



## ORIGINAL STUDY ARTICLE

DOI: <https://doi.org/10.36233/0507-4088-327>

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# Results of a blood serum examination of residents of Moscow and the Moscow region after the end of the West Nile fever outbreak in 2021

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## Abstract

The **aim** of this study was to determine the level of humoral immunity to the West Nile virus (WNV) in the Moscow region population after the end of the outbreak in October 2021, as well as to confirm the specificity of antibodies to WNV by comparatively testing patient sera for antibodies to the antigenically related endemic tick-borne encephalitis orthoflavivirus (TBEV) using ELISA-IgM, ELISA-IgG, and 50% plaque reduction neutralization test.

**Materials and methods.** We analyzed 1,594 sera from outpatients-residents of Moscow and the surrounding region—who underwent outpatient examination in the winter of 2021 at Infectious Diseases Clinical Hospital No. 1 in Moscow (IKB No. 1) and medical institutions of the Moscow Regional Research Institute named after M.F. Vladimirovsky Regional Research Institute (MONIKI) conducted a study without any connection to WNV infection in the summer-autumn of 2021.

**Results.** All samples were negative in ELISA-IgM test with WNV and TBEV antigens. In an ELISA-IgG test with the WNV antigen, antibodies were detected in 64 samples (4.0%). All samples were tested for IgG antibodies to TBEV in an ELISA and neutralizing antibodies to WNV and TBEV in a 50% plaque reduction neutralization test. Specific antibodies to WNV were detected in 44 samples (68.8%), to TBEV in eleven (17.2%), and group-specific antibodies in nine (14.0%). According to the total data from the test of 1,594 sera from residents of the Moscow region, specific antibodies to WNV were detected in 2.8% of cases, to TBEV in 0.7%, and group-specific antibodies in 0.6%. Ten of the 11 individuals with specific IgG antibodies to TBEV were undergoing outpatient examination at Moscow's Infectious Diseases Clinical Hospital No. 1 for a history of tick-borne encephalitis or for post-vaccination immunity testing following vaccination against this infection. The detection rate of specific antibodies to WNV in similar studies conducted in the same region in 2013 was 0.2%, while in 2021 it was 2.8%. The difference between these rates is statistically significant ( $p < 0.01$ ).

**Conclusion.** Based on these data, it can be concluded that sporadic undiagnosed cases of WNV infection occurred in Moscow and the surrounding region between 2013 and 2021.

**Keywords:** *West Nile fever; West Nile virus; tick-borne encephalitis virus; ELISA; neutralization test; retrospective serological examination of patients*

**For citation:** Butenko A.M., Kholodilov I.S., Dailak I.V., Baranets M.S., Karganova G.G., Bazarova M.V., Ivanova I.V., Barsukova N.A., Bogomolov P.O. Results of a blood serum examination of residents of Moscow and the Moscow region after the end of the West Nile fever outbreak in 2021. *Problems of Virology (Voprosy Virusologii)*. 2026; 71(1): 32–41. DOI: <https://doi.org/10.36233/0507-4088-327> EDN: <https://elibrary.ru/cpnsbc>

**Financing.** This study was not supported by any external sources of funding.

**Conflict of interest.** The authors declare no apparent or potential conflicts of interest related to the publication of this article.

**Ethics approval.** The study was conducted with the informed consent of the patients. The research protocol by the Ethics Committee of the The National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya of the Ministry of Health of the Russian Federation No. 2 dated 29 December 2021.

ОРИГИНАЛЬНЫЕ ИССЛЕДОВАНИЯ

DOI: <https://doi.org/10.36233/0507-4088-327>

## Результаты исследования сывороток крови жителей Москвы и Московской области после окончания вспышки лихорадки Западного Нила в 2021 году

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### Резюме

**Цель** работы заключалась в определении показателей иммунной прослойки к вирусу Западного Нила (ВЗН) у населения Московского региона после окончания вспышки этой инфекции в октябре 2021 г., а также подтверждении специфичности антител к ВЗН при сравнительном исследовании сывороток крови пациентов на антитела к антигенно родственному эндемичному ортофлави вирусу клещевого энцефалита (ВКЭ) методами иммуноферментного анализа (ИФА) для выявления антител класса IgM (ИФА-IgM), класса IgG (ИФА-IgG) и реакции нейтрализации.

