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Serological monitoring of avian influenza and Newcastle disease in the Russian Federation in 2020

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SUMMARY
Within the framework of the Rosselkhoznadzor measures aimed at control of highly dangerous diseases and development of timely recommendations for disease prevention and control, 36,986 serum samples to be tested for the presence of avian influenza virus antibodies and 30,325 serum samples to be tested for the presence of Newcastle disease virus antibodies were submitted to the FGBI "ARRIAH" Reference Laboratory for Avian Viral Diseases in 2020. The samples were collected from domestic, wild and synanthropic birds in 60 Subjects of the Russian Federation. As a result of the laboratory diagnosis, antibodies against type A influenza virus were found in vaccinated chickens from two poultry farms in the Primorsky Krai. Typing of sample sera using hemagglutination inhibition test showed that the detected antibodies were specific to the haemagglutinin subtype of the vaccine antigen (A/H9). Antibodies to the H9 subtype avian influenza virus were detected in sera of non-vaccinated geese from two poultry farms in the Kurgan Oblast and from one poultry farm in the Republic of Bashkortostan. As for the backyards where scheduled vaccination against avian influenza A/H5 is carried out, a low level of immunity was seen in the Republics of Adygea and Chechnya (0 and 15%, respectively), while a high immunity level was observed in the Rostov Oblast (74%). High seroprevalence of Newcastle disease virus was found in adult poultry in indoor industrial farms, which was associated with mass vaccination against the disease. In broiler chickens, post-vaccination antibodies were observed, on average, in 44% of the tested sera samples. The antibodies against Newcastle disease virus and avian influenza virus subtype H5 detected in wild and synanthropic birds indicate the circulation of these viruses in the Russian Federation. The insufficient level of post-vaccination antibodies suggests that the risk of epidemic among poultry in industrial poultry farms and backyards remains.

Keywords: monitoring, avian influenza, Newcastle disease, blood sera, antibodies, hemagglutination inhibition test, ELISA

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Серологический мониторинг гриппа птиц и ньюкаслской болезни на территории Российской Федерации в 2020 г.

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РЕЗЮМЕ

В рамках реализации мероприятий Россельхознадзора с целью осуществления контроля за особо опасными болезнями и для своевременной выработки рекомендаций по профилактике и борьбе с ними в референтную лабораторию вирусных болезней птиц ФГБУ «ВНИИЗЖ» в течение 2020 г. было доставлено 36 986 проб сыворотки крови для исследования на наличие антител к вирусу гриппа птиц и 30 325 проб – на наличие антител к вирусу ньюкаслской болезни. Пробы были отобраны от домашних, диких и синантропных птиц из 60 субъектов Российской Федерации. В результате лабораторных исследований антитела к вирусу гриппа типа А были обнаружены у вакцинированных кур из двух птицеводческих предприятий Приморского края. При типировании проб сыворотки крови в реакции торможения гемагглютинации установили, что выявленные антитела соответствовали вакцинному антигену по подтипу гемагглютинина (A/H9). Антитела к вирусу гриппа птиц подтипа H9 были обнаружены в сыворотках крови от невакцинированных гусей с двух птицефабрик Курганской области и одной птицефабрики Республики Башкортостан. В личных подсобных хозяйствах граждан, где проводится плановая вакцинопрофилактика гриппа птиц A/H5, низкий уровень иммунитета установлен в Республике Адыгея и Чеченской Республике (0 и 15% соответственно) и высокий уровень – в Ростовской области (74%). Высокий уровень антител к вирусу ньюкаслской болезни был установлен у взрослой птицы в промышленных хозяйствах закрытого типа, что связано с массовой вакцинацией против данного заболевания. У цыплят-бройлеров отмечали наличие поствакцинальных антител в среднем в 44% исследованных проб сыворотки крови. Выявленные антитела к вирусам ньюкаслской болезни и гриппу птиц подтипа H5 среди диких и синантропных птиц свидетельствуют о циркуляции данных вирусов на территории Российской Федерации. Недостаточный уровень поствакцинальных антител указывает на сохранение риска возникновения эпизоотий среди домашних птиц промышленных птицеводческих предприятий и личных подсобных хозяйств.

Ключевые слова: мониторинг, грипп птиц, ньюкаслская болезнь, сыворотки крови, антитела, реакция торможения гемагглютинации, иммуноферментный анализ

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INTRODUCTION

Avian influenza and Newcastle disease are the most significant, from both epidemic and economic perspective, avian diseases that cause huge damage to poultry farming worldwide [1–5].

