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Results of reconnaissance epizootiological monitoring for West Nile fever in certain regions of European Russia and the Urals in 2024

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Abstract

Introduction. Climate warming contributes to the intensification of epizootic and epidemic processes of West Nile fever (WNF). In southern Russia, the activity of the epizootic process is recorded annually, but in the central region of the European part of the country and in the Urals, the enzootic circulation of the West Nile virus (WNV) has not been confirmed in the territory of 20 subjects.

The aim of the study is to investigate zoological and entomological material for WNV infection to confirm the ongoing epizootic process in old WNV foci and in previously non-endemic areas.

Materials and methods. Field samples were collected in 2024 in 19 subjects in accordance with the methods regulated in normative documents. The material was studied using the reverse transcription polymerase chain reaction method.

Results. In total, during the 2024 field season, 5,419 samples of field samples were examined: 684 samples of birds from 74 species, 455 samples of small mammals from 13 species, 45 samples of frogs from 1 species, 3,665 samples of blood-sucking mosquitoes from 33 species (93,438 specimens), and 570 samples of ixodid ticks from 17 species (4,809 specimens). Markers of WNV in field samples were detected in 7 subjects from 3 federal districts. In the Kirov and Chelyabinsk regions and the Republic of Mordovia, evidence of the ongoing epizootic process of WNF has been obtained for the first time. WNV RNA was detected in 6 (0.5%) out of 1184 tested samples of vertebrate animals and in 27 (0.6%) out of 4235 samples of arthropods. The level of individual infection was 0.03% in blood-sucking mosquitoes, 0.06% in ixodid ticks, and 0.9% in birds.

Conclusion. The results of the studies confirm the enzootic circulation of WNV in the territories of the Southern, Volga and Ural Federal Districts.

Keywords: *West Nile fever, West Nile virus, epizootic process, infection, natural reservoir, vectors*

Ethics approval. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July, 2010). The study was approved by the Bioethics Committee of the Volgograd Plague Control Research Institute (Protocol No. 1 dated January 14, 2024).

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ORIGINAL RESEARCHES

Оригинальное исследование
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Итоги рекогносцировочного эпизоотологического мониторинга лихорадки Западного Нила на отдельных территориях европейской части России и Урала в 2024 году

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Аннотация

Актуальность. Потепление климата способствует интенсификации эпизоотического и эпидемического процессов лихорадки Западного Нила (ЛЗН). На юге России активность эпизоотического процесса регистрируют ежегодно, но в центральном регионе европейской части страны и на Урале энзоотическая циркуляция вируса Западного Нила (ВЗН) не подтверждена на территории 20 субъектов.

Цель работы — исследовать зоолого-энтомологический материал на инфицированность ВЗН для подтверждения течения эпизоотического процесса в «старых» очагах ЛЗН и на ранее неэндемичных территориях.

Материалы и методы. Полевой материал собирали в 2024 г. в 19 субъектах в соответствии с регламентированными в нормативных документах методами. Исследование материала проводили методом полимеразной цепной реакции с обратной транскрипцией.

Результаты. Всего в полевой сезон 2024 г. исследовано 5419 проб полевого материала: 684 пробы птиц 74 видов, 455 проб мелких млекопитающих 13 видов, 45 проб лягушек 1 вида, 3665 проб кровососущих комаров 33 видов (93 438 экземпляров), 570 проб иксодовых клещей 17 видов (4809 экземпляров). Маркеры ВЗН в полевом материале обнаружены в 7 субъектах из 3 федеральных округов. В Кировской, Челябинской областях и Республике Мордовия доказательства течения эпизоотического процесса ЛЗН получены впервые. РНК ВЗН выявлена в 6 (0,5%) из 1184 исследованных проб позвоночных животных и в 27 (0,6%) из 4235 проб членистоногих. Уровень индивидуальной заражённости кровососущих комаров составил 0,03%, иксодовых клещей — 0,06%, птиц — 0,9%.

Выводы. Результаты исследований подтверждают энзоотичную циркуляцию ВЗН на территории Южного, Приволжского и Уральского федеральных округов.

Ключевые слова: *лихорадка Западного Нила, вирус Западного Нила, эпизоотический процесс, инфицированность, природный резервуар, переносчики*

Этическое утверждение. Авторы подтверждают соблюдение институциональных и национальных стандартов по использованию лабораторных животных в соответствии с «Consensus Author Guidelines for Animal Use» (IAVES, 23.07.2010). Исследование одобрено комитетом по биоэтике Волгоградского научно-исследовательского противочумного института Роспотребнадзора (протокол № 1 от 14.01.2024).