**Материалы и методы.** Были исследованы 1594 сыворотки амбулаторных пациентов – жителей Москвы и Московской области, которые проходили в зимний период 2021 г. амбулаторное обследование в Инфекционной клинической больнице № 1 г. Москвы (ИКБ № 1) и лечебных учреждениях Московского областного научно-исследовательского института им. М.Ф. Владимирского (МОНИКИ) без какой-либо связи с заболеванием лихорадкой Западного Нила (ЛЗН) в летне-осенний период 2021 г.

**Результаты.** Во всех пробах результаты исследования методом ИФА-IgM с антигенами ВЗН и ВКЭ оказались отрицательными. В ИФА-IgG с антигеном ВЗН антитела были обнаружены в 64 пробах (4,0%). Все они были протестированы на антитела класса IgG к ВКЭ в ИФА и нейтрализующие антитела к ВЗН и ВКЭ в реакции нейтрализации. Специфичность антител к ВЗН установлена в 44 пробах (68,8%), к ВКЭ – в 11 (17,2%), группоспецифичность – в 9 (14,0%). По суммарным данным исследования 1594 сывороток жителей Московского региона специфические антитела к ВЗН были обнаружены в 2,8% случаев, к ВКЭ – в 0,7%, группоспецифические антитела – в 0,6%. Из 11 пациентов, имеющих специфические IgG к ВКЭ, 10 проходили амбулаторное обследование в ИКБ № 1 г. Москвы по поводу ранее перенесенного клещевого энцефалита или исследования поствакцинального иммунитета после вакцинации против этой инфекции. Частота обнаружения специфических антител к ВЗН при проведении аналогичных исследований в том же регионе в 2013 г. составляла 0,2%, в 2021 г. – 2,8%. Разница между этими показателями статистически достоверна ( $p < 0,01$ ).

**Заключение.** На основании полученных данных можно заключить, что в период с 2013 по 2021 г. в Москве и Московской области имели место спорадические недиагностированные случаи ЛЗН.

**Ключевые слова:** лихорадка Западного Нила; вирус Западного Нила; вирус клещевого энцефалита; ИФА; реакция нейтрализации; ретроспективное серологическое обследование пациентов

**Для цитирования:** Бутенко А.М., Холодильов И.С., Дайлак И.В., Баранец М.С., Карганова Г.Г., Базарова М.В., Иванова И.В., Барсукова Н.А., Богомолов П.О. Результаты исследования сывороток крови жителей Москвы и Московской области после окончания вспышки лихорадки Западного Нила в 2021 году. *Вопросы вирусологии*. 2026; 71(1): 32–41. DOI: <https://doi.org/10.36233/0507-4088-327> EDN: <https://elibrary.ru/cpnsbc>

**Финансирование.** Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

**Конфликт интересов.** Авторы заявляют об отсутствии конфликта интересов.

**Этическое утверждение.** Исследование проводилось при добровольном информированном согласии пациентов. Протокол локального Этического комитета №2 ФГБУ «НИЦЭМ им. Н.Ф. Гамалеи» МЗ РФ от 29.12.2021.

## Introduction

West Nile fever (WNF), associated with various genotypes of the West Nile virus (WNV), is endemic to all inhabited continents. In the USSR, the first WNV strains were isolated in 1963 from preimaginal *Hyalomma plumbeum* ticks (now known as *H. marginatum*) collected from rooks in the Astrakhan region [1]. In 1967–1970, they were isolated from two species of mosquitoes, two species of birds, and from the blood of a brown hare in the Astrakhan region [2]. Also in 1967, 11 cases of this infection were verified for the first time in the Astrakhan region [3], and three strains of WNV were isolated from hospitalized patients [2].

