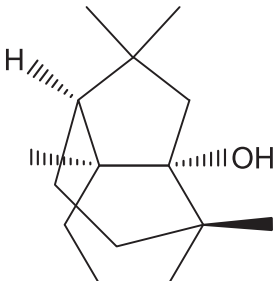

Figure 18. Ginsenol structure

Pathogenetic properties of the resistant virus were studied in experiments on a model of influenza pneumonia in white mice. Groups of animals were infected with the same doses of the original (embryonic) virus, the virus passaged in a cell culture without drugs, and the virus passaged in the presence of a ginsenol derivative. The level of specific mortality of animals, changes of their weight, as well as the size of foci of post-influenza pneumonia in the lungs were studied as pathogenicity criteria. The conducted studies showed that during the passages in the culture of MDCK cells, the pathogenicity of the virus decreases as compared to the embryonic virus. This was manifested by a statistically significant decrease in animal mortality (Fig. 20A), improvement of their weight (Fig. 20B), as well as a significant decrease in the size of lung lesions at the stage of post-influenza pneumonia (Fig. 19B).

The search for effective inhibitors of influenza virus reproduction among chemical libraries of compounds of other structural classes is continued. Thus, studies revealed a high potential of compounds of the azole-azine classes and verdazil derivatives against the Coxsackievirus B3. Compounds of the benzodithiols and azole-azines classes showed high inhibitory activity against the influenza virus.

In the group of benzodithiols (Fig. 21), 33 compounds were studied, of which 14 (42%) had a selectivity index above 10. The leader compound with a piperidine substituent had a selectivity index of 29.

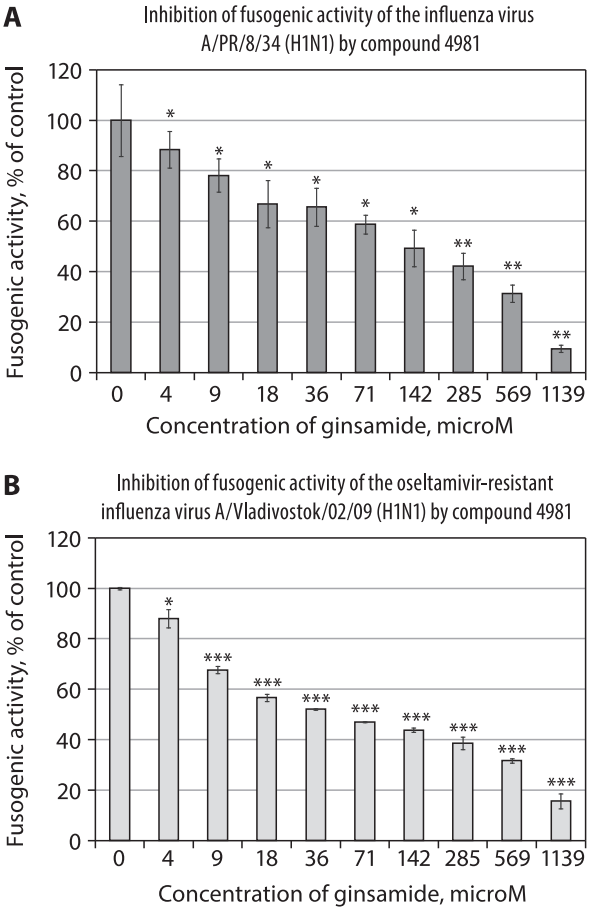

Figure 19. Direct anti-fusogenic activity of compound 4981 against hemagglutinin of influenza virus A/Puerto Rico/8/34 (H1N1) (A) and oseltamivir-resistant strain of influenza virus A/Vladivostok/02/09 (H1N1) (B)

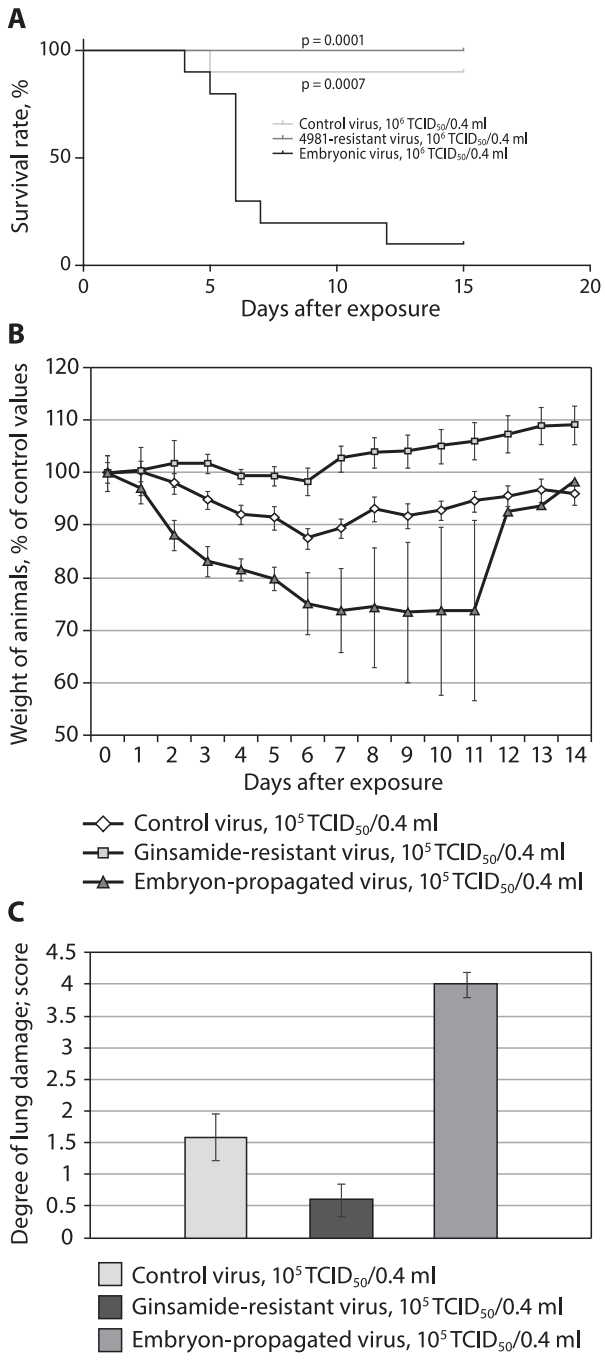

Figure 20. Differences in the death rates of laboratory animals (A), in the weight (B) and the degree of lung damage (C) during the clinical course of influenza pneumonia caused by the embryonic, control and ginsamide-resistant influenza viruses A/Puerto Rico/8/34 (H1N1)

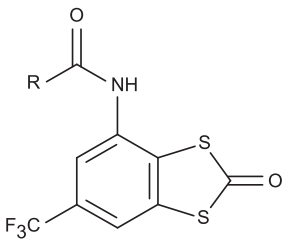

Figure 21. General structure of benzodithiol derivatives

For a more detailed study of the activity of the leader compound of this group, 4d, a series of experiments were conducted to assess their effect on the viral production in a cell culture, depending on the time of their administration. The obtained results are summarized in Fig. 22.

As can be seen from the above data, the compound 4d showed the greatest activity at the early stages of the viral cycle (0–2 hours after exposure), which indicates that its most likely targets are viral proteins that play a decisive role at this stage of viral reproduction, i.e. HA and M2. To test this hypothesis, the ability of 4d to inhibit fusogenic activity of viral hemagglutinin was studied. The obtained data are presented in Fig. 23.

As follows from the above data, the compound 4d showed dose-dependent inhibitory activity against viral hemagglutinin, which explains its antiviral effect; therefore, the group of benzodithiol derivatives can be considered promising for further modification of their chemical structures in order to obtain substances with best pharmacological properties.

Anti-influenza activity of chemical libraries based on purine derivatives is characterized. It was shown that the compounds of this series, having moderate virus-inhibitory activity, are able to block the reproduction of both influenza A and influenza B viruses, thus having a wide range of activity and having a good potential for further research into their biological properties.

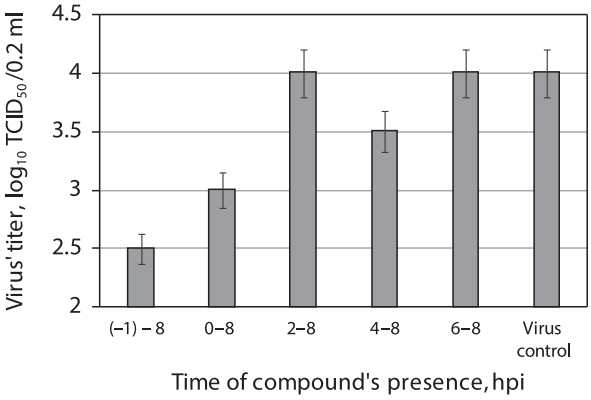

Figure 22. Infectious activity of influenza virus A/Puerto Rico/8/34 (H1N1) depending on the time of administration of the compound 4d to the medium

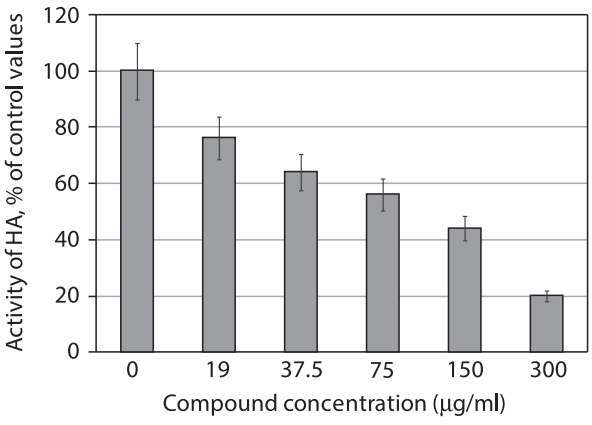

Figure 23. Inhibitory activity of the compound 4d against hemagglutinin of influenza virus A/Puerto Rico/8/34 (H1N1)

Publications

1. *Andreeva O.V., Garifullin B.F., Zarubaev V.V., Slita A.V., Yesaulkova I.L., Saifina L.F., Shulaeva M.M., Belenok M.G., Semenov V.E., Kataev V.E. Synthesis of 1,2,3-triazolyl nucleoside analogues and their antiviral activity // Mol. Divers. 2020; 25 (1): 473–490. doi: 10.1007/s11030-020-10141-y*
2. *Efremova M.M., Molchanov A.P., Starova G.L., Muryleva A.A., Slita A.V., Zarubaev V.V. 1,3-Dipolar cycloaddition of N-allyl substituted polycyclic derivatives of isoindole-1,3-dione with nitrones and nitrile oxides: an experimental and theoretical investigation // Tetrahedron. 2020; 76 (15): 131104. doi: 10.1016/j.tet.2020.131104*
3. *Elkina N.A., Burgart Y.V., Shchegolkov E.V., Krasnykh O.P., Maslova V.V., Triandafilova G.A., Solodnikov S.S., Muryleva A.A., Misiurina M.S., Slita A.V., Zarubaev V.V., Saloutin V.I. Competitive routes to cyclizations of polyfluoroalkyl-containing 2-tolylhydrazinylidene-1,3-diketones with 3-aminopyrazoles into bioactive pyrazoloazines // J. Fluor. Chem. 2020; 240: 109648. doi: 10.1016/j.jfluchem.2020.109648*
4. *Esaulkova Ya.L., Muryleva A.A., Sinegubova E.O., Belyaevskaya S.V., Garshinina A.V., Kireeva M.A., Volobueva A.S., Slita A.V., Kadyrova R.A., Zarubaev V.V. Mechanisms of antiviral activity of sage-leaved rock-rose extract (Cistus salviifolius) against human respiratory viruses // Antibiotics and Chemotherapy. 2020; 65 (7–8): 8–17. (In Russ.) doi: 10.37489/0235-2990-2020-65-7-8-8-17*
5. *Ilyina I.V., Patrusheva O.S., Zarubaev V.V., Misiurina M.A., Slita A.V., Esaulkova I.L., Korchagina D.V., Gatilov Y.V., Borisevich S.S., Volcho K.P., Salakhutdinov N.F. Influenza antiviral activity of F- and OH-containing isopulegol-derived octahydro-2H-chromens // Bioorg. Med. Chem. Lett. 2020; 31: 127677. doi: 10.1016/j.bmcl.2020.127677*
6. *Khomenko T.M., Zarubaev V.V., Lantseva K.S., Volobueva A.S., Slita A.V., Borisevich S.S., Korchagina D.V., Komarova N.I., Volcho K.P., Salakhutdinov N.F. New type of anti-influenza agents with benzo[d][1,3]dithiol core // Bioorg. Med. Chem. Lett. 2020; 30 (24): 127653. doi: 10.1016/j.bmcl.2020.127653*
7. *Shcherbakov K.V., Artemyeva M.A., Burgart Y.V., Saloutin V.I., Volobueva A.S., Misiurina M.A., Esaulkova I.L., Sinegubova E.O., Zarubaev V.V. 7-Imidazolyl-substituted 4′-methoxy and 3′,4′-dimethoxy-containing polyfluoroflavones as promising antiviral agents // J. Fluor. Chem. 2020; 240: 109657. doi: 10.1016/j.jfluchem.2020.109657*
8. *Ulomskiy E.N., Ivanova A.V., Gorbunov E.B., Esaulkova I.L., Slita A.V., Sinegubova E.O., Voinkov E.K., Drokin R.A., Butorin I.I., Gazizullina E.R., Gerasimova E.L., Zarubaev V.V., Rusinov V.L. Synthesis and biological evaluation of 6-nitro-1,2,4-triazoloazines containing polyphenol fragments possessing antioxidant and antiviral activity // Bioorg. Med. Chem. Lett. 2020; 30 (13): 127216. doi: 10.1016/j.bmcl.2020.127216*
9. *Volobueva A.S., Egorova A,. Galochkina A.S., Ekins S., Zarubaev V.V., Makarov V.A. The Evolution of Pleconaril: modified O-alkyl linker analogs have biological activity towards Coxsackievirus B3 Nancy // Molecules. 2020; 25 (6): E1345. doi: 10.3390/molecules25061345*
10. *Volobueva A.S., Zarubaev V.V., Lantseva K.S. Development of antiviral therapeutics for Coxsackievirus type B3 infection // Russian Journal of Infection and Immunity. 2020. 2021; 11 (1): 57–67. doi: 10.15789/2220-7619-DOA-1273*
11. *Zarubaev V.V., Garshinina A.V., Slita A.V., Belyaevskaya S.V., Lavrentieva I.N. Antiviral activity of Kagocel on the model of experimental lethal influenza infection // Antibiotics and Chemotherapy. 2020; 65 (1–2): 15–20. (In Russ.) doi: 10.37489/0235-2990-2020-65-1-2-15-20*
12. *Zarubaev V.V., Slita A.V., Sinegubova E.O., Muryleva A.A., Lavrentieva I.N. Antiviral activity of enisamium iodide against influenza and ARVI viruses in vitro on various cell lines // Ter. Arkh. 2020; 92 (11): 45–50. doi: 10.26442/00403660.2020.11.000872*

Department of Immunology

LABORATORY FOR PATHOGEN IDENTIFICATION

Head of the Laboratory: Alyona Svarval
Researchers: N. Roschina, L. Kouliasheva, D. Starkova, R. Ferman

1. The objective of our work was to study the seroepidemiological features of *H. pylori* infection in the population of St. Petersburg at the present time, as well as the molecular and biological properties of *H. pylori* strains circulating in the Northwestern Federal District, and to study their sensitivity to antibacterial and probiotic drugs

To study the prevalence of *H. pylori* infection in the pediatric population of St. Petersburg in 2007–2018, 1969 children and adolescents aged 0 to 19 years were screened for the presence of *H. pylori* (961 persons in 2007–2011 and 1008 persons in 2012–2018). For the screening, persons residing in St. Petersburg without clinical manifestations of *H. pylori* infection were selected. IgG screening for *H. pylori* antibodies and *H. pylori* CagA was performed using ELISA test systems manufactured by DRG (Germany) and Biohit (Finland). The screening showed that in 2007–2011, a total of 39.54±1.6% of the examined children and adolescents had antibodies to the bacterial antigen of *H. pylori*. At the same time, over these years, antibodies to the toxin-associated CagA protein of *H. pylori* were detected in 37.77±1.6% of the examined children and adolescents. In 2012–2018, the screening results showed that a total of 19.54±1.2% of the examined children and adolescents had antibodies to the bacterial antigen of *H. pylori*. Antibodies to the toxin-associated CagA protein of *H. pylori* were detected in 17.76±1.2% of the individuals examined. Therefore, the current epidemic situation with regard to *H. pylori* shows a decrease in infection rates among the younger generation: the study revealed signs of the so-called "generation effect", which is observed in some countries of Western Europe, North America, and in Japan. These features of the current epidemic situation warrant further investigation.

Genomic polymorphism of *H. pylori* strains isolated from patients of various groups in St. Petersburg was analyzed. Genetic heterogeneity of 58 *H. pylori* strains was found: 40 (68.9%) strains were *cagA*-positive, 37 (63.8%) — *oipA*-positive; the *vacA* gene in various allelic variants was detected in all strains (100%). In patients with chronic gastritis, the proportion of *cagA*+ strains of *H. pylori* was 57.1%, in patients with duodenal ulcer disease and stomach cancer — 82.6% and 100%, respectively. *oipA*+ strains were found in almost equal proportions in patients with chronic gastritis (57.1%) and ulcer disease (60.9%). The dominant alleles of the *vacA* gene were *vacAs1* (82.7%), *vacAm1* (53.4%) and *vacAi1* (60.3%). All *cagA*-positive strains were carriers of the *vacAs1* allele, and vice versa, none of the strains of the vacAs2 genotype had the *cagA* gene. The proportion of *cagA+/vacAs1* genotype strains in patients with chronic gastritis was 57.1% as compared to 82.6% and 100% in patients with ulcer disease and stomach cancer. Of the 23 strains isolated from patients with ulcer disease, only one had the *vacAs2 (cagA–)* genotype. Nine combined genotypes were identified, the most common of which was *cagA+/oipA+/s1/m1/i1* (37.9%), which combined 22 strains isolated from patients with chronic gastritis, duodenal ulcer disease and stomach cancer.

The study of antibiotic resistance of isolated *H. pylori* strains showed that 40 (30%) of them were resistant to clarithromycin, 31 (23.3%) — to levofloxacin, 6 (4.5%) — to amoxicillin, and 1 (0.8%) — to tetracycline. Sequencing of the 23S rRNA Clar gene region of *H. pylori* isolates revealed point mutations in the following positions: G1513A, A1821G, G1826A, T1830C, A2142G, A2143G, T2182C, and T2244C. The T2244C mutation was found in all clarithromycin resistant *H. pylori* strains. Therefore, a high level of resistance of *H. pylori* strains to clarithromycin and levofloxacin was revealed, requiring individual testing of their sensitivity before prescribing eradication treatment. The analysis of point mutations in the 23S rRNA region revealed genetic heterogeneity of *H. pylori* isolates resistant to clarithromycin. On 18.12.2019, nucleotide sequences of *Helicobacter pylori* 23S ribosomal RNA gene were deposited to the GenBank: *Helicobacter pylori* strain HP30Rus 23S ribosomal RNA gene, partial sequence GenBank: MN822715.1; *Helicobacter pylori* strain HP246Rus 23S ribosomal RNA gene, partial sequence GenBank: MN822718.1; *Helicobacter pylori* strain HP64Rus 23S ribosomal RNA gene, partial sequence GenBank: MN822719.1; *Helicobacter pylori* strain HP156Rus 23S ribosomal RNA gene, partial sequence GenBank: MN822724.1; *Helicobacter pylori* strain HP80Rus 23S ribosomal RNA gene, partial sequence GenBank: MN822794.1; *Helicobacter pylori* strain HP199Rus 23S ribosomal RNA gene, partial sequence GenBank: MN822799.1; *Helicobacter pylori* strain HP202Rus 23S ribosomal RNA gene, partial sequence GenBank: MN822927.1; *Helicobacter pylori* strain HP229Rus 23S ribosomal RNA gene, partial sequence GenBank: MN822928.1).

Taking into account the development of *H. pylori* resistance to antibiotics used for its eradication, searching for new drugs with a bactericidal or bacteriostatic effect against this organism remains relevant. Our objective was to study the effect of metformil on the growth of *H. pylori in vitro*. 4 strains of *H. pylori* with typical properties were selected for the study. It was found that when used in concentrations of more than 1 mg/ml, metformil had an inhibitory effect on the growth of *H. pylori*. The potential of using metformil as an anti-helicobacter drug requires further research.

The study of the effect of probiotics and autoprobiotics on the growth of *H. pylori* was continued. It is known that probiotics used to treat *H. pylori* are not always effective. They can have low anti-helicobacter activity and be quickly eliminated from the system. Autoprobiotics — nonpathogenic indigenous bacteria isolated from the patient and administered to him for therapeutic purposes — have a number of advantages over probiotics, i.e. immunological tolerance and lack of antagonism toward the microbiota. The effect of probiotic strains of *Enterococcus faecium L3* (L3), *E. faecium SF-68* (SF) and 10 *E. faecium* autoprobiotics (APs) isolated from the stomach and intestines of patients with helicobacter infection on the growth of *H. pylori*

strains was studied. Growth inhibition zones (3–15 mm) were measured after the incubation of *H. pylori* under microaerophilic conditions. *H. pylori* strains showed different sensitivity to probiotics and autoprobiotics. L3, SF, and APs inhibited the growth of *H. pylori* in 79%, 64% and 60–78%, respectively. Two strains of *E. faecium* autoprobiotics were more active than probiotics (the inhibition zone was 13–15 mm in diameter). These findings are indicative of high activity of probiotic strains *Enterococcus faecium L3* (L3), *E. faecium SF-68* (SF) and 10 autoprobiotics *E. faecium* (APs) and highlight the need to select the most active drugs against each individual strain of *H. pylori*.

Using the PCR, five INDEL markers in the DNA of 20 *H. pylori* strains isolated in St. Petersburg were identified. Clustering of the identified INDEL genotypes was carried out together with the genotypes of 21 strains from the GenBank database; the Minimum spanning tree (MST) algorithm of BioNumerics 7.6 software package was used to construct a phylogenetic tree. 13 individual genotypes were identified in 20 strains from St. Petersburg, 17 strains belonging to the European cluster (*hpEurope*), 2 strains belonging to the *hspEAsia* cluster (SP980, SP994) and one strain belonging to the *hspWAfrica* cluster (SP981). The most common genotype identified in the European cluster includes 6 strains from St. Petersburg and 2 strains from the GenBank database. This was a joint work with the federal government health care institution Rostov-on-Don Anti-Plague Institute of Rospotrebnadzor.

2. The study of the current epidemic situation of sexually transmitted infections in the Northwestern Federal District and characterization of pathogen populations based on the genomic and proteomic approach was continued

Research into the spectrum of human papillomavirus (HPV) DNA genotypes was continued) (types 16, 18, 26, 31, 33, 35, 38, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, and 82), as well as into its prevalence and viral load. For this purpose, 578 persons aged 18 to 50 years were examined. When looking into the DNA of the papillomavirus with high carcinogenic risk of 3 phylogenetic groups with the determination of the total viral load, 20% of the examined samples were positive. HPV phylogenetic group A9 was the one that was found most often (genotypes 16, 31, 33, 35, 52, and 58) — in 7.14% of cases, followed by the HPV-phylogenetic group A7 (genotypes 18, 39, 45, and 59) — 6.9% of cases, and HPV-phylogenetic groups A5 and A6 (genotypes 51 and 56) — 5.9% of cases. Further study revealed that the structure of HPV genotypes in females in St. Petersburg was dominated by genotype 16, and the group of dominant genotypes also included genotypes 51, 53 and 56. In males, the genotypes most often detected were 16, 52, and 53 as well. In females, monoinfection was found in 55.9% of cases, whereas multi-infection with 2 or more genotypes — in 44.12%. In males, monoinfection was detected in 57.14% of cases, while in 42.86% 2 or more genotypes were detected. The viral load in females ranged from 1.6 to 7.7 log of HPV per 10^5 cells. In males, the viral load ranged from 1.9 to 5.5 log of HPV per 10^5 cells. Persons aged 20–29 years were considered to constitute the age group of risk (in this group, HPV of high carcinogenic risk was identified in more then 50%). Therefore, new data on the dominant genotypes of the human papillomavirus, prevalence and viral load in different age categories in females and males in St. Petersburg will be useful for the development of measures for epidemiological surveillance, treatment and prevention of papillomavirus infection.

Epidemiological study of the prevalence of herpes virus infections (HSV1,2, HHV6, EBV, CMV) in the population of St. Petersburg was continued. The studies were carried out using the real-time PCR. Blood, saliva, and urogenital tract material specimens were used as test material. DNA was extracted using the DNA-sorb-AM reagent kit (AmpliSens, Moscow). AmpliSens HSV I, II-FL and AmpliSens EBV/CMV/HHV6-screen-FL (AmpliSens, Moscow) reagent kits were used for the study.

410 persons aged 18 to 50 years were examined for the presence of herpes simplex virus type 1 and 2 in the urogenital tract material. HSV1 and 2 was detected in 8 samples (1.95%).

1134 persons with various forms of herpetic infections were examined. The HHV6 virus was detected in 704 persons examined (62.08%). The EBV virus was detected in 432 persons (38.09%). The CMV virus was detected in 54 cases (4.76%). Most often, co-infection with HHV6 and EBV was seen ($p < 0.005$). When studying the age-specific patterns of the herpetic infection distribution, it was found that children from 0 to 6 years old are the most vulnerable to these infections. In this age group, the herpes virus type 6 was most often detected (68.38% of cases), the EBV virus was detected in 38.4% of the persons, and the CMV virus — in 24.7%. The prevalence of cytomegalovirus infection in children 0 to 6 years old is the highest among all age categories. Among young and middle-aged persons (18–44 years), HHV6 and EBV viruses were most common — 66.04% and 39.9%, respectively, which was the highest value among adults. The study of seroepidemiological features of HVIs and the direction of their time trend, assessment of prevalence in the population in risk groups, study of the HVI manifestation (comparison of seropositivity and virus shedding data), and improvement of methods aimed at early detection of infections of this group are essential for the development of measures for their prevention and treatment.

Together with the Institute of Experimental Medicine (St. Petersburg), research was continued to study the antiviral activity of enterocins and probiotics on a biological model (Vero and Hep-2 cell cultures). It has been shown that enterocins and *E. faecium* L3 strain inhibit the reproduction of the herpes simplex virus type 2, similarly to the antiviral activity of acyclovir.

It is known that dysbiotic changes caused by opportunistic microorganisms increase the risk of sexually transmitted infections, which are associated with adverse effects on reproduction, including miscarriage. Vaginal microbiota was examined before *in vitro* fertilization in groups of women with different outcomes of the assisted reproductive technology (ART). To assess the qualitative and quantitative composition of microorganisms in the vaginal biocenosis, multiplex quantitative real time polymerase chain reaction (PCR) was performed using the Femoflor-16 reagent kit (NGO DNK-technologiya, Russia). The vaginal microbiota in the compared groups had different composition. In the group of women who did not end up with a clinical pregnancy, the counts of *Ureaplasma urealiticum*/*Ureaplasma parvum*, *Gardnerella vaginalis*/*Prevotella bivia*/*Porphyromonas* spp., and *Mobiluncus* spp./*Corynebacterium* spp. were on average higher than in the group of women with a subsequent pregnancy. *Mycoplasma hominis*, as well as *Sneathia* spp./*Sneathia* spp./*Fusobacterium* spp. were only detected in women with an ART failure. It was also found that in the group of pa-

tients, in whom *in vitro* fertilization did not result in a clinical pregnancy, the vaginal microbiome was dominated by strict and facultative anaerobes, in particular, *Gardnerella* spp., *Atopobium* spp., and *Prevotella* spp., which are the main causative agents of bacterial vaginosis. This is a joint work with the federal state budgetary institution of higher education Mechnikov North-Western State Medical University of the Ministry of Health of Russia.