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Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

West Nile fever (WNF) is an enzootic, natural focal, vector-borne infectious disease caused by the West Nile virus (WNV) from the genus *Orthoflavivirus*. Certain bird species are the reservoir host of the pathogen, while the vectors are blood-sucking mosquitoes [1].

Climate warming over the past decades has contributed to the transformation of many ecosystems on the planet, leading to changes in the natural habitats of various animal species, including reservoirs and vectors of zoonotic infections. The increase in temperature leads to an increase in the replication rate of the pathogen, an extension of the stay of migratory birds in nesting areas, as well as accelerated development, extended activity periods, and the expansion of the ranges of blood-sucking mosquito vectors [2, 3]. All of the mentioned contributes to the intensification of epizootic and epidemic processes of WNF in natural focal areas and the spread of WNV.

Targeted monitoring for the infection rates in reservoir and vector populations makes it possible to identify signs of the activation of the epizootic process, promptly carry out measures to reduce vector populations, and inform the public about the necessity to use individual and collective protection measures against mosquito bites. Moreover, effective monitoring allows for dynamic observations of the activity of natural foci of zoonotic infections.

At the beginning of the study of WNF, it was commonly accepted that the range of WNV was limited to the territories of the equatorial, subequatorial, tropical, subtropical and southern parts of the temperate climate zones. On the territory of the former USSR, it covered the southern part of the European part of Russia, Belarus, Moldova, Ukraine, Azerbaijan, Georgia, Tajikistan, Kyrgyzstan, Kazakhstan and Turkmenistan. For the first time in the USSR, the virus was isolated in 1963 from *Hyalomma plumbeum* ixodid ticks (now *Hyalomma marginatum*) in the Astrakhan region, as well as from a sandpiper and a blackbird from Azerbaijan [4]. In the 1980s, the pathogen was detected in regions located significantly further north: in rooks and nidicolous birds from their nests in the Omsk region, in nidicolous birds from the Novosibirsk region, and in *Aedes vexans* mosquitoes from the Republic of Tatarstan. During the WNF outbreak in Moscow in 2021, the WNV RNA was detected in 14.2% of samples from the total number of examined blood-sucking mosquitoes, 68.0% of dead birds, and 32.0% of live birds [7]. The obtained data indicated a broader territorial spread of the WNF pathogen than was previously accepted [8]. As of early 2024, markers of WNF have been identified in samples from carriers and vectors in 52 entities across the territory of the Russian Federation. In southern Russia, the activity of the epizootic process is recorded annually. At the same time, the absence of positive findings in certain regions of the central part of European Russia and in the Urals draws attention — endemic circulation of WNV has not been confirmed in 10 entities of the Central Federal District (CFD), 6 in the Volga Federal District (VFD) and 4 in the Ural Federal District (UFD). In certain non-endemic territories, local cases of human WNF have been identified, indicating the presence of foci of this arboviral infection. Therefore, conducting active reconnaissance surveys aimed at clarifying the nosoareal is relevant.

The aim of this study is to investigate the zoological-entomological material for the presence of WNF to confirm the ongoing epizootic process in old foci of WNF and in previously non-endemic areas.

Materials and methods

The collection of field samples for research at the Reference Center for monitoring the WNF pathogen in the 2024 season was carried out from April to November in 19 entities of the Russian Federation (**Figure**) by employees of the Volgograd Research Anti-Plague Institute of Rospotrebnadzor, as well as by anti-plague institutions and the Centers for Hygiene and Epidemiology in the entities of the Russian Federation.

The capture of small mammals was carried out with the help of snap traps. Birds were hunted by employees of hunting farms through shooting, and the collection of fallen individuals was carried out by staff of zoological groups and researchers. Sampling of blood-sucking mosquitoes in open biotopes in household plots, along water bodies, in cemeteries, and in forests was carried out using automatic traps such as BG-sentinel-2 (Biogents AG), LovKom (ProTechnoSystems), Mosquito Magnet Executive (Woodstream), Black Kill M3000 (Black Kill), and an entomological net. Mosquitoes were captured in enclosed biotopes (chicken coops, pigsties, basements of multi-story buildings) with the help of battery-operated vacuum cleaners (BLV 18-200, Karcher and Tefal X-PERT 3.60 Versatile Handstick TY6975WO, Tefal) and exhausters. The collection of ixodid ticks was carried out using classical methods: in nature — with an entomological flag on vegetation, in populated areas — from animals (small and large livestock, dogs, cats).