Until the end of the 1990s, cases of WNF were registered exclusively in the Astrakhan and Volgograd regions [4]. In 1999, according to Rospotrebnadzor, 560 cases were registered during an epidemic outbreak of WNF: 380 in the Volgograd region, 95 in the Astrakhan region, and retrospectively 85 cases in the Krasnodar region [5]. In 2000, the first cases of the disease outside the known endemic southern regions of the European part of Russia were registered in the Rostov region, and in 2006 – in the Ulyanovsk region [4]. By 2009, cases of WNF had been reported in four regions of the Russian Federation, while circulation of WNV had been recorded in 22 regions of the country [4].

According to Rospotrebnadzor, during the 2021 epidemic season, 72 cases of WNF were registered in nine regions of the Russian Federation: 26 in Moscow and the Moscow Region, 25 in the Voronezh Region, 13 in the Volgograd Region, 2 each in the Rostov Region and the Republic of Dagestan, one each in the Lipetsk and Tula Regions, the Krasnodar Territory, as well as the Republic of Crimea. Cases in the Moscow region were diagnosed for the first time in the history of registration in the USSR and Russia, which indicates the continuing expansion of the WNF area in the European part of the country. Cases of WNF in the Moscow region in 2021 were registered from mid-July to mid-October [6]. During the outbreak, two strains of WNV were isolated from a patient with WNF. Based on one of them, a candidate inactivated whole-virus vaccine against WNF was created [7]. In 2022, only one case of the disease was diagnosed.

This study used blood serum samples from outpatients, which were kindly provided in December 2021 by Moscow Infectious Diseases Hospital No. 1 (City Clinical Hospital No. 1) and the M.F. Vladimirsky Moscow Research Clinical Institute (MONIKI). Blood serum samples were taken in the winter of 2021 from patients with no connection to any possible previous case of WNF disease.

**Moral aim** of the study was to determine the level of humoral immunity to WNV in the population of the Moscow region after the end of the outbreak of this infection in October 2021, as well as to confirm the specificity of antibodies to WNV in a comparative examination of patients' blood sera for antibodies to the antigenically related endemic tick-borne encephalitis orthoflavivirus (TBEV) using enzyme-linked immunosorbent assay (ELISA) meth-

ods to detect IgM antibodies (ELISA-IgM), IgG antibodies (ELISA-IgG) and neutralization reaction.

## Materials and methods

### *Virus strains used for antigen preparation and neutralization reaction*

- TBEV – strain MOS-152-N-2017 (European subtype), isolated from nymphs of *Ixodes ricinus* ticks collected in Moscow in 2017 (GenBank numbers OQ673267 and MN663429) [8];
- TBEV – strain 4072 (Siberian subtype) from the collection of the Laboratory of Biology and Indication of Arthropod-Borne Viruses of the N.F. Gamaleya National Research Center for Epidemiology and Microbiology;
- WNV – strain SHUA-1 (genotype 2), isolated from the saliva of a patient with WNF (Moscow Region, 2021) (GenBank number PQ679039) [7];
- WNV – strain Hp-94 (genotype 1a), isolated from *Hyalomma marginatum* ticks collected in the Astrakhan Region in 1963 (GenBank number JX041633) [1];
- WNV – strain Ast-986 (genotype 1a), isolated from the blood of a patient with WNF (Astrakhan Region, 1999) (GenBank number JX041634) [9].

### *Blood serum*

In this study, blood serum samples were collected from 1,594 outpatients residing in the Moscow region during the winter of 2021 by employees of Moscow Clinical Hospital No. 1 and the M.F. Vladimirsky Research Institute of Clinical Pathology and Immunology. The patients examined did not show any signs of WNF disease in the summer-autumn period of the same year.

### *Enzyme-Linked Immunosorbent Assay (ELISA)*

To perform ELISA for the detection of IgM and IgG antibodies to WNV, sucrose-acetone antigens of WNV (strain Ast-986) and TBEV (strain 4072) prepared from the brains of infected newborn white mice were used [10].

The ELISA-IgG method was performed according to the description by J.M. Meegan and L.W. Le Duc [11].

The ELISA-IgM (MAC-ELISA) method corresponded to the technique described in a study by C.H. Calisher et al. [12].