Publications

1. *Ermolenko E.I., Molostova A.S., Gladyshev N.S., Svarval A.V., Dubosarsky Yu.S., Kolomina E.A., Gusev A.S., Varzin S.A. An integrated approach to diagnostics and therapy // Proceedings of the 14th Russian scientific-practical conference with international participation Health as the Basis of Human Potential: Challenges and Solutions. St. Petersburg, November 21–23, 2019; 14 (2): 574–585. (In Russ.)*
2. *Gladyshev N.S., Molostova A.S., Svarval A.V., Varzin S.A., Ermolenko E.I. Gastric and non-gastric diseases associated with Helicobacter pylori infection // Proceedings of the 14th Russian scientific-practical conference with international participation Health as the Basis of Human Potential: challenges and solutions. St. Petersburg, November 21–23, 2019; 4 (2): 535–548. (In Russ.)*
3. *Kouliasheva L., Roschina N., Nikitina T.V., Soultanov V.S. Anti-protozoal Activity of Conifer Green Needle Complex against Trichomonas vaginalis // Natural Product Communications. 2019; 14 (1): 147–150.*
4. *Kuleshov K.V., Svarval A.V., Kaftyreva L.A. Helicobacteriosis // L125 Laboratory diagnostics of infectious diseases / Ed. by V.G. Akimkin, M.G. Tvorogova. Moscow: Central Research Institute of Epidemiology, 2020: 196–199. (In Russ.) doi: 10.36233/978-5-9900432-0-6*
5. *Lebedeva E.A., Rishchuk S.V., Dushenkova T.A., Mokhov A.S., Desyatova M.V., Ermolenko E.I., Leontyeva G.F., Svarval A.V., Shchederkina E.E., Kolodzhieva V.V., Nilova L.Yu., Orishak E.A., Goncharov A.E. Altered microbiota in the female reproductive tract as a risk factor for failure of assisted reproductive technologies // Russian Journal of Infection and Immunity. 2021; 11 (2): 365–370. doi: 10.15789/2220-7619-CIT-1551*
6. *Molostova A.S., Gladyshev N.S., Svarval A.V., Ferman R.S., Karaseva A.B., Lavrenova N.S., Kashchenko V.A., Varzin S.A., Ermolenko E.I. Diagnosis of helicobacteriosis: issues and prospects // The Medical Alphabet. 2020; 17: 54–59. (In Russ.) doi: 10.33667/2078-5631-2020-17-54-59*
7. *Pegasheva I.L., Pavlovich I.M., Gordienko A.V., Chirsky V.S., Erokhina A.A., Svarval A.V. Influence of the pathogenicity factors of Helicobacter pylori (CagA and vacA) on precancerous changes in the gastric mucosa in patients with chronic gastritis // Experimental and Clinical Gastroenterology. 2019; 165 (5): 67–71. (In Russ.) doi: 10.31146/1682-8658-ecg-165-5-61-71*
8. *Svarval A.V., Starkova D.A., Roschina N.G., Ferman R.S., Gladyshev N.S. Epidemiology and laboratory diagnosis of Helicobacter pylori infection. Analytical review. St. Petersburg: St. Petersburg Pasteur Institute, 2020. 60 p. (In Russ.)*
9. *Uspensky Yu.P., Baryshnikova N.V., Ermolenko E.I., Suvorov A.N., Svarval A.V. A vaccine against Helicobacter pylori: Myth or reality? // Russian Journal of Infection and Immunity. 2019; 9 (3–4): 457–466. (In Russ.)*

LABORATORY OF MOLECULAR IMMUNOLOGY (RESOURCE SHARING CENTRE)

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

The Laboratory of Molecular Immunology is engaged in research in the field of clinical immunology, in particular the immunology of infectious diseases. The laboratory research interests include infectious agents. The head of the laboratory is Professor Areg A. Totolian, the director of the Institute, academician of the Russian Academy of Sciences, PhD in Medical Sciences. The laboratory staff consists of highly skilled specialists representing various biomedical disciplines: medical doctors, biologists, including geneticists and immunologists, biotechnologists. A resource sharing center works on the base of the Pasteur Institute. The center houses equipment enabling cutting-edge research. The devices used are: NovoCyte (ACEA Biosciences) and FACS Canto II (Beckton Dickinson) flow cytometers, MagPix (Millipore) multiplex assay based on Luminex xMAP technology, MALDI-TOF Microflex (Brucker Daltonics), matrix-assisted laser desorption/ionization time-of-flight mass-spectrometer, GenomeLab XP (Beckman Coulter) genetic analysis instrument, Pyromark Q24 (Qiagen) genetic analysis system, Ettan IPGphor 3 IEF System and Ettan DALTsix Large Vertical System (GE Healthcare), 2D-Electrophoresis Systems, Biomek 4000 (Beckman Coulter) Laboratory Automation Workstation, NextSeq genome sequencer, etc.

The laboratory team is engaged in the following research projects. A study and comparative analysis of the content of cytokines and chemokines involved in the antiviral immune response in the peripheral blood of chronic viral hepatitis B (CVHB) patients, including in groups of patients with different stages of liver fibrosis, is carried out. Mechanisms of chronicity and features of the immune response against the hepatitis B virus are researched. Chemokine receptor expression on various lymphocyte subpopulations is studied. Further studying the chemokine receptors expression and activation markers on the surface of key immune system cells involved in the control of viral infection will provide a deeper understanding of the interaction between the microbe and the immune system. An algorithm enabling to identify the cause of the initial form of fibrosis F0–F1 chronic hepatitis B or chronic hepatitis C by the content of IFNγ, CCL2/MCP-1 and CCL8/MCP-2 cytokines in blood plasma, was developed. Its diagnostic efficiency is 89.4%.

There is also research conducted to determine the content of cytokines/chemokines in the blood plasma of patients with autoimmune liver conditions. The results of scientific research on this topic were presented at various scientific conferences.

We continue to search for laboratory markers of liver damage of different origins. This research project will be a multi-year effort and includes two methodological approaches, genomic and proteomic. The study of markers of liver damage in patients with chronic hepatitis C and with varying degrees of liver fibrosis is underway. The markers were obtained during antiviral therapy with interferon-containing regimens. The study measured plasma concentrations of cytokines/chemokines. The study confirmed the role of the TNFα, CCL2/MCP-1 and CXCL11/ITAC cytokines/chemokines in the cellular immunity activation and the elimination of the hepatitis C virus. Positive prognostic variables in the effectiveness of interferon therapy were revealed. Based on the detection of cytokines/chemokines concentration in the blood serum with a multiplex assay, it became possible to clarify the immunopathogenesis of CHC, as well as to isolate and propose the CXCL10/IP-10 and CCL20/MIP3α chemokines as biological markers for monitoring the effectiveness of therapy in patients with F3–F4 and recurrent HCV infection during treatment.

The research resulted in a successful PhD thesis defense in Medicine on the topic "Clinical and immunological criteria for evaluating the effectiveness of chronic hepatitis C antiviral therapy".

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

Prevalence assessment of HBsAg-negative (occult) HBV in both the population and various high-risk patient groups as well as in blood donors is another line of research. It is of particular importance due to the high infectivity of the pathogen even with low viral loads.

A method for detecting HBV DNA in peripheral blood at low viral load using real-time PCR has been developed, and its significance in the identification of HBsAg-negative viral hepatitis B detection has been evaluated. At the first stage of the developed method, HBV DNA is amplified with the oligonucleotides flanking the genome region 2932–3182 ... 1–1846 nt. At the second stage, two oligonucleotide pairs are used in two virus regions (gene S and gene X) along with corresponding oligonucleotide fluorescently labeled probes complementary to the amplified fragment regions carrying fluorophores on the 5'-end, and non-fluorescent quenchers at the 3'-end. The channel corresponding to the FAM fluorophore detects the HBV DNA S-region amplification product, and the channel corresponding to the ROX fluorophore detects the HBV DNA X-region amplification product. The method sensitivity for DNA extraction from 100 μL plasma was 10 IU/ml. Obtaining the threshold cycle Ct for only one FAM or ROX fluorophore may indicate the HBV DNA presence in a sample with a load of less than 10 IU/ml. Notably, HBV detection is possible with a repeated PCR method of the corresponding sample with the HBV DNA extraction from an increased plasma volume (200–1000 μl). The developed method makes it possible to identify various HBV genetic variations, both typical and rare for the Russian Federation, those, circulating in other regions of the world. The method can be used to detect HBV in risk groups, in the population, as well as in blood donors screening to ensure the blood transfusions safety.

In December 2019, a new disease was reported; its causative agent was the SARS-CoV-2 β-coronavirus. The infection caused by this virus spread to almost every country of the world in a short time, resulting in a pandemic of the new COVID-19 disease (coronavirus disease 2019). During

the COVID-19 epidemic, a laboratory-based assessment of herd immunity to SARS-CoV-2 in St. Petersburg and the Leningrad Oblast inhabitants was carried out. SARS-CoV-2 specific antibodies tests were performed on 2713 people aged from 1 to 70 years and older in St. Petersburg and on 3130 people aged from 1 to 70 years and older in Leningrad Oblast.

Moreover, biomaterial was sampled and banked from new coronavirus infection patients who were in inpatient treatment at the new coronavirus infection COVID-19 treatment center of The First Pavlov State Medical University. Research of the immune parameters of the acute phase in recovered and deceased patients is currently underway.

The following research is being carried out in conjunction with other laboratories of the Institute. Genome analysis of hepatitis B virus strains circulating on the territory of the Northwestern Federal District of the Russian Federation is conducted in collaboration with the Viral Hepatitis Laboratory. Sequencing and comparative phylogenetic analysis of HBV strains is carried out. Sequencing of core/pre-core and pre-S/S areas of the HBV genome enables the genotyping of the isolated virus and the research into HBV primary and secondary resistance to antiviral drugs. Accumulation of data on HBV molecular epidemiology is part of the program for AHBV elimination in the Northwestern Federal District. Sequencing of HBV strains resulted in a thesis paper for a PhD degree in Biological Sciences.

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

To assess the spread of infection, parvovirus infection markers and measles detection is carried out namely in risk groups in the Northwestern Federal District of Russia. It is jointly conducted with the Laboratory of Experimental Virology. It has been found that parvovirus infection is widespread in the North-West of Russia. The obtained results indicate the advisability of conducting a laboratory examination of pregnant women who are exposed to exanthematous diseases, for markers of parvovirus infection; the importance of screening donated blood for PVB19 DNA, further rejecting the blood pools with a high viral load; the need for differential diagnosis between rubella and parvovirus infection by laboratory medicine.

We carried out a study of sarcoidosis cytokine profile features together with The First Pavlov State Medical University of St. Petersburg, the Ministry of Health of the Russian Federation. Sarcoidosis is an inflammatory disease of unknown etiology with damage to the lungs and other organs, which characterizes by non-necrotizing granulomas. In this case, the immune system cells are activated, namely in T-lymphocytes, and a wide range of cytokines is produced. The level of 46 cytokines/chemokines in the blood plasma sarcoidosis patients was detected with a multiplex assay based on xMAP magnetic bead technology. Cytokine profile features identification in sarcoidosis patients may indicate their important role in the granulomas formation and the outcome.

The laboratory team study prevalence of viral (A, B, C, D, E) hepatitides serological and molecular biological markers and HIV among conditionally healthy population of the Republic of Guinea and high-risk population. The work is part of the Government of the Russian Federation on Russian-Guinean scientific and technical cooperation order implementation. The analysis of the genotype/subgenotype structures and mutations of the detected viruses is underway. The prevalence of HBsAg-negative (occult) viral hepatitis B is estimated.

In addition, the xMAP multiplex assay revealed interpopulation differences in the cytokines/chemokines content in blood plasma between the residents of the Republic of Guinea and the citizens of the Russian Federation. The standards for the Republic of Guinea on a wide range of cytokines/chemokines content in blood plasma were established. Differences in the cytokine/chemokine levels can be attributed to different habitats, the circulation of infectious diseases, the intestinal microbiota, skin, and mucosa content, as well as to genetic differences.

Research within Russian-Vietnamese cooperation is also primarily on viral hepatitis and HIV, socially significant diseases. Work is underway to assess the prevalence of enteric and parenteral viral hepatitis markers, to analyze the genetic structure of hepatitis, and HIV viruses in Vietnam.

Resource Share Center is engaged in joint scientific projects with the following organizations:

- Institute of Experimental Medicine, FSBI, NWB RAMS (Department of Immunology), St. Petersburg;
- St. Petersburg State Pediatric Medical University, SBEI HPE (Subdepartment of Epidemiology and Infectious Diseases in Adults), St. Petersburg;
- The Pavlov St. Petersburg State Medical University, SBEI HPE (subdepartment of Infectious Diseases, Pulmonology, Otolaryngology, Center for Molecular Medicine), St. Petersburg;
- Russian State Medical University, GBOU HPE (Subdepartment of Pediatrics), Moscow;
- The Kirov S.M. Military Medical Academy, MoD, RF, FGOP HPE (Subdepartment of Infectious Diseases), St. Petersburg.

The laboratory scheduled two theses to be completed for a PhD degree in Biological Sciences.

The laboratory team actively presents the outcomes of the scientific activities at leading Russian and international conferences.

Publications

1. *Arsentyeva N.A., Batsunov O.K., Kudryavtsev I.V., Semyonov A.V., Totolian A.A. CD32a receptor and its role in well-being and disease // Med. Immunol. 2020; 22 (3): 433–442. (In Russ.) doi: 10.15789/1563-0625-CRI-2029*
2. *Arsentyeva N.A., Lyubimova N.E., Batsunov O.K., Semyonov A.V., Totolian A.A. Analysis of plasma cytokine profile in healthy residents of the Republic Of Guinea // New features of current infections in the Republic of Guinea / Ed. by A.Yu. Popova. St. Petersburg: St. Petersburg Pasteur Institute, 2020: 98–108.*
3. *Arsentyeva N.A., Lyubimova N.E., Batsunov O.K., Semyonov A.V., Totolian A.A. Cytokine profile features of blood plasma in healthy residents of the Republic of Guinea // Med. Immunol. 2020; 22 (4): 765–778. (In Russ.) doi: 10.15789/1563-0625-AOB-2073*
4. *Arsentyeva N.A., Semyonov A.V., Zhebrun D.A., Vasilyeva E.V., Totolian A.A. The role of the chemokine receptor CXCR3 and its ligands in some immunopathological conditions // Medical Immunology (Russia). 2019; 21 (4): 617–632. (In Russ.) doi: 10.15789/1563-0625-2019-4-617-632* ***Scopus, RSCI, HAC***

5. *Basina V.V., Arsentyeva N.A., Batsunov O.K., Lyubimova N.E., Semyonov A.V., Esaulenko E.V., Totolian Areg A. Features of chemokine receptors CXCR3 and CCR6 expression and their ligands in the peripheral blood of patients with chronic hepatitis C during antiviral therapy with pegylated interferons // Med. Immunol. 2019; 21 (1): 107–120. (In Russ.) doi: 10.15789/1563-0625-2019-1-107-120* ***Scopus***
6. *Basina V.V., Arsentyeva N.A., Lyubimova N.E., Semyonov A.V., Esaulenko E.V., Totolian A.A. Clinical and immunological characteristics of difficult cases of chronic hepatitis C during antiviral therapy // Bulletin of the Yaroslav the Wise Novgorod State University. 2020; 3 (119): 25–31. doi: 10.34680/2076-8052.2020.1(117).25-31* ***RSCI, HAC***
7. *Basina V.V., Peradze Kh.D., Avdovskaya A.K., Petrova O.A. Surgical complications in patients with salmonellosis: case series analysis // Bulletin of the Dagestan State Medical Academy. 2019; 1 (30): 28–33. (In Russ.)* ***RSCI***
8. *Batsunov O.K., Arsentyeva N.A., Lyubimova N.E., Esaulenko E.V., Semyonov A.V., Totolian A.A. The content of some cytokines and chemokines in the blood chronic hepatitis B patients in the early stages of liver fibrosis // Med. Immunol. 2020; 22 (2): 291–300. (In Russ.) doi: 10.15789/1563-0625-COC-1964*
9. *Lavrentyeva I.N., Antipova A.Yu., Bichurina M.A., Khamitova I.V., Nikishov O.N., Kuzin A.A. Markers of parvovirus infection in individuals with exanthemic diseases and risk groups // Journal Infectology. 2019; 11 (3): 110–117. (In Russ.) doi: 10.22625/2072-6732-2019-11-3-110-117* ***Scopus***
10. *Lavrentyeva I.N., Bichurina M.A., Antipova A.Yu., Khamitova I.V., Zheleznova N.V., Magassuba N.F., Camara J. Epidemiological surveillance of measles and other exanthemic infections in the Republic of Guinea and other African countries. Analytical Review. St. Petersburg: St. Petersburg Pasteur Institute, 2019. 64 p. (In Russ.)*
11. *Lavrentyeva I.N., Khamitova I.V., Camara J., Antipova A.Yu., Bichurina M.A., Magassouba F.N., Nikishov O.N., Kuzin A.A., Semyonov A.V. Humoral immunity to B19 parvovirus in the population of certain geographic regions // J. Microbiol. Immunol. Infect. 2020; 97 (3): 233–241. doi: 10.36233/0372-9311-2020-97-3-5*
12. *Lavrentyeva I.N., Khamitova I.V., Slita A.V., Levkovskiy A.E., Diallo A.A., Diallo A.K., Sow T.C., Naydenova E.V., Agafonov D.A., Senichkina A.M. Impact of coinfection of PV B19 on the course and prognosis of malaria caused by Plasmodium falciparum// New features of current infections in the Republic of Guinea / Ed. by A.Yu. Popova. St. Petersburg: St. Petersburg Pasteur Institute: 209–212.*
13. *Lazareva N.M., Baranova O.P., Kudryavtsev I.V., Arsentyeva N.A., Lyubimova N.E., Ses T.P., Ilkovich M.M., Totolian Areg A. Features of the cytokine profile in sarcoidosis // Med. Immunol. 2020; 22 (5): 993–1002. doi: 10.15789/1563-0625-FOC-2064*
14. *Lyubimova N.E., Semyonov A.V. Frequency of protective alleles of the CCR5 and CCR2 genes in the sample groups of children in St. Petersburg // Topical Issues of HIV infection. Mother-child Health Protection: conference proceedings. September 14–15, 2020. St. Petersburg: Person and Their Health Publishing House, 2020: 33–39. (In Russ.)*
15. *Milyukhina I.V., Usenko T.S., Senkevich K.A., Nikolayev M.A., Timofeyeva A.A., Agapova E.A., Semyonov A.V., Lyubimova N.E., Totolian A.A., Pchelina S.N. Plasma cytokine profile of patients with GBA-associated Parkinson's disease // Bulletin of Experimental Biology and Medicine. 2019; 168 (10): 404–408. (In Russ.)* ***WoS***
16. *Nikishov O.N., Kuzin A.A., Zobov A.E., Lavrentyeva I.N., Antipova A.Yu., Ostankova Yu.V., Khamitova I.V., Nikishov S. N. Research findings of prevalence and parvovirus B19 activity of circulation (Parvoviridae, Parvovirinae, Erythroparvovirus, Primate erythroparvovirus 1) in socially significant categories of the population // Problems of Virology. 2020; 65 (3): 143–149. (In Russ.) doi: 10.36233/0507-4088-2020-65-3-143-149*
17. *Ostankova Yu.V., Nogoybayeva K.A., Zuyeva E.B., Kasymbekova K.T., Tobokalova S.T., Semyonov A.V. Phylogenetic analysis and characterization of full-length hepatitis delta virus genome sequences isolated from patients with chronic viral B/D hepatitis in the Kyrgyz Republic // Problems of Especially Dangerous Infections. 2020; (1): 124–132. doi: 10.21055/0370-1069-2020-1-124-132.*
18. *Ostankova Yu.V., Semyonov A.V, Totolian Areg A. Quantitative assessment method of covalently closed circular HBV DNA in liver biopsy slide // Russian Clinical Laboratory Diagnostics. 2019; 64 (9): 565–570. doi: 10.18821/0869-2084-2019-64-9-565-570* ***Scopus***
19. *Ostankova Yu.V., Semyonov A.V, Zuyeva E.B, Totolian Areg A. Hepatitis B virus detection and molecular genetic characterization in HIV-infected patients in Arkhangel // Problems of Virology. 2019; 64 (3): 105–111. (In Russ.) doi: 10.18821/0507-4088-2019-64-3-105-111* ***Scopus***
20. *Ostankova Yu.V., Semyonov A.V., Serikova E.N., Zuyeva E.B., Schemelev A.N., Naydenova E.V., Scherbakova S.A., Boumbaly S., Barry M., Boiro M.Y., Totolian Areg A. HBV genotype prevalence in Russian-Guinea hospital patients in Kindia, the Republic of Guinea // New features of current infections in the Republic of Guinea / Ed. by A.Yu. Popova. St. Petersburg: St. Petersburg Pasteur Institute, 2020: 120–126.*
21. *Ostankova Yu.V., Semyonov A.V., Zuyeva E.B., Gabdrakhmanov I.A., Kozlov K.V., Zhdanov K.V., Totolian Areg A. Diversity of hepatitis B virus genetic variation in military personnel // Journal Infectology. 2019; 11 (3): 46–53. (In Russ.) doi: 10.22625/2072-6732-2019-11-3-46-53* ***Scopus***
22. *Ostankova Yu.V., Semyonov A.V., Zuyeva E.B., Nogoybayeva K.A., Kasymbekova K.T., Tobokalova S.T., Totolian Areg A. Prevalence of clinically significant virus mutations in patients with chronic viral hepatitis B // Russian Clinical Laboratory Diagnostics. 2020; 65 (1): 61–66. (In Russ.)*
23. *Ostankova Yu.V., Semyonov A.V., Zuyeva E.B., Totolian A.A. Prevalence of occult hepatitis B among HBsAg-negative persons with HIV in Veliky Novgorod // HIV Infection and Immunosuppression. 2019; 11 (1): 64–70. (In Russ.) doi: 10.22328/2077-9828-2019-11-1-64-70* ***Scopus***
24. *Ostankova Yu.V., Valutite D.E., Zuyeva E.B., Serikova E.N., Schemelev A.N., Boumbaly S., Balde T.A.L., Semyonov A.V. Primary mutations of hepatitis C drug resistance in patients with newly diagnosed HIV infection // Problems of Especially Dangerous Infections. 2020; (3): 97–105. doi: 10.21055/0370-1069-2020-3-97-105*
25. *Peradze Kh.D., Petrova O.A., Tsertsvadze G. K. Erysipelas in the clinical practice of infectious disease doctor and surgeon // Bulletin of the Dagestan State Medical Academy. 2019; 4 (33): 16–21. (In Russ.)*
26. *Popova A.Yu., Yezhlova E.B., Melnikova A.A., Bashketova N.S., Fridman R.K., Lyalina L.V., Smirnov V.S., Chkhindzheriya I.G., Grechaninova T.A., Agapov K.A., Arsentyeva N.A., Bazhenova N.A., Batsunov O.K., Danilova E.M., Zuyeva E.V., Komkova D.V., Kuznetsova R.N., Lyubimova N.E., Markova A.N., Khamitova I.V., Lomonosova V.I., Vetrov V.V., Milichkina A.M., Dedkov V.G., Totolian A.A. Herd immunity to SARS-CoV-2 in St. Petersburg during the COVID-19 epidemic // Problems of Especially Dangerous Infections. 2020; (3): 124–130. doi: 10.21055/0370-1069-2020-3-124-130*
27. *Popova A.Yu., Yezhlova E.B., Melnikova A.A., Istorik O.A., Mosevich O.S., Lyalina L.V., Smirnov V.S., Cherniy M.A., Balabysheva N S., Loginova I.S., Vladimirova O.S., Samoglyadova I.S., Vasev N.A., Rumyantseva S.V., Chupalova E.Yu., Selivanova G.V., Muravyova M.V., Timofeyeva L.V., Khankishiyeva E.N., Tylchevskaya V.D., Nikitenko N.D., Kostenitskaya T.I., Virkunen N.V., Klimkina I.M., Kuzmina T.M.,*

Degtyarenko N.V., Bazunova A.I., Filippova L.A., Palchikova N.A., Kukshkin A.V., Arsentyeva N.A., Batsunov O.K., Bogumilchik E.A., Voskresenskaya E.A., Drobyshevskaya V.G., Zuyeva E.V., Kokorina G.I., Kurova N.N., Lyubimova N.E., Ferman R.S., Khamdulayeva G.N., Khamitova I.V., Khorkova E.V., Milichkina A.M., Dedkov V.G., Totolian A.A. Assessment of herd immunity to SARS-CoV-2 in Leningrad Oblast during the COVID-19 epidemic // Problems of Especially Dangerous Infections. 2020; 3: 114–123. doi: 10.21055/0370-1069-2020-3-114-123

28. *Porin A.A., Makarova M.A., Balde R., Buaro M.Y., Boumbaly S., Matveyeva Z.N., Zuyeva E.V., Kaftyreva L.A. Campylobacteriosis. Antimicrobial resistance of thermotolerant Campylobacter isolated in the Republic of Guinea // New features of current infections in the Republic of Guinea / Ed. by A.Yu. Popova. St. Petersburg: St. Petersburg Pasteur Institute: 173–178.*
29. *Samoilov A.E., Stoyanova N.A., Tokarevich N.K., Evengard B., Zueva E.V., Panferova Y.A., Ostankova Y.V., Zueva E.B., Valutite D.E., Kovalev E.V., Litovko A.R., Goncharov A.U., Semenov A.V., Khafizov K., Dedkov V.G. Lethal outcome of Leptospirosis in Southern Russia: characterization of Leptospira interrogators isolated from a deceased teenager // Int. J. Environ. Res.Public Health. 2020; 17 (12): 4238.*
30. *Schemelev A.N., Ostankova Yu.V., Zuyeva E.B., Boumbaly S., Balde T.A., Semyonov A.V. Characterization of hepatitis B virus and human immunodeficiency virus among HIV/HBV coinfected patients from the Republic of Guinea // Problems of Especially Dangerous Infections. 2019 3: 118–124. (In Russ.) doi: 10.21055/0370-1069-2019-3-118-124* ***Scopus***
31. *Schemelev A.N., Ostankova Yu.V., Zuyeva E.B., Khanh, Thu Huinh Kh., Semyonov A.V. Genotypic and pharmacoresistant characteristics in HIV patients in the Socialist Republic of Vietnam // HIV Infection and Immunosuppression. 2020; 12 (2): 56–68. (In Russ.) doi: 10.22328/2077-9828-2020-12-2-56-68*
32. *Semyonov A.V., Ostankova Yu.V., Schemelev A.N., Serikova E.N., Zuyeva E.B., Boumbaly S., Balde T.A.L., Totolian Areg A. Characterization of HBV and HIV among HIV/HBV co-infected patients from the Republic of Guinea // New features of current infections in the Republic of Guinea / Ed. by A.Yu. Popova. St. Petersburg: St. Petersburg Pasteur Institute, 2020: 127–133.*
33. *Semyonov A.V., Ostankova Yu.V., Serikova E.N., Zuyeva E.B., Totolian Areg A. Optimization of the algorithm for diagnosing markers of chronic hepatitis B in newly diagnosed patients with HIV infection // Russian Clinical Laboratory Diagnostics. 2020; 65 (9): 574–579. (In Russ.) doi: 10.18821/ 0869-2084-2020-65-9-574-579*
34. *Semyonov A.V., Ostankova Yu.V., Zuyeva E.B., Serikova E.N., Schemelev A.N., Boumbaly S., Barry M., Boiro M.Y., Totolian Areg A. Hepatitis B epidemiology in African countries // New features of current infections in the Republic of Guinea / Ed. by A.Yu. Popova. St. Petersburg: St. Petersburg Pasteur Institute, 2020: 134–142.*
35. *Semyonov A.V., Ostankova Yu.V., Zuyeva E.B., Serikova E.N., Schemelev A.N., Naydenova E.V., Scherbakova S.A., Boumbaly S., Barry M., Boiro M.Y., Totolian Areg A. Hepatitis B markers prevalence in Russian-Guinea hospital patients in Kindia, the Republic of Guinea // New features of current infections in the Republic of Guinea / Ed. by A.Yu. Popova. St. Petersburg: St. Petersburg Pasteur Institute, 2020: 143–148.*
36. *Semyonov A.V., Ostankova Yu.V. Occult (latent) hepatitis B: problems of laboratory diagnostics // Infectious Diseases: News, Opinions, Training. 2019; 8 (3): 60–69. (In Russ.) doi: 10.24411 / 2305-3496-2019-13010* ***RSCI***
37. *Usenko T.S., Nikolayev M.A., Miliukhina I.V., Bezrukova A.I., Senkevich K.A., Gomzyakova N.A., Beltseva Y.A., Zalutskaya N.M., Gracheva E.V., Timofeyeva A.A., Petrova O.A., Semyonov A.V., Lubimova N.E., Totolian A.A., Pchelina S.N. Plasma cytokine profile in synucleinopathies with dementia // J. Clin. Neurosci. 2020; 78: 323–326. doi: 10.1016/j.jocn.2020.04.058* ***WoS***
38. *Voytenkova E.V., Zabrovskaya A.V., Suzhayeva L.V., Zuyeva E.V., Kaftyreva L.A. Difficulties in identification of Comamonas kerstersii strains isolated from intestinal microbiota of residents of the Republic of Guinea // New features of current infections in the Republic of Guinea / Ed. by A.Yu. Popova. St. Petersburg: St. Petersburg Pasteur Institute: 184–186.*
39. *Yakovlev A., Sulima D., Larionov V., Koryagin V., Sharipova M., Fedunyak I., Musatov V., Kachenya G., Doguzhiyeva E., Sokolova O., Gorchakova O., Semyonov A., Zuyeva E., Ostankova Yu., Prosvernitsyn S., Kiyashko S., Chornoguz Yu., Valutite D. Low-level aviremic replication of HCV RNA in PBMC/WBC immune blood cells as one of the results of primary interferon-free DAA regimens in real clinical practice in AVT-naive patients with chronic HCV RNA viremia // Doctor. 2020; (2): 57–64. (In Russ.) doi: 10.29296/25877305-2020-02-13*
40. *Zhdanov K.V., Semyonov A.V., Karyakin S.S., Kozlov K.V., Sukachev V.S., Ostankova Yu.V., Valutite D.E., Zuyeva E.B., Sidorov R.S., Saulevich A.V., Bulankov Yu.I., Lyashenko Yu.I., Ivanov K.S. S MadCAM-1 as an immunological marker in the intestine-liver system in chronic hepatitis C patients and obese patients // Journal Infectology. 2019; 11 (2): 63–70. (In Russ.) doi: 10.22625/2072-6732-2019-11-2-63-70* ***Scopus***

Department of Epidemiology

LABORATORY OF EPIDEMIOLOGY OF INFECTIOUS AND NON-COMMUNICABLE DISEASES

Head of the Laboratory: Liudmila Lyalina
Researchers: V. Vetrov, V. Kaziakhmedova, V. Zaguzov

In 2019–2020, the Laboratory of Epidemiology of Infectious and Non-Communicable Diseases participated in research work in the following areas:

- Monitoring of the incidence of COVID-19 in the territories of the North-West of the Russian Federation (2020);
- Epidemiological substantiation and evaluation of the effectiveness of vaccination against papillomavirus infection and against HPV-associated malignant neoplasms;
- Epidemiology of measles and rubella at the stage of their elimination in the North-West of Russia;
- Implementation of the acute viral hepatitis B elimination program in the Northwestern Federal District of Russia;
- Epidemiological surveillance of polio, diseases with acute flaccid paralysis syndrome and enterovirus (non-polio) infection in 14 regions of Russia in accordance with the National Action Plan for Maintaining the Polio-Free Status of the Russian Federation.