The collected arthropods were delivered to the laboratory in thermal containers with cold packs, identified using the Stemi 2000C (Karl Zeiss) and MSP-1 (LOMO) stereomicroscopes on a cooled surface to the species level according to standard keys [9–12]. They were placed in 2 mL cryotubes.

Field samples were transported on dry ice or in car refrigerators at –20°C. For the analysis of climatic factors, data from the Federal Service for Hydrometeo-

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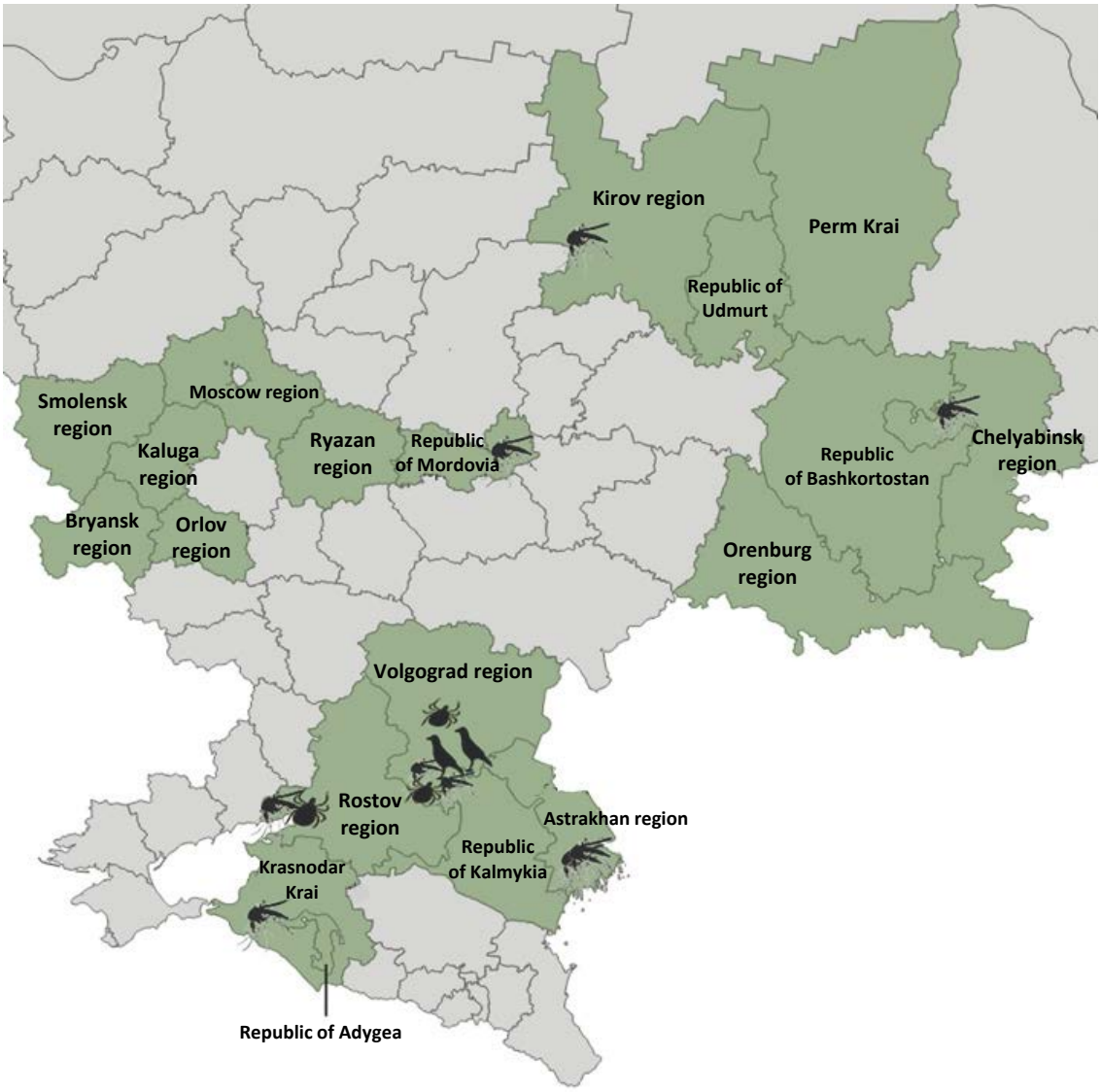

Entities where zoological and entomological material sampling was conducted in 2024, and points of positive WNV RNA findings from birds and arthropods.

rology and Environmental Monitoring of the Russian Federation were used[1].