### *Neutralization reaction*

The detection of antibodies to WNV and TBEV was carried out by a neutralization reaction, based on the reduction of 50% of plaques in a monolayer culture of SPEV cells (porcine embryonic kidney cell culture) under an agar cover. To serial 4-fold dilutions of sera in medium 199 on Earl's solution (M.P. Chumakov Federal Research Center for Infectious Diseases and Vaccines, Russian Academy of Sciences), equal volumes of virus suspensions at a concentration of approximately 40 PFU/0.2 mL were added. The virus titer was calculated in plaque-forming units (PFU). The virus-serum dilutions were incubated for 60 min at 37 °C, after which 100 µL was added to 24-well panels with a monolayer of SPEV cells and incubated for 60 min at 37°C. After incubation, the cells were covered with a 1.26%

solution of methylcellulose in medium 199 on a solution of Hanks (M.P. Chumakov Federal Research Center for Infectious and Parasitic Diseases, Russian Academy of Sciences) and Earl (2 : 1) with the addition of streptomycin and penicillin (PanEco, Russia) and 5% fetal calf serum (Gibco, Invitrogen, USA) and incubated in a CO<sub>2</sub> atmosphere at 37 °C. After 6 days, the coating was removed, the cells were washed with medium 199 in Earl's solution and fixed with 96% ethanol. Staining was performed with a 0.4% solution of gentian violet in 10% ethanol. After drying the plates, the number of plaques was counted. Serums that neutralized at least 50% of the plaques compared to the negative control were considered positive. The antibody titer was calculated using the Reed and Mench method [13].

### Results

In the first stage of the study, using the ELISA-IgG method, where the Ast-986 strain was used as an antigen, 967 blood sera samples from outpatients obtained from the Moscow City Clinical Hospital No. 1 were examined. The results were positive in 40 cases (4.1%). The antibody titers were: 1 : 100 (7 samples), 1 : 200 (8 samples), 1 : 400 (6 samples), 1 : 800 (10 samples), 1 : 1,600 (3 samples), and 1 : ≥ 3,200 (6 samples).

Among 627 patients undergoing outpatient examination at the M.F. Vladimirsky MONIKI, positive results were observed in 24 (3.8%). Antibody titers were 1 : 100 (3 samples), 1 : 200 (5 samples), 1 : 400 (2 samples), 1 : 800 (4 samples), 1 : 1600 (1 sample), 1 : 3,200 (3 samples), and 1 : ≥ 6,400 (6 samples).

Overall, according to the results of the ELISA-IgG test with the WNV antigen of 1,594 blood serum samples from residents of Moscow and the Moscow region at the end of 2021, 64 (4.0%) were positive, with no IgM antibodies present.

In order to confirm the specificity of the antibodies detected in 64 positive samples in the ELISA-IgG with the WNV antigen, these samples were tested using an ELISA-IgG with TBEV antigen (Siberian subtype) and neutralization reactions with WNV (genotype 2 and partially genotype 1a) and TBEV (European subtype).

The specificity of antibodies to WNV and TBEV was determined according to the criteria adopted in serodiagnosis of antigenically related orthoflavivirus infections: 1) absence of IgG and neutralizing antibodies to one of these agents; 2) 4-fold or greater predominance of homologous antibody titers; 3) equal titers of homologous and heterologous antibodies. In the latter case, the presence of group-specific antibodies could be associated with previous vaccination of patients against tick-borne encephalitis, yellow fever, Japanese encephalitis, dengue fever, or previous infections associated with related orthoflaviviruses (e.g., dengue, Zika, Usutu, Omsk hemorrhagic fever, Powassan, etc.)

When analyzing the results of testing 40 blood sera from the City Clinical Hospital No. 1 in Moscow using ELISA-IgG methods with antibodies to WNV and TBEV and neutralization reactions with these viruses, WNV-specific antibodies were detected in 24 sera (60.0%),

and TBEV-specific antibodies were detected in 10 sera (25.0%); group-specific antibodies were detected in 6 sera (15.0%) (**Table 1**).