The novel coronavirus infection (COVID-19)

The incidence of COVID-19 in 2020 developed into a pandemic and became the object of close attention of the state and health authorities in most countries of the world, including the Russian Federation. The International Committee on Taxonomy of Viruses assigned the name SARS-CoV-2 to the novel coronavirus. It is a single-stranded RNA-containing virus, which belongs to the Beta-CoV B lineage of the *Coronaviridae* family. The virus is assigned to group II pathogenicity, as are some other representatives of this family (SARS-CoV, MERS-CoV). According to published data, the genetic sequence of SARS-CoV-2 is at least 79% identical to the sequence of SARS-CoV.

In Russia, the first case of COVID-19 was registered on March 1, 2020. The results of the analysis of COVID-19 incidence in the territories of the Northwestern Federal District showed that the first cases of the disease were imported from various countries, and then the infection began to spread quickly among the local population. In 2020, 486 908 laboratory-confirmed cases of infection were diagnosed in the District. The largest number of cases was found in the metropolitan city of St. Petersburg (242 124 cases), which accounted for 49.7% of the total number of cases registered in the Northwestern Federal District, and also in the Arkhangelsk Region (43 327 cases, 8.9%) and the Murmansk Region (36 106 cases, 7.4%).

The incidence rate of COVID-19 in the Northwestern Federal District as a whole was 3489.9 per 100 000 population (95% CI 3480.0–3499.7). In the regions of the district, the incidence varied significantly. Fig. 24 shows the ranking of the territories of the Northwestern Federal District by the incidence of the disease in 2020.

At the end of the year, the highest incidence of COVID-19 was observed in the Murmansk Region, with 4791.4 per 100 000 population (95% CI 4741.5–4841.3), St. Petersburg, 4524.1 per 100 000 (95% CI 4506.1–4541.9), and the Republic of Karelia, 4425.0 per 100 000 (95% CI 4372.3–4477.7). The incidence rates were also high in the Arkhangelsk Region, at 3899.7 per 100 000 population (95% CI 3863.2–3936.1), the Pskov Region, at 3659.6 per 100 000 (95% CI 3611.9–3707.3), the Komi Republic, 3609.7 per 100 000 (95% CI 3568.3–3651.1), and the Novgorod Region, 3018.9 per 100 000 (95% CI 2974.4–3063.4). In other territories of the District, the incidence of COVID-19 was lower: Vologda Oblast had 1991.6 cases per 100 000 population (95% CI 1965.6–2017.7), the Nenets Autonomous Okrug, 1795.6 per 100 000 (95% CI 1669.5–1921.6), the Kaliningrad Region, 1788.9 per 100 000 (95% CI 1762.6–1815.1), the Leningrad Region, 1313.9 per 100 000 population (95% CI 1297.4–1330.5).

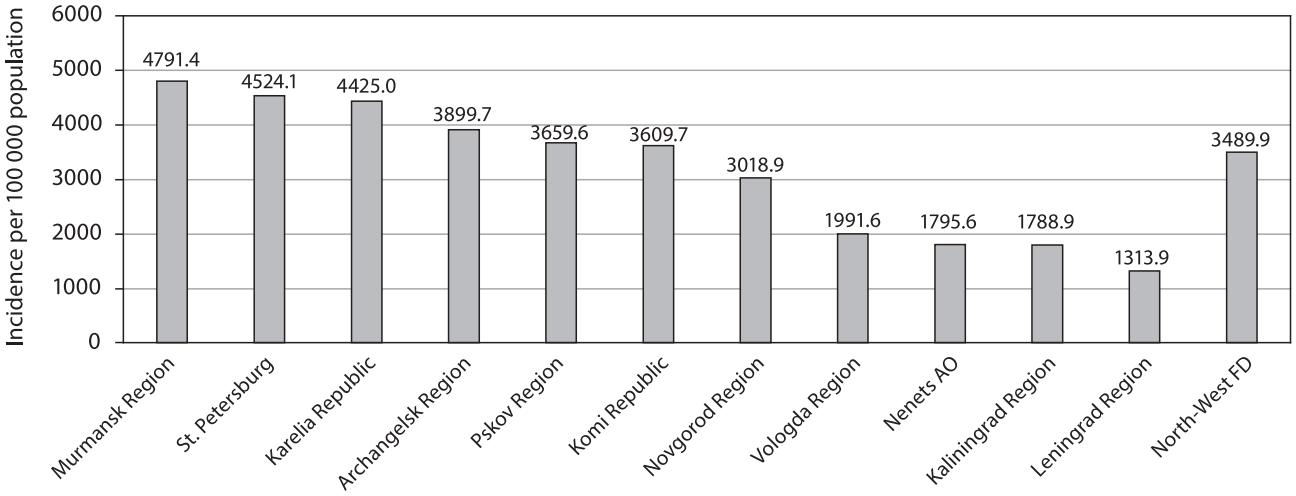

Figure 24. The ranking of the territories of the Northwestern Federal District by the incidence of COVID-19 in 2020

The findings of the analysis of COVID-19 mortality in the territories of the Northwestern Federal District in 2020 showed that a total of 10 571 deaths from this infection were registered; The mortality rate in the District as a whole was 2.17% (95% CI 2.16–2.18). the largest number of deaths was registered in St. Petersburg (7694 cases, 72.8% of the total number of deaths in the Northwestern Federal District), the Murmansk Region (651 cases, 6.2%), and the Komi Republic (575 cases, 5.4%). The ranking of the territories of the Northwestern Federal District by the mortality rate in 2020 is shown in Fig. 25.

The highest mortality was observed in St. Petersburg, at 4.78% (95% CI 4.76–4.80). In other Regions, the mortality rates were as follows: Komi Republic, 1.56% (95% CI 1.53–1.59), the Arkhangelsk Region, 1.4% (95% CI 1.38–1.42), the Murmansk Region, 1.39% (95% CI 1.36–1.42), the Kaliningrad Region, 1.28% (95% CI 1.26–1.3), the Vologda Region, 1.27% (95% CI 1.25–1.29), the Novgorod Region, 1.1% (95% CI 1.07–1.13), the Leningrad Region, 0.94% (95% CI 0.93–0.95), the Pskov Region, 0.86% (95% CI 0.84–0.88), the Republic of Karelia, 0.61% (95% CI 0.59–0.63). No deaths from COVID-19 had place in the Nenets Autonomous Okrug in 2020.

Papillomavirus infection

In the Russian Federation, vaccination against papillomavirus infection has been provided since 2007, but it is not included in the national calendar of preventive vaccinations. In 2019–2020, work continued to study the epidemiological specifics of diseases associated with the human papillomavirus (HPV) in the territories of the Northwestern Federal District in the conditions of low HPV vaccination coverage. Vaccination was carried out mainly at the expense of the persons who were informed of the importance of the preventive measure. In 2019, immunization against papillomavirus infection was included in Regional health development programs in St. Petersburg, the Leningrad Region, the Republic of Karelia, and the Komi Republic. In most Regions, this contributed to an increase in vaccination rates against papillomavirus infection. In St. Petersburg in 2020, 1074 people received a complete course of vaccination, in 2019, 2641 people (mostly women), in the Leningrad Region, 274 people, in 2019, according to reports of state medical organizations, only 6 people were vaccinated. In Karelia and Komi Republics, the number of people vaccinated in 2020 was 304 and 118 people, respectively. Such indicators contributed to the prevention of HPV-associated diseases among vaccinated individuals, but did not significantly affect the incidence and prevalence of infection at the population level.

The incidence of condyloma acuminata is one of the early criteria for evaluating the effectiveness of HPV vaccination. Fig. 26 shows the incidence of condyloma acuminata in the Russian Federation, St. Petersburg, Leningrad Region, and the Republic of Karelia over a 10-year period. The morbidity rates among the population in the conditions of the metropolitan city of St. Petersburg have always been higher compared to the level in Russia as a whole, amounting in 2019 to 46.2 and 21.7 per 100 000 population, respectively. In the Leningrad Region, the incidence of condyloma acuminata was at the level of 19.5 per 100 000, in the Republic of Karelia, 31.4 per 100 000 population. The specifics of morbidity should be taken into account in the substantiation of Regional vaccination programs and in the evaluation of their effectiveness.

Condyloma acuminata cases were registered among both men and women (Fig. 27).

In St. Petersburg, higher incidence rates were among women, whereas in the Republic of Karelia, among men. The main risk group is young people aged 18–29 years. These data indicate the expediency of a gender-neutral HPV vaccination strategy provided the financial resources are available.

The early criteria for evaluating the effectiveness of vaccination against human papillomavirus also include the prevalence of high-risk HPV types. Fig. 28 shows the results of a study of the prevalence of HPV (16 genotypes in total) among patients of an STD clinic in St. Petersburg. Laboratory diagnostics was performed with the use of Russian test systems; the research method was real-time PCR. The results of the study showed that the frequency of HPV detection in 2016–2020 ranged from 20.0 to 24.7 per 100 examined people. In 2019 and 2020, there were no significant differences in the prevalence of HPV among patients.

Human papilloma viruses were detected among both male and female patients (Fig. 29). The prevalence of HPV among men was usually higher than among women, the 2020 figures being 20.4% and 19.7%, respectively, but

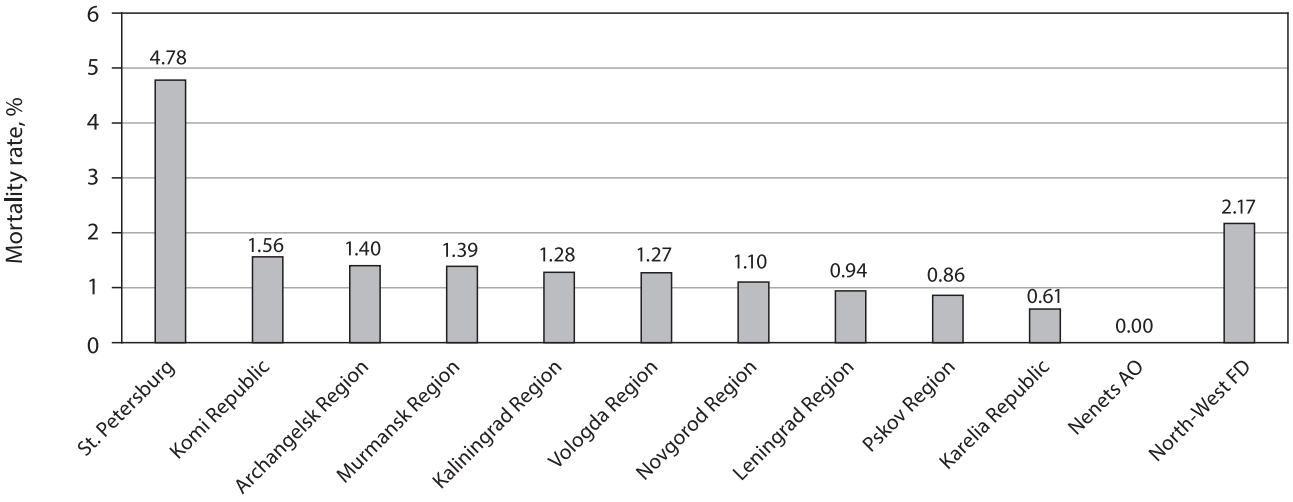

Figure 25. The ranking of the territories of the Northwestern Federal District by COVID-19 mortality rate in 2020

the differences are not statistically significant ($p > 0.05$). These data also indicate the feasibility of a gender-neutral approach to HPV vaccination.

No significant differences were also found in the prevalence of specific HPV genotypes among men and women. The most prevalent HPV genotype in St. Petersburg, as well as in other regions of Russia and other countries, is type 16. In 2019, the prevalence was 9.4% among women and 7.7% among men.

In other territories of the Russian Federation where similar studies were conducted, the prevalence rates of specific genotypes differed, which indicates the need to study

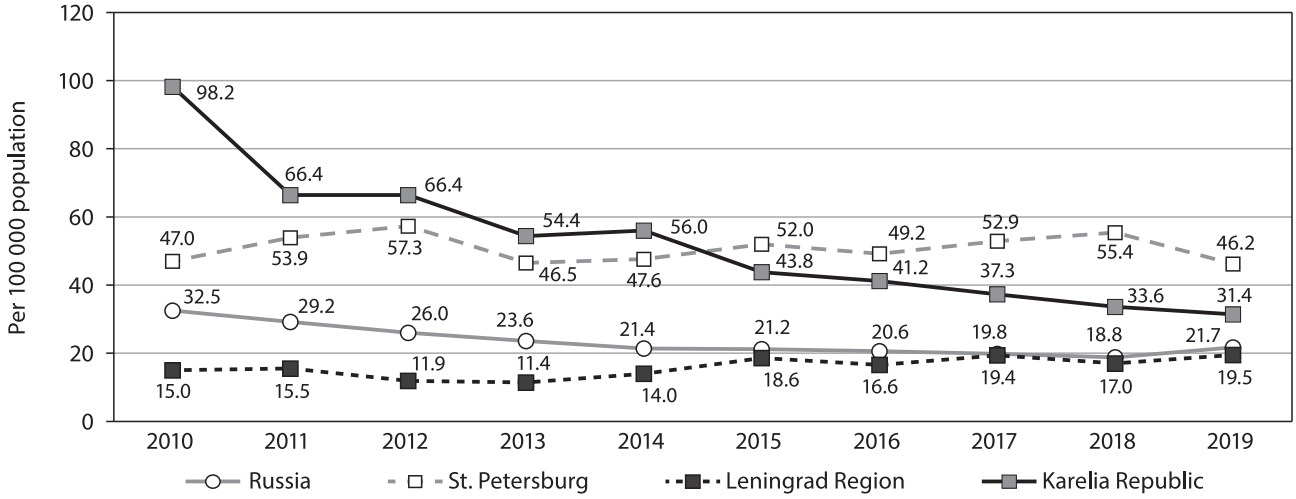

Figure 26. The incidence of condyloma acuminata in the Russian Federation, St. Petersburg, the Republic of Karelia, and the Leningrad Region in 2010–2019

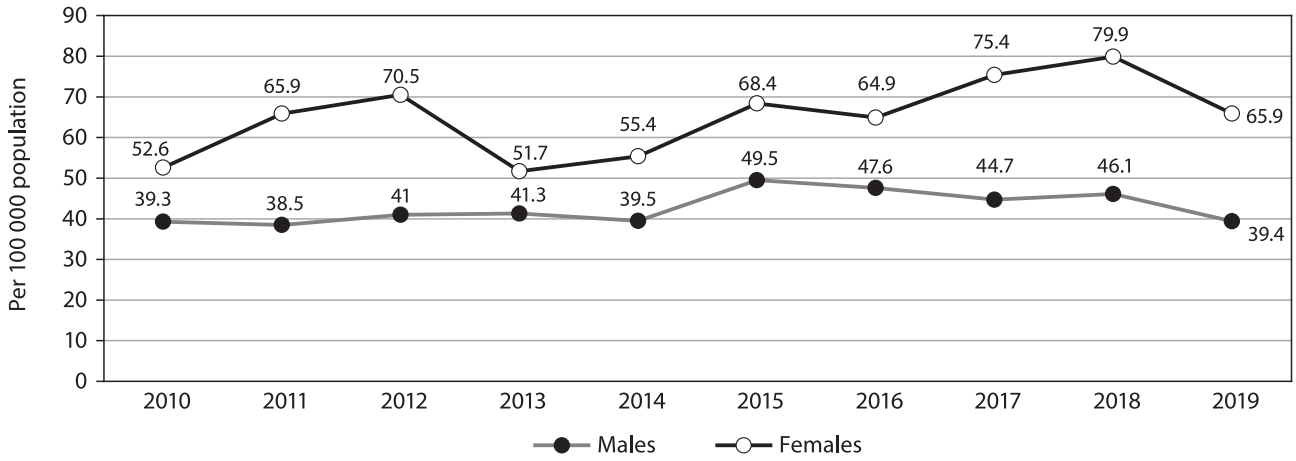

Figure 27. The incidence of condyloma acuminata in male and female population in St. Petersburg, 2010–2019

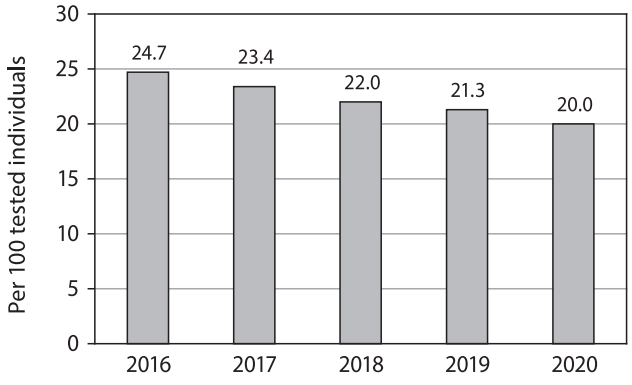

Figure 28. The prevalence of HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 83) among patients of an STD clinic in St. Petersburg, 2016–2020

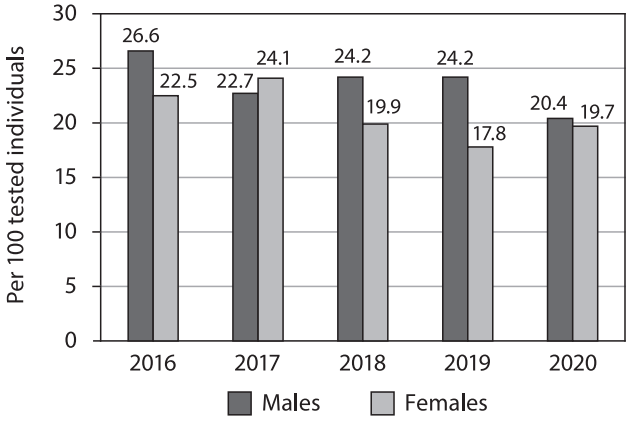

Figure 29. The prevalence of high-risk HPV among male and female patients of an STD clinic in St. Petersburg, 2016–2020

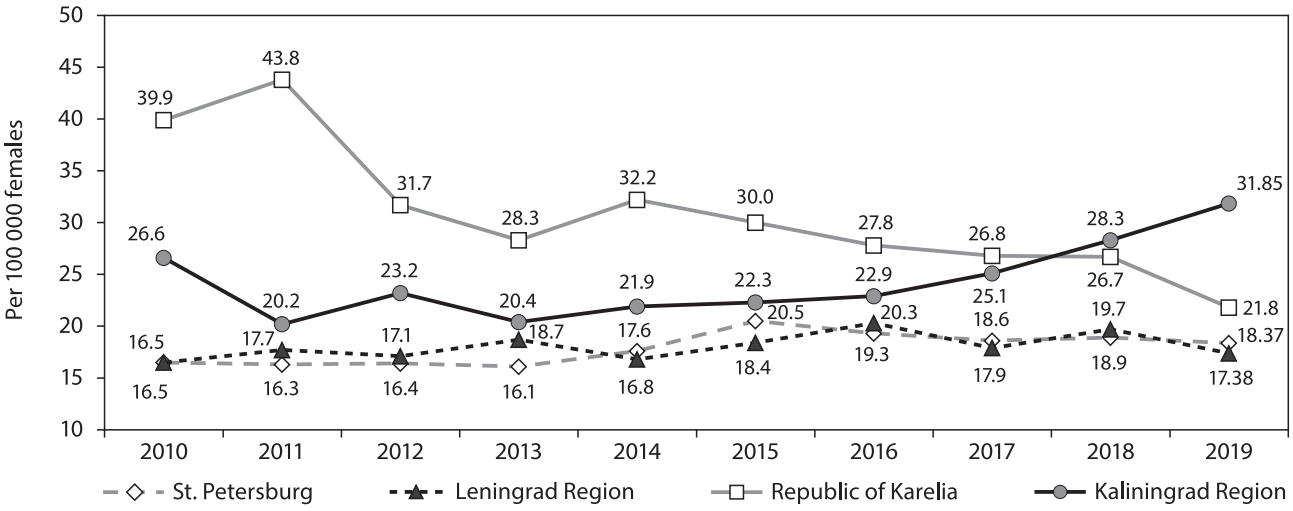

Figure 30. The incidence of cervical cancer in St. Petersburg, the Republic of Karelia, the Leningrad Region, and the Kaliningrad Region in 2010–2019 ("rough" indicators)

these data in each Region to substantiate the relevance of the problem, its social significance, and to assess the effectiveness of vaccination against papillomavirus infection.

Cervical cancer is one of the urgent problems of public health in the Russian Federation and in the territories of the Northwestern Federal District of Russia. In respect of HPV vaccination, the incidence of cervical cancer is one of the late criteria for evaluating the effectiveness of vaccination. Fig. 30 shows the incidence of cervical cancer in St. Petersburg, the Republic of Karelia, Leningrad and Kaliningrad Regions in 2010–2019. The most pronounced trend towards an increase in morbidity is observed in the Kaliningrad Region, where in 2019 the indicator was 31.85 per 100 000 of female population. In the Republic of Karelia, St. Petersburg, and the Leningrad Region, the incidence rate was lower and amounted to 21.8; 18.37; and 17.38 per 100 000, respectively.

Measles and rubella

In 2019–2020, work continued under the measles and rubella elimination program in the Russian Federation as part of the activities of the St. Petersburg Regional Center for the Supervision of Measles and Rubella, which is based in St. Petersburg Pasteur Institute. The Center supervises 11 territories of the Northwestern Federal District of Russia with a population of 13.5 million people. The duties of the research staff of the Laboratory of Epidemiology of Infectious and Non-Communicable Diseases include:

- collecting data from the Regions and preparing monthly reports on the registration of measles and rubella in the District, sending these reports to the National Research and Methodological Center for the Supervision of Measles and Rubella (Moscow),
- visits to supervised territories with scheduled inspections of the activities of Rospotrebnadzor and healthcare institutions under the measles and rubella elimination program (in 2020, visits were canceled due to the COVID-19 epidemic),
- provision of advisory assistance in the event of major outbreaks of infections,
- entering data from the epidemiological survey records of measles and rubella foci into the Centralized Information System for Infectious Diseases (CISID),
- analysis of the incidence of measles and rubella in the territories of the Northwestern Federal District of Russia,
- reports on the state of epidemiological surveillance over measles and rubella in the supervised territories at the annual meetings of specialists of the Russian Federation,

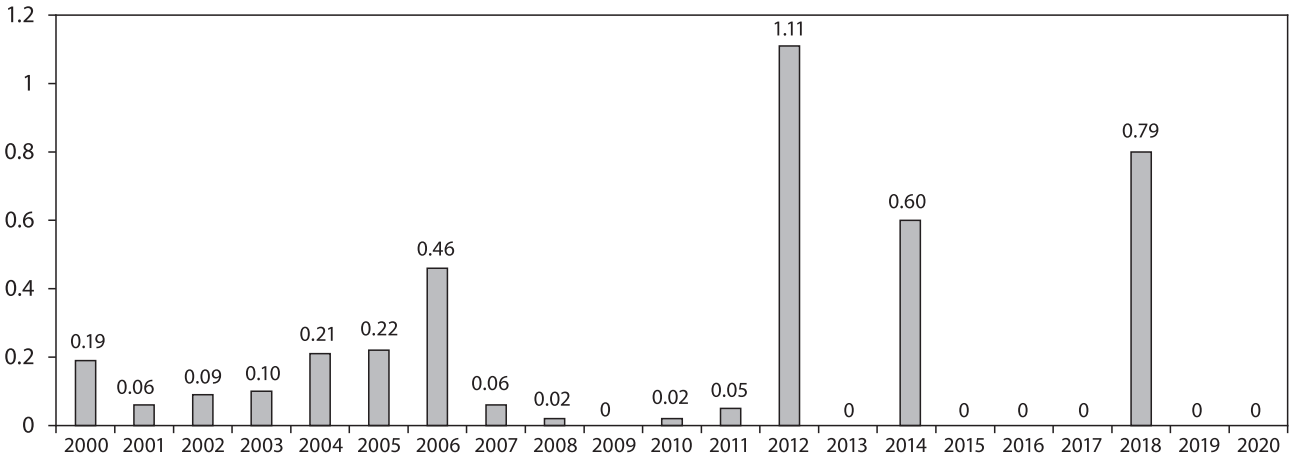

Figure 31. Measles incidence in the territories of the Northwestern Federal District of Russia in 2000–2020 (per 100 000 population)

- preparation of annual reports on the implementation of the measles and rubella elimination program in the territories of the Northwestern Federal District of Russia.

Fig. 31 shows the trends and incidence of measles in the Northwestern Federal District as a whole in 2000–2020. The analysis of these data showed the presence of periodic increases in the prevalence with intervals of 3 to 5 years in the District as a whole, in the conditions of high rates of vaccination coverage against measles (more than 95% according to reports from the regions). In some territories of the District, no measles cases have been registered for 10 years or longer. The last increase in morbidity rate was in 2018–2019, whereas in 2020 there was a decrease to 0.46 per 100 000 population. Since June 2020, no cases of measles have been detected in any of the 11 territories of the District, in the presence of the ongoing surveillance system for exanthemic diseases.

The age structure of patients registered in the Northwestern Federal District of Russia in 2019 and 2020 was dominated by the adult population aged 18 and older (66% and 72%). Children and adolescents accounted for 34% and 28% of cases, respectively (Fig. 32).

The analysis of the vaccination status of measles patients showed that the majority have not been vaccinated against this infection, or their vaccination status is unknown; the proportion of such patients in 2020 reached 76.6% and 20.4% (Fig. 33). The proportion of twice-vaccinated against measles among the sick in 2020 decreased to 3%.

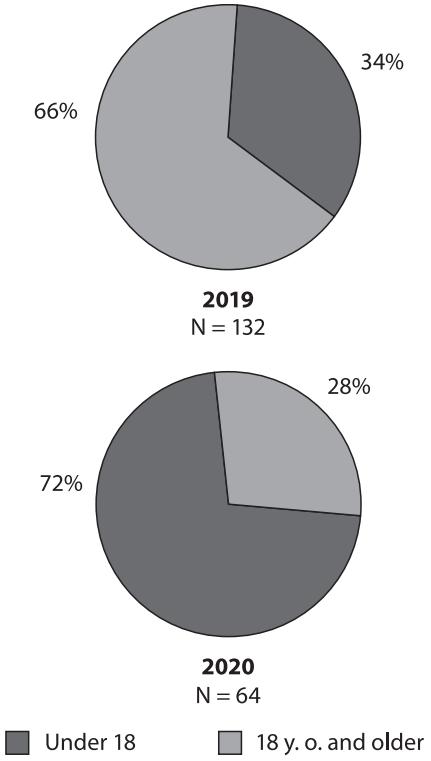

Figure 32. The age structure of measles patients in the territories of the Northwestern Federal District in 2019 and 2020

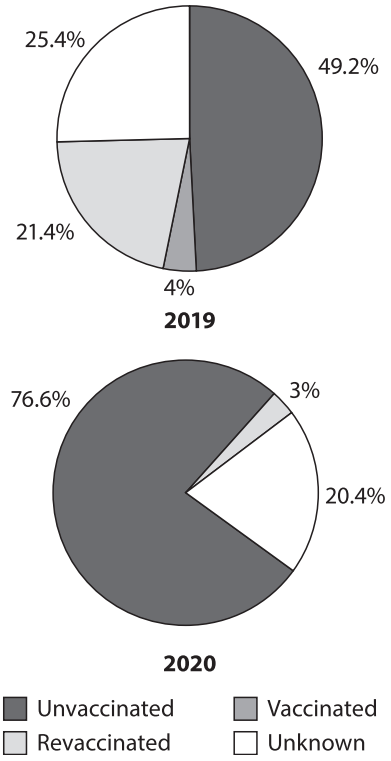

Figure 33. Distribution of measles patients depending on vaccination status in the territories of the Northwestern Federal District of Russia in 2019–2020

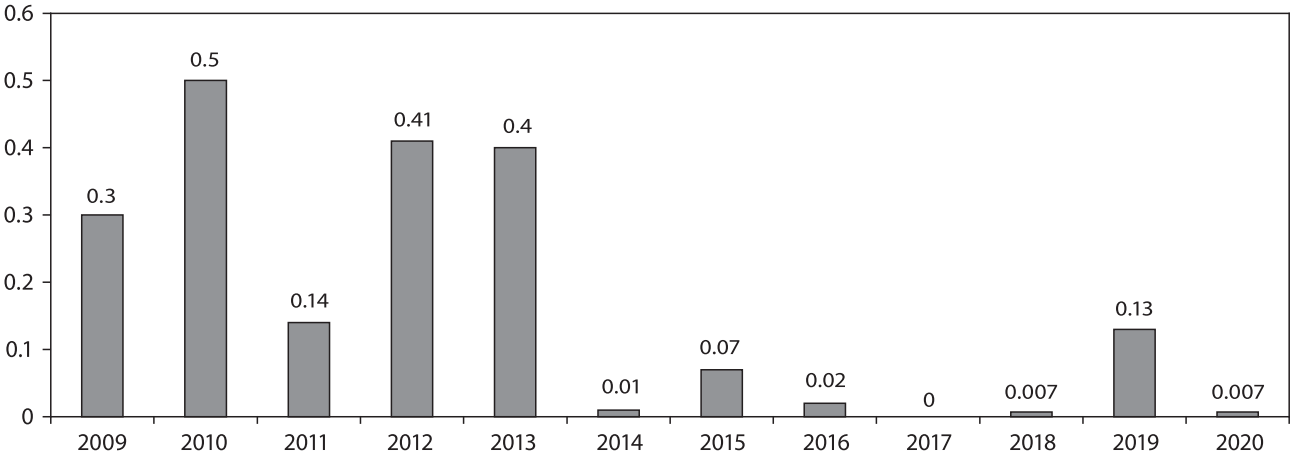

Figure 34. The incidence of rubella in the Northwestern Federal District of Russia in 2009–2020

Since 2014, the incidence of rubella in the territories of the Northwestern Federal District of Russia has been less than 1 per 1 million population, with the exception of 2019 (Fig. 34).