Avian influenza (AI) is induced by type A influenza virus belonging to the family *Orthomyxoviridae*. It is a segmented RNA virus that is classified into 18 hemagglutinating (H1–H18) and 11 neuraminidase (N1–N11) subtypes based on the antigenic difference of two surface glycoproteins [6]. Avian influenza virus (AIV) is an important zoonotic pathogen resulting in mass death of affected birds and causing serious damage to industrial poultry farming and small-scale farms [7–10]. Serious diseases were most frequently caused by AIV belonging to subtypes H5 and H7.

In 2020 the World Animal Health Organization received notifications on outbreaks of highly pathogenic avian influenza (HPAI) from 36 countries around the world. The disease was most often registered in Europe. There were 484 notifications of outbreaks in poultry and 618 notifications of outbreaks among wild birds. The vast majority of the disease cases were caused by HPAI virus subtype H5N8. However, outbreaks caused by HPAI virus of subtypes H5N1 and H5N5 among poultry and HPAI virus of subtypes H5N3 and H5N5 among wild birds were also reported. No distinct prevalence of HPAI subtype H5N8 was observed in Asia. There were 228 outbreaks among poultry caused by various variations of HPAI virus subtype H5 (N1, N2, N5, N6, N8), and 68 outbreaks among wild birds caused by HPAI virus subtypes H5N1, H5N6 and H5N8. HPAI subtype H5N8 outbreaks among poultry were also recorded in South Africa. Notifications on HPAI subtype H7 reported in poultry were received from Australia and the USA [11].

Highly pathogenic AIV H5N8 was first detected in industrial poultry in China in 2010 [12]. In July 2020 an outbreak caused by HPAI virus subtype H5N8 was also reported among wild birds in the Russian Federation. Since August, HPAI virus subtype H5N8 infection started to be registered in Russia among domestic birds both in poultry holdings and in small-scale poultry farms. The most frequent outbreak notifications were received from the Subjects of the Russian Federation bordering on Kazakhstan (the Omsk, Tyumen, Kurgan and Chelyabinsk Oblasts) [13]. Besides, single outbreaks were reported in the Volga, Southern, North Caucasian and Central Federal Districts.

Newcastle disease (ND) is an avian viral disease characterized by pneumonia, encephalitis and multifocal hemorrhages of internal organs. The causative agent is an RNA-virus belonging to the family *Paramyxoviridae*. The ND virus is potentially contagious for most avian species, and it often causes mortality of susceptible domestic birds (mainly gallinaceous species) [14]. At least four panzootics

Table 1
Detection of antibodies to NDV in chicken sera submitted from industrial poultry farms, using ELISA and HI test

Federal District	Parent flock, up to 100 days old		Parent flock, over 100 days old		Broiler chickens	
	number of positives / total number of samples	number of p/f	number of positives / total number of samples	number of p/f	number of positives / total number of samples	number of p/f
Central	392/868	11	4,226/4,365	29	288/935	7
Northwestern	574/924	9	530/531	8	233/1165	3
Southern	241/410	6	1,649/1,696	11	219/630	3
North Caucasian	283/450	3	196/200	1	260/320	4
Volga	509/675	7	3,114/3,314	35	1,272/2,302	18
Ural	39/136	2	382/382	9	110/174	7
Siberian	193/370	7	1,097/1,185	18	243/527	7
Far Eastern	208/250	3	882/1,035	7	197/350	4

p/f – poultry farm.

were recognized [15], negatively affecting not only the economy as a whole, but also human welfare due to food supply reduction [16].

According to the veterinary services of the Subjects of the Russian Federation, ND outbreaks were recorded in the Republic of Ingushetia, in the Vladimir and Kursk Oblasts (11 infected settlements) in 2020 [17].

The necessity of avian influenza and Newcastle disease monitoring studies is determined by the risk of introduction of new virus strains into the country, the risk of pathogen introduction into commercial poultry farms, the emergence of epidemics that lead to great economic losses [18].

The aim of the work was to conduct seromonitoring studies among domestic, wild and synanthropic birds within the framework of the Rosselkhoznadzor measures aimed at control of highly dangerous diseases and timely development of recommendations for avian influenza and Newcastle disease prevention and control.