Among 24 sera obtained from the M.F. Vladimirsky MONIKI, antibodies specific to WNV were detected in 20 sera (83.3%), to TBEV in one serum (4.2%), and group-specific antibodies in three (**Table 2**).

In total, out of 64 sera positive in ELISA for IgG antibodies with WNV antigen, obtained from two medical institutions, specific antibodies to WNV were detected in ELISA and neutralization reactions in 44 sera (68.8%), in 11 (17.2%) – to TBEV, and in 9 cases (14.0%) – group-specific antibodies. Based on the results of testing 1,594 sera using ELISA-IgG and neutralization reactions, specific antibodies to WNV were detected in the population of the Moscow region in 2.8% of cases, to TBEV in 0.7%, and group-specific antibodies in 0.6%.

### Discussion

WNF is a viral zoonotic disease with a vector-borne transmission, in most cases asymptomatic and only in 1% of cases manifesting as an acute febrile illness with symptoms of general intoxication, in some cases leading to damage to the central nervous system with the development of meningitis, encephalitis, or acute flaccid paralysis [14].

In the Moscow region, WNF disease began to be registered in 2013 in the form of sporadic cases [15]. In 2021, 27 cases of WNF were registered for the first time in Moscow against the backdrop of extremely intense epizootics among birds, accompanied by their mass death [16]. At the same time, the number of infected people could be close to 7,000, since it has been previously shown that for every one neuroinvasive case of WNF, there are 256 asymptomatic cases [17].

This paper presents the results of a study of 1,594 blood serum samples from patients in Moscow and the Moscow region with no history of WNF in their medical records, obtained three months after the end of the WNF outbreak in the Moscow region in the summer of 2021. The objective of this study was to conduct a serological examination of this population cohort using ELISA-IgG, ELISA-IgM, and neutralization tests to determine the indicators of humoral immunity to WNV during the 2021 epidemic season or previous years and to confirm the specificity of antibodies to another antigenically related virus, TBEV. According to the summary data of the examination of 1,594 sera, specific antibodies to WNV were detected in 2.8% of cases, to TBEV in 0.7%, and group-specific antibodies in 0.6%. Ten patients with specific antibodies to TBEV underwent outpatient examination at the First Clinical Hospital in Moscow for tick-borne encephalitis or post-vaccination immunity testing after vaccination against this infection. The frequency of detection of specific antibodies to WNV in similar studies in the same region in 2013 was 0.2% [18], and in 2021 – 2.8%. The difference between these indicators is statistically significant ( $p < 0.01$ ). Based on these data, it can be concluded that between 2013 and 2021, there were sporadic undiagnosed cases of WNF in Moscow and the

**Table 1.** Results of examination of blood serum of outpatients without a history of West Nile fever from the City Clinical Hospital No. 1 of Moscow using ELISA-IgG and 50% plaque reduction neutralization test for the presence of antibodies to West Nile virus and tick-borne encephalitis virus

**Таблица 1.** Результаты исследования сывороток крови амбулаторных пациентов (без ЛЗН в анамнезе) из ИКБ № 1 г. Москвы в ИФА-IgG и реакции нейтрализации на наличие антител к вирусам Западного Нила и клещевого энцефалита