In 2017, no cases of rubella were registered in the territories of the District. In 2018, one case was identified in the Leningrad Region in an adult not vaccinated against this infection in the age group of 20–24 years. In 2019, 18 cases of the disease were diagnosed in St. Petersburg. Since June 2020, rubella has not been registered in any of the 11 territories.

Publications

1. *Kholopov D.V., Lyalina L.V., Khizha V.V., Topuzov E.E. Epidemiological characteristics of malignant neoplasms associated with the human papillomavirus in St. Petersburg in 2011–2018 // Malignant Tumors. 2020; 10 (3), s1: 124. (In Russ.)*
2. *Lyalina L.V., Kaziakhmedova V.V., Kasatkin E.V., Kovelenov A.Yu., Goryaev E.A., Katkiavichene E.V., Yurova A.Yu., Molchanova Zh.R. Clinical and epidemiological substantiation of a gender-neutral approach to vaccination against papillomavirus infection in the territories of Northwest Russia // Journal Infectology. 2020; 12 (2), 1: 77. (In Russ.)*
3. *Popova A.Yu., Ezhlova E.B., Melnikova A.A., Babura E.A., Mikheenko O.P., Lyalina L.V., Smirnov V.S., Molchanova Zh.R., Gorbatova Ya.V., Kharitonova M.N., Zubova A.N., Pogrebnaya T.N., Danilova V.I., Kukharchuk S.V., Dudinskaya E.V., Arbuzova T.V., Lomonosova V.I., Totolian A.A. Herd immunity of SARS-CoV-2 among the population of Kalinigrad region amid the COVID-19 epidemic // Journal Infectology. 2020;12 (5): 62–71. (In Russ.) doi: 10.22625/2072-6732-2020-12-5-62-71*
4. *Popova A.Yu., Ezhlova E.B., Melnikova A.A., Bashketova N.S., Fridman R.K., Lyalina L.V., Smirnov V.S., Chkhindzheriya I.G., Grechaninova T.A., Agapov K.A., Arsent'eva N.A., Bazhenova N.A., Batsunov O.K., Danilova E.M., Zueva E.V., Komkova D.V., Kuznetsova R.N., Lyubimova N.E., Markova A.N., Khamitova I.V., Lomonosova V.I., Vetrov V.V., Milichkina A.M., Dedkov V.G., Totolian A.A. Herd immunity to SARS-CoV-2 among the population in Saint-Petersburg during the COVID-19 epidemic // Problems of Particularly Dangerous Infections. 2020; 3: 124–130. (In Russ.) doi: 10.21055/0370-1069-2020-3-124-130*
5. *Popova A.Yu., Ezhlova E.B., Mel'nikova A.A., Historik O.A., Mosevich O.S., Lyalina L.V., Smirnov V.S., Cherny M.A., Balabysheva N.S., Loginova I.S., Vladimirova O.S., Samoglyadova I.S., Vasev N.A., Rumyantseva S.V., Chupalova E.Yu., Selivanova G.V., Muraviova M.V., Timofeeva L.V., Khankishieva E.N., Tylchevskaya V.D., Nikitenko N.D., Kostenitskaya T.I., Virkunen N.V., Klimkina I.M., Kuzmina T.M., Degtyarenko N.V., Bazunova A.I., Filippova L.A., Palchikova N.A., Kukshkin A.V., Arsentieva N.A., Batsunov O.K., Bogumilchik E.A., Voskresenskaya E.A., Drobyshevskaya V.G., Zueva E.V., Kokorina G.I., Kurova N.N., Lyubimova N.E., Ferman R.S., Khamdulaeva G.N., Khamitova I.V., Khorkova E.V., Milichkina A.M., Dedkov V.G., Totolian A.A. Assessment of population immunity to SARS-CoV-2 among the population of the Leningrad Region during the COVID-19 epidemic // Problems of Particularly Dangerous Infections. 2020; 3: 114–123. (In Russ.) doi: 10.21055/0370-1069-2020-3-114-123*

LABORATORY OF VIRAL HEPATITIS

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Acute viral hepatitides

The epidemic process of acute viral hepatitis (AVH) in the Russian Federation (RF) is steadily declining. The decrease in the incidence is evident in almost all the etiological entities of the AVH group, with the exception of hepatitis E (HEV). In 2020, 4383 new cases of AVH were registered (3.02 per 100 000 persons), which amounted to 10% of the entire structure of viral hepatitides in the Russian Federation that year.

Hepatitis A (HA) is one of the most common among hepatitides in the world, occupying a leading position in the general structure of AVH. The incidence decreased almost 3.8 times (from 7.3 to 1.9 per 100 000 people) from 2009 to 2020. It should be noted that the general trend towards a decrease in the HA incidence is observed in both adults and children. In 2020, children under 14 years of age had the lowest incidence of 3.01 per 100 000 people. However, the incidence rate in children still exceeded that in the entire population — 1.9 per 100 000. In 2019–2020, 19 HA epidemic foci in varying intensity were recorded in Russian federal constituent entities, with a total of 481 people affected. In 2020, no large outbreaks were recorded in the Russian Federation, whereas in 2019, three large HA outbreaks (over 50 people) were recorded with a total of 328 people affected, of which 116 were children under the age of 14. The share of children in the outbreak incidence averaged 35.4%. In 2019, outbreaks were recorded in the Perm Territory, in the Republic of Tatarstan, and in the Republic of Altai.

The HEV cases were first formally recorded in the Russian Federation in 2013, and the average annual HEV incidence rate is demonstrating the continued upward trend. There are within-country differences in the intensity of the HEV epidemic process. The detection of the HEV in patients in various regions of the Russian Federation indicated that it was widespread. In 2019, the average annual incidence increased compared to 2018 and amounted to 0.12 per 100 000 (n = 173). HEV cases were recorded in 33 federal constituent entities and in 5 federal districts. The poor situation with HEV persisted in Central Federal District (0.22 per 100 000, n = 93), namely, in Voronezh Oblast (0.9 per 10 000, n = 21), in Kursk Oblast (1.21 per 100 000, n = 13). There were no HEV cases recorded in four federal districts: in the Southern Federal District, in the Crimean Federal District, in the Far Eastern Federal District, in the North Caucasus Federal District.

Acute hepatitis B (HBV) incidence showed a positive trend in all federal districts in 2019–2020, it was less than 1.0 per 100 000. It was found that the lowest incidence was observed in the Far Eastern Federal District. By 2019, the hepatitis B incidence declined by 0.12 and amounted to 0.56 per 100 000 people. Analysis of the incidence ranking data revealed that this rate was 0 per 100 000 in 18 federal constituent entities. It was in the range of 0.01 to 1.0 per 100 000 in 58 federal constituent entities, and it exceeded 1.0 per 100 000 only in 9 federal constituent entities (in Moscow, in Kursk Oblast, in the Republic of Crimea, in the Republic of North Ossetia-Alania, in Rostov Oblast, in Saratov Oblast, in Sevastopol, in Tver Oblast, in Tyumen Oblast).

Acute hepatitis C (AHVC) incidence in federal districts continued to decline in 2019–2020. Over the 2019, the incidence was above the national in four federal districts: in the Ural Federal District (FD) (1.73 per 100 000), in the Northwestern FD (1.22 per 100 000), and in the Southern FD (1.15 per 100 000).

In 2019, the AHVC incidence exceeded the national in 27 federal constituent entities, 9 territories had the excess twofold or more: Voronezh Oblast (2.36 per 100 thousand population), Kaluga Oblast (2.08 per 100 000), the Republic of Crimea (2.35 per 100 000), the Republic of North Ossetia (2.71 per 100 000), Kurgan Oblast (3.09 per 100 000), Tyumen Oblast (2.32 per 100 000), Yamalo-Nenets Autonomous Okrug (2.04 per 100 000), Magadan Oblast (2.08 per 100 000), Chukotka Autonomous Okrug (2.02 per 100 000).

The hepatitis C virus (HCV) displays high genetic heterogeneity, which greatly affects the course of infectious and epidemic processes. Analysis of 50 857 HCV samples, carried out in 2018–2019, demonstrates the prevalence of genotype 1 (24 524 samples — 48%), including those with subtype 1b (12 417 samples — 50.6%); the second most common was genotype 3 (21 806 samples — 42.9%), genotype 2 was detected in 4255 samples (8.4%); genotypes 5 and 6 were extremely rare — in 36 (0.1%) and in 14 (0%) samples, respectively.

Chronic viral hepatitides

There are no significant positive trends in the epidemic process of chronic viral hepatitides B and C (CHB, CHC). In recent years, there has been a steady trend towards an increase in the cumulative cases of chronic liver diseases of viral etiology. It should be conceded that, given the number of patients with a confirmed diagnosis, compared to CHB, CHC is an issue of more significant concern for the Russian Federation. Its share is almost 77% of all newly diagnosed cases in the general etiological structure of chronic viral hepatitides.

In 2020, the total number of CHC patients in the Russian Federation was 621 468 people (0.5% of the population). The incidence of CHC varies within same federal district. In 2019, the Northwestern Federal District was the worst in terms of the incidence of CHC, St. Petersburg had an incidence of 80.79 per 100 000, Kamchatka Territory, Far Eastern Federal District, — 57.43 per 100 000, the Novosibirsk Region, the Siberian Federal District — 61.66 per 100 000, Moscow, the Central Federal District — 52.96 per 100 000, Yamalo-Nenets Autonomous Okrug, the Ural Federal District — 48.52 per 100 000. In the listed territories of the Northwestern Federal District, the Far Eastern Federal District, and the Siberian Federal District, the incidence was over 2 times the all-Russian in 2019, which amounted to 30.9 per 100 000.

The analysis of the average annual incidence in the federal districts of new cases of CHB demonstrated that they were uneven, the highest incidence was revealed in the

Northwestern Federal District and in the Far Eastern Federal District. The incidence was 21.00 per 100 000 in the Northwestern Federal District in 2019, however, this figure exceeds more than twofold the average annual rate for the Russian Federation. In the Far Eastern Federal District, the CHB detection rate was 10.28 per 100 000 in 2019.

Publications

1. *Basina V.V., Arsentyeva N.A., Batsunov O.K., Lyubimova N.E., Semyonov A.V., Esaulenko E.V., Totolian A.A. Expression patterns of chemokine receptors CXCR3 and CCR6 and their ligands in the peripheral blood of chronic hepatitis C patients during antiviral therapy with pegylated interferons // Medical Immunology (Russia). 2019; 21 (1): 107–120. (In Russ.)*
2. *Basina V.V., Arsentyeva N.A., Lyubimova N.E., Semyonov A.V., Esaulenko E.V., Totolian A.A. Clinical and immunological characteristics of difficult chronic hepatitis C cases during antiviral therapy // Bulletin of the Novgorod State University. 2020; 3 (119): 25–31. (In Russ.)*
3. *Batsunov O.K., Arsentyeva N.A., Lyubimova N.E., Esaulenko E.V., Semyonov A.V., Totolian A.A. The content of some cytokines and chemokines in the blood of chronic hepatitis B patients in the early stages of liver fibrosis // Medical Immunology (Russia). 2020; 22 (2): 291–300. (In Russ.)*
4. *Bushmanova A.D., Novak K.E., Esaulenko E.V., Ostankova Yu.V., Danilova E.M. Molecular biological methods for diagnosing hepatitis A // Bulletin of the Novgorod State University. 2020; 3 (119): 32–38. (In Russ.)*
5. *Esaulenko E.V., Bushmanova A.D. Epidemiological features of hepatitis A in the Russian Federation // Topical issues of infectious disease pathology in the South of Russia: proceedings of the XII applied research conference, 2019: 175–176. (In Russ.)*
6. *Esaulenko E.V., Dzemova A.A., Trifonova G.F. Chronic hepatitis C in the Northwestern Federal District during the global elimination of viral hepatitis // Topical issues of socially significant infectious and parasitic diseases: proceedings of the 20th Russian-Italian conference. Veliky Novgorod, 2020: 76–81. (In Russ.)*
7. *Esaulenko E.V., Ganchenko R.A. Modeling the epidemiological situation of hepatitis C in the Northwestern Federal District and St. Petersburg until 2030 // Infect. Dis. 2020; 18 (3): 48–55. (In Russ.)*

LABORATORY OF MOLECULAR EPIDEMIOLOGY AND EVOLUTIONARY GENETICS

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Tuberculosis and mycobacteria

Projects and collaborations

Funded projects

- Russian Foundation for Basic Research project No. 17-54-30020 "A personalized approach to fight the HIV and drug resistant TB epidemic in Irkutsk, Siberia" (project PI — O. Ogarkov, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia), 2017–2019.
- Russian Foundation for Basic Research project 18-04-01035 "Investigation of the role of the repeat element *IS*6110 in the micro- and macroevolution of *Mycobacterium tuberculosis* phylogenetic lineage 2" (PI — E. Shitikov, Center of Physico-Chemical Medicine, Moscow), 2018–2019.
- Russian Foundation for Basic Research, Project #19-04-00263 "Pathogenomic features and epidemic potential of highly resistant strains of ancient sublineage of *Mycobacterium tuberculosis* Beijing genotype" (PI — I. Mokrousov), 2019–2020.
- 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.
- 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). 2019–2021.
- 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.

International collaborations

National Institute for Public Health and the Environment, RIVM (2018–2021), Beijing Children's Hospital, China (2017–2021), North Estonian Medical Centre (Tallinn Estonia), Biomedical Research and Study Centre, University of Riga (Latvia), 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 (Lisboa, Portugal), Instituto de Investigación Sanitaria Gregorio Marañón (Madrid, Spain).

National collaborations

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

Major research results

Genomic analysis of clinical *M. bovis* BCG isolates

Background. The only licensed live Bacille Calmette–Guérin (BCG) vaccine used to prevent severe childhood tuberculosis comprises genetically divergent strains with variable protective efficacy and rates of BCG-induced adverse events. The whole-genome sequencing (WGS) allowed evaluating the genome stability of BCG strains and the impact of spontaneous heterogeneity in seed and commercial lots on the efficacy of BCG-vaccines in different countries. Our study aimed to assess sequence variations and their putative effects on genes and protein functions in the BCG-1 (Russia) seed lots compared to their progeny isolates available from immunocompetent children with BCG-induced disease (mainly, osteitis).

Results. Based on the WGS data, we analyzed the links between seed lots 361, 367, and 368 used for vaccine manufacture in Russia in different periods, and their nine progeny isolates recovered from immunocompetent children with BCG-induced disease (Fig. 35). The complete catalog of variants in genes relative to the reference genome (GenBank: CP013741) included 4 synonymous and 8 nonsynonymous single nucleotide polymorphisms, and 3 frameshift deletions. Seed lot 361 shared variants with 2 of 6 descendant isolates that had higher proportions of such polymorphisms in several genes, including *ppsC*, *eccD5*, and *eccA5* involved in metabolism and cell wall processes and reportedly associated with virulence in mycobacteria. One isolate

preserved variants of its parent seed lot 361 without gain of further changes in the sequence profile within 14 years.

Conclusion. The background genomic information allowed us for the first time to follow the BCG diversity starting from the freeze-dried seed lots to descendant clinical isolates. Sequence variations in several genes of seed lot 361 did not alter the genomic stability and viability of the vaccine and appeared accumulated in isolates during the survival in the human organism. The impact of the observed variations in the context of association with the development of BCG-induced disease should be evaluated in parallel with the immune status and host genetics. Comparative genomic studies of BCG seed lots and their descendant clinical isolates represent a beneficial approach to better understand the molecular bases of efficacy and adverse events during the long-term survival of BCG in the host organism.

Rectangle boxes correspond to the original seed lots with dates of lyophilization shown in parentheses. Rectangle shadowed boxes depict the sequenced seed lots (dates of lyophilization shown in parentheses). Dashed circles show commercial vaccine lots (not available for sequencing) used for immunization with dates of inoculation in parentheses. Square boxes mark sequenced BCG clinical isolates (dates of BCG culture isolation shown in parentheses). Gray-scale small boxes depict sequence variants in genes related to particular proteins (proportions shown in parentheses) in seed lots 361, 367, 368, and their progeny clinical isolates.

Molecular insight into *Mycobacterium tuberculosis* bedaquiline resistance

Emergence and spread of multi- and extensively drug resistant (MDR/XDR) *Mycobacterium tuberculosis* strains is a global concern and novel drugs are required. Here, we analyzed genetic variation underlying development of *M. tuberculosis* resistance to bedaquiline in the Russian province of Kaliningrad with a high rate of primary MDR-TB (30.5%). We hypothesized that whole-genome sequencing analysis of consecutive isolates from the same patient spanning long time period would permit to gain comprehensive and quantitative view on the real-time mycobacterial adaptation to the human host.

Bedaquiline susceptibility testing is only in the process of implementation in Russia and the phenotypic susceptibility data were not available for the studied isolates neither in the regional laboratory (Kaliningrad) nor in the supervising reference center (St. Petersburg). However, mutations in *Rv0678c, atpE, pepQ* genes were robustly correlated with bedaquiline resistance in the previous studies (Somoskovi et al., 2015; Veziris et al., 2017; Zimenkov et al., 2017; de Vos et al., 2019) and we considered them as sufficiently reliable genotypic proxy of the bedaquiline resistance.

In total, 43 *M. tuberculosis* isolates were recovered from 11 patients infected with mainly XDR strains. All isolates were assigned to the Beijing genotype. Nine and two patients were infected with the Russian successful clone B0/W148 and Central Asia Outbreak strain, respectively. We identified ten unique mutations in the bedaquiline re-

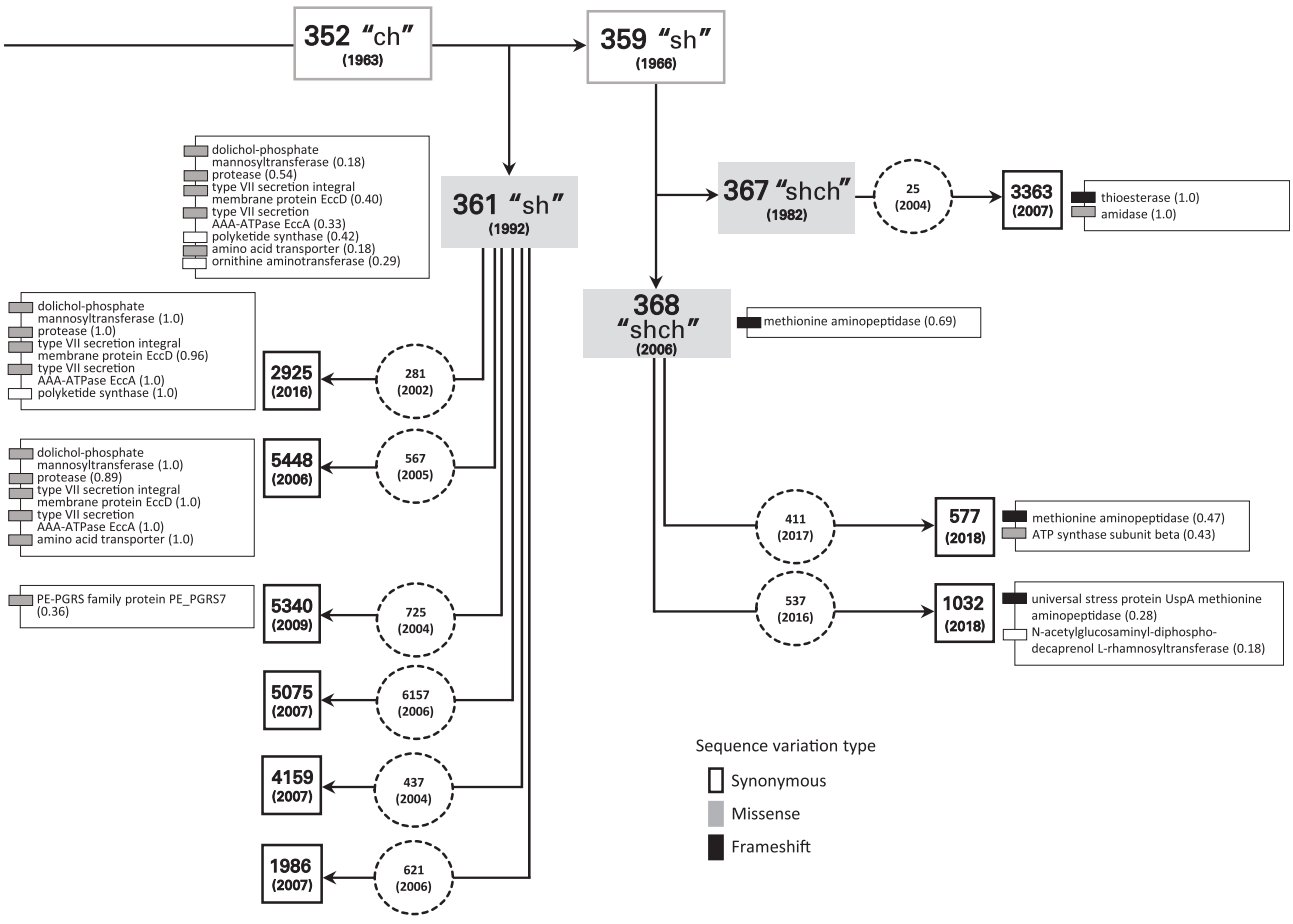


Figure 35. Schematic view of relationships between vaccine strain BCG-1 (Russia) parent seed lots, commercial vaccine lots, and their progeny clinical strains

sistance genes in 6 patients. In particular, five mutations were frameshift and three mutations had no or little effect on the protein structure. Mutations in the efflux involved gene *Rv0678* were found in isolates from 5 patients, and a mutation in *atpE* coding for the bedaquiline target ATP synthase, was detected in one case. Both heteroresistance and fluctuating prevalence of mutations observed in both genes including emergence of mutations several months after stop of treatment (Fig. 36).

Effects of the other mutations in *Rv0678* and *atpE* on protein structures were assessed using in silico structural analysis of electrostatic potential around mutated amino acids and accessible surface area (Fig. 37).

In terms of molecular epidemiology, the studied collection was totally dominated by the Beijing genotype, furthermore its Russian and Central Asian epidemic clones B0/W148 and CAO that both demonstrated a capacity to rapidly acquire bedaquiline resistance mutations. This can have serious negative impact on the MDR/XDR-TB control in this region as well as Russia and former Soviet Union countries, on the whole, where these MDR/XDR-associated strains are endemically dominant.

Importantly, the bedaquiline treatment was efficient only in the patients whose *M. tuberculosis* isolates did not harbor any bedaquiline resistance mutations. In contrast, treatment outcome was negative in all patients with *Rv0678* or *atpE* mutant isolates, regardless of were these mutations pre-existing or emerged, dominant or minor, with or without significant effect on the protein structure.

Molecular insight into *Mycobacterium tuberculosis* perchlozone resistance

Perchlozone® (4-thioureido-iminomethylpyridinium perchlorate [PCZ]) is a new thiosemicarbazone approved in Russia, along with bedaquiline and delamanid, for treatment of MDR/XDR tuberculosis (TB). The drug was also approved for use or is in the process of registration in some other countries. PCZ is similar to thiacetazone (TAC) and differs from it by the side chain attached to the thiosemicarbazone moiety. Thiacetazone (TAC) was formerly used in combination with isoniazid to treat patients infected with MDR *M. tuberculosis* strains but was removed from the antitubercular chemotherapy due to its secondary toxic effects. TAC is a prodrug that is activated by the flavin-containing monooxygenase EthA to exert its antimycobacterial activity, and mutations in *ethA* are associated with TAC resistance in *M. tuberculosis*. Upon activation, TAC binds to the HadA component of the HadABC dehydratase complex, leading to inhibition of mycolic acid biosynthesis. PCZ is a prodrug that is activated by EthA and inhibits the HadABC complex. EthA is known to activate second-line drugs ethionamide (ETH) and prothionamide (PTH) whereas *ethA* or *ethR* mutations were described as one of the ETH/PTH resistance mechanisms.

In this study, we aimed to gain insight into the molecular basis of PCZ resistance including dynamic changes in *M. tuberculosis* genome during long-term treatment. To this end, we applied next-generation, whole-genome sequencing to the isolates consecutively recovered from patients who received PCZ as part of their chemotherapy regimen.

This prospective study included patients admitted in 2018–2019 to the regional tuberculosis dispensary, Kaliningrad, Russia, whose treatment regimen included PCZ. Multiple *M. tuberculosis* isolates were recovered during PCZ treatment, and the bacterial DNA was subjected to WGS followed by bioinformatics analysis.

In total, 35 isolates were recovered from 9 patients who received PCZ (2–6 isolates from each, median 4 isolates per patient). First available isolates were resistant to 6 to 11 drugs, including ETH or PTH. One patient was MDR, one was pre-XDR and 7 were XDR.

We identified mutations in the genes putatively associated with PCZ resistance, *ethA* and *hadA*. The most frequent one was a frameshift *ethA* 106GA>G (7 of 9 patients) and most of the other mutations were also likely present before PCZ treatment. In one patient, a frameshift mutation *ethA* 702CT>C emerged after 6 months of PCZ treatment (Fig. 38).

In conclusion, the frequent presence of cross-resistance mutations to both PCZ and ETH/PTH presents an especially worrisome finding of this study. This situation raises a major concern with regard to the non-efficiency of PCZ in the treatment of a significant number of MDR-TB cases whose isolates may be additionally resistant to ETH/PTH. In view of the high and increasing burden of MDR-TB in Russia, ETH and PTH are frequently used to treat such patients. The ETH/PTH regimen can take at least 18–24 months as recommended by national and international guidelines and ETH/PTH resistance can emerge quite frequently in clinical isolates, especially in MDR isolates.

Treatment regimens of the XDR-TB patients include several drugs and the presence of PCZ resistance mutations is not necessarily associated with treatment failure. However, the inclusion of additional and non-effective drug in the treatment regimen is impractical and may be adverse for patient's health and well-being. To adequately assess the association of the identified mutations with PCZ resistance, an implementation of the phenotypic PCZ susceptibility testing is urgently needed. A large prospective study of the diverse *M. tuberculosis* collection is warranted to formulate the recommendations for optimal use of PCZ taking into consideration possible ETH/PTH resistance of the isolates.

Central Asia Outbreak Clade (project in collaboration with Federal Research and Clinical Centre of Physical-Chemical Medicine, Moscow)

Central Asia Outbreak (CAO) clade is a branch of the *Mycobacterium tuberculosis* Beijing genotype that is associated with multidrug-resistance, increased transmissibility and epidemic spread in parts of the former Soviet Union. Furthermore, migration flows bring these strains far beyond their areas of origin. We aimed to find a specific molecular marker of the Beijing CAO clade and develop a simple and affordable method of its detection. Based on the bioinformatics analysis of the large *M. tuberculosis* whole-genome sequencing (WGS) dataset (n = 1398), we identified *IS*6110 insertion in the *Rv1359-Rv1360* intergenic region as a specific molecular marker of the CAO clade. We further designed and optimized a multiplex PCR method to detect this insertion. The method was validated in silico with recently published WGS dataset from Central Asia (n = 277) and experimentally, with *M. tuberculosis* isolates from European and Asian parts of Russia, former Soviet Union and East Asia (n = 319). The developed molecular assay may be recommended for rapid screening of retrospective collections and for prospective surveillance, when comprehensive but expensive WGS is not available or practical. The assay may be espe-

cially useful in the high MDR-TB burden countries of the former Soviet Union and in the countries with respective immigrant communities.