Field samples were examined using the reverse transcription polymerase chain reaction method at the stationary laboratory of the Volgograd Research Anti-Plague Institute of Rospotrebnadzor. For the extraction of WNV RNA, suspensions of blood-sucking arthropods, as well as organs from birds, small mammals, and frogs (in a pooled sample from each individual – brain, kidneys, spleen) were prepared. The detection of WNV RNA was carried out using the AmpliSens WNV-FL reagent kit (Central Research Institute of Epidemiology of Rospotrebnadzor) according to the manufacturer's instructions. The determination of the WNV lineage in positive samples was carried out using the Ampligen-WNV-genotype-1/2/4 reagent kit (Volgograd Research Anti-Plague Institute of Rospotrebnadzor).

The infection rate of vertebrates was determined by calculating the proportion of positive samples from the total number of samples examined (%), and the individual infection rate of arthropods was calculated using the formula by V.N. Beklemishev [13]. Statistical processing of the materials and calculations were carried out using the Microsoft Excel program.

Results

A total of 5,419 field samples were examined during the 2024 field season: 684 bird samples from 74 species, 455 small mammal samples from 13 species, 45 frog samples from 1 species, 3,665 blood-sucking

[1] Newsletter of the Federal Service for Hydrometeorology and Environmental Monitoring: overview of the state and trends of climate change in Russia 2024 (December 2023 — November 2024). URL: http://downloads.igce.ru/climate_change_2/monitoring-klimata/Russia/2024/2024.pdf (In Russ.)

Table 1. Amount of field samples collected in 2024

Entity	Birds (specimens)	Small mammals (specimens)	Mosquitos		Ixodid ticks	
			specimens	samples	specimens	samples
Astrakhan region	–	–	7.231	254	559	81
Volgograd region	332	–	11.355	423	350	75
Rostov region	–	–	3.189	124	5	2
Republic of Kalmykia	–	–	598	25	–	
Republic of Adygea	7	9	2.926	112	370	67
Krasnodar Krai	1	–	9.736	341	–	–
Chelyabinsk region	–	–	2.430	97	–	–
Orenburg region	20	35	8.855	312	230	23
Republic of Bashkortostan	5	30	4.102	158	400	40
Perm Krai	24	54	3.034	142	779	70
Kirov region	–	75	7.067	276	200	21
Republic of Mordovia	44	60	5.252	254	109	24
Republic of Udmurt	23	73	2.995	120	222	20
Bryansk region	166	–	2.493	97	–	–
Smolensk region	4	35	5.650	214	400	20
Orlov region	20	15	2.736	117	310	33
Moscow region	17	18	4.054	168	57	8
Kaluga region	21	16	3.941	180	403	43
Ryazan region	0	35	5.794	251	415	43
Total	684	455	93.438	3665	4809	570

mosquito samples from 33 species (93,438 specimens), and 570 ixodid tick samples from 17 species (4,809 specimens). **Table 1** presents the amount of birds, small mammals, mosquitoes and ixodid ticks in each entity. In the Volgograd region, 45 frogs were also collected for the study.

The species composition, amount of samples and results of the studies are presented in **Table 2** and **Table 3**.

Markers of the WNF pathogen were found in field samples in 7 entities from 3 federal districts (figure), including in 3 entities (Kirov, Chelyabinsk regions and the Republic of Mordovia), where evidence of the epizootic process of WNF was obtained for the first time. In the specified territories, local cases of WNF were registered in the Chelyabinsk region in 2010 and 2011 [14], however, from 2010 to 2023, WNV markers were not detected in field samples.

WNV RNA was detected in 6 (0.5%) out of 1184 tested samples of vertebrate animals and in 27 (0.6%) out of 4235 samples of arthropods. In 1 sample, the RNA was typed as lineage 1 (Rostov region), in 25 samples as lineage 2 (Rostov, Volgograd, Kirov, Chelyabinsk, Astrakhan regions, Republic of Mordovia, Krasnodar Krai), and in 6 samples as lineage 4 (Volgograd region).

