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Serological monitoring of avian influenza and Newcastle disease in the Russian Federation in 2019

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SUMMARY
More than 30,000 samples of blood serum from domestic, wild and synanthropic birds from 50 regions of the Russian Federation were submitted to the FGBI "ARRIAH" (Vladimir) Reference Laboratory for Avian Viral Diseases to be tested for avian influenza and Newcastle disease within the framework of monitoring activities conducted by the Rosselkhoznadzor in 2019. As a result of the laboratory diagnosis, antibodies to type A influenza virus were detected in vaccinated chickens from two poultry farms in the Perm and Primorsky Krais (A/N9). The detected antibodies were specific to the haemagglutinin subtype of the vaccine antigen. As for the backyards in the RF Subjects, where scheduled vaccination against avian influenza A/H5 is carried out, a low level of immunity was seen in the Rostov and Astrakhan Oblasts (35 and 44%, respectively) while a high level of immunity was observed in the Republic of Altai, Krasnodar Krai, the Chechen Republic and the Primorsky Krai (69, 78, 80 and 88%, respectively). High seroprevalence of Newcastle disease virus in adult poultry in indoor holdings was associated with mass vaccination against the disease. In broiler chickens, post-vaccination antibodies were observed, on average, in 42% of the studied blood serum samples. Antibodies to the Newcastle disease virus were detected in 39% of samples from backyard chickens. Seroprevalence in wild and synanthropic birds was high. The obtained results suggest that the risk of introduction and spread of avian influenza and Newcastle disease in industrial poultry farms and in backyards remains.

Key words: avian influenza, Newcastle disease, epizootology, monitoring, poultry, wild birds.

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Серологический мониторинг гриппа птиц и ньюкаслской болезни в Российской Федерации в 2019 году

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РЕЗЮМЕ
В рамках мониторинговых мероприятий, проводимых Россельхознадзором, в референтную лабораторию вирусных болезней птиц ФГБУ «ВНИИЗЖ» (г. Владимир) в течение 2019 года было доставлено более 30 000 проб сыворотки крови от домашних, диких и синантропных птиц из 50 регионов

Российской Федерации для исследования на грипп птиц и ньюкаслскую болезнь. В результате лабораторной диагностики антитела к вирусу гриппа типа А были выявлены у вакцинированных кур из двух птицеводческих предприятий Пермского и Приморского краев (A/H9). Выявленные антитела были специфичны вакцинному антигену по подтипу гемагглютинина. В личных подсобных хозяйствах граждан субъектов Российской Федерации, где проводится плановая вакцинопрофилактика гриппа птиц A/H5, установлен низкий уровень иммунитета в Ростовской и Астраханской областях (35 и 44% соответственно) и высокий уровень – в Республике Алтай, Краснодарском крае, Чеченской Республике и Приморском крае (69, 78, 80 и 88% соответственно). Высокая серопревалентность ньюкаслской болезни была установлена для 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 42% исследованных проб сыворотки крови. В 39% проб от кур из личных подсобных хозяйств были обнаружены антитела к вирусу ньюкаслской болезни. Серопревалентность у диких и синантропных птиц была высокой. Полученные результаты свидетельствуют о сохранении риска заноса и распространения гриппа птиц и ньюкаслской болезни в промышленных птицеводческих хозяйствах и личных подсобных хозяйствах граждан.

Ключевые слова: грипп птиц, ньюкаслская болезнь, эпизоотология, мониторинг, домашняя птица, дикая птица.

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INTRODUCTION

Highly pathogenic avian influenza (HPAI) and Newcastle disease (ND) are the World Organisation for Animal Health (OIE) listed diseases that are subject to mandatory notification when detected in the territory of the country.

In poultry, these diseases can cause damage to vital organs and the subsequent death of all the unvaccinated flock, thus, leading to large economic losses to poultry farms [1–3].