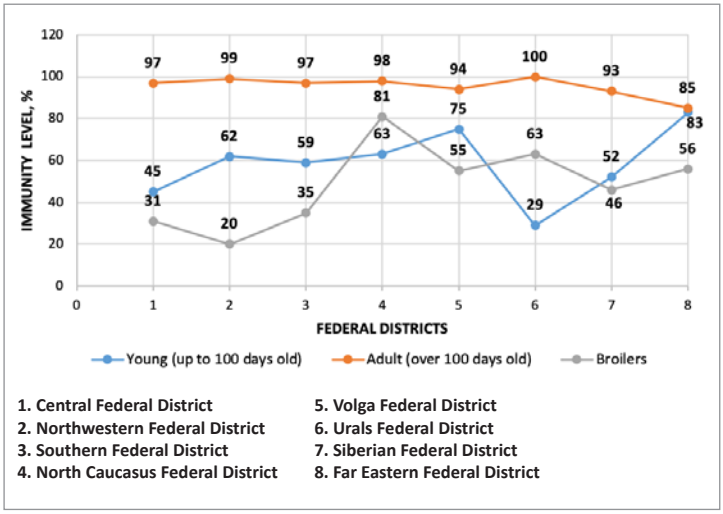

Fig. 1. Intensity of post-vaccination immunity to the ND virus in chickens from industrial poultry farms

MATERIALS AND METHODS

Test samples. Bird sera submitted by the Rosselkhoznadzor Territorial Administrations to the FGBI "ARRIAH" in 2020 were tested.

Test kits and reagents:

– Single serum dilution ELISA kit for detection of antibodies to avian influenza virus (FGBI "ARRIAH");

– Single serum dilution ELISA kit for detection of antibodies to Newcastle disease virus (FGBI "ARRIAH");

– HI test kit for detection of antibodies to avian influenza virus subtype H5 (FGBI "ARRIAH");

– HI test kit for detection of antibodies to avian influenza virus subtype H9 (FGBI "ARRIAH");

– HI test kit for detection of antibodies to Newcastle disease virus (FGBI "ARRIAH");

– reference antigens of AI virus subtypes H5, H7 and H9 and corresponding positive sera produced by IZSVe (Italy);

– reference antigens of AI virus subtypes H5, H7 and H9 and corresponding positive sera produced by GD (Netherlands).

All tests were carried out using commercial ELISA kits according to the manufacturer's instructions. The hemagglutination inhibition assay (HI assay) using reference components was carried out in accordance with the "Methodical Guidelines for HI identification of avian influenza and Newcastle disease viruses"[1].

Treatment of test sera samples. To remove the thermolabile inhibitors, all samples were inactivated in a water bath for 30 minutes at 56 °C.

RESULTS AND DISCUSSION

Pursuant to Rosselkhoznadzor Order No. 1423 of December 25, 2019 "On laboratory testing within the Rosselkhoznadzor activities for ensuring compliance with the World Trade Organization (WTO) Sanitary and

[1] MG 27-16 Methodical Guidelines for HI identification of avian influenza and Newcastle disease viruses: approved by the Rosselkhoznadzor on June 06, 2016. Vladimir: FGBI "ARRIAH". 2016. 14 p.

Phytosanitary (SPS) Agreement requirements upon Russia's accession to the WTO for 2020", 30,325 sera samples collected from poultry, wild and synanthropic birds were tested for the presence of antibodies to the Newcastle disease virus. The samples were received from 60 Subjects of the Russian Federation. The chicken sera were tested using ELISA and HI assay. Serum samples of other species of domestic, wild and synanthropic birds were tested using HI assay.

4,083 serum samples from young chickens under the age of 100 days obtained from 24 Subjects of the Russian Federation were tested. The minimum percentage of seropositive young poultry from commercial and parent stock was in the Urals Federal District, and the maximum percentage was in the Far Eastern Federal District. On average, in the Russian Federation, the immunity level for this group was low and amounted to 60%. Such a low percentage can be explained by the fact that the sera were collected from birds of various ages (1–100 days), and vaccination schemes used in poultry farms do not always provide a sufficient level of antibodies by the time of sampling.

12,708 serum samples obtained from adult commercial poultry in 44 Subjects of the Russian Federation were tested. The maximum percentage of seropositive birds was observed in the Urals Federal District (100%), and the minimum – in the Far Eastern (85%) Federal District. Test results for sera taken from adult commercial poultry in all Federal Districts of the Russian Federation showed persistent and intense immunity (on average 95%).