The overall level of individual infection rate (infection of each individual) among blood-sucking mosquitoes was 0.03%, among ixodid ticks it was 0.06%, and among birds it was 0.9%. When examining samples from small mammals and frogs, no markers of WNV were found.

From the CFD with negative results, 228 birds, 119 small mammals, 1,027 mosquito samples and 147 ixodid tick samples were examined.

The largest number of samples from the total number examined came from the Southern Federal District (SFD) and the VFD. From the SFD, 340 birds, 9 small mammals, 45 frogs, 1279 mosquito samples, and 225 ixodid tick samples were tested. WNV RNA was detected in 20 samples of mosquitoes (individual infection rate was 0.06%), 3 samples of ixodid ticks (0.24%), and 6 samples of birds (1.76%). Vertebrates from the VFD in the studies were represented by 116 birds and 327 small mammals. No positive findings from vertebrates were detected. Out of 1,262 mosquito samples collected in this district, WNV RNA was detected in 3 (individual infection rate — 0.01%). The results of the studies on 198 samples of ixodid ticks are negative.

97 samples of mosquitoes were delivered from the UFD for research. RNA markers of WNV were detected in 1 sample. The level of individual mosquito infection rate was 0.04%.

Discussion

One of the leading factors contributing to the activation of the epizootic process of WNF is the high

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Table 2. Results of WNV RNA testing in vertebrate species in 2024

Species	Amount of studied specimens	Amount of positive specimens
Birds		
White stork — *Ciconia ciconia* Linnaeus, 1758	1	0
Gray partridge — *Perdix perdix* Linnaeus, 1758	4	0
Common pheasant — *Phasianus colchicus* Linnaeus, 1758	5	0
Black grouse — *Lyrurus tetrix* Linnaeus, 1758	1	0
Rock dove — *Columba livia* Gmelin, 1789	84	0
Wood pigeon — *Columba palumbus* Linnaeus, 1758	2	0
Mottled duck — *Anas fulvigula* Ridgway, 1874	2	0
Gadwall — *Mareca strepera* Linnaeus, 1758	4	0
Tufted duck — *Aythya fuligula* Linnaeus, 1758	1	0
Greater white-fronted goose — *Anser albifrons* Scopoli, 1769	12	0
Greylag goose — *Anser anser* Linnaeus, 1758	6	0
Garganey — *Spatula querquedula* Linnaeus, 1758	7	0
Eurasian teal — *Anas crecca* Linnaeus, 1758	12	0
Mallard — *Anas platyrhynchos* Linnaeus, 1758	157	0
Common pochard — *Aythya ferina* Linnaeus, 1758	3	0
Northern Shoveler — *Spatula clypeata* Linnaeus, 1758	2	0
Eurasian Wigeon — *Mareca penelope* Linnaeus, 1758	2	0
Common merganser — *Mergus merganser* Linnaeus, 1758	2	0
Ruddy shelduck — *Tadorna ferruginea* Pallas, 1764	1	0
Common goldeneye — *Bucephala clangula* Linnaeus, 1758	3	0
Eurasian woodcock — *Scolopax rusticola* Linnaeus, 1758	54	0
Common snipe — *Gallinago gallinago* Linnaeus, 1758	1	0
Black-headed gull — *Chroicocephalus ridibundus* Linnaeus, 1766	9	0
Common tern — *Sterna hirundo* Linnaeus, 1758	1	0
Little tern — *Sternula albifrons* Pallas, 1764	2	0
Caspian gull — *Larus cachinnans* Pallas, 1811	1	0
Northern Lapwing — *Vanellus vanellus* Linnaeus, 1758	1	0
Eurasian dotterel — *Eudromias morinellus* Linnaeus, 1758	1	0
Common sandpiper — *Actitis hypoleucos* Linnaeus, 1758	2	0
Corncrake — *Crex crex* Linnaeus, 1758	1	0
Eurasian coot — *Fulica atra* Linnaeus, 1758	17	0
Gray heron — *Ardea cinerea* Linnaeus, 1758	12	2
Black-crowned Night heron — *Nycticorax nycticorax* Linnaeus, 1758	2	0
Purple heron — *Ardea purpurea* Linnaeus, 1766	4	0
Glossy ibis — *Plegadis falcinellus* Linnaeus, 1766	1	0
Great cormorant — *Phalacrocorax carbo* Linnaeus, 1758	43	2
Hooded crow — *Corvus cornix* Linnaeus, 1758	50	1
Common raven — *Corvus corax* Linnaeus, 1758	1	0
Rook — *Corvus frugilegus* Linnaeus, 1758	79	0
Eurasian magpie — *Pica pica* Linnaeus, 1758	22	0
Western jackdaw — *Coloeus monedula* Linnaeus, 1758	2	0
Eurasian jay — *Garrulus glandarius* Linnaeus, 1758	6	0
Eurasian tree sparrow — *Passer montanus* Linnaeus, 1758	3	0
House sparrow — *Passer domesticus* Linnaeus, 1758	3	0
Great tit — *Parus major* Linnaeus, 1758	7	0
Red-backed shrike — *Lanius collurio* Linnaeus, 1758	1	0