Newcastle disease has become widespread in many parts of the world [4–7]. In 2019, Newcastle disease outbreaks notified to the OIE occurred in the United States, Mexico, Israel, Kazakhstan, and Russia. According to the immediate notifications made by the Veterinary Services of the Russian Federation, ND outbreaks were reported in 9 regions of the country (18 affected areas) in 2019. Newcastle disease epidemics caused by Newcastle disease virus (NDV) subgenotype VII(L) were large-scale in terms of their geographical distribution (Krasnodar Krai, Chechen Republic, Stavropol Krai, Primorsky Krai, Saratov oblast, Zabaykalsky Krai, Altai Krai, Omsk and Kursk oblasts) and long-term (from January to December 2019).

Recently, there has been a steady decline in the number of countries reporting HPAI outbreaks. According to the OIE, in 2018 and 2019, a total of 33 and 20 countries, respectively, reported on HPAI outbreaks. HPAI was mainly caused by a highly virulent HPAI virus of the H5 subtype with various neuraminidases, with H5N1 and H5N8 subtypes being the most prevalent [8–11].

According to the immediate notifications received from the Veterinary Services of the Subjects of the Russian Federation, in 2018, HPAI virus of the H5 subtype was detected in poultry flocks in 15 regions (more than 80 cases), in 2019 – only in the Rostov oblast (2 cases), which was reported to the OIE.

However, since the beginning of 2020, the opposite trend demonstrating the increased HPAI activity has been observed. In January 2020, the OIE received reports on HPAI outbreaks from 12 countries (China, Germany, Romania, Slovakia, Ukraine, Czech Republic, Hungary, Poland, Taiwan, Saudi Arabia, Israel and Vietnam). Since a number of countries has common borders with the Russian Federation, and Russia is involved in trade relations with other countries, there is a real risk of disease introduction into the territory of the Russian Federation.

The necessity of monitoring studies on avian influenza (AI) and Newcastle disease 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 [8, 9, 12, 13].

This paper presents the results of the serological monitoring of Newcastle disease and avian influenza in poultry, synanthropic and wild birds conducted in the Russian Federation in 2019 within the framework of the Rosselkhoznadzor diagnostic and preventive measures for highly dangerous animal diseases, aimed at protecting the territory of the Russian Federation against the introduction of animal diseases from abroad and against their further spread.

MATERIALS AND METHODS

Biological material (serum samples) was provided for the study by the Rosselkhoznadzor Territorial Administrations.

The study was carried out using commercial kits for the detection of antibodies to Newcastle disease and avian influenza viruses, produced by the FGBI "ARRIAH", according to the manufacturer's instructions and diagnostic materials (avian influenza virus antigens of subtypes H5, H7 and H9 and homologous sera) produced by GD (the Netherlands) and IZSVe (Italy) according to the standard method [14]. Enzyme-linked immunosorbent assay (ELISA) Kits were used to test sera from chicken and turkey for avian influenza and from chicken for ND; haemagglutination inhibition (HI) kits were used to test poultry sera (from chicken, turkey, duck, goose, quail and pheasant) as well as sera of wild and synanthropic birds. The received

serum samples were inactivated by heating at 56 °C for 30 min. HI test results were considered positive if the serum titer was ≥ 4.0 $\log_2$ for avian influenza and ≥ 3.0 $\log_2$ for Newcastle disease.

RESULTS AND DISCUSSION

As part of the State epidemic monitoring (Rosselkhoznadzor Order No. 1519 of 28.12.2018 "On laboratory tests within the Rosselkhoznadzor activities for ensuring compliance with the World Trade Organization (WTO) Sanitary and phytosanitary (SPS) Agreement requirements upon Russia accession to the WTO for 2020"), in 2019, the poultry sera (28,262 samples) received from 49 regions of the Russian Federation were tested for the presence of antibodies to the Newcastle disease virus by HI test and ELISA. The total of 20,374 samples of poultry serum (from chicken, turkey, quail and goose) were delivered from 139 commercial poultry farms located in 41 regions of the Russian Federation (Fig. 1).