Tests were conducted for 6,403 serum samples obtained from broiler chickens in 28 Subjects of the Russian Federation. The minimum percentage of seropositive broilers was observed in the Northwestern Federal District, and the maximum – in the North Caucasian Federal District. On average, in the Russian Federation the immunity level for this group was 44%. A low average percentage of positive samples in broiler chicken can be explained by the fact that farms used vaccination schemes that did not allow development of sufficient post-vaccination antibody levels by the time of blood sampling.

The test results are presented in Figure 1 and Table 1.

1,086 serum samples of turkeys, geese and ducks obtained from industrial poultry farms of the Northwestern, North Caucasian, Volga, Urals and Siberian Federal Districts were tested (Fig. 2). The intensity of the immune response exceeding 80% was observed in turkeys in three poultry farms located in the Udmurtia Republic (100%), Orenburg (84%) and Tyumen (83%) Oblasts. The percentage of seropositive birds from the Stavropol Krai, the Republic of Mordovia, Leningrad, Samara and Omsk Oblasts was low and ranged from 0 to 45%.

The low average percentages of seropositive birds among geese (60%) and ducks (32%) can be explained by the fact that not all poultry farms carry out vaccination against the ND virus (according to the accompanying documentation).

In 2020, within monitoring studies to detect antibodies to the ND virus, 5,658 samples of poultry sera were received from backyards and small-scale farms: chickens, geese, ducks, turkeys, quails and guinea fowl. Antibodies to the ND virus were found only in the blood of chickens, geese, quails and turkeys (Table 2).

In 2020, 26,357 samples of chicken sera delivered from 180 industrial poultry farms of the Russian Federation were examined for the presence of antibodies to the AI virus. Four positive samples from Primorsky Krai were detected. As the data in accompanying documents show, the antibodies were induced after immunization with the vaccine against AI subtype H9. No antibodies to avian influenza virus were found in other samples (Fig. 3).

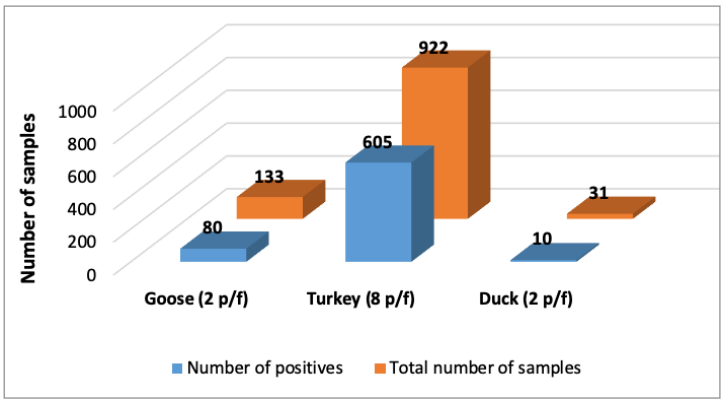

Fig. 2. Detection of antibodies against NDV in turkey, goose and duck sera submitted from industrial poultry farms

Table 2
Detection of antibodies to NDV in poultry sera submitted from backyards and small-scale farms

Federal District	Number of positives / total number of samples			
	chickens	geese	turkeys	quails
Central	191/560	0/10	–	–
Northwestern	28/119	–	–	–
Southern	471/1,588	0/12	3/15	0/10
North Caucasian	674/959	–	50/50	–
Volga	104/203	6/45	12/25	1/5
Urals	24/140	0/39	–	–
Siberian	82/336	3/11	1/3	–
Far Eastern	583/1,116	0/4	225/292	–

"–" no samples were submitted for testing.

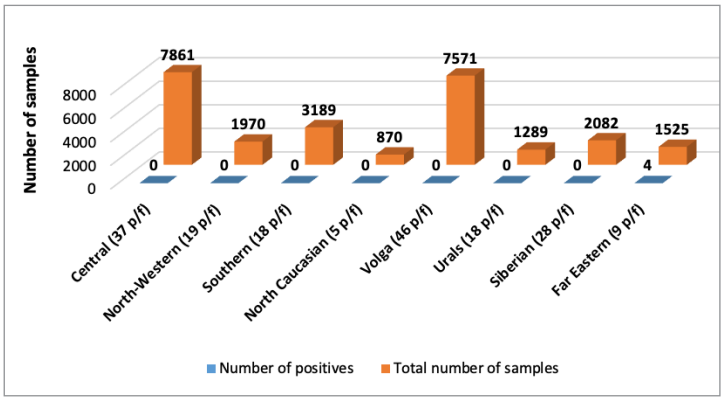

Fig. 3. Detection of antibodies to AIV in chicken sera submitted from industrial poultry farms