End of the Table 2

Species	Amount of studied specimens	Amount of positive specimens
Song thrush — *Turdus philomelos* Brehm, 1831	2	0
American robin — *Turdus migratorius* Linnaeus, 1766	1	0
Common blackbird — *Turdus merula* Linnaeus, 1758	2	0
Fieldfare — *Turdus pilaris* Linnaeus, 1758	7	0
Garden warbler — *Sylvia borin* Boddaert, 1783	1	0
Common redstart — *Phoenicurus phoenicurus* Linnaeus, 1758	1	0
European greenfinch — *Chloris chloris* Linnaeus, 1758	1	0
Bohemian waxwing — *Bombycilla garrulus* Linnaeus, 1758	1	0
Barn swallow — *Hirundo rustica* Linnaeus, 1758	1	0
Yellowhammer — *Emberiza citrinella* Linnaeus, 1758	1	0
European robin — *Erithacus rubecula* Linnaeus, 1758	5	0
Eurasian chaffinch — *Fringilla coelebs* Linnaeus, 1758	1	0
Blyth's reed warbler — *Acrocephalus dumetorum* Blyth, 1849	2	0
Arctic warbler — *Phylloscopus borealis* Blasius, 1858	2	0
Eurasian wren — *Troglodytes troglodytes* Linnaeus, 1758	1	0
Common grasshopper warbler — *Locustella naevia* Boddaert, 1783	1	1
Spotted flycatcher — *Muscicapa striata* Pallas, 1764	1	0
Barred warbler — *Curruca nisoria* Bechstein, 1795	1	0
Common swift — *Apus apus* Linnaeus, 1758	2	0
Black woodpecker — *Dryocopus martius* Linnaeus, 1758	1	0
Great spotted woodpecker — *Dendrocopos major* Linnaeus, 1758	2	0
European Nightjar — *Caprimulgus europaeus* Linnaeus, 1758	1	0
Ural owl — *Strix uralensis* Pallas, 1771	1	0
Tawny owl — *Strix aluco* Linnaeus, 1758	4	0
Red-footed falcon — *Falco vespertinus* Linnaeus, 1766	1	0
Western marsh harrier — *Circus aeruginosus* Linnaeus, 1758	1	0
Black kite — *Milvus migrans* Boddaert, 1783	1	0
Common buzzard — *Buteo buteo* Linnaeus, 1758	1	0
Total	684	6
Small mammals		
Bank vole — *Myodes glareolusu* Schreber, 1780	186	0
East European vole — *Microtus majori* Satunin, 1907	1	0
Common vole — *Microtus arvalis* Pallas, 1779	40	0
Feldmäuse – *Microtus* Schrank, 1798, sp.	7	0
European water vole — *Arvicola amphibius* Linnaeus, 1758	1	0
House mouse — *Mus musculus* Linnaeus, 1758	8	0
Yellow-necked mouse — *Apodemus flavicollis* Melchior, 1834	9	0
Ural field mouse — *Sylvaemus uralensis* Pallas, 1811	134	0
Striped field mouse — *Apodemus agrarius* Pallas, 1771	35	0
Short-tailed field vole — *Microtus agrestis* Linnaeus, 1761	1	0
Brown rat — *Rattus norvegicus* Berkenhout, 1769	5	0
European mole — *Talpa europaea* Linnaeus, 1758	1	0
Common shrew — *Sorex araneus* Linnaeus, 1758	27	0
Total	455	0
Amphibians		
Marsh frog — Pelophylax ridibundus Pallas, 1771	45	0