1,315 serum samples from young chicken in commercial and parent flocks (up to 100 days old) were tested, and Newcastle disease virus specific antibodies were detected in 936 samples. The percentage of seropositive poultry in the commercial and parent flocks was minimal – 48% – in the Central (CFD) and maximum – 98% – in the Southern (SFD) Federal Districts. Up to 60% of positive samples were found in poultry from the Siberian (SiFD) and the Far Eastern (FEFD) Federal Districts. In the Privolzhsky (PFD), the Ural (UFD), and the Northwestern (NWFD) Districts the number of positive samples ranged from 87 to 92%.

The study of 13,389 serum samples collected from adult poultry in all the Federal Districts of the Russian Federation revealed 11,749 positive results. The lowest number of positive samples (49%) was observed in the Northwestern Federal District. In other districts, the number of positive samples ranged from 79% in the Privolzhsky Federal District (PFD) to 100% in the Ural Federal District (UFD).

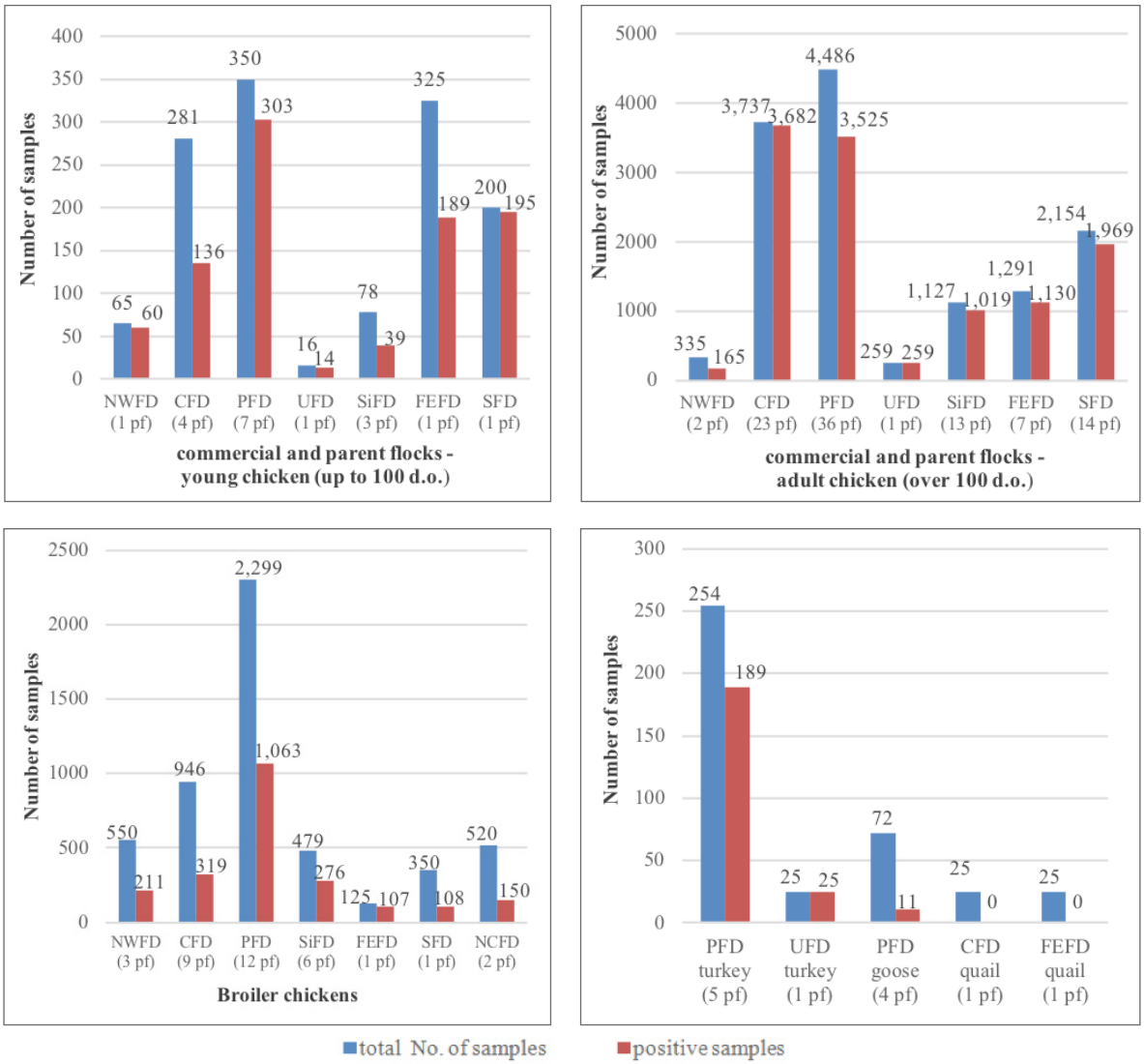

Fig. 1. Detection of antibodies to NDV in poultry sera, submitted from Russian industrial poultry farms, using HI test and ELISA.
The number of poultry farms from which samples were delivered is shown in parentheses.

Рис. 1. Результаты исследований сывороток крови птиц из промышленных птицеводческих предприятий РФ в РТГА и ИФА на наличие антител к вирусу ньюкаслской болезни.
В скобках указано количество птицеводческих хозяйств, из которых доставляли пробы.

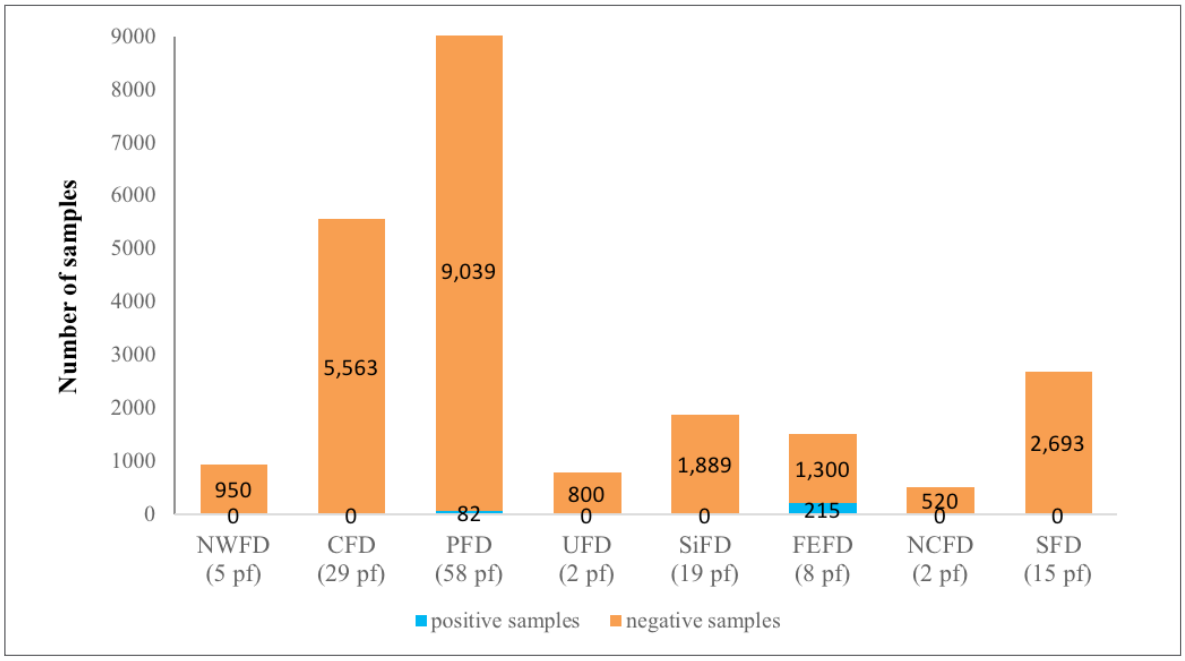

Fig. 2. Detection of antibodies to AI virus in poultry sera, submitted from Russian industrial poultry farms, using ELISA and HI test in 2019.
The number of poultry farms from which samples were delivered is shown in parentheses.