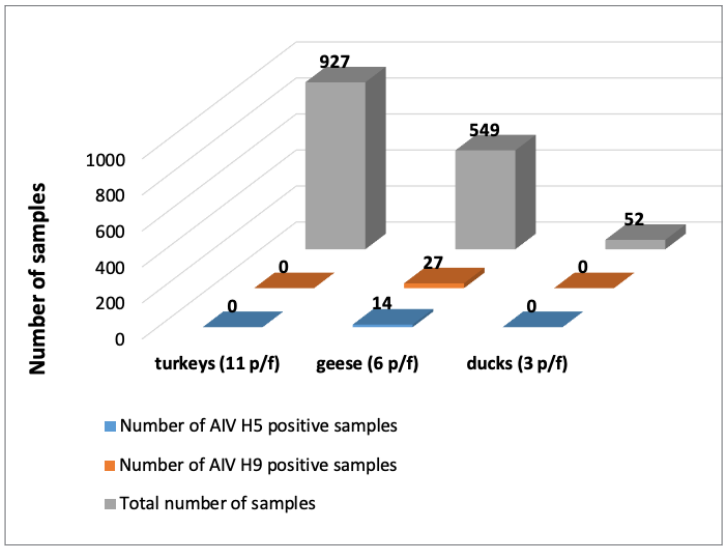

Fig. 4. Detection of antibodies to H5 and H9 AIV in turkey, goose and duck sera submitted from industrial poultry farms

Table 3
Detection of antibodies against H5 AIV in poultry sera submitted from backyards and small-scale farms

Federal District	Number of positives/total number of samples	
	chickens	quails
Central	0/2,334	–
Northwestern	0/119	–
Southern	537/2,122	3/20
North Caucasian	63/959	–
Volga	0/245	0/5
Urals	0/140	–
Siberian	0/336	–
Far Eastern	0/1,642	–

"–" no sera were submitted for testing.

The FGBI "ARRIAH" received 1,528 serum samples from ducks, geese and turkeys from the Northwestern, North Caucasian, Volga, Urals and Siberian Federal Districts. All samples were HI tested for the presence of antibodies to the AI virus subtypes H5, H7 and H9. Antibodies to the AI virus subtype H7 were not detected in all tested samples. No antibodies to the AI virus subtypes H5 and H9 were detected in the sera of turkeys and ducks.

Antibodies to the AI subtype H5 virus were found in the sera of geese received from a poultry farm located in the Kurgan Oblast. According to the data available in the accompanying documentation, the poultry were immunized with a vaccine against AI subtype H5. Antibodies to the AI virus of the H9 subtype were found in sera of geese delivered from two poultry farms of the Kurgan Oblast and one poultry farm in the Republic of Bashkortostan. Vaccination against AI subtype H9 virus on these poultry farms was not stated in the accompanying documentation (Fig. 4).

In 2020 8,704 bird serum samples from backyards and small-scale farms were delivered for monitoring studies to be tested for antibodies to the AI subtype H5 virus. The sera were obtained from chickens, geese, ducks, turkeys, quails and guinea fowls (Table 3). Antibodies to the AIV were found only in chicken and quail sera. According to

Table 4
Detection of antibodies against AI and ND viruses in sera of wild and synanthropic birds using HI test

Federal District	Bird species	The number of positives/ total number of samples			
		ND	AI (H5)	AI (H7)	AI (H9)
Central	synanthropic birds	0/10	0/20	0/20	0/20
	wild birds	0/25	0/25	0/25	0/25
Northwestern	wild ducks	5/120	5/120	0/120	0/120
	crows	4/4	0/4	0/4	0/4
	seagulls	0/16	0/16	0/16	0/16
	wild geese	0/4	0/4	0/4	0/4
	pigeons	10/10	0/10	0/10	0/10
Volga	pigeons	66/80	0/80	0/80	0/80
Siberian	pigeons	35/85	0/85	0/85	0/85
	decorative and wild birds	0/25	0/25	0/25	0/25
	mallard duck	0/3	0/3	0/3	0/3
	wild ducks	0/5	0/5	0/5	0/5