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Table 3. Results WNV RNA testing in arthropod species in 2024

Species	Amount of specimens	Amount of studied samples	Amount of positive samples
Blood-sucking mosquitoes			
Anopheles algeriensis Theobald, 1903	172	7	0
Anopheles claviger Meigen, 1804	695	36	0
Anopheles hyrcanus Pallas, 1771	4096	146	0
к. Anopheles maculipennis Meigen, 1818	38 292	1577	2
Anopheles plumbeus Stephens, 1828	8	2	0
Aedes albopictus Skuse, 1895	30	1	0
Aedes annulipes Meigen, 1830	111	6	0
Aedes behningi Martini, 1926	201	11	0
Aedes cantans Meigen, 1818	4687	164	0
Aedes caspius Pallas, 1771	2865	109	0
Aedes cataphylla Dyar, 1916	204	8	0
Aedes cinereus Meigen, 1818	2066	80	1
Aedes communis De Geer, 1776	964	36	0
Aedes cyprius Ludlow, 1920	5	1	0
Aedes dorsalis Meigen, 1830	878	34	0
Aedes excrucians Walker, 1856	709	25	0
Aedes flavescens Muller, 1764	1014	52	0
Aedes geniculatus Olivier, 1791	214	11	0
Aedes intrudens Dyar, 1919	380	14	0
Aedes nigrinus Eckstein, 1918	15	1	0
Aedes pulcritarsis Rondani, 1872	83	4	0
Aedes punctor Kirby, 1837	132	5	0
Aedes riparius Dyar et Knab, 1907	63	3	0
Aedes sticticus Meigen, 1838	3974	143	0
Aedes subdiversus Martini, 1926	1	1	0
Aedes vexans Meigen, 1830	8344	293	0
Culex modestus Ficalbi, 1890	5197	188	2
Culex pipiens Linnaeus, 1758	13 428	477	11
Culiseta alaskaensis Ludlow, 1906	322	24	0
Culiseta annulata Schrank, 1776	480	46	0
Culiseta longiareolata Macquart, 1838	59	6	0
Coquillettidia richiardii Ficalbi, 1889	3573	146	2
Uranotaenia unguiculata Edwards, 1913	176	8	6
Total	93 438	3665	24
Ixodid ticks			
Dermacentor marginatus Sulzer, 1776	96	13	0
Dermacentor niveus Neumann, 1897	33	3	0
Dermacentor reticulatus Fabricius, 1794	1688	193	0
Haemaphysalis punctata Canestrini and Fanzago, 1878	9	1	0
Hyalomma detritum Schulze, 1919	20	1	0
Hyalomma marginatum Koch, 1844	197	50	2
Hyalomma scupense Schulze, 1919	181	39	0
Ixodes persulcatus Schulze, 1930	960	86	0

End of the Table 3

Species	Amount of specimens	Amount of studied samples	Amount of positive samples
Ixodes ricinus Linnaeus, 1758	895	77	0
Rhipicephalus annulatus Say, 1821	342	64	0
Rhipicephalus niveus Yamazaki (1919)	15	2	0
Rhipicephalus rossicus Yakimov et Kohl-Yakimova, 1911	169	18	1
Rhipicephalus sanguineus Latreille, 1806	66	9	0
Rhipicephalus turanicus Pomerantsev 1936	20	2	0
Rhipicephalus pumilio Schulze, 1935	118	12	0
Total	4809	570	3

air temperature readings. In 2024, averaged anomalies were significant across most of Russia in April, throughout the summer months, and in September, which contributed to an increase in the replication rate of the pathogen, accelerated the development stages of vectors, and prolonged the stay of migratory birds in their breeding areas.

When examining the material from birds, all positive samples were found only in the Volgograd region. Among them, there are sedentary birds (gray crow) and migratory birds (common cricket, great cormorants, gray herons). A fallen common cricket was discovered by us in the area of high-rise buildings in the center of Volgograd in mid-August during the period of maximum activity of the pathogen and *Culex* mosquitoes. At the same time, over the many years of studying WNF in southern Russia, official epizootics among birds with fatal outcomes (in Volgograd and Astrakhan regions) have not been recorded. This fact was explained by the possible adaptation of local bird populations as a result of long-term interaction with the pathogen population [15]. Confirmation of the etiological role of the WNF pathogen in the occurrence of fatal disease in birds in the territory of the old disease focus in the 2024 season suggests that active monitoring for the morbidity of wild and synanthropic birds, as well as targeted examination for the presence of WNV markers in deceased individuals in accordance with regulatory documents, is not being carried out[2].