Highly resistant *M. tuberculosis* strains of the early ancient sublineage of the Beijing genotype in Russia

The *Mycobacterium tuberculosis* Beijing genotype is a clinically and epidemiologically important lineage further subdivided into ancient/ancestral and modern strains. In our previous study in western Siberia, we identified VNTR-based clusters within the early ancient sublineage of the Beijing genotype characterized by an unexpectedly high rate of extensive drug resistance (XDR). Here, we analyzed next generation sequencing data in order to gain insight into genomic signatures underlying drug resistance of these strains. In total, 184 genomes of the Beijing early ancient sublineage from Russia, China, Japan, Korea, Vietnam, Thailand were used for phylogenetic analysis. The drug-resistant profile was deduced genotypically. The Russian isolates were distributed in two clusters and were all drug resistant, mainly pre-XDR and XDR. The largest of these clusters included only Russian isolates from remote locations in both Asian and European parts of the country (Fig. 39). All isolates had a quadruple drug resistance (to isoniazid, rifampin, ethambutol and streptomycin) due to the 6-mutation signature (KatG Ser315Thr, KatG Ile335Val, RpoB Ser450Leu, RpoC Asp485Asn, EmbB Gln497Arg, RpsL Lys43Arg). In most samples, it was complemented with additional and different *pncA*, *gyr*A, or *rrs* mutations leading to the pre-XDR/XDR genotype. Phylogenomic analysis suggests a distant origin of this Russian resistant cluster in the early 1970s but location and circumstances are yet to be clarified.

Circulation of these resistant isolates with identical mutational signature in very distant locations of Russia underlines an importance of their close monitoring in view of the potentially wider dissemination.

In *M. tuberculosis* research, an SNP barcode system for lineages, genotypes and subtypes is being developed but it is not clear how new codes can be proposed and agreed upon by the scientific community. Undoubtedly, it would be desirable to assign specific SNP-barcodes to the newly identified and epidemiologically significant clusters. However, the existing barcode system is of low-resolution for the Beijing genotype. Analysis of more strains from more locations will hopefully pave the way to the more comprehensive and more discriminatory classification.

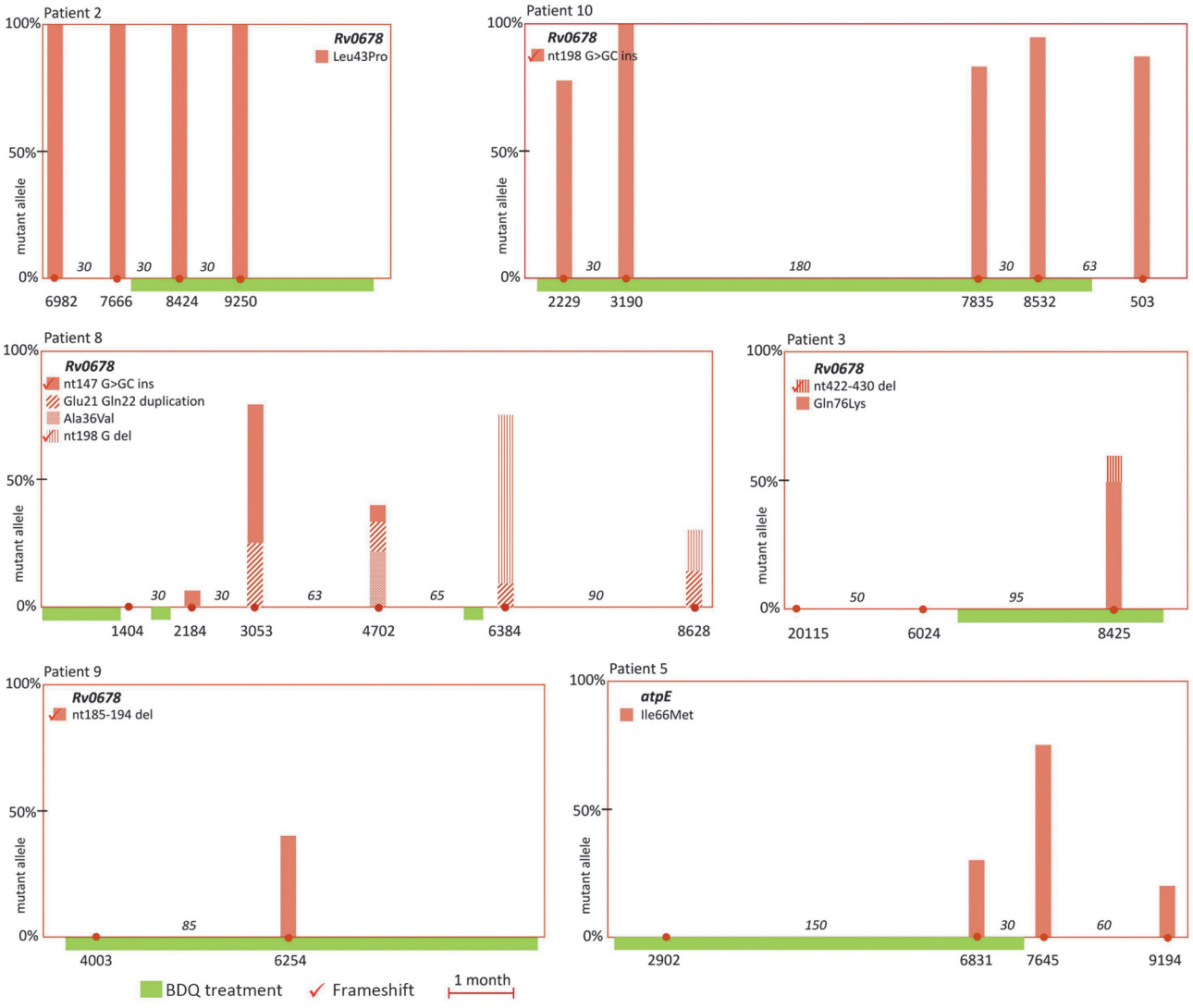


Figure 36. Timelines of treatment of the XDR TB patients with negative treatment outcome whose consecutive *M. tuberculosis* isolates harbored *Rv0678* or *atpE*

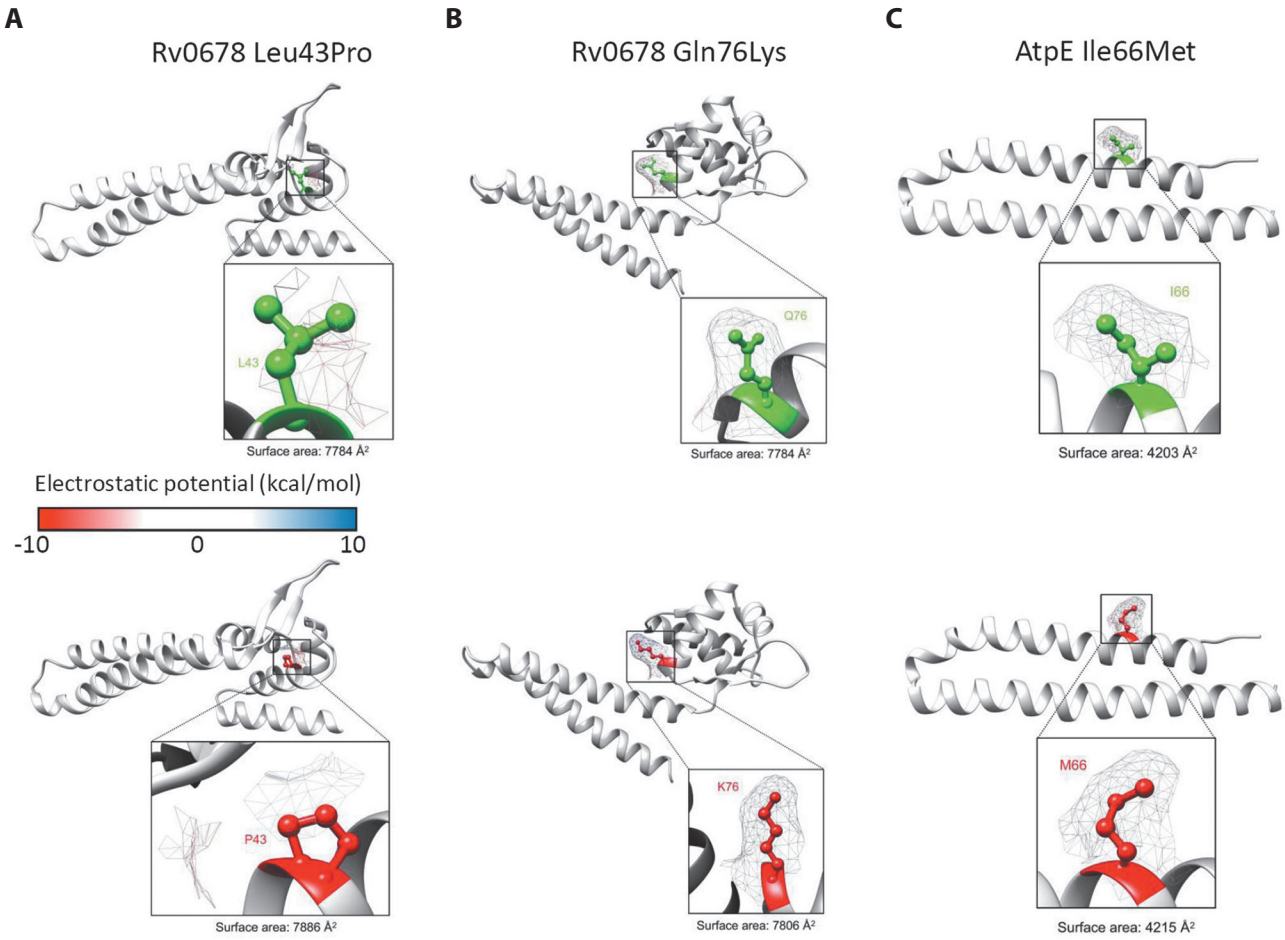


Figure 37. Structures of the MmpR5 (Rv0678) and AtpE (Rv1305) proteins in wild type and mutant forms for some of the identified substitutions in *M. tuberculosis* isolates. A. Rv0678 Leu43Pro; B. Rv0678 Glu76Lys; C. Rv1305 Ile66Met

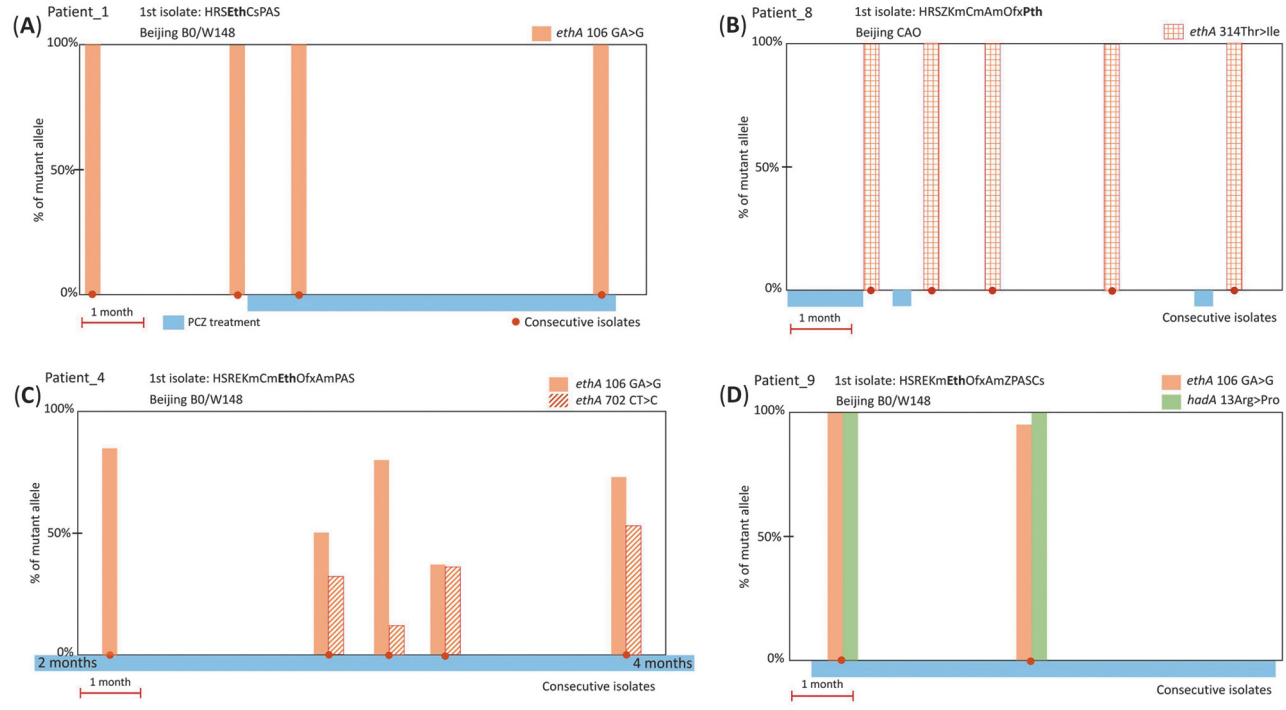


Figure 38. Representative examples of timelines of PCZ treatment, and *ethA* or *hadA* mutations in *M. tuberculosis* isolates

(A) Frameshift *ethA* 106 GA>G mutation present in PCZ pre-treatment isolates (5 patients); (B) Substitution ethA 314 ACC>ATC/Thr>Ile (2 patients); (C) Frameshift *ethA* 702 CT>C emerged during long-term PCZ treatment, in addition to the likely pre-existing *ethA* 106 GA>G (1 patient); (D) Substitution *hadA* 13 CGG>CCC/Arg>Pro and frameshift *ethA* 106 GA>G (1 patient).

In order to study the evolution and the hypothetical origin of these Russian resistant clusters on a larger timescale, a further study is warranted with geographically more diverse and more exhaustive collections including susceptible and pre-MDR isolates.

Molecular epidemiology of tuberculosis in the Komi Republic, northwestern Russia

The local situation with tuberculosis (TB) is shaped by the complex interplay of multiple factors related to both human host and *Mycobacterium tuberculosis*. We hypothesized that TB epidemiology in the rural regions in the Soviet Union was impacted by construction of the Gulag camps and significant incoming migration.

The Komi Republic is located in the northernmost part of the European Russia. The population is ~850 000, with a population density of 2.1 people per sq.km. The urban population is 78%, and the main ethnic groups are Russians (60%) and Komi (25%). The industrial development of Komi in the 20th century started with construction of the forced-labor concentration camps in 1929 which impacted the demography of the region in three ways. First, the population increased 4-fold during the last 100 years from 207 000 in 1926 to 830 000 in 2019. The so-called "camp cities" of Ukhta, Vorkuta, Pechora, Inta, and Sosnogorsk emerged from the Gulag camps in the 1930s–1956. Their population initially consisted of the released prisoners and workers employed in the forced-labor camps and grew due to incoming migration from other Russian regions. Second, ethnic population structure dramatically changed as the percentage of the Finno-Ugric Komi people decreased from 92% in 1926 to 24% in 2010, as a result of influx from other Russian regions, urbanization, and Gulag-driven industrial development. Third, the particular feature of the Soviet (and Russian) penitentiary system is that the prisoners are sent to prisons outside their regions of residence. In Komi, the former prisoners mostly stayed in the Komi region after their release and thus make a visible proportion of the current population of the region.

In this study, In this study, more than half (56.2%) of *M. tuberculosis* isolates were assigned to the Beijing genotype, which is a characteristic feature of other regions of northwestern Russia and Russia as a whole where the Beijing genotype was identified in 40–70% of the local populations in most regions (Fig. 40). MDR was detected in 30.8% isolates; eight were extensively drug resistant. The main Beijing subtypes B0/W148 and 94–32 differed in the MDR rate, 83.3% and 27.2%, respectively. The non-Beijing isolates represented five genotypes (LAM, Ural, Haarlem, X, T). The proportion of Beijing B0/W148 in the "camp" cities was twice as large as in other districts of the Komi Republic. The *M. tuberculosis* population in the Komi Republic in northern Russia is relatively heterogeneous and is represented by the global genetic families Beijing, T, LAM, Ural, and Haarlem. The Beijing genotype is predominant (56.2%), and its prevalence rate increased by 14.3% over the last decade. The Beijing strains mainly belong to the major Russian clusters of its modern sublineage — B0/W148 (32.9%) and 94–32 (61.1%). Circulation of the MDR isolates of the Beijing genotype and especially its B0/W148 cluster critically and adversely impacts the current situation with the MDR-TB in the Komi Republic. The increased prevalence of the MDR-associated Beijing B0/W148 in the urban setting on the whole, and in the "camp cities" (transformed from the Gulag camps), in particular, highlights an increased transmission capacity of this successful Russian variant of *M. tuberculosis*. A long-term continuous molecular monitoring of the *M. tuberculosis* population is required to confirm the found differences and to predict the epidemic trends of tuberculosis in this region in the northern Russia.

Figure 39. Enlarged view and additional information on clusters with Russian isolates

M. tuberculosis population structure in Albania (in collaboration with Dr. Silva Tafaj, National Reference Laboratory in Mycobacteria, Tirana, Albania)

Albania is a Balkan country with moderate to low incidence of tuberculosis (TB) and very low prevalence of drug resistant TB. Here, we analyzed a country-wide multi-year *Mycobacterium tuberculosis* collection in order to detect possible dynamic trends of TB in Albania, with a focus on drug resistance and endemic/epidemic clones.

In total, 743 isolates collected in 2007 to 2011 were divided into 107 spoligotypes and 351 MIRU-types. Based on the MIRU-VNTR phylogenetic analysis, the isolates were assigned to the following lineages/families: animal ecotypes (5 *M. bovis* and 2 *M. caprae* isolates), Lineage 2 (5 Beijing isolates), Lineage 3 (1 CAS-Delhi isolate) and, mostly and overwhelmingly, Lineage 4 (Cameroon, Uganda, Ghana and related; NEW-1-related; Ural, Haarlem, LAM, S, TUR; and unclassified isolates). Most of the isolates (452/743) were intermediately located on the global VNTR tree and did not cluster with any reference profile; they were distantly related to different families within Lineage 4 and we designated them as "unclassified L4" isolates. The significantly higher proportion of drug resistance was observed in (i) Beijing genotype compared to all other isolates (60%, P = 0.008), (ii) "unclassified L4" compared to all other isolates (13.9%, P = 0.04) and (iii) SIT2936 compared to other "unclassified L4" (34.3%, P = 0.0006). Analysis of the yearly collections revealed (i) some decrease of the large heterogeneous "unclassified L4" from 65% to 57%; (ii) steadily increasing gradient of LAM from 3.4 to 13.3%; (iii) stable prevalence of Haarlem (15–20%); and (iv) decrease of TUR with only 1.1% in 2011. Most of the LAM (33/49) and Beijing (3/5) isolates belonged to the VNTR types specific for Russia and former Soviet Union countries.

Our results highlight a peculiar nature of *M. tuberculosis* population in Albania that is dominated by local and unclassified genotypes within Lineage 4, and also features European genotypes and epidemically relevant clones originating from the former Soviet Union countries. At the same time, these imported clones remain drug susceptible and prevalence of drug resistance on a whole is low.

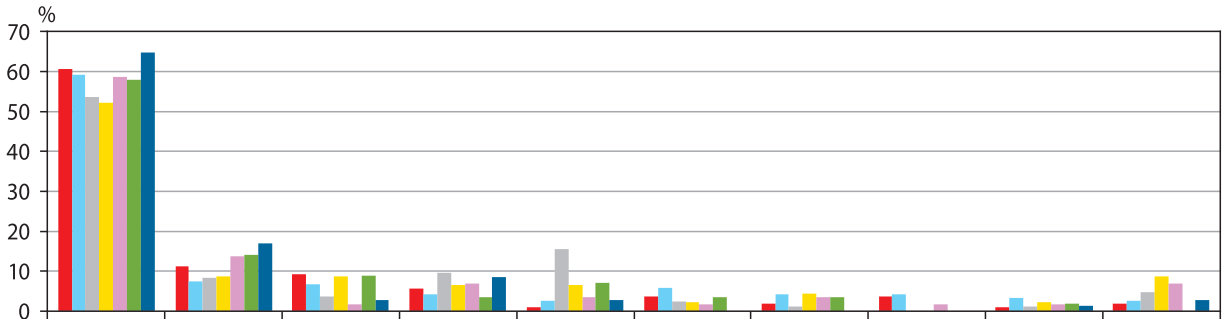
Species diversity of non-tuberculous mycobacteria in patients with mycobacteriosis in the North-Western Federal District of Russia

Aim of the study: to analyze the structure and trends in non-tuberculous mycobacteria (NTBM) causing diseases in the North-Western Federal District of the Russian Federation.

In total, 745 clinical NTM strains were identified. All clinical strains were isolated from patients with mycobacteriosis in 2012–2018. Analysis of the structure of the NTM population showed the predominance of the *M. avium* species (56.4%) (Fig. 41).

During the treatment of patients from St. Petersburg and the Leningrad region, 585 strains of NTM were isolated, and 340 (58.1%) belonged to the *M. avium*. Less frequently, other types of slow- and fast-growing NTM were detected: *M. intracellulare* (11.3%), *M. fortuitum* (6.7%), *M. chelonae* (5.8%) and *M. gordonae* (5.0%) and others. In the Kaliningrad region, over the same period, among 61 NTM strains 61% *M. avium*, 11% *M. fortuitum*, 10% *M. gordonae*, were found. In the Republic of Karelia, as well as in the Vologda, Pskov, and Novgorod regions, a smaller proportion of *M. avium* was isolated. In the Republic of Karelia *M. avium* (39%), *M. gordonae* (26%) were found. In the Pskov region 48% *M. avium*, 16% *M. fortuitum*, 12% *M. peregrinum* were isolated; in the Vologda Oblast — 40% *M. avium*, *M. fortuitum* and *M. abscessus* 20% each, *M. intracellulare* and *M. pere-*

Figure 40. Percent of the Beijing genotype in *M. tuberculosis* local populations across Federal Districts (https://en.wikipedia.org/wiki/Federal_districts_of_Russia) and regions of the Russian Federation



	M. avium	*M. intracellulare*	*M. fortuitum*	*M. chelonae*	*M. gordonae*	*M. abscessus*	*M. lentiflavum*	*M. peregrinum*	*M. kansasii*	Others*
2012	60.7	11.2	9.3	5.6	0.9	3.7	1.9	3.7	0.9	1.9
2013	59.2	7.5	6.7	4.2	2.5	5.8	4.2	4.2	3.3	2.5
2014	53.6	8.3	3.6	9.5	15.5	2.4	1.2	0	1.2	4.8
2015	52.2	8.7	8.7	6.5	6.5	2.2	4.3	0	2.2	8.7
2015	58.6	13.8	1.7	6.9	3.4	1.7	3.4	1.7	1.7	6.9
2017	57.9	14	8.8	3.5	7	3.5	3.5	0	1.8	0
2018	64.8	16.9	2.8	8.5	2.8	0	0	0	1.4	2.8

Figure 41. Frequency of the occurrence of NTM species in patients with mycobacteriosis in St. Petersburg and the Leningrad Region (2012–2018)

*Others: slow-growing mycobacteria — *M. malmoense, M. xenopi, M. celatum, M. marinum, M. scrofulaceum, M. szulgai*; fast growing mycobacteria — *M. smegmatis*.

grinum 10% each; in the Novgorod region — 50% *M. avium*, 25% each *M. kansasii* and *M. intracellulare*. In contrast to these regions, where *M. avium* is the dominant species, other types of NTM have predominated in the Komi Republic and the Arkhangelsk Region: *M. lentiflavum* (44%) and *M. gordonae* (34%) dominated in the Komi Republic and Arkhangelsk respectively.

Over the past 12 years, there has been an increase in the detection of NTM in immunocompetent and HIV-infected individuals in St. Petersburg. For the period 2006–2011 only 22 *M. avium* strains and 20 strains of other NTM species (*M. intracellulare*, *M. fortuitum*, *M. kansasii*, *M. abscessus*, and *M. peregrinum*) were isolated, while for the period 2012–2018 were identified already 306 clinical isolates of *M. avium* and 224 isolates of the large group of NTM (*M. intracellulare*, *M. fortuitum*, *M. chelonae*, *M. gordonae*, *M. abscessus*, *M. lentiflavum*, *M. peregrinum*, *M. kansasii*, *M. malmoense*, *M. xenopi*, *M. celatum*, *M. smegmatis*, *M. marinum*, *M. scrofulaceum*, *M. szulgai*). This can be explained by the fact that doctors began to pay more attention to the etiological diagnosis of mycobacterial infections, especially with the increase of the prevalence of immunosuppressive conditions (drug therapy, pulmonary diseases, HIV infection). Secondly, the introduction of molecular methods for the mycobacteria identification into clinical laboratory practice played an important role, which greatly simplified the NTM identification scheme. The steady increase in the detection of NTM demands the formation of national clinical guidelines for the diagnosis of mycobacteriosis of various localization and registration of each case of NTM identification.

Unlike other regions of Russia, in the Northwestern region, there was a consistently low level of detection of *M. kansasii* and *M. xenopi*. For the last 12 years in St. Petersburg and Leningrad region, the variety NTM in patients with different immune status has been growing but the portion of *M. avium* remains to be stably high exceeding 50%.

Thus, our data indicate a change in the distribution of NTM species throughout the entire period of the study, possibly as a reflection of migration processes or a different level of examination of risk groups.

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

IN ENGLISH: 24 articles: 13 in Q1

1. *Acosta F., Norman A., Sambrano D., Batista V., Mokrousov I., Shitikov E., Jurado J., Mayrena M., Luque O., Garay M., Solís L., Muñoz P., Folkvardsen D.B., Lillebaek T., Pérez-Lago L., Goodridge A., García de Viedma D. Probable long-term prevalence for a predominant Mycobacterium tuberculosis clone of a Beijing genotype in Colon, Panama // Transbound. Emerg. Dis. 2020; 68 (4): 2229–2238. doi: 10.1111/tbed.13875*
2. *Arikawa K., Ichijo T., Nakajima S., Nishiuchi Y., Yano H., Tamaru A., Yoshida S., Maruyama F., Ota A., Nasu M., Starkova D.A., Mokrousov I., Narvskaya O.V., Iwamoto T. Genetic relatedness of Mycobacterium avium subsp. hominissuis isolates from bathrooms of healthy volunteers, rivers, and soils in Japan with human clinical isolates from different geographical areas // Infect. Genet. Evol., 2019; 74: 103923. doi: 10.1016/j.meegid.2019.103923*
3. *Bespyatykh J., Shitikov E., Guliaev A., Smolyakov A., Klimina K., Veselovsky V., Malakhova M., Arapidi G., Dogonadze M., Manicheva O., Bespiatykh D., Mokrousov I., Zhuravlev V., Ilina E., Govorun V. System OMICs analysis of Mycobacterium tuberculosis Beijing B0/W148 cluster // Scientific Reports. 2019; 9: 19255. doi: 10.1038/s41598-019-55896-z*
4. *Chernyaeva E., Rotkevich M., Krasheninnikova K., Lapidus A., Polev D.E., Solovieva N., Zhuravlev V., Yablonsky P., O'Brien S.J. Genomic variations in drug resistant Mycobacterium tuberculosis strains collected from patients with different localization of infection // Antibiotics (Basel). 2020; 10 (1): 27. doi: 10.3390/antibiotics10010027*
5. *Jagielski T., Mokrousov I. Special Issue on Molecular aspects of mycobacterial infections // Infect. Genet. Evol. 2019; 72: 1–3. doi: 10.1016/j.meegid.2019.03.014*
6. *Mokrousov I., Akhmedova G., Molchanov V., Fundovnaya E., Kozlova E., Ostankova Y., Semenov A., Maslennikova N., Leontev D., Zhuravlev V., Turkin E., Vyazovaya A. Frequent acquisition of bedaquiline resistance by epidemic extensively drug-resistant Mycobacterium tuberculosis strains in Russia during long-term treatment // Clin. Microbiol. Infect. 2020; 27 (3): 478–480. doi: 10.1016/j.cmi.2020.08.030*