No П/п	Sample number № проб	Antibodies to viruses Антитела к вирусам					Specificity of antibodies to the virus Специфичность антител к вирусу
		West Nile Западного Нила			tick-borne encephalitis клещевого энцефалита		
		lineage 1a (AST.986) генотип 1a (ACT.986)	lineage 1a (Hr-94) генотип 1a (Hr-94)	lineage 2 (SHUA-1) генотип 2 (SHUA-1)	subtype Sib. (4072) подтип Сиб. (4072)	subtype Europ. (MOS-152-N-2017) подтип Европ. (MOS-152-N-2017)	
		IgG	PH	PH	IgG	PH	
1	19	1 : 100	–	Negative Отр.	1 : 800	1 : 139	TBEV БКЭ
2	29	1 : 200	–	Negative Отр.	1 : 400	Negative Отр.	Group-specific Группоспец.
3	53	> 1 : 3200	–	1 : 104	1 : 400	1 : 64	WNV ВЗН
4	56	1 : 400	–	1 : 27	Отр. Negative	Negative Отр.	WNV ВЗН
5	57	> 1 : 3200	–	1 : 104	1 : 200	Negative Отр.	WNV ВЗН
6	60	1 : 200	–	Negative Отр.	1 : 800	> 1 : 640	TBEV БКЭ
7	72	1 : 200	–	Negative Отр.	1 : 100	Negative Отр.	Group-specific Группоспец.
8	111	1 : 100	–	Negative Отр.	1 : 800	1 : 93	TBEV БКЭ
9	188	1 : 100	–	Negative Отр.	1 : 200	1 : 116	TBEV БКЭ
10	194	1 : 400	–	Negative Отр.	1 : 800	Negative Отр.	Group-specific Группоспец.
11	215	1 : 800	–	1 : 55	Отр. Negative	Negative Отр.	WNV ВЗН
12	221	> 1 : 3200	–	> 1 : 640	1 : 100	Negative Отр.	WNV ВЗН
13	241	1 : 400	–	Negative Отр.	> 1 : 3200	1 : 309	TBEV БКЭ
14	253	1 : 1600	–	> 1 : 640	1 : 100	Negative Отр.	WNV ВЗН
15	288	> 1 : 3200	–	1 : 302	Отр. Negative	Negative Отр.	WNV ВЗН
16	331	1 : 200	1 : 93	Negative Отр.	Отр. Negative	1 : 30	WNV ВЗН
17	496	1 : 200	–	Negative Отр.	1 : 100	Negative Отр.	Group-specific Группоспец.
18	514	1 : 200	–	Negative Отр.	1 : 100	Negative Отр.	Group-specific Группоспец.
19	525	1 : 1600	–	1 : 640	1 : 200	1 : 40	WNV ВЗН
20	533	1 : 800	–	1 : 55	Negative Отр.	Negative Отр.	WNV ВЗН
21	566	1 : 100	–	Negative Отр.	1 : 400	1 : 79	TBEV БКЭ
22	573	1 : 800	1 : 24	Negative Отр.	1 : 3200	> 1 : 640	TBEV БКЭ

Продолжение табл. 1 см. на стр. 37.

No П/п	Sample number № проб	Antibodies to viruses Антитела к вирусам					Specificity of antibodies to the virus Специфичность антител к вирусу
		West Nile Западного Нила			tick-borne encephalitis клещевого энцефалита		
		lineage 1a (AST.986) генотип 1a (ACT.986)	lineage 1a (Hr-94) генотип 1a (Hr-94)	lineage 2 (SHUA-1) генотип 2 (SHUA-1)	subtype Sib. (4072) подтип Сиб. (4072)	subtype Europ. (MOS-152-N-2017) подтип Европ. (MOS-152-N-2017)	
		IgG	PH	PH	IgG	PH	
23	605	1 : 200	–	1 : 290	Negative Отр.	Negative Отр.	WNV ВЗН
24	616	1 : 800	–	1 : 182	1 : 100	Negative Отр.	WNV ВЗН
25	637	1 : 800	–	1 : 640	1 : 100	Negative Отр.	WNV ВЗН
26	642	1 : 800	–	1 : 275	1 : 100	Negative Отр.	WNV ВЗН
27	651	1 : 800	–	1 : 30	1 : 100	Negative Отр.	WNV ВЗН
28	690	1 : 100	–	Negative Отр.	1 : 100	Negative Отр.	Group-spec. Группоспец.
29	697	1 : 400	–	1 : 53	1 : 100	Negative Отр.	WNV ВЗН
30	698	1 : 3200	–	1 : 363	1 : 100	Отр Negative	WNV ВЗН
31	701	1 : 200	–	Negative Отр.	Negative Отр.	Отр Negative	WNV ВЗН
32	723	1 : 400	–	Negative Отр.	Negative Отр.	Отр Negative	WNV ВЗН
33	788	1 : 100	–	Negative Отр.	1 : 800	1 : 269	TBEV БКЭ
34	795	1 : 800	–	1 : 371	1 : 100	Negative Отр.	WNV ВЗН
35	801	1 : 3200	–	1 : 333	1 : 200	Отр Negative	WNV ВЗН
36	807	1 : 800	–	1 : 87	1 : 200	Negative Отр.	WNV ВЗН
37	896	1 : 100	–	Negative Отр.	1 : 400	1 : 93	TBEV БКЭ
38	906	1 : 1600	1 : 87	Negative Отр.	> 1 : 3200	1 : 389	TBEV БКЭ
39	916	1 : 800	–	1 : 344	1 : 100	Negative Отр.	WNV ВЗН
40	951	1 : 400	–	1 : 429	1 : 100	Negative Отр.	WNV ВЗН