The remaining birds with detected WNV RNA were captured in fish farming ponds and lakes of the Volga-Akhtuba floodplain in the Volgograd region from September 28 to November 3. Great cormorants and gray herons use these biotopes as stops for rest and feeding during the autumn migration to wintering grounds, while resident gray crows are attracted to these water bodies due to the constant presence of food remnants from other birds on their shores.

Markers of WNV in Russia have been found in mosquitoes of 19 species over the entire observation period [16–18]. According to the results of our research in 2024, mosquitoes of 6 species tested positive for the presence of WNV RNA. Among the positive findings, *C. pipiens* accounted for 45.8%, *U. unguiculata* for 25.0%, *C. richiardii*, *Cx. modestus* and *A. maculipennis* mosquitoes for 8.3% each, and *Ae. cinereus* for 4.2%.

The level of individual infection rate in mosquitoes feeding on birds and mammals, including humans, was 0.08% for *C. pipiens*, 0.06% for *C. richiardii*, 0.04% each for *Cx. modestus* and *Ae. cinereus*, and 0.006% for *An. maculipennis*. This indicator for *Uranotaenia unguiculata* mosquitoes, whose main hosts are frogs — carriers of the lineage 4 WNV — reached 6.3%. The pathogenicity of lineage 4 WNV for humans remains unproven.

All *C. pipiens*, *An. maculipennis* and *C. richiardii* mosquitoes in which WNV markers were detected were collected in populated areas. *Ae. cinereus* and *Cx. modestus* mosquitoes were caught on the shores of water bodies in areas where waterfowl concentrate. This indicates a high risk of WNV infection for the population in both urbanized and natural habitats.

Territorially, as expected, the maximum number (20) of positive findings from mosquitoes was identified in the SFD: 8 in Astrakhan region, 10 in Volgograd region, and 1 each in Rostov region and Krasnodar Krai. In the VFD, WNV markers were found in mosquitoes: in 2 samples from the Republic of Mordovia and 1 from the Kirov region. Out of 97 samples collected in the Southern UFD in the Chelyabinsk region, the pathogen was found in 1 sample from *C. modestus* thermophilic mosquitoes, which reach high numbers only in the Southern UFD. In central Russia, however, they are usually found in small numbers and not in all regions. And although the average summer temperatures in the Southern Urals are 2.0–3.5°C lower than in the CFD as a whole, the presence of one of the main carriers of WNV in Europe in this area and the detection of the pathogen RNA from it indicate a sufficiently high risk of infection for the population in the Chelyabinsk region.

[2] Paragraphs 5.18, 5.5, 8.5.1 Epidemiological surveillance, laboratory diagnostics and prevention of West Nile fever. MU 3.1/4.2.4063-24. Moscow; 2024. 46 p. (In Russ.)

In the other surveyed regions, infected carriers of WNV were not found, which does not rule out the presence of WNV foci and requires conducting repeated studies with the selection of other biotopes for field sample collection.

Positive samples for the presence of WNV RNA from ixodid ticks were found only in the SFD: 2 from *H. marginatum* in the Volgograd region and 1 from *Rhipicephalus rossicus* in the Rostov region. Their individual infection rates were 1.0% and 1.8%, respectively.

The detection of WNV RNA in mid-April from ticks and in June from mosquitoes and ticks indicates an early activation of the epizootic process in 2024. Moreover, the April findings may also indicate the preservation of WNV in ticks during the winter period.

The established combined presence of lineages 1 and 2 WNV in the Rostov region is of scientific interest.

Conclusion

In southern Russia, the enzootic circulation of WNV in 2024 has been confirmed in the territories of Volgograd, Astrakhan, Rostov regions and Krasnodar Krai. The beginning of the epizootic process of WNF was registered in these territories (with the exception of Astrakhan region) during the spring–early summer period, which was a precursor to possible epidemiological instability. The presence of positive findings in the Republic of Mordovia, Kirov and Chelyabinsk regions confirms the circulation of the pathogen in the territories of the VFD and the UFD. Information on the spread of WNV in Russia has been supplemented with data from three new regions, and the Kirov region was the northernmost point where the pathogen RNA was detected in field samples in our study.

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