Рис. 2. Выявление антител к вирусу гриппа птиц в сыворотках крови птиц из промышленных птицеводческих хозяйств РФ в ИФА и РТГА в 2019 г.
В скобках указано количество птицеводческих хозяйств, из которых доставляли пробы.

The study of 5,269 serum samples collected from broiler chicken in 7 Federal Districts of the Russian Federation revealed the presence of Newcastle disease virus specific antibodies in 2,234 samples. The lowest number of positive samples was found on the farms of the North Caucasus Federal District (NCFD) (29%), and the highest number – in the Far Eastern Federal District (86%), in the remaining districts the number of positive samples ranged from 31% to 58%. A relatively low average percentage of positive samples in broiler chicken can be explained by the fact that different farms used different vaccination schedules, and that the level of post-vaccination antibodies was not always high enough by the time of blood sampling (at 30–45 days of age).

In the study of serum samples collected from turkey in the PFD and UFD, antibodies to the Newcastle disease virus were detected in 74% and 100% of samples from vaccinated birds, respectively. No antibodies to the Newcastle disease virus were found in the quail sera. When studying goose sera, specific antibodies were detected in birds from two regions – the Republic of Mari El and the Republic of Tatarstan.

According to the information available in the accompanying documents, the antibodies detected in serum samples collected from poultry on commercial poultry farms were induced by the Newcastle disease virus vaccine strains contained in live or inactivated vaccines. On some of the farms vaccination did not result in a satisfactory antibody response in broiler chickens.

The main goal of serological monitoring studies on influenza type A in poultry is the control of the infection in unvaccinated flocks, as well as the control of post-vaccination immunity in vaccinated flocks.

In 2019, 22,754 poultry serum samples (from chicken, turkey, duck, goose, quail) were delivered from 138 commercial poultry farms, located in 41 regions and 8 Federal Districts of the Russian Federation, to be tested for avian influenza (Fig. 2).

Antibodies to the avian influenza virus were found in chicken from one of the farms in the Perm Krai (subtype H9) and in chicken from two poultry farms in the Primorsky Krai (also subtype H9). Vaccination against avian influenza was carried out on all the commercial farms. The detected antibodies were specific to the vaccine antigen by the hemagglutinin subtype. No avian influenza virus antibodies were detected in other poultry species.

In 2019, 8,699 poultry serum samples (from chicken, turkey, goose, duck, quail, pheasant) received from backyards and small scale farms located in 29 regions of the Russian Federation were tested for avian influenza (Tables 1 and 2).

Avian influenza virus specific antibodies were detected only in the samples collected from chicken vaccinated against influenza; samples from other poultry species showed negative results (Tables 1 and 2).

According to the accompanying documents, vaccination against avian influenza was carried out using inactivated vaccines containing the antigen of the homologous influenza virus A/H5. The number of positive results in vaccinated flocks in various regions ranged from 35% to 80%. Low levels of post-vaccination antibodies were observed in the Rostov and Astrakhan oblasts (35 and 44%, respectively), and high levels (69, 78, 80, 88%) – in the Republic of Altai, Krasnodar Krai, Chechen Republic and Primorsky Krai, respectively.

In 2019, 7,888 poultry serum samples received from backyards and small scale farms located in 27 regions in 7 Federal Districts of the Russian Federation were tested for antibodies to the Newcastle disease virus (Tables 1 and 2).

Table 1
Detection of antibodies to ND and AI viruses in poultry sera, submitted from backyards and small scale farms in the Russian Federation, using ELISA and HI test in 2019

Таблица 1
Выявление антител к вирусам ньюкаслской болезни и гриппа птиц в сыворотках крови домашних птиц из ЛПХ и