**Note.** NR – neutralization reaction; TBEV – antibodies against tick-borne encephalitis virus; WNV – antibodies against West Nile virus; group-spec. – antibodies against both TBEV and WNV simultaneously; negative – negative result.

**Примечание.** PH – реакция нейтрализации; БКЭ – антитела против вируса клещевого энцефалита; ВЗН – антитела против вируса Западного Нила; группоспец. – антитела против БКЭ и ВЗН одновременно; отр. – отрицательный результат.

Moscow region.

Among the sera studied, three samples were found to have antibodies to WNV-1a and no antibodies WNV-2, based on the results of the neutralization reaction. These data show that the neutralization reaction allows us to differentiate antibodies to different WNV lineages, while ELISA can detect antibodies but cannot differentiate them. Based on the ELISA alone, these three patients would be

classified as infected in the Moscow metropolitan area, while the neutralization test results indicate that the infection most likely occurred in another region of Russia, or that not only WNV-2 but also WNV-1a is circulating in Moscow and the Moscow region.

According to a survey of 163 practically healthy residents of Astrakhan in 1999 during an epidemic outbreak of WNF, 5 of them were found to have IgM

**Table 2.** Results of examination of blood serum of outpatients without a history of West Nile fever from Moscow Regional Research and Clinical Institute (MONIKI) using ELISA-IgG and 50% plaque reduction neutralization test for the presence of antibodies to West Nile virus and tick-borne encephalitis virus

**Таблица 2.** Результаты исследования сывороток крови амбулаторных пациентов (без ЛЗН в анамнезе) из МОНИКИ им. М.Ф. Владимирского в ИФА-IgG и реакции нейтрализации на наличие антител к вирусам Западного Нила и клещевого энцефалита