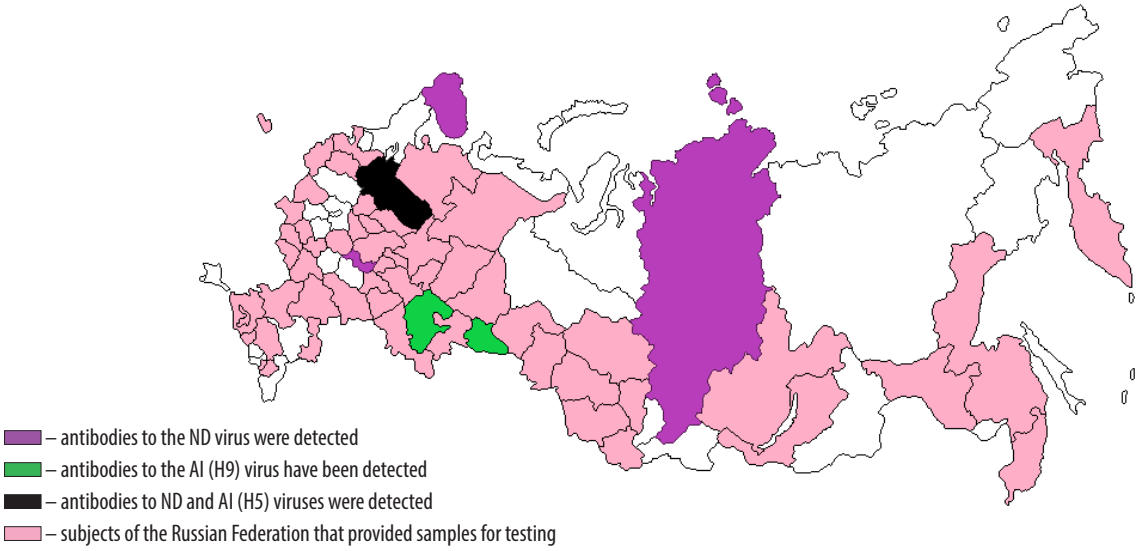

Fig. 5. RF Subjects where antibodies against AI and ND viruses were detected in non-vaccinated commercial, wild and synanthropic birds in 2020

the accompanying documentation, vaccination of chickens against the AI subtype H5 virus was carried out in the Republic of Adygea, the Rostov Oblast and the Chechnya Republic (immunity level in these Subjects of the Russian Federation was 0; 74 and 15%, respectively). Vaccination of quails was carried out only in the Astrakhan Oblast (the percentage of seropositive sera was 30%). No antibodies to the AI subtype H5 virus were detected in the sera from unvaccinated birds.

Many species of wild and synanthropic birds are natural reservoirs of AI and ND pathogens, therefore, monitoring the epidemic situation among birds of this group helps to predict and control the diseases.

In 2020 the FGBI "ARRIAH" received 397 serum samples of wild and synanthropic birds from 9 Subjects of the Russian Federation to be tested for avian influenza. All sera were tested for the presence of antibodies to the AI virus subtypes H5, H7, H9. Based on test results antibodies to the AI subtype H5 virus were detected in 5 wild ducks from the Vologda Oblast. Antibodies to the AIV subtypes H7 and H9 were not detected.

387 serum samples from wild and synanthropic birds were tested for the presence of antibodies to the ND virus. Antibodies to the ND virus were found in 111 sera from pigeons delivered from the Murmansk Oblast, the Krasnoyarsk Krai and the Republic of Mordovia. Antibodies to the ND virus were also detected in sera from wild ducks and crows from the Vologda Oblast (Table 4). The circulation of the ND agent among synanthropic birds, that were, for most part, sampled near poultry farms, indicates a high risk of infection entry in flocks of farmed birds.

Figure 5 shows the location of the Subjects of the Russian Federation, where antibodies to AI and ND viruses were detected in blood sera from unvaccinated commercial, wild and synanthropic birds.

CONCLUSION

Based on monitoring results conducted in 2020, the conclusion can be made on the circulation of avian influenza A/H5 and Newcastle disease viruses among wild and synanthropic birds, as well as avian influenza A/H9 virus among domestic birds in the territory of the Russian Federation. In the future, outbreaks of Newcastle disease and highly pathogenic influenza may occur among birds in the wild population, which also causes a high risk of infection of the above diseases in poultry farms with a low biocontainment safety. Therefore, it is very important to conduct timely monitoring studies to detect the entry of the pathogen and the spread of highly pathogenic influenza and Newcastle disease in wild and synanthropic bird populations, as well as to assess the level of post-vaccination antibodies among domestic birds in order to carry out adequate anti-epidemic and preventive measures.

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