7. *Mokrousov I., Akhmedova G., Polev D., Molchanov V., Vyazovaya A. Acquisition of bedaquiline resistance by extensively drug-resistant Mycobacterium tuberculosis strain of Central Asian Outbreak clade // Clin. Microbiol. Infect. 2019; 25 (10): 1295–1297. doi: 10.1016/j.cmi.2019.06.014*
8. *Mokrousov I., Sinkov V., Vyazovaya A., Pasechnik O., Solovieva N., Khromova P., Zhuravlev V., Ogarkov O. Genomic signatures of drug resistance in highly resistant Mycobacterium tuberculosis strains of the early ancient sublineage of Beijing genotype in Russia // Int. J. Antimicrob. Agents. 2020; 56 (2): 106036. doi: 10.1016/j.ijantimicag.2020.106036*
9. *Mokrousov I., Vyazovaya A., Levina K., Gerasimova A., Zhuravlev V., Viiklepp P., Kütt M. Spatiotemporal dynamics of drug-resistant Mycobacterium tuberculosis: Contrasting trends and implications for tuberculosis control in EU high-priority country // Transbound Emerg. Dis. 2020; 68 (2): 896–906. doi: 10.1111/tbed.13758*
10. *Mokrousov I., Vyazovaya A., Pasechnik O., Gerasimova A., Dymova M., Chernyaeva E., Tatarintseva M., Stasenko V. Early ancient sublineages of Mycobacterium tuberculosis Beijing genotype: unexpected clues from phylogenomics of the pathogen and human history // Clin. Microbiol. Infect. 2019; 25 (8): 1039.e1–1039.e6. doi: 10.1016/j.cmi.2018.11.024*
11. *Mokrousov I. Current topics of molecular mycobacteriology // Infect. Genet. Evol. 2019; 73: 132–138. doi: 10.1016/j.meegid.2019.04.027*
12. *Mokrousov I. Ubiquitous and multifaceted: SIT53 spoligotype does not correlate with any particular family of Mycobacterium tuberculosis // Tuberculosis (Edinb). 2021; 126: 102024. doi: 10.1016/j.tube.2020.102024*
13. *Mokrousov I., Vyazovaya A., Akhmedova G., Solovieva N., Turkin E., Zhuravlev V. Genetic variation putatively associated with Mycobacterium tuberculosis resistance to perchlozone, a new thiosemicarbazone: clues from whole genome sequencing and implications for treatment of multidrug-resistant tuberculosis // Antibiotics. 2020; 9 (10): 669. doi: 10.3390/antibiotics9100669*
14. *Narvskaya O., Starkova D., Levi D., Alexandrova N., Molchanov V., Chernyaeva E., Vyazovaya A., Mushkin A., Zhuravlev V., Solovieva N., Vishnevskiy B., Mokrousov I. First insight into the whole-genome sequence variations in Mycobacterium bovis BCG-1 (Russia) vaccine seed lots and their progeny clinical isolates from children with BCG-induced adverse events // BMC Genomics. 2020; 21 (1): 567. doi: 10.1186/s12864-020-06973-5*
15. *Perdigão J., Silva C., Diniz J., Pereira C., Machado D., Ramos J., Silva H., Abilleira F., Brum C., Reis A.J., Macedo M., Scaini J.L., Silva A.B., Esteves L., Macedo R., Maltez F., Clemente S., Coelho E., Viegas S., Rabna P., Rodrigues A., Taveira N., Jordao L., Kritski A., Lapa E., Silva J.R., Mokrousov I., Couvin D., Rastogi N., Couto I., Pain A., McNerney R., Clark T.G., von Groll A., Dalla-Costa E.R., Rossetti M.L., Silva P.E.A., Viveiros M., Portugal I. Clonal expansion across the seas as seen through CPLP-TB database: a joint effort in cataloguing Mycobacterium tuberculosis genetic diversity in Portuguese-speaking countries // Infect. Genet. Evol. 2019; 72: 44–58.*
16. *Perdigão J., Silva C., Maltez F., Machado D., Miranda A., Couto I., Rabna P., Florez de Sessions P., Phelan J., Pain A., McNerney R., Hibberd M.L., Mokrousov I., Clark T.G., Viveiros M., Portugal I. Emergence of multidrug-resistant Mycobacterium tuberculosis of the Beijing lineage in Portugal and Guinea-Bissau: a snapshot of moving clones by whole-genome sequencing // Emerg. Microbes Infect. 2020; 9 (1): 1342–1353. doi: 10.1080/22221751.2020.1774425*
17. *Shitikov E., Guliaev A., Bespyatykh J., Malakhova M., Kolchenko S., Smirnov G., Merker M., Niemann S., Mokrousov I., Ilina E., Govorun V. The role of IS6110 in micro- and macroevolution of Mycobacterium tuberculosis lineage 2 // Mol. Phylogenet. Evol. 2019; 139: 106559. doi: 10.1016/j.ympev.2019.106559*
18. *Shitikov E., Vyazovaya A., Malakhova M., Guliaev A., Bespyatykh J., Proshina E., Pasechnik O., Mokrousov I. Simple assay for detection of the Central Asia outbreak clade of the Mycobacterium tuberculosis Beijing genotype // J. Clin. Microbiol. 2019; 57 (7). pii: e00215-19. doi: 10.1128/JCM.00215-19*
19. *Skiba Y., Mokrousov I., Nabirova D., Vyazovaya A., Maltseva E., Malakhova N., Ismagulova G., Pole I., Ranka R., Sapiyeva Z., Ismailov S., Moffett D. Mycobacterium tuberculosis RD-Rio Strain in Kazakhstan // Emerg. Infect. Dis. 2019; 25 (3): 604–606. doi: 10.3201/eid2503.181179*
20. *Sun L., Zhang L., Wang T., Jiao W., Li Q., Yin Q., Li J., Qi H., Xu F., Shen C., Xiao J., Liu S., Mokrousov I., Huang H., Shen A. Mutations of Mycobacterium tuberculosis induced by anti-tuberculosis treatment result in metabolism changes and elevation of ethambutol resistance // Infect. Genet. Evol. 2019; 72: 151–158. doi: 10.1016/j.meegid.2018.09.027*
21. *Tafaj S., Mokrousov I., Borroni E., Trovato A., Kapisyzi P., Bardhi D., Hafizi H., Bala S., Bulo A., Bino S., Rastogi N., Cirillo D. Peculiar features of the Mycobacterium tuberculosis population structure in Albania // Infect. Genet. Evol. 2020; 78: 104136. doi: 10.1016/j.meegid.2019.104136*
22. *Umpeleva T., Belousova K., Golubeva L., Boteva T., Morozova I., Vyazovaya A., Mokrousov I., Eremeeva N., Vakhrusheva D. Molecular characteristics of Mycobacterium tuberculosis in the "closed" Russian town with limited population migration // Infect. Genet. Evol. 2020; 79: 104174. doi: 10.1016/j.meegid.2020.104174*
23. *Valcheva V., Savova-Lalkovska T., Vyazovaya A., Dimitrova A., Bonovska M., Najdenski H. First insight into phylogeography of Mycobacterium bovis and M. caprae from cattle in Bulgaria // Infect. Genet. Evol. 2020; 81: 104240. doi: 10.1016/j.meegid.2020.104240*
24. *Vyazovaya A., Proshina E., Gerasimova A., Avadenii I., Solovieva N., Zhuravlev V., Narvskaya O., Mokrousov I. Increased transmissibility of Russian successful strain Beijing B0/W148 of Mycobacterium tuberculosis: Indirect clues from history and demographics // Tuberculosis (Edinb). 2020; 122: 101937. doi: 10.1016/j.tube.2020.101937*

IN RUSSIAN: 8 articles

1. *Vyazovaya A.A., Pasechnik O.A., Gerasimova A.A., Mokrousov I.V. The population structure of Beijing family of Mycobacterium tuberculosis in Western Siberia // Tuberculosis and Lung Diseases. 2020; 98 (5): 32–36. doi: 10.21292/2075-1230-2020-98-5-32-36*
2. *Gerasimova A., Panteleev A., Mokrousov I. HIV-associated tuberculosis with central nervous system involvement (literature review) // Medical Alliance. 2020; 8 (4): 25–31. doi: 10.36422/23076348-2020-8-4-25-31*
3. *Mokrousov I., Chernyaeva E., Vyazovaya A., Zhuravlev V. The use of whole-genome analysis for identifying molecular markers of significant genetic clusters of Mycobacterium tuberculosis in Russia // Pathogenesis. 2019; 17 (4): 43–49. doi: 10.25557/2310-0435.2019.04.43-49*
4. *Pasechnik O.A., Vyazovaya A.A., Dymova M.A., Blokh A.I., Stasenko V.L., Tatarintseva M.P., Mokrousov I.V. Tuberculosis outcomes related to the Mycobacterium tuberculosis genotype // Russian Journal of Infection and Immunity. 2019; 9 (3–4): 531–538. doi: 10.15789/2220-7619-2019-3-4-531-538*
5. *Pasechnik O.A., Vyazovaya A.A., Bloch A.I., Yarusova I.V., Tatarintseva M.P., Mokrousov I.V. Assessment of the Prevalence and epidemic spread of strains of ancient, and modern sublineages of the Mycobacterium tuberculosis Beijing genotype in Omsk Region // Epidemiology and Vaccinal Prevention. 2020; 19 (4): 20–29. doi: 10.31631/2073-3046-2020-19-4-20-29*

6. *Starkova D.A., Vyazovaya A.A., Narvskaya O.V., Iwamoto T., Molchanov V.M., Zhuravlev V.Y., Vishnevsky B.I. Single nucleotide polymorphism in HSP65 and MACPPE12 genes of Mycobacterium avium subsp. Hominissuis // Russian Journal of Genetics. 2019; 55 (5): 544–550. doi: 10.1134/S0016675819050126*
7. *Starkova D.A., Zhuravlev V.Yu., Vyazovaya A.A., Solovieva N.S., Kulikova O.N., Narvskaya O.V. Species diversity of non-tuberculous mycobacteria in patients with mycobacteriosis in the North-Western Federal District of Russia // Tuberculosis and Lung Diseases. 2019; 97 (6): 16–22. doi: 10.21292/2075-1230-2019-97-6-16-22*
8. *Starkova D.A., Narvskaya O.V. Genetic determinants of virulence and drug resistance of Mycobacterium avium subsp. hominissuis — a causative agent of mycobacteriosis in humans // Russian Journal of Infection and Immunity. 2020; 10 (1): 26–34. doi: 10.15789/2220-7619-GDO-1220*

Articles on other topics

1. *Lyytinen O.L., Starkova D., Poranen M.P. Microbial production of lipid-protein vesicles using enveloped bacteriophage phi6 // Microb. Cell Fact. 2019; 18: 29. doi: 10.1186/s12934-019-1079-z*
2. *Nikolsky M.A., Vyazovaya A.A., Lioznov D.A., Narvskaya O.V., Zolotova M.A., Knyazeva E.S. Clinical and laboratory features of human herpes virus type 7 infection in children // Immunopathology, Allergology, Infectology. 2019; 4: 68–73. (In Russ.) doi: 10.14427/jipai.2019.4.68*
3. *Nikolsky M.A., Vyazovaya A.A., Vedernikov V.E., Narvskaya O.V., Lioznov D.A., Smirnova N.N., Polunina A.V., Burmistrova A.G., Zolotova M.A.. Molecular and biological characteristics of human herpes virus type 6 in patients with different variants of the disease course // Pediatrics. 2019; 98 (1): 53–56. (In Russ.) doi: 10.24110/0031-403X-2019-98-1-53-56*
4. *Sharma N.C., Efstratiou A., Mokrousov I., Mutreja A., Das B., Ramamurthy T. Diphtheria // Nature Reviews Disease Primers. 2019; 5: 81. doi: 10.1038/s41572-019-0131-y*

Patents and databases

1. *Patent RU2684314: Mokrousov I., Vyazovaya A., Zhuravlev V., Solovieva N., Vishnevsky B., Narvskaya O. Method of detection of Mycobacterium tuberculosis Beijing genotype B0-cluster by real-time PCR. Applicant and copyright holder: St. Petersburg State Research Institute of Phthisiopulmonology, St. Petersburg Pasteur Institute. Registered in State Register of Inventions of Russian Federation: 05.04.2019. Priority: 30.06.2017.*
2. *Patent RU2689800: Mokrousov I., Vyazovaya A., Chernyaeva E., Solovieva N., Narvskaya O., Zhuravlev V., Method of detection of Mycobacterium tuberculosis Beijing genotype 94-32-cluster by real-time PCR. Applicant and copyright holder: St. Petersburg State Research Institute of Phthisiopulmonology, St. Petersburg Pasteur Institute. Registered in State Register of Inventions of Russian Federation: 29.05.2019. Priority: 11.12.2017.*
3. *Patent RU2689801: Mokrousov I., Vyazovaya A., Solovieva N., Mushkin A.Y., Vishnevsky B.I., Narvskaya O., Zhuravlev V., Method of detection of Mycobacterium bovis BCG strains by real-time PCR. Applicant and copyright holder: St. Petersburg State Research Institute of Phthisiopulmonology, St. Petersburg Pasteur Institute. Registered in State Register of Inventions of Russian Federation: 29.05.2019. Priority: 06.06.2018.*
4. *Patent RU2735415: Mokrousov I.V., Shitikov E.A., Vyazovaya A.A., Skiba Yu.A., Malakhova M.V., Bespyatykh Yu.A., Solovieva N.S., Zhuravlev V.Yu. Method for detecting Mycobacterium tuberculosis of the Central Asian epidemic cluster of the Beijing genotype. Applicant and copyright holder: St. Petersburg State Research Institute of Phthisiopulmonology, St. Petersburg Pasteur Institute, Federal Research And Clinical Center of Physical-Chemical Medicine Federal. Registered in State Register of Inventions of Russian Federation: 02.11.2020. Priority: 15.11.2019.*
5. *Patent RU2743365: Mokrousov I., Vyazovaya A., Gerasimova A., Solovieva N., Zhuravlev V. Method for detection of phylogenetic sublineages of the Mycobacterium tuberculosis Beijing genotype by real-time PCR. Applicant and copyright holder: St. Petersburg State Research Institute of Phthisiopulmonology, St. Petersburg Pasteur Institute. Registered in State Register of Inventions of Russian Federation: 17.02.2021. Priority: 12.05.2020.*
6. *Certificate of registration for database RU2019620542: Mokrousov I.V., Vyazovaya A.A., Narvskaya O.V. Spoligoprofiles of Mycobacterium tuberculosis strains circulating in Vietnam. The date of state registration in the register of databases 04.08.2019.*
7. *Certificate of registration for database RU2019622064: Mokrousov I.V., Vyazovaya A.A., Narvskaya O.V., Gerasimova A.A., Proshina E.E., Solovieva N.S., Zhuravlev V.Yu. Spoligoprofiles of Mycobacterium tuberculosis strains circulating in the Komi Republic. The date of state registration in the register of databases 11.13.2019.*

VIRAL HEPATITES

In 2019–2020, cooperation was continued with the Joint Russian-Vietnamese Tropical Research and Technological Center in Hanoi (Vietnam) to study the prevalence and genetic structure of the hepatitis virus population among the inhabitants of various regions of Vietnam. The project studied the prevalence of viral hepatitis B, C and E among the indigenous population of the Northern Provinces of Vietnam, as well as among the young population of Vietnam.

A molecular genetic study was conducted to discover the structure of the hepatitis B virus population circulating among the youth of Thai Nguyen province and Da Nang city. The samples positive for HBV DNA (n = 21) underwent whole genome sequencing. The recombinant HBV genotype and subgenotype were determined. Only two HBV subgenotypes were identified: the predominant genotype was subgenotype B4 (81%; 17/21), followed by subgenotype C1 (19%, 4/21). Phylogenetic analysis revealed that Vietnamese HBV strains belonging to the B4 subgenotype exhibited high heterogeneity, forming a distinct branch divided into different groups, and were separate from other strains isolated in Asia. Similarity plots showed that 16 of the 17 HBV isolates identified as B4 subgenotype were variants recombinant in the preC/C region of the genome, formed by widely circulating variants of the B and C genotypes. Based on the analysis of the "a" determinant in the S region of HBV strains were classified into three subtypes: ayw1, adr, and adrq–. Amino acid substitutions in the preS/S region of the strains studied, which might be associated with drug-resistant and vaccine escape mutations, were not found.

Conclusion. The high genetic diversity of HBV strains circulating in the younger population of Vietnam shows that the sources of infection are multiple and that there is a range

of virus variants that spread successfully, and it provides insights into the patterns that may be supporting the epidemic process in Vietnam. The dominance of the B4 subgenotype in the spatiotemporal data indicates its epidemic significance for Vietnam.

* * *

The problem of enteric viral hepatitis is still urgent all over the world. Vietnam belongs to the territories of Southeast Asia where hepatitis E virus (HEV) is endemic. However, the data on the prevalence of HEV infection among the indigenous population of Vietnam are limited, and there are no data available for various minor ethnic groups. As the country spans a long distance north to south, the provinces of Vietnam differ in climatic and socio-economic conditions. According to the 2019 census, there are 54 ethnic groups living in Vietnam; the country's total population is about 96 million, of which about 63.0% is rural. The population of the Northern province of Hà Giang is ethnically diverse, with about 22 ethnicities that have preserved their identity, which determines their specific lifestyle and economic activity. We conducted a cross-sectional study to assess the prevalence of serological markers of HEV infection among the indigenous inhabitants of Ha Giang, a northern province of Vietnam. The study included 1127 conventionally healthy indigenous residents aged 18 to 83 years (average age 42.8±1.5) who lived in three districts (Yên Minh, BắC Mê and Đông Văn) of Hà Giang province in 2019, each of which was characterized by a unique ethnic composition of the local population. All blood plasma samples obtained were marked with code names according to the Declaration of Helsinki. The presence of HEV-specific IgG class antibodies (anti-HEV IgG) was determined by ELISA method with commercial DS-ELISA-ANTI-HEV-G kits in accordance with the instructions of the manufacturer ("NPO Diagnostic Systems", Nizhny Novgorod, Russia).

The incidence of anti-HEV IgG among the conventionally healthy indigenous population in the Northern Province of Ha Giang was 74.4% (838/1127; 95% CI 71.7–76.8). The highest incidence of anti-HEV IgG (87.6%) among the residents who participated in the study was found in the Đông Văn district, which is significantly higher compared to that in the districts of BắC Mê ($\chi^2 = 16.37$, $p = 0.000052$) and Yên Minh ($\chi^2 = 214.64$, p Yên Minh District was characterized by the lowest percentage of people involved in the epidemic process ($\chi^2 = 77.55$, $p < 0.00001$). No significant differences were found in the detection of anti-HEV depending on gender, either in general or in individual districts. Taking into account that all the three districts were mostly populated by two ethnicities, H'mong and/or Tay, representatives of the remaining, minor ethnicities, were assigned to one general comparison group (hereinafter referred to as "the others"). The highest incidence of anti-HEV IgG (85.9%) was found in the H'mong ethnic group, which was significantly higher than in the Tay ethnic groups ($\chi^2 = 77.32$, $p < 0.00001$) and "others" ($\chi^2 = 63.44$, $p < 0.00001$).

Conclusion. The findings of this study indicate a highly active HEV epidemic process among the indigenous inhabitants of Ha Giang, a province of Northern Vietnam, belonging to minor ethnic groups that still maintain their traditional lifestyle. The reasons of the successful spread of HEV in the region, with the involvement of all age groups of the population in the epidemic process, include the location of Ha Giang province in remote mountainous areas, some of which are still difficult to access, its low economic status, poor sanitary and hygienic living conditions, lack of high-quality water supply, multi-ethnic population with ethnic groups having different lifestyles, and constant contact of the population with natural potential sources of HEV infection, including various wild and domestic animals. Ha Giang Province is experiencing the rapid development of the tourism industry, in particular in Đông Văn District, where the Đông Văn Karst Plateau, a UNESCO Global Geopark, is located. In this connection, the results obtained emphasize the need to plan and implement measures aimed at preventing and monitoring HEV infection in endemic regions of the country in order to reduce the risk of the spread of HEV both in the country and across its borders.

Publications

1. *Kalinina O.V., Lichnaya E.V., Pham T.H.G., Bui T.L.A., Vo V.C., Bui T.T.N., Pham N.Q., Starkova D.A., Karandashova I.V., Chulanov V.P., Dmitriev A.V. The prevalence of parenteral viral hepatitis in Vietnam // Current trends and prospects of Russian-Vietnamese cooperation in the field of ensuring sanitary and epidemiological well-being: a collective monograph / Ed. by A.Yu. Popova. St. Petersburg, 2019: 272–284.*
2. *Kalinina O.V., Lichnaya E.V., Pham T.H.G., Vo V.C., Bui T.T.N., Dmitriev A.V., Chulanov V.P. Evaluation of the effectiveness of vaccination against hepatitis B in young adult population in two provinces of Vietnam // Current trends and prospects of Russian-Vietnamese cooperation in the field of ensuring sanitary and epidemiological well-being: a collective monograph / Ed. by A.Yu. Popova. St. Petersburg, 2019: 308–313.*
3. *Lichnaya E.V., Pham T.H.G., Bui T.L.A., Bui T.T.N., Vo V.C., Pham N.Q., Karandashova I.V., Chulanov V.P., Dmitriev A.V., Kalinina O.V. The genetic structure of the HBV population in Vietnam // Current trends and prospects of Russian-Vietnamese cooperation in the field of ensuring sanitary and epidemiological well-being: a collective monograph / Ed. by A.Yu. Popova. St. Petersburg, 2019: 314–325.*

Northwestern District Centre for AIDS Prevention and Control

LABORATORY OF HIV IMMUNOLOGY AND VIROLOGY

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Continued study of HIV epidemic features in the Northwestern Federal District (NWFD) of the Russian Federation in 2019–2020

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

The NWFD includes 11 entities of the Russian Federation with the total population of 13.9 million (9.5% of the Russian population).

In 2020, the population of the NWFD as compared to 2019 decreased by 0.4% (or 61 734 persons). In St. Petersburg and Kaliningrad Oblast, the number of inhabitants started to grow due to increased birth rate and population migration (+0.3% and +1.4%, respectively). In the remaining territories of the NWFD, the population declined. Significant natural population decline in the District is to a large extent due to the age structure of the population with a high proportion of elderly persons. There are already 1.5 times more senior people in the NWFD than there are children under 16. The Pskov Oblast and Novgorod Oblast have particularly old population due to the outflow of young people from these regions that has lasted for the past decades. In the NWFD, only St. Petersburg, Leningrad Oblast and Kaliningrad Oblast have a stable migration inflow. These regions always have a positive migration balance, both with regard to other regions of the district, and to most of the other entities of the Russian Federation and the New Independent States. The relative migration inflow to the Kaliningrad Oblast is particularly intense, and it is often higher than the natural population decline. Therefore, the population of this region has increased as compared to the early 1990s, while in all other regions of the NWFD it has decreased.

The overall screening coverage of the Russian Federation (RF) citizens for HIV antibodies in 2020 decreased as compared to 2019 by 18.7%, whereas in 2019 it showed an increase of +5.8% (Fig. 42). The number of persons who underwent HIV tests in the NWFD amounted to: RF citizens — 2 940 675 persons, foreign citizens — 265 284 persons. The number of tested foreign citizens in 2020 (265 284) decreased as compared to 2019 (400 727 persons) by 33.8%. Of the overall number of tested persons, in 2020 foreign citizens accounted for 8.3%, in 2019 — for 11.6%, whereas in 2005 it was 2.1%. The decrease in the overall screening coverage of Russian and foreign citizens is associated with the reduced amount of HIV examinations and a decrease in the uptake of services in the Centres for AIDS Prevention and Control in the midst of anti-epidemic measures to prevent the spread of the COVID-19 coronavirus infection.

In vulnerable groups, or the so-called risk groups (drug users, MSMs, persons with STDs and persons deprived of liberty), the amount of tests in the NWFD as a whole in 2019 increased by 2.1% as compared to 2018 (Fig. 43). However, if we compare this figure with that of 2007, we will see a decrease of 40%.

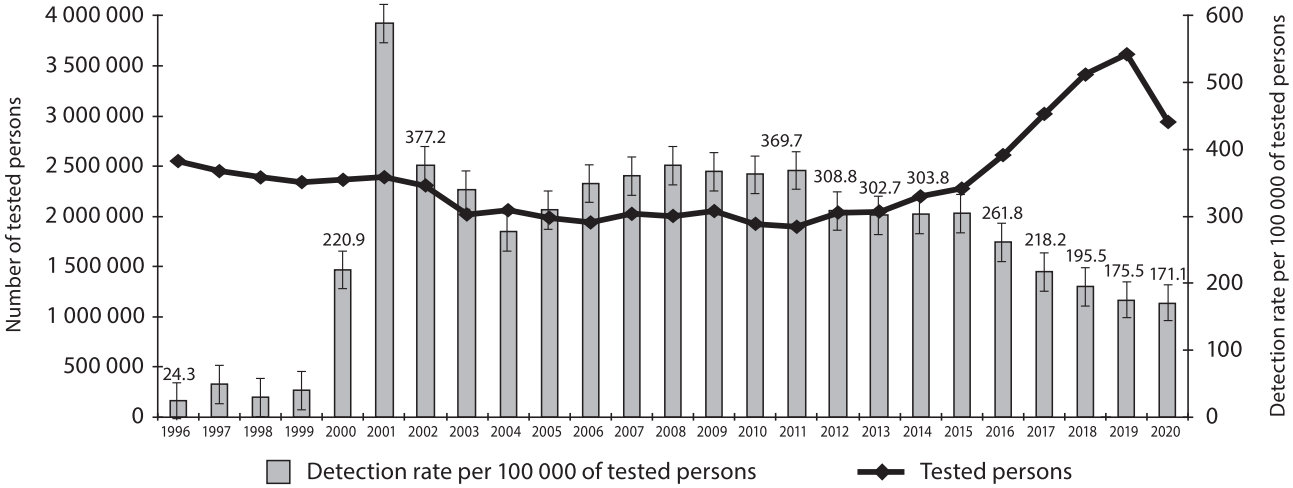


Figure 42. Number of tests for HIV antibodies and detection rate per 100 000 of tested persons in the NWFD, 1996–2020

The average detection rate for the district (code 100) in 2020 was 171.1 per 100 000 of tested persons (in 2019 it was 175.3, in 2018 — 195.5, in 2017 — 218.2, whereas in 2016 — 261.8), a decrease of 2.4%.

Analysis of screening effectiveness for individual groups (according to the codes of Form No. 4) in the NWFD as a whole showed that in 2019, the detection rate in the group of drug users (code 102) was 1.9% (in 2008 — 5.2%). In the group of persons examined during the epidemiological survey (code 120), the detection rate was 2.8% (in 2008 — 7.4%). The detection rate in the group of persons deprived of liberty (code 112) was 1.5% (in 2008 — 3.6%). In the group of homosexuals and bisexuals, in 2019 this parameter was 4.5% (Tabl. 12).

In 2019, the detection rate in the group of drug users (code 102) was more than 5% and more than the district average in the Vologda Oblast — 5.1%. In 2019, the detection rate in the group of homosexuals and bisexuals (code 103) was more than 5% in the Murmansk Oblast (33.3%), Vologda Oblast (25.0%), Kaliningrad Oblast (12.0%), in St. Petersburg (4.6%). In the Pskov Oblast, HIV detection rate among persons deprived of liberty in 2019 was 30.3%.

During the entire reporting period (as of 31.12.2020), a total of 147 731 cases of HIV infection were registered in 11 territories of the NWFD. This is 10% of the total number of persons living with HIV registered in the Russian Federation by the specified date (1 510 000 persons). With the exception of deceased (34 160 persons), by the end of 2020, 113 571 persons with HIV lived in the NWFD.

In 2020, 5031 new cases of HIV infection were registered among the citizens of the RF in 11 territories of the NWFD, which is 20.6% less than in 2019 (Fig. 44, Tabl. 13).

A decrease in the number of new HIV cases was seen in all 11 territories of the NWFD.

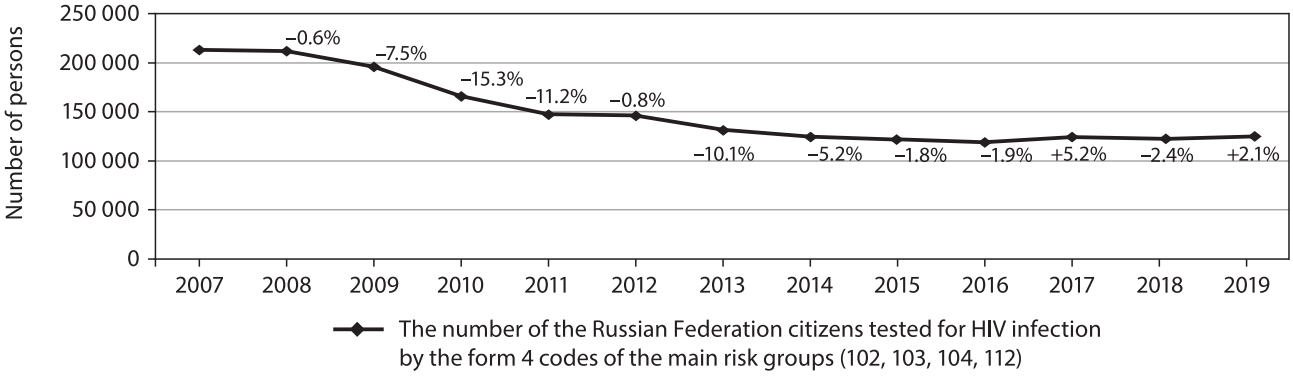


Figure 43. Changes in the number of persons examined for HIV infection in the NWFD by year, the most active risk groups (codes 102, 103, 104, and 112) in 2007–2019

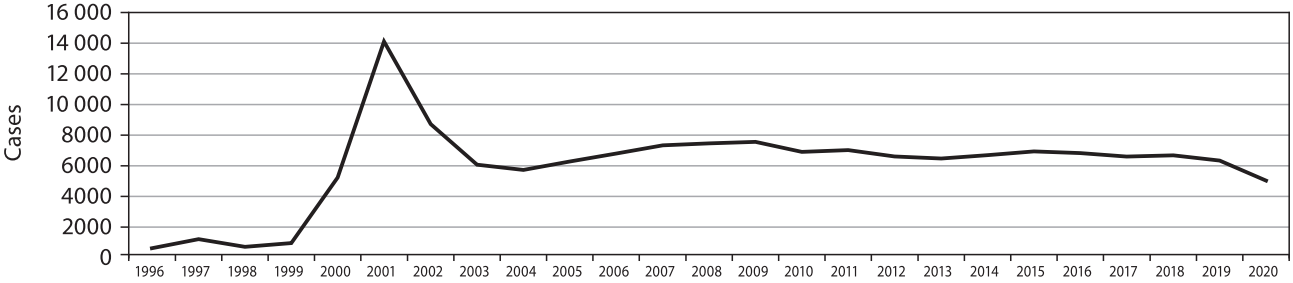
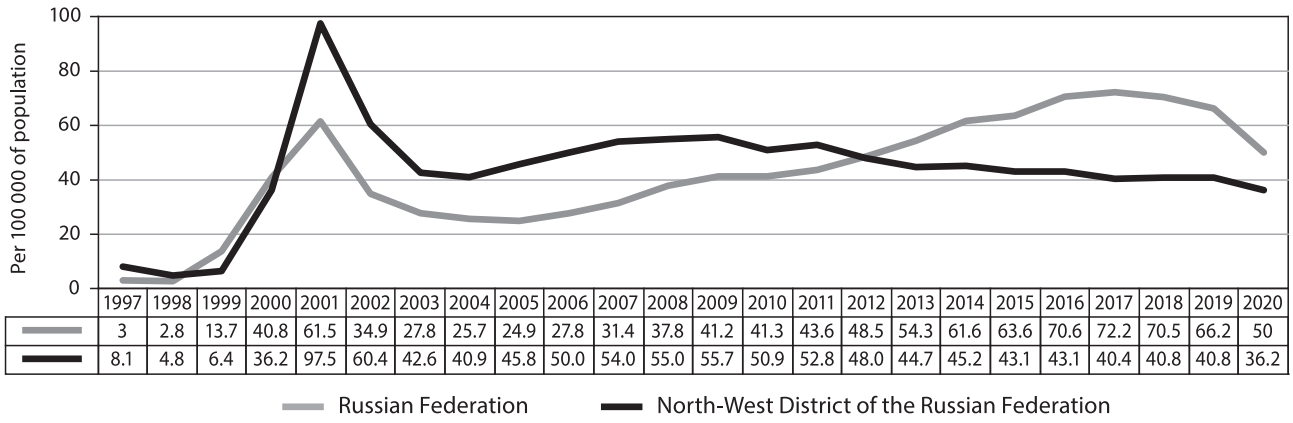


Figure 44. Annual dynamics of newly detected HIV cases in the NWFD (1996–2020)



	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
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	50
North-West District of the Russian Federation	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	36.2

Figure 45. The incidence of HIV in the Russian Federation and in the NWFD, 1997–2020

Over many years of observation, the incidence of HIV in the NWFD of the RF had exceeded the national average (up to 1.5 times). However, since 2009, there has been a trend to the incidence reduction, and in 2013, the incidence rate was already lower than the national average (44.7 and 54.3 per 100 000 of population, respectively). In 2020, in the NWFD, the incidence rate among people with newly diagnosed HIV decreased as compared to 2019 (40.8) and amounted to 36.2 per 100 000 of population, which is lower than the Russian average (45.0 per 100 000 of population) (Fig. 45).