No П/п	Sample number № проб	Antibodies to viruses Антитела к вирусам					Specificity of antibodies to the virus Специфичность антител к вирусу
		West Nile virus Западного Нила			tick-borne encephalitis virus Клещевого энцефалита		
		lineage 1a (AST.986) генотип 1a (ACT.986)	lineage 1a (Hr-94) генотип 1a (Hr-94)	lineage 2 (SHUA-1) генотип 2 (SHUA-1)	subtype Sib. (4072) подтип Сиб. (4072)	subtype Europ. (MOS-152-N-2017) подтип Европ. (MOS-152-N-2017)	
		IgG	PH	PH	IgG	PH	
1	1/12	> 1 : 6400	–	1 : 332	1 : 400	Negative Отр.	WNV ВЗН
2	1/25	1 : 800	–	> 1 : 640	1 : 200	Negative Отр.	WNV ВЗН
3	1/36	1 : 6400	–	> 1 : 640	1 : 100	1 : 39	WNV ВЗН
4	1/39	1 : 6400	–	1 : 43	Negative Отр.	1 : 10	WNV ВЗН
5	1/57	1 : 800	–	1 : 331	1 : 200	1 : 21	WNV ВЗН
6	1/83	1 : 6400	–	1 : 501	1 : 200	Negative Отр.	WNV ВЗН
7	1/94	1 : 3200	–	> 1 : 640	1 : 100	Negative Отр.	WNV ВЗН
8	1/151	1 : 3200	–	1 : 251	1 : 800	Negative Отр.	WNV ВЗН
9	1/153	1 : 200	–	1 : 138	Negative Отр.	Negative Отр.	WNV ВЗН
10	1/167	1 : 400	–	1 : 47	Negative Отр.	Negative Отр.	WNV ВЗН
11	1/222	1 : 400	–	Negative Отр.	1 : 100	Negative Отр.	WNV ВЗН
12	1/238	1 : 1600	–	> 1 : 640	1 : 400	Negative Отр.	WNV ВЗН
13	1/250	1 : 200	–	Negative Отр.	1 : 100	Negative Отр.	Group-spec. Группоспец.
14	1/262	1 : 6400	–	1 : 98	1 : 200	Negative Отр.	WNV ВЗН
15	1/284	1 : 800	–	1 : 17	1 : 100	1 : 12	Group-spec. Группоспец.
16	1/317	1 : 6400	–	1 : 195	1 : 400	Negative Отр.	WNV ВЗН
17	1/318	1 : 200	–	1 : 93	1 : 100	Negative Отр.	WNV ВЗН
18	1/378	1 : 3200	–	1 : 87	1 : 200	Negative Отр.	WNV ВЗН
19	2/28	1 : 100	–	Negative Отр.	1 : 800	1 : 320	TBEV ВКЭ
20	5/122	1 : 100	–	Negative Отр.	Negative Отр.	Negative Отр.	WNV ВЗН
21	6/6	1 : 200	1 : 21	1 : 23	Negative Отр.	1 : 18	Group-spec. Группоспец.
22	6/11	1 : 100	–	1 : 31	Negative Отр.	1 : 15	WNV ВЗН
23	6/15	1 : 200	–	1 : 20	Negative Отр.	Negative Отр.	ВЗН WNV
24	6/16	1 : 800	–	1 : 27	Negative Отр.	Negative Отр.	ВЗН WNV

**Note.** NR – neutralization reaction; TBEV – antibodies against tick-borne encephalitis virus; WNV – antibodies against West Nile virus; group-spec. – antibodies against both TBEV and WNV; negative – negative result.

**Примечание.** PH – реакция нейтрализации; ВКЭ – антитела против вируса клещевого энцефалита; ВЗН – антитела против вируса Западного Нила, группоспец. – антитела против ВКЭ и ВЗН одновременно; отр. – отрицательный результат.

antibodies to WNV, which indicated a recent inapparent form of WNF. A comparison of these results with the relative incidence rates per 100,000 population of the city and region (12.2%) allowed us to determine the approximate ratio of manifest and inapparent cases of WNF in 1999 as 1 : 300 [19].

A study by M. Busch et al. [17] showed that in the United States, there were 256 asymptomatic cases for every one neuroinvasive case of WNF. Given these similar figures and the number of registered cases of WNF in 2021 in the Moscow region, it can be estimated that the number of people infected during the WNF epidemic outbreak was in the range of approximately 6,700–7,800.

The question remains open as to what extent the outbreak was caused by the introduction of the virus by birds and favorable weather conditions or by the activation of a long-standing source of infection. In any case, close monitoring of the situation is required, as the example of New York shows the possible consequences of the virus entering a metropolis and forming an urban source of infection.

### Conclusion

Based on the data obtained, it can be assumed that sporadic cases of WNF occurred in the Moscow region, but they remained undiagnosed. In the differential diagnosis of orthoflavivirus infection, the application of ELISA alone may not be sufficient for a correct diagnosis.

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**Contribution:** Butenko A.M. – study concept, experiments, data interpretation, preparation of the article text for publication; Kholodilov I.S. – experiments, data interpretation, preparation of the article text; Dailak I.V. – experiments; Baranets M.S. – experiments; Karganova G.G. – data interpretation, preparation of the article text for publication; Bazarova M.V. – data collection; Ivanova I. V. – data collection; Barsukova N.A. – data collection; Bogomolov P.O. – data collection.

Received 12 November 2025

Accepted 09 January 2026

Published 28 February 2026

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Поступила 12.11.2025

Принята в печать 09.01.2026

Опубликована 28.02.2026