Based on HIV epidemic monitoring in 11 territories of the NWFD, the following trends of the epidemic in the District can be noted.

Breakdown of age groups by gender shows that in recent years, HIV in the NWFD and in the RF as a whole has been diagnosed in older age groups. In 2019, the highest incidence rates in males were seen in the age group of 35–39 years (164.5 per 100 000 of population), whereas in females it was in the age group of 30–34 years (75.4 per 100 000 of population). Despite the relatively low incidence rates, adolescent girls and young women remain the most vulnerable to HIV infection.

In 2019, the proportion of young people among those newly diagnosed with HIV continued to decrease. In 2005, the age group of 15–19 years accounted for 8.4%, in 2019 — for 1.1%; the group of 20–24 years in 2005 accounted for 30.6%, in 2019 — for 4.2%.

The general structure of people living with HIV in the NWFD in 2019 was dominated by males (60.7%). The proportion of women in the total structure of people living with HIV has grown from 18.9% in 1995 to 26.2% in 2000 to 40.3% in 2019.

In 2019, the transmission of the virus during heterosexual contacts was registered in 69.9% (the RF average is 60.8%), via intravenous drug administration — in 25.2% of cases (the RF average is 35.6%). In 2019, the transmission of the virus through intravenous drug injections did not exceed 50% in any territory of the NWFD (Fig. 46, 47).

Table 12. Explanation of codes used in HIV testing of the population

Cohort of tested persons	Code of the cohort
Citizens of the Russian Federation	100
Donors (of blood, biological fluids, organs and tissues)	108
Healthcare workers working with HIV-positive patients or infected material	115
Drug users	102
Homosexuals and bisexuals	103
Persons with STDs	104
Persons deprived of liberty	112
Persons examined for clinical reasons	113
Pregnant women (donors of placental and miscarriage-derived blood)	109
Others	118
Persons examined as part of an epidemiological survey	120
Foreign citizens	200

Table 13. Registration of new HIV cases in the territories of the NWFD in 2020 as compared to 2019

Territory	2019	2020	Growth/decline (%)
Arkhangelsk Oblast	363	301	–17.1
Vologda Oblast	404	365	–9.7
Kaliningrad Oblast	416	349	–16.1
Republic of Karelia	241	180	–25.3
Komi Republic	397	383	–3.5
Leningrad Oblast	1023	719	–29.7
Murmansk Oblast	426	387	–9.2
Novgorod Oblast	352	249	–29.3
Pskov Oblast	108	94	–13.0
Nenets Autonomous Okrug (NAO)	7	7	0.0
St. Petersburg	2601	1997	–23.2
NWFD	6338	5031	–20.6

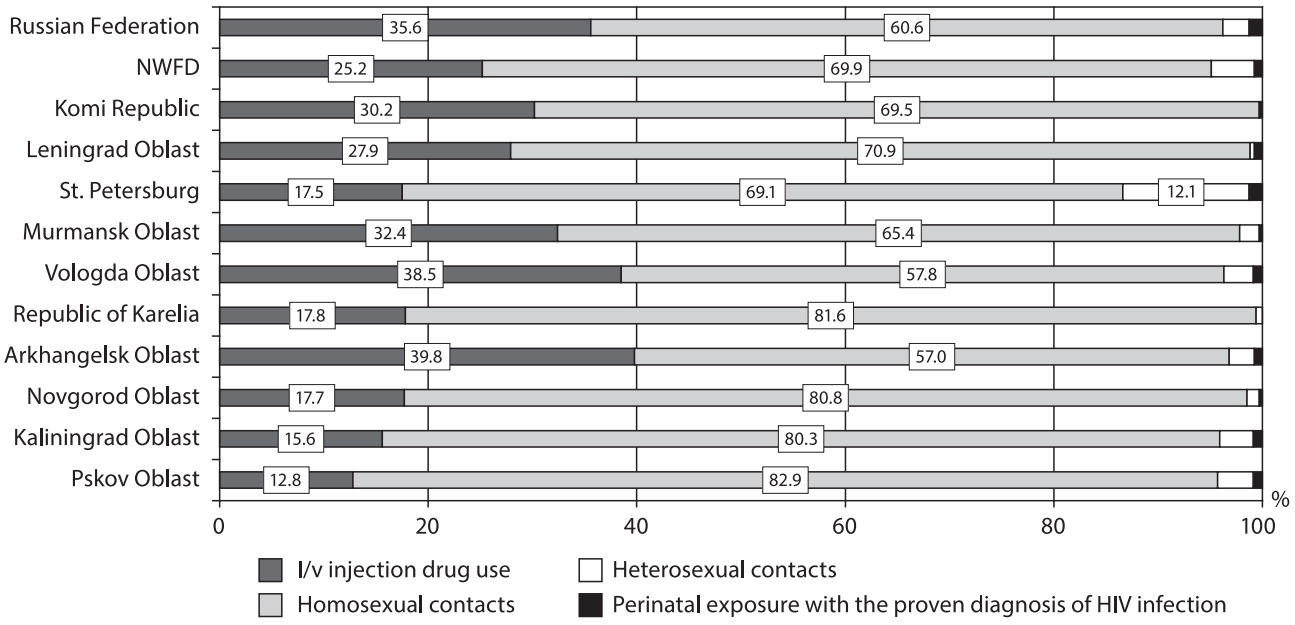


Figure 46. Distribution of HIV-positive persons in the NWFD by risk factors of infection (excluding cases where these factors were unknown) in individual territories in 2019

Just as in the Russian Federation as a whole, in the NWFD over the past five years of monitoring the HIV epidemic, the proportion of the MSM group has increased: in 2019 it amounted to 2.5% (in 2018 — 3.8%, in 2012 — 1.2%). As for individual territories in 2019, the proportion of the MSM group in St. Petersburg was higher than the district average (10.1%) (Fig. 46).

Analysis of the distribution of HIV cases by risk factors separately among males and females showed that heterosexual contact is the major route for pathogen transmission in females.

Due to the late detection and seeking medical help, as well as increased number of patients with concomitant diseases, in 2019 the number of deaths in the district increased, including those diagnosed with AIDS (Fig. 48).

In the NWFD, 1541 HIV-positive persons died in 2020 for various reasons unrelated to HIV (in 2019 — 1554, in 2018 — 1501, in 2017 — 1490, in 2016 — 1252). 755 deaths of persons diagnosed with AIDS were registered (in 2019 — 908, in 2018 — 919, in 2017 — 1039, in 2016 — 1328). The causes of death in patients with AIDS were mainly tuberculosis (50%), pneumonia, lymphoma, or meningoencephalitis. In total, since the beginning of HIV infection registration from 1987 to 2020, 34 160 HIV-positive persons have died, of which 12 301 were diagnosed with AIDS.

Active involvement of women in the epidemic process has contributed to an increased number of children born to HIV-infected mothers, but over the past three years there has been a trend towards decrease in both the annual number of children born to HIV-infected mothers and the number of children diagnosed with HIV infection (Fig. 49). Since the beginning of the registration of HIV infection in 1987, 19 655 children in the district have had perinatal HIV exposure. The overall number of children with a confirmed diagnosis of HIV due to perinatal transmission was 991 by the end of 2019. It should be noted that more than half of the children who were diagnosed with HIV in 2019 were residents of St. Petersburg and the Leningrad Oblast. Perinatal infection of children in the NWFD decreased from 25 to 1.2%, in individual territories it varied from 0 to 2.4%.

The coverage of three-stage chemoprophylaxis of HIV transmission from mother to child remained at the same level. In 2019, 89.2% of mother-child pairs received a full course of three-stage chemoprophylaxis of HIV transmission from mother to child, in 2018 it was 88.9%, in 2017 — 88.9%, and in 2006 — 72,5%.

The number of HIV-positive persons under regular medical check-ups at AIDS Centres of the NWFD increases annually. At the end of 2019, a total of 68 219 HIV-positive persons underwent regular medical check-ups at 11 territorial AIDS centres, which is 87.8% of those subject to observation. Every 10th or 11th person with detected HIV is lost to follow-up. This figure tends to decrease, but it is necessary to take into account that a significant part of people are still unaware of their disease and their total number is comparable to the number of persons undergoing check-ups.

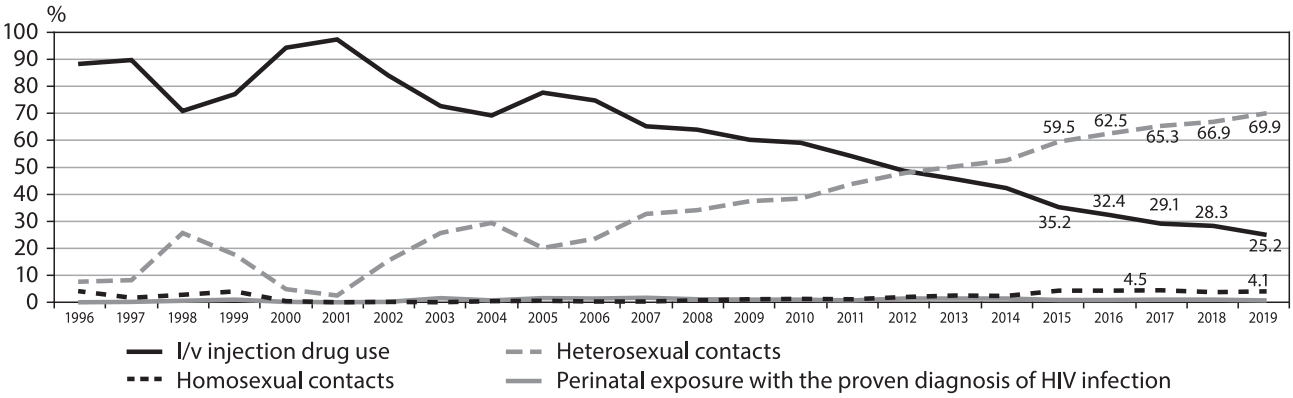


Figure 47. Distribution of HIV cases in the NWFD by risk factors of infection from 1996 to 2019 (excluding cases where these factors were unknown)

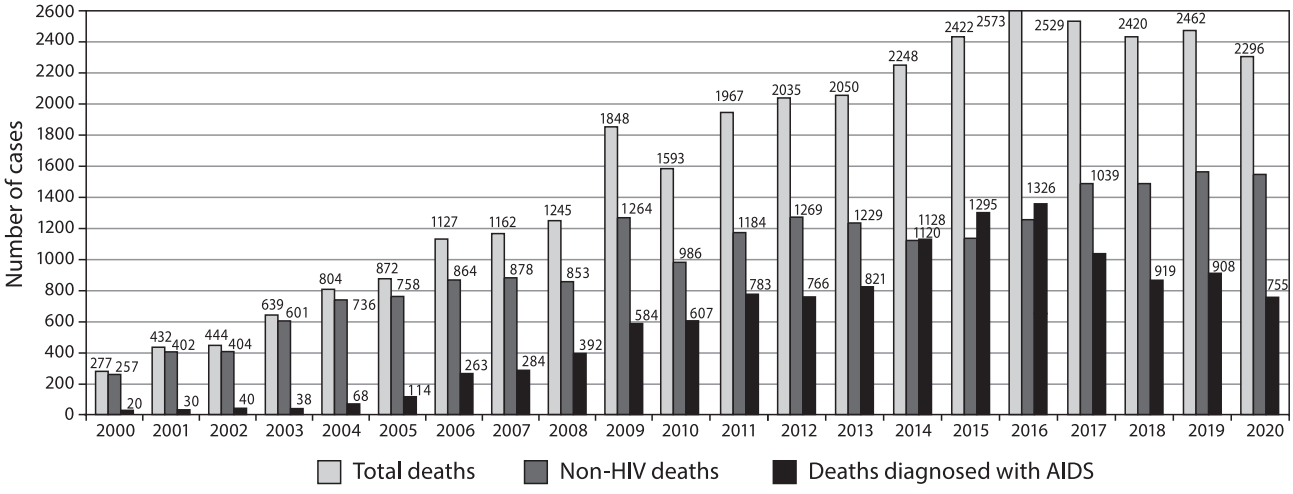


Figure 48. Registered deaths of HIV-positive and AIDS patients in the territories of the NWFD, 2000–2019

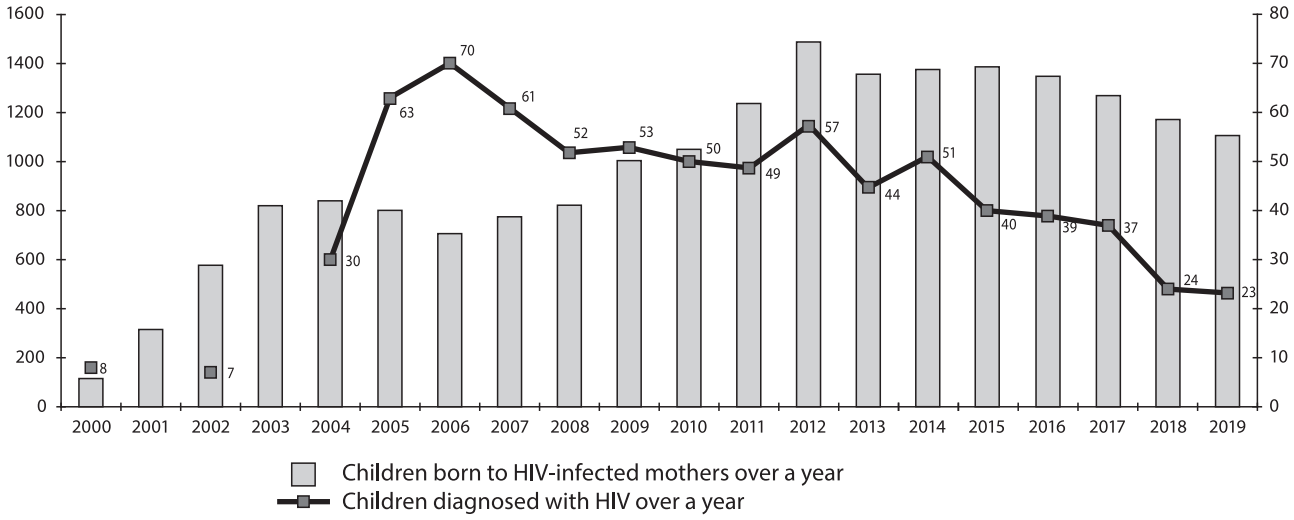


Figure 49. Children born to HIV-infected mothers in the territories of the NWFD, 2000–2019

In 34.1% of the individuals under regular check-ups, HIV infection was at the subclinical (latent) stage 3. The stage of secondary manifestations (4A, 4B, 4C) was diagnosed in 63.1% of patients (in 2018 — 61.1%, in 2017 — 61.1%, in 2016 — 66.3%).

Antiretroviral therapy of HIV patients

In 2019, 48 719 HIV patients received specific antiretroviral therapy (in 2018 — 41 081, in 2017 — 34 180, in 2016 — 29 816), which is 71.6% of those under regular check-ups and 43.9% of those living with HIV (Fig. 50).

As of 31.12.2019, 2944 HIV-positive persons placed in detention within the Federal Penitentiary Service of Russia received ART (in 2018 — 2210, in 2017 — 2900, in 2016 — 2614).

Laboratory of HIV Immunology and Virology

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

In 2019–2020, 276 studies were carried out aimed at detecting resistance to antiretroviral drugs. A number of mutations causing multiple resistance to NRTI, NNRTI and PI drugs were revealed. The most common mutations are: M184V (53.98%), K103N (16.3%), L74V (14.85%), K101E (12.31%), A62V (10.5%), and G190S (9.42%), the remaining mutations are found in less than 10% of cases. All of the mutations occurred during the ongoing treatment with antiretroviral drugs.

Findings of the study in the population of HIV-positive persons in the NWFD are put together into a uniform database for subsequent retrospective analysis of trends in primary and secondary HIV resistance to antiretroviral drugs for the 11-year period from 2009 to 2020.

To assess the molecular and epidemiological structure and drug-resistant variants of HIV among persons with virological failure of antiretroviral therapy from the Arkhangelsk Oblast, 76 patients were examined. In the examined group, HIV of genotype A, variant A6 (IDU-A) prevailed (89.5%) as compared to genotype B (9.2%), in one case (1.3%) the variant CRF03_AB was detected. Resistance to one or more drugs was found in 86.8% of patients. Mutations associated with resistance to protease inhibitors were found in 33.3%, to reverse transcriptase inhibitors — in 92.4%. Isolates with drug resistance only to NRTI accounted for 16.6%, to NNRTI — for 1.5%, to PI — for 10.6%, to both PI and NRTI — for 12.1%, to both NRTI and NNRTI — 46.96%, to all three groups of drugs simultaneously — 12.1%. The most common mutations are those of drug resistance to lamivu-

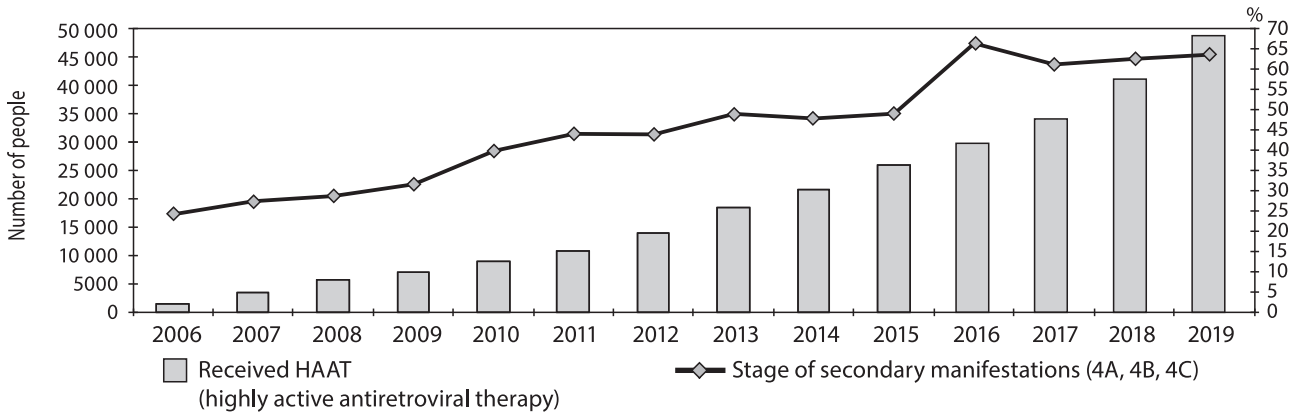


Figure 50. The number of HIV-patients receiving ART and the proportion of patients with severe stages of the disease in the period from 2006 to 2019

dine and emtricitabine (M184V), delavirdine (K103N), thymidine analogues (T215F/Y and/or K219Q/E and/or D67N), nevirapine and efavirenz (G190S), and non-thymidine nucleoside analogues (L74V).

Under the agreement on scientific cooperation, drug resistant HIV variants were evaluated in 42 HIV-positive persons (4 people with newly diagnosed HIV and 38 with virological failure of antiretroviral therapy) in Ho Chi Minh City (Socialist Republic of Vietnam). In the examined group, HIV of the circulating recombinant form CRF01_AE prevailed (92.2%) as compared to genotype B (5.3%), whereas CRF08_BC was detected in one patient (2.6%). Among people with newly diagnosed HIV, three persons had the CRF01_AE genetic variant and one person had genotype B. Mutations of drug resistance were detected in 76.2% of patients. Among the isolates with revealed drug resistance, 43.75% had single mutations. Mutations to RTI were more common (84.8%) than mutations to PI (15.2%). The most common mutations were NNRTI — 47.8%, followed by NRTI (37%) and PI (15.2%). Isolates with drug resistance only to NRTI accounted for 9.4% (7.1% of the total group), only to NNRTI — for 28.1% (21.4% of the total group), only to PI — for 12.5% (9.5% of the total group), to both PI and NRTI — for 6.25% (4.8% of the total group), to PI and NNRTI — for 3.1% (2.4% of the total group), to NRTI and NNRTI — for 37.5% (28.6% of the total group), and no isolates with simultaneous resistance-associated mutations to all three groups of drugs were detected. Among the mutations of drug resistance to PI, the major M46I/L and minor K20T mutations were found in 28.6% each (2.4% of all mutations) of cases. In the reverse transcriptase region, the K103N NNRTI resistance mutation was found most often — in 12.98% (11.9% of all mutations), followed by the M184V NRTI and V106I NNRTI resistance mutations in equal proportions — 11.7% (10.7% of all mutations) each, respectively, followed by the Y181C NNRTI resistance mutation — 10.4% (9.5% of all mutations). The occurrence of ≥ 3 mutations of drug resistance in patients with two/three ART protocols was significantly higher than in patients after one protocol, regardless of the duration of therapy — $\chi^2 = 8.960$, $p = 0.003$, $df = 1$, the normalized value of the Pearson coefficient of contingency $C' = 0.676$, which indicates a strong relationship between the increase in the number of ART protocols and the frequency of drug resistance mutations in the analyzed region. The number of natural polymorphic variants in the analyzed region among patients receiving ART ranged from 17 to 35 mutations and also depended on the number of ART protocols. It was shown that ≥ 23 polymorphic variants in patients with two/three ART protocols are more common than in patients with one protocol — $\chi^2 = 3.149$, $p = 0.035$, $df = 1$, the normalized value of the Pearson coefficient of contingency $C' = 0.459$, which indicates a relatively strong relationship between the increase in the number of ART protocols and the number of polymorphic variants in the presented pol gene fragment.

The priority research areas of the laboratory include the identification of markers of hepatitis B (HBV), C (HCV) and D (HDV) viruses in various risk groups, as well as molecular genetic analysis of isolates of these viruses during monoinfection and co-infection with HIV. An optimized algorithm for the diagnosis of chronic hepatitis B has been tested and applied in the laboratory, which allows the identification of the occult (HBsAg-negative) form of the disease. The prevalence of occult hepatitis B among HBsAg-negative HIV-positive individuals with failed ART in Veliky Novgorod and Arkhangelsk was analyzed. In the group from Arkhangelsk, HBsAg-negative (occult) hepatitis B was detected in 43.8% of HIV-postitive patients. Only HBV of genotype D was detected, and HBV of D1 subgenotype prevailed (39.28%) as compared to HBV subgenotypes D2 (32.1%) and D3 (28.6%). Serological markers were found in 42.8% of patients with detected HBV DNA. Two HBV isolates were revealed with drug resistance mutations in the polymerase gene leading to amino acid substitution (L180M, M204V) associated with the development of resistance to lamivudine, entecavir, telbivudine and tenofovir. In the group from Veliky Novgorod, occult hepatitis B was detected in 57.89% of cases, while anti-HBcore IgG and anti-HBe IgG were detected in 13.63% of individuals. Phylogenetic analysis showed that only genotype D was represented in the examined group, and subgenotype D2 (47.72%) prevailed as compared to subgenotypes D1 (34.09%) and subgenotype D3 (18.18%). Only one isolate of the hepatitis B virus with mutations of drug resistance to the therapy with nucleotide/nucleoside analogues was identified, mutations causing an amino acid substitution in the virus polymerase gene (L180M, M204V) associated with the development of resistance to lamivudine, entecavir, telbivudine, and tenofovir.

The prevalence of HBV and HCV markers in patients with newly diagnosed HIV was analyzed. Serological markers of HBV were detected in 79.6% of cases. At the same time, HBsAg was detected in 5.6% of patients. Anti-HBcore IgG were found in 62.24% of cases, anti-HBe IgG — in 27.55%, and anti-HBs IgG — in 52.55% of cases. HBV DNA was found in 18.36% of HIV-positive persons, including 12.75% of cases of HBsAg-negative form of the disease. In the examined group, HBV of genotype D prevailed (91.7%), genotype A was detected in 8.3% of cases. Subgenotypes accounted for the following proportions: D2 — 55.6%, D1 — 22.2%, D3 —13.9%, and A2 — 8.3%. Mutations in the reverse transcriptase (RT) region were detected in 91.6% of patients, in the SHB region — in 83.3%, in the Core and Precore regions — in 72.2% and 27.7% of patients, respectively. 8.3% of cases of HBV with mutations of drug resistance to lamivudine, entecavir, telbivudine and tenofovir were identified, mutations causing amino acid substitutions in the HBV polymerase gene at positions L180M, T184A, and M204V. Vaccine escape mutations were detected in 61.1% of patients. In all samples with drug resistance mutations, escape mutants were also present. Analysis of the regions of the basal nucleus promoter, Precore and Core revealed 22.2% of patients with the A1762T/G1764A double mutation, 25% with the G1896A mutation, and all three substitutions were detected in one person. In the Core region, 77.7% of patients had mutations in one of the hot spots of 87, 97, 112 and 130 codon substitutions, which can play a role in immunomodulation in the chronic HCV infection. HCV antibodies were found in 18.87% of the group members. HCV RNA was detected in 18.36% of patients, including 89.18% of anti-HCV-positive and 1.88% of anti-HCV-negative patients. When analyzing sex-related patterns of HCV detection in HIV-positive persons, it was shown that co-infection was more common in males (77.8%) as compared to females (22.2%) — $\chi^2 = 3.996$ at $p = 0.0456$, $df = 2$. The difference in the HIV viral load between the groups with HIV monoinfection and with HIV + HCV co-infection was revealed ($\chi^2 = 6.284$ at $p = 0.0432$, $df = 2$). A significant difference between the groups in the number

of $CD4^+$ lymphocytes was shown: $\chi^2 = 8.187$ at $p = 0.0167$, $df = 2$. Phylogenetic analysis showed the following distribution of HCV subtypes: HCV 1b — 47.2%, HCV 3a — 30.6%, HCV 1a —13.9%, HCV 2a — 5.5% and one single sample was determined as HCV 2k. In 25% of the samples, NS5b mutations were found in positions associated with the development of HCV drug resistance, including in 1a and 3a HCV genotypes (5.6% of the total HIV + HCV group each), as well as in HCV 1b (13.9% of the total group). Mutations in HCV 1a were substitutions of C316Y and N444D. Substitutions of C316N, C451S, and S556N/G were identified in cases of HCV 1b. In patients with HCV 3a, samples with the D310N mutation associated with poor prognosis were identified.

Under the agreement on scientific cooperation, molecular genetic characterization of HBV and HIV was performed in patients with HIV/HBV co-infection living in the Republic of Guinea. Serological markers of HIV were detected in 11.02% of individuals. HIV RNA was detected in 12.9% of patients in the seropositive group (1.43% of the total group). Serological markers of HBV in the HIV RNA-positive individuals were detected in 29.03% of patients, including 16.12% of HBsAg and 12.9% of anti-HBcore IgG. HBV DNA was detected in all HBsAg-positive and in two anti-HBcore IgG-positive patients, as well as in 12 people negative for all serological markers of HBV analyzed in the work. Thus, HBV DNA was found in 61.29% of HIV RNA-positive persons. Based on the nucleotide sequence analysis of the pol gene fragment of 19 HIV samples, it was shown that the circulating recombinant form of HIV CRF02_AG (52.63%) prevails in the examined group as compared to HIV A1 (42.1%), and one sample was an independent recombinant of the A1 and G genotypes. Phylogenetic analysis of HBV in the studied samples showed that the group was dominated by HBV genotype E — 47.36%, as compared to HBV D1 — 21.05%, D2 — 15.78%, D3 — 10.52%, and A2 — 5.26%. Samples of HIV and HBV with mutations of drug resistance were found, despite the absence of antiretroviral therapy.

Under the agreement on scientific cooperation, a group of patients with HBV/HDV co-infection from the Kyrgyz Republic was analyzed. In the examined group, only the virus of genotype D was represented, HBV of subgenotype D1 (68.75%) prevailed as compared to HBV of subgenotype D2 (18.75%) and subgenotype D3 (12.5%). Several independent sources of infection were obvious for all subgenotypes; subclusters including isolates from Kyrgyzstan, Kazakhstan and Uzbekistan, were revealed, as well as subclusters including isolates only from Kyrgyzstan that have less similarity with isolates previously deposited in the international database, which probably is evidence of independent homologous evolution of HBV in the region. Clinically significant mutations were detected in 26.5% of patients, including 12.5% with escape mutations that prevent the detection of the virus and/or allow the virus to replicate despite the vaccine (122K, 128V, 133I, 134N). Another 12.5% of isolates have mutations independently associated with the development of liver cirrhosis and hepatocellular carcinoma, including 21, 24 and 27 nucleotide deletions in the Pre-S2 region and the S11F mutation in the PreCore region. In one case, unusual mutations of 236S and 250P were found in positions described as drug resistance sites of the P-region associated with the development of resistance to adefovir, tenofovir, and entecavir. Based on the phylogenetic analysis of the HDV nucleotide sequences, it was shown that the virus of genotype 1 (96.9%) prevails in the examined group, as compared to genotype 2 (3.1%). When assessing the divergence of the examined genotype 1 samples, the maximum genetic distance was 12.49%, and the minimum was 7.41%, while within individual clusters the genetic distance was from 2.6% to 8.5%. The analysis of the amino acid sequence of the posttranslational phosphorylation site in the S-HDAg protein revealed serine at positions 2, 123 and 177 responsible for the synthesis of the genomic RNA of the HDV by interaction with cellular RNA polymerase II. At the acetylation site, the lysine residue was present in all isolates (K-72). The requirements for changes in the amino acid sequence for the RNA binding function may be less strict: position 81 is of interest (most have residue V, a smaller amount — residue I, and one case had A and E), 97 (mainly lysine, and there are also samples with R, Q, E), different amino acid residues in some samples are found in the positions 83, 86, 88–90, 93, 95, 96, and 100, and one sample with substitutions in positions 101 and 107 was found. In position 131, residue K prevailed in the examined group, in 8 cases residue R was represented, residues M, L, and G were found in one case each. At position 135 within the HLH region, each of the amino acid residues R and T was shown in one case, whereas in the rest, residue E was found. At position 139 at the beginning of the ARM region, the R variant prevailed, ten patients had the K amino acid, and two cases — the E residue. At position 142 of the ARM region, the amino acid R was predominant in our strains, while E was detected in the sample Kyr39. At the beginning of the HDAg epitope region at position 148, three samples had the amino acid R, while the others only had the amino acid P. In the same region, at position 151, G residue was the main amino acid. Unusual mutations were shown in position 149: T residue prevailed, P was found in 10 cases, V — in 5 cases, and Q residue was found in only one sample. Analysis of the position 202 in the highly conserved C-terminus of L-HDAg of HDV-1 made it possible to divide the samples into groups of sequences: one with a serine residue (corresponding to the UCC codon), the other with alanine (corresponding to the GCC codon). In our samples, the A-202 residue was detected in 56.5% of cases, whereas the S-202 residue was detected in 29% of cases. Of all the sequences we analyzed, in one case, the valine residue V was represented at position 202. We found especially interesting the samples with residues P-202 (proline) and E-203 (glutamic acid), which accounted for 11.3% of the total number of samples studied. All the studied isolates express CRPQ region at the C-terminal end of L-HDAg. The CRPQ residues are specific for genotype 1 and are used to differentiate it from other HDV genotypes.

A promising line of research is the determination of genetic markers of disease progression and response to therapy for HIV, HBV, and HCV. For example, deletion polymorphism of genes of xenobiotics biotransformation system of the glutathione-S-transferase family (GSTT1 and GSTM1) was analyzed in HIV-positive individuals with failed antiretroviral therapy who received two or more ART protocols, as well as in patients with newly diagnosed HIV. The control group included persons without HBV, HCV, HIV and clinical manifestations of chronic and/or acute diseases. It was shown that the frequency of homozygous deletions of GSTM1 and GSTT1 in HIV-positive patients with failed ART were 41.47% and 44.88%, respectively, including the combined genotype GSTM1 0/0 + GSTT1 0/0 — 22.15% of the group. In the control group, GSTM1 0/0 accounted

for 41.46%, GSTT1 0/0 — for 22.76%, including the combined genotype GSTM1 0/0 + GSTT1 0/0 — 12.19% of the group. Analysis of genotype distribution (GSTM1 0/0 + GSTT1 +/–, GSTM1 +/– + GSTT1 0/0, GSTM1 0/0 + GSTT1 0/0, GSTM1 +/– + GSTT1 +/–) revealed significant differences between the control group and HIV-positive individuals with ART failure — $\chi^2 = 18.103$, df = 3, p = 0.0004. The distribution of genotypes of GST genes in the group of patients with newly diagnosed HIV infection did not differ from that in the control group. The risk of ART failure in cases with the combined genotype GSTM1 0/0 + GSTT1 0/0 was shown: OR = 2.05, p = 0.0323, CI = 1.073–3.914. It should be noted that we did not find any differences in the distribution of genotypes of GST genes depending on the number or features of ART protocols.

Conclusion

According to the results of the study and the data of multiple publications, prolonged ART did not lead to significant changes in the epidemic process of HIV infection and at the population level did not demonstrate the clinical effectiveness as estimated according to the distribution of the clinical stages of the disease, as well as the mortality of patients against the backdrop of HIV-induced immunosuppression.

In addition to medicines provision, there is a number of other factors that influence the HIV epidemic directly or indirectly. They affect various components of professional medical activities, as well as other areas of life of society and are not in line with the main funding streams of the fight against the epidemic. Therefore, it confirms the thesis that some diseases are a social issue, i.e., they affect many institutions of the society. HIV infection, viral hepatitis and tuberculosis are socially significant infections (Decree of the Government of the Russian Federation No. 715 dated 01.12.2004).

There is a contradiction between understanding the existing situation, which creates a new epidemic round of severe and comorbid forms of the disease, on the one hand, and lacking financial support for the necessary comprehensive measures to counter the pandemic, which are therefore not implemented.

Publications

Guidelines

1. *Belyakov N.A., Rassokhin V.V. HIV infection and comorbid conditions. St. Petersburg: Baltic Medical Educational Centre, 2020: 680.*

Monographs

1. *Belyakov N.A., Rassokhin V.V. Comorbid conditions in HIV infection. Part 2. Secondary and concomitant infections. St. Petersburg: Baltic Medical Educational Centre, 2019: 252.*
2. *Belyakov N.A., Rassokhin V.V. Comorbid conditions in HIV infection. Part 3. Somatic diseases and disorders. St. Petersburg: Baltic Medical Educational Centre, 2019. 252 p.*
3. *Bagnenko S.F., Belyakov N.A., Rassokhin V.V., Trofimova T.N. The beginning of the COVID-19 epidemic. St. Petersburg: Baltic Medical Educational Centre, 2020. 360 p.*
4. *Belyakov N.A., Rassokhin V.V., Trofimova T.N. Personalized HIV medicine. St. Petersburg: Baltic Medical Educational Centre, 2020. 320 p.*

Analytical reviews

1. *HIV infection and comorbid conditions in the Northwestern Federal District of the Russian Federation in 2018. Analytical review / Ed. by Belyakov N.A. St. Petersburg: St. Petersburg Pasteur Institute, 2019: 56.*

Articles

1. *Arsentieva N.A., Batsunov O.K., Kudryavtsev I.V., Semenov A.V., Totolian A.A. CD32A receptor and its role in normal and pathological conditions // Medical Immunology (Russia). 2020; 22 (3): 433–442. doi: 10.15789/1563-0625-CRI-2029*
2. *Arsentieva N.A., Lyubimova N.E., Batsunov O.K., Semenov A.V., Totolian A.A. Features of the cytokine profile of the blood plasma of healthy inhabitants of the Republic of Guinea // Medical Immunology (Russia). 2020; 22 (4): 765–778. doi: 10.15789/1563-0625-AOB-2073*
3. *Arsentieva N.A., Semenov A.V., Zhebrun D.A., Vasilyeva E.V., Totolian A.A. The role of the CXCR3 chemokine receptor and its ligands in some pathological immune conditions // Medical Immunology (Russia). 2019; 21 (4): 617–632. doi: 10.15789/1563-0625-2019-4-617-632*
4. *Azovtseva O.V., Panteleev A.M., Karpov A.V., Arkhipov G.S., Weber V.R., Belyakov N.A., Arkhipova E.I. Analysis of medical and social factors affecting the formation and course of HIV, tuberculosis and viral hepatitis co-infection // Russian Journal of Infection and Immunity. 2019; 9 (5–6): 787–799.*
5. *Bagnenko S.F., Rassokhin V.V., Belyakov N.A., Boeva E.V., Yastrebova E.B. Coronavirus infection COVID-19. Treatment and prevention // HIV and Immunosuppressive Disorders. 2020; 12 (2): 31–55.*
6. *Basina V.V., Arsentieva N.A., Lyubimova N.E., Semenov A.V., Esaulenko E.V., Totolian A.A. Clinical and immunological characteristics of "difficult" patients with chronic hepatitis C during antiviral therapy // Bulletin of the Novgorod State University. 2020; 3 (119): 25–31. doi: 10.34680/2076-8052.2020.1(117).25-31*
7. *Belyakov N.A., Ogurtsova S.V., Azovtseva O.V., Kurganova T.Yu., Melnikova Z.N., Leonova O.N., Stepanova E.V., Kovelenov A.Yu., Asadullayev M.R., Rassokhin V.V. Analysis of the main epidemiological indicators of HIV Infection and results of multi-year application of antiretroviral therapy (by the materials from the North-West of Russia) // Infectious Diseases: News, Views, Education. 2020; 9 (1; 32): 19–27.*
8. *Belyakov N.A., Rassokhin V.V., Kolbin A.S., DiClemente R.J., Panteleev A.M., Azovtseva O.V., Ogurtsova S.V., Simakina O.E., Stepanova E.V., Vyaltsin S.V., Zholobov V.E., Kovelenov A.Yu., Melnikova T.N., Kurganova T.Yu., Ulumbekova G.E. Epidemiological, clinical and financial components of the results of long-term antiretroviral therapy in patients with HIV infection // HIV Infection and Immunosuppressive Disorders. 2019; 11 (4): 7–19.*
9. *Belyakov N.A., Rassokhin V.V., Rosenthal V.V., Ogurtsova S.V., Stepanova E.V., Melnikova T.N., Kurganova T.Yu., Azovtseva O.V., Simakina O.E., Totolian A.A. Epidemiology of HIV infection. The role of monitoring, scientific and sentinel surveillance, modelling and situational forecasting // HIV Infection and Immunosuppressive Disorders. 2019; 11 (2): 7–26.*
10. *Belyakov N.A., Rassokhin V.V., Simakina O.E., Ogurtsova S.V., Khalezova N.B. The role of drug use in the spread and course of HIV infection: a comprehensive view of the problem // Medical-biological and Socio-psychological Problems of Safety in Emergency Situations. 2020 (2): 69–83.*

11. *Belyakov N.A., Rassokhin V.V., Simakina O.E. HIV infection, migration processes and the coastal regions of Russia // Marine Medicine. 2019; 5 (3): 77–89.*
12. *Belyakov N.A., Rassokhin V.V., Stepanova E.V., Sizova N.V., Samarina A.V., Yastrebova E.B., Boeva E.V., Khalezova N.B., Gutova L.V., Ogurtsova S.V., Kovelenov A.Yu., Panteleev A.M., Leonova O.N., Azovtseva O.V., Melnikova T.N., Kurganova T.Yu., Buzunova S.A., Di Clemente R. Personalized approach to the treatment of a patient with HIV infection // HIV Infection and Immunosuppressive Disorders. 2020; 12 (3): 7–34.*
13. *Belyakov N.A., Rassokhin V.V., Yastrebova E.B. Coronavirus infection COVID-19. The nature of the virus, pathogenesis, clinical manifestations. Message 1 // HIV and Immunosuppressive Disorders. 2020; 12 (1): 7–21.*
14. *Belyakov N.A., Trofimova T.N., Rassokhin V.V., Shelomov A.S., Magonov E.P., Bogdan A.A., Bakulina E.G., Gromova E.A., Khalezova N.B., Neznanov N.G., Kataeva G.V. Interdisciplinary personalized approach and technologies of brain studies in HIV infection // Radiation Diagnostics and Therapy. 2020; 11, (2): 7–28.*
15. *Bushmanova A.D., Novak K.E., Esaulenko E.V., Ostankova Yu.V., Danilova E.M. Molecular biological methods for the diagnosis of hepatitis A // Bulletin of the Yaroslav the Wise Novgorod State University. 2020; (119): 32–38.*
16. *Chumakov E.M., Petrova N.N., Rassokhin V.V. Compulsive sexual behaviour as a risk factor for HIV infection // HIV Infection and Immunosuppressive Disorders. 2019; 11 (1): 7–15.*
17. *Egorova S.A., Kaftyreva L.A., Suzhaeva L.V., Zabrovskaya A.V., Voitenkova E.V., Matveeva Z.N., Ostankova Yu.V., Likhachev I.V., Satosova N.V., Kitsbabashvili R.V., Smirnova E.V., Semchenkova L.I., Bystraya T.E., Sokolnik S.E., Utkina N.P., Sikhando L.Yu. Antimicrobial resistance and clinically significant mechanisms of resistance of Salmonella strains isolated in 2014–2018 in St. Petersburg, Russia // Russian Clinical Laboratory Diagnostics. 2019; 64 (10): 620–626. doi: 10.18821/0869-2084-2019-64-10-620-626*
18. *Gordon E.O., Posokhova L.A., Podymova A.S., Yastrebova E.B. Substantiation and development of a family planning algorithm in HIV-serodiscordant couples // HIV Infection and Immunosuppressive Disorders. 2019; 11 (1): 38–45.*
19. *Gromova E.A., Kataeva G.V., Khomenko Yu.G., Kotomin I.A., Bogdan A.A., Kosykh A.V., Rassokhin V.V., Belyakov N.A., Trofimova T.N. Psychoemotional state and cognitive functions in HIV patients and the functional state of brain structures (according to positron emission tomography and magnetic resonance spectroscopy) // Clinical and Special Psychology. 2020; 9 (1): 78–103.*
20. *Gusev D.A., Samarina A.V., Yastrebova E.B., Mozaleva O.L. Modern aspects of prevention of perinatal HIV transmission in St. Petersburg // Journal Infectology. 2019; 11 (1): 58–64.*
21. *Kazachek A.V., Melnikova T.N., Samarina A.V., Rassokhin V.V. Analysis of the epidemiological situation and perinatal HIV infection in the Vologda Oblast // HIV Infection and Immunosuppressive Disorders. 2020; 12 (3): 69–76.*
22. *Khalezova N.B., Boeva E.V., Rassokhin V.V., Gutova L.V., Di Clemente R.J., Belyakov N.A. Clinical and personal characteristics of females with HIV/HCV co-infection, history of alcohol and drug use during the disease // HIV Infection and Immunosuppressive Disorders. 2019; 11 (4): 38–49.*
23. *Kireev D.E., Shipulin G.A., Semenov A.V., Tivanova E.V., Chulanov V.P., Kolyasnikova N.M., Zueva E.B., Galli C., Pokrovsky V.V. Comparative evaluation of the 4th generation of ELISA/CLIA test systems used in the Russian Federation for the diagnosis of HIV infection // HIV Infection and Immunosuppressive Disorders. 2019; 11 (2): 103–113. doi: 10.22328/2077-9828-2019-11-2-103-113*
24. *Miliukhina I.V., Senkevich K.A., Pchelina S.N., Usenko T.S., Nikolaev M.A., Timofeeva A.A., Semenov A.V., Totolian A.A., Agapova E.A., Lubimova N.E. Plasma cytokines profile in patients with parkinson's disease associated with mutations in GBA gene // Bulletin of Experimental Biology and Medicine. 2020; 168 (4): 423–426. doi: 10.1007/s10517-020-04723-x*
25. *Nikishov O.N., Kuzin A.A., Zobov A.E., Lavrentieva I.N., Antipova A.Yu., Ostankova Yu.V., Khamitova I.V., Nikishov S.N. Results of a study of parvovirus B19 (Parvoviridae, Parvovirinae, Erythroparvovirus, Primate erythroparvovirus 1) prevalence and circulation activity in socially significant categories of the population // Problems of Virology. 2020; 65 (3): 143–149. doi: 10.36233/0507-4088-2020-65-3-143-149*
26. *Novak K.E., Nikiforova A.O., Ingabire T., Zueva E.B., Shchemelev A.N., Esaulenko E.V., Semenov A.V. Optimization of prevention of HIV-1 drug resistance mutations in patients with virological failure of antiretroviral drugs // Bulletin of the Novgorod State University. 2020; 3 (119): 47–51. doi: 10.34680/2076-8052.2020.3(119).47-51*
27. *Ogurtsova S.V., Konovalova N.V., Shchemelev A.N. Milestones of the creation of the Northwestern District AIDS Centre // HIV Infection and Immunosuppressive Disorders. 2019; 11 (2): 114–119.*
28. *Ostankova Yu.V., Nogoibaeva K.A., Zueva E.B., Kasymbekova K.T., Tobokalova S.T., Semenov A.V. Phylogenetic analysis and characterization of full-size genome sequences of hepatitis Delta virus isolated from patients with chronic viral hepatitis B/D in the Kyrgyz Republic // Problems of Especially Dangerous Infections. 2020; (1): 124–132. doi: 10.21055/0370-1069-2020-1-124-132*
29. *Ostankova Yu.V., Semenov A.V., Totolian Areg A. A method for quantifying the covalently closed circular DNA of HBV in puncture liver biopsy samples // Russian Clinical Laboratory Diagnostics. 2019; 64 (9): 565–570. doi: 10.18821/0869-2084-2019-64-9-565-570*
30. *Ostankova Yu.V., Semenov A.V., Totolian Areg A. Detection of hepatitis B virus in blood plasma at low viral load // Russian Clinical Laboratory Diagnostics. 2019; 64 (10): 635–640. doi: 10.18821/0869-2084-2019-64-10-635-640*
31. *Ostankova Yu.V., Semenov A.V., Zueva E.B., Gabdrakhmanov I.A., Kozlov K.V., Zhdanov K.V., Totolian Areg A. The variety of hepatitis B virus genetic variants in military personnel // Journal Infectology. 2019; 11 (3): 46–53. doi: 10.22625/2072-6732-2019-11-3-46-53*
32. *Ostankova Yu.V., Semenov A.V., Zueva E.B., Nogoibaeva K.A., Kasymbekova K.T., Tobokalova S.T., Totolian A.A. The prevalence clinically significant virus mutations among patients with chronic viral hepatitis B // Russian Clinical Laboratory Diagnostics. 2020. 65 (1): 61–66. doi: 10.18821/0869-2084-2020-65-1-61-66*
33. *Ostankova Yu.V., Semenov A.V., Zueva E.B., Totolian A.A. The prevalence of occult hepatitis B in HBsAg-negative HIV patients in Veliky Novgorod // HIV infection and Immunosuppressive disorders. 2019; 11 (1): 64–70. doi: 10.22328/2077-9828-2019-11-1-64-70*
34. *Ostankova Yu.V., Semenov A.V., Zueva E.B., Totolian Areg A. Detection and molecular genetic characteristics of the hepatitis B virus in HIV patients in Arkhangelsk // Problems of Virology. 2019; 64 (3): 105–111. doi: 10.18821/0507-4088-2019-64-3-105-111*
35. *Ostankova Yu.V., Shchemelev A.N., Zueva E.B., Churina M.A., Valutite D.E., Semenov A.V. Molecular epidemiology and drug resistance of HIV in patients with virological failure of antiretroviral therapy in the Arkhangelsk Oblast // HIV Infection and Immunosuppressive Disorders. 2019; 11 (4): 65–72. doi: 10.22328/2077-9828-2019-11-4-79-90*
36. *Ostankova Yu.V., Shchemelev A.N., Zueva E.B., Huinh H.K.T., Semenov A.V. Incidence of viral hepatitis markers among inhabitants of South Vietnam. Current trends and prospects of Russian-Vietnamese cooperation in the field of ensuring sanitary and epidemio-*

logical safety: a joint monograph / Ed. by A.Yu. Popova; Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing. Volgograd: Volga-Press Publishing House, LLC, 2019: 285–307.

37. *Ostankova Yu.V., Valutite D.E., Zueva E.B., Serikova E.N., Shchemelev A.N., Boumbaly S., Balde T.A.L., Semenov A.V. Primary mutations of drug resistance of the hepatitis C virus in patients with newly diagnosed HIV infection // Problems of Especially Dangerous Infections. 2020; (3): 97–105. doi: 10.21055/0370-1069-2020-3-97-105*
38. *Priyma E.N., Ostankova Yu.V., Butskaya M.Yu., Semenov A.V. Modern aspects of specific laboratory diagnostics of HBV infection // Bulletin of the Yaroslav the Wise Novgorod State University. 2020; 3 (119): 56–61.*
39. *Rassokhin V.V., Boeva E.V. Issues of epidemiology and pathogenesis of HCV and HIV co-infection // HIV Infection and Immunosuppressive Disorders. 2020; 12 (1): 32–46.*
40. *Rassokhin V.V., Boeva E.V. Review of the 5th Conference on viral hepatitis and HIV infection in the countries of Central and Eastern Europe // HIV Infection and Immunosuppressive Disorders. 2019; 11 (4): 102–105.*
41. *Rassokhin V.V., Leonova O.N., Boeva E.V., Stepanova E.V., Belyakov N.A., Di Clemente R.J. HIV infection, secondary conditions and comorbidities. Part 2. Concomitant diseases // Medical Academic Journal. 2019; 19 (1): 5–16.*
42. *Rassokhin V.V., Samarina A.V., Belyakov N.A., Trofimova T.N., Lukina O.V., Gavrilov P.V., Grinenko O.A. Epidemiology, clinical signs, diagnosis, assessment of the severity of the COVID-19 disease, taking into account concomitant // HIV Infection and Immunosuppression. 2020; 12 (2): 7–30.*
43. *Samarina A.V., Dyldina N.S., Fertikh E.K., Yastrebova E.B., Abramova I.A., Gusev D.A. Correction of lipid metabolism disorders in children against the background of antiretroviral therapy using an integrase inhibitor // Journal Infectology. 2019; 11 (3): 63–68.*
44. *Samoilov A.E., Stoyanova N.A., Tokarevich N.K., Evengard B., Zueva E.V., Panferova Y.A., Ostankova Y.V., Zueva E.B., Valutite D.E., Kovalev E.V., Litovko A.R., Goncharov A.U., Semenov A.V., Khafizov K., Dedkov V.G. Lethal outcome of Leptospirosis in Southern Russia: characterization of Leptospira interrogators isolated from a deceased teenager // Int. J. Environ. Res. Public Health. 2020; 17 (12): 4238. doi: 10.3390/ijerph17124238*
45. *Semenov A.V., Ostankova Yu. V., Serikova E.N., Zueva E.B., Totolian Areg A. Optimization of the algorithm for diagnosing markers of chronic hepatitis B in patients with newly diagnosed HIV infection // Russian Clinical Laboratory Diagnostics. 2020; 65 (9): 574–579. doi: 10.18821/0869-2084-2020-65-9-574-579*
46. *Semenov A.V., Ostankova Yu.V. Occult (latent) hepatitis B: Issues of laboratory diagnostics // Infectious Diseases: News, Opinions, Training. 2019; 8, (3): 60–69. doi: 10.24411/2305-3496-2019-13010*
47. *Semenov A.V., Pshenichnaya N.Yu. Born in Wuhan: Lessons learnt from the COVID-19 epidemic in China // Russian Journal of Infection and Immunity. 2020; 10 (2): 210–220. doi: 10.15789/2220-7619-BIW-1453*
48. *Semenov A.V., Pshenichnaya N.Yu. Lessons learnt from the COVID-19 epidemic in Italy // Russian Journal of Infection and Immunity. 2020; 10 (3): 410–420. doi: 10.15789/2220-7619-LTL-1468*
49. *Shchemelev A.N., Ostankova Yu.V., Zueva E.B., Boumbaly S., Balde T.A., Semenov A.V. Characteristics of hepatitis B virus and human immunodeficiency virus among patients with HIV/HBV co-infection from the Republic of Guinea // Problems of Especially Dangerous Infections. 2019; (3): 118–124. doi: 10.21055/0370-1069-2019-3-118-124*
50. *Shchemelev A.N., Ostankova Yu.V., Zueva E.B., Huinh Hoang Khanh Thu, Semenov A.V. Features of HIV infection in the territory of the Socialist Republic of Vietnam. Current trends and prospects of Russian-Vietnamese cooperation in the field of ensuring sanitary and epidemiological safety: a joint monograph / Ed. by A.Yu. Popova; Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing. Volgograd: Volga-Press Publishing House, LLC, 2019: 326–334.*
51. *Shchemelev A.N., Ostankova Yu. V., Zueva E.B., Huynh Hoang Khanh Thu, Semenov A.V. Characteristics of HIV genotype and drug resistance in patients in the Socialist Republic of Vietnam // HIV Infection and Immunosuppressive Disorders. 2020; 12 (2): 56–68. doi: 10.22328/2077-9828-2020-12-2-56-68*
52. *Simakina O.E., Belyakov N.A., Rassokhin V.V., Khalezova N.B. Role of drug addiction in the spread and formation of the HIV epidemic // Marine Medicine. 2020; 6 (2): 7–24.*
53. *Simakina O.E., Voshcheva M.S., Ogurtsova S.V. The tenth anniversary of the journal HIV Infection and Immunosuppressive Disorders and the 10th Anniversary Conference // HIV Infection and Immunosuppressive Disorders. 2019; 11 (1): 109–111.*
54. *Trofimova T.N., Bakulina E.G., Rassokhin V.V., Azovtseva O.V., Belyakov N.A. Radiation semiotics of brain lesions in HIV infection, taking into account the immune status and antiretroviral therapy // Pacific Medical Journal. 2019; 3 (77): 60–66.*
55. *Usenko T.S., Nikolaev M.A., Miliukhina I.V., Bezrukova A.I., Senkevich K.A., Pchelina S.N., Timofeeva A.A., Petrova O.A., Semenov A.V., Totolian A.A., Gracheva E.V., Gomzyakova N.A., Beltceva Y.A., Zalutskaya N.M., Lubimova N.E. Plasma cytokine profile in synucleinophaties with dementia // Journal of Clinical Neuroscience. 2020; 78: 323–326. doi: 10.1016/j.jocn.2020.04.058*
56. *Vetrova M.V., Aleksandrova O.V., Paschenko A.E., Toropov S.E., Rassokhin V.V., Abyshev R.A., Levina O.S., Niccolai L.M., Heimer R. Physician and patient prediction of adherence to antiretroviral therapy in HIV positive people in Saint-Petersburg, Russia // AIDS Care. 2020. 33 (4): 473–477.*
57. *Yakovlev A., Sulima D., Larionov V., Koryagin V., Sharipova M., Fedunyak I., Musatov V., Kachenya G., Doguzhieva E., Sokolova O., Gorchakova O., Semenov A., Zueva E., Ostankova Yu., Prosvernitsyn S., Kiyashko S., Chornoguz Yu., Valutite D. Aviremic low-level replication of HCV RNA in PBMC/WBC immune blood cells (secondary occult HCV infection) as one of the results of primary interferon-free DAA therapy in real clinical setting in antiviral therapy naive patients with chronic RNA HCV viremia (description of a case series) // Doctor. 2020; 31 (2): 57–64. doi: 10.29296/25877305-2020-02-13*
58. *Yastrebova E.B., Samarina A.V., Fertikh E.K., Gutova L.V. Pediatric aspects of HIV infection and ways to deal with them in St. Petersburg // HIV Infection and Immunosuppressive Disorders. 2019; 11 (1): 31–37.*
59. *Zagdyn Z.M., Verbitskaya E.V., Sokolovich E.G., Belyakov N.A. Comprehensive assessment of the effectiveness of the system for countering the spread of HIV/tuberculosis in the Northwest of Russia // Tuberculosis and Lung Diseases. 2019; 97 (3): 6–15.*
60. *Zhdanov K.V., Semenov A.V., Karyakin S.S., Kozlov K.V., Sukachev V.S., Ostankova Yu.V., Valutite D.E., Zueva E.B., Sidorov R.S., Saulevich A.V., Bulankov Yu.I., Lyashenko Yu.I., Ivanov K.S. S MAdCAM-1 as an immunological marker in the intestine–liver system in overweight patients with chronic hepatitis C // Journal Infectology. 2019; 11 (2): 63–70. doi: 10.22625/2072-6732-2019-11-2-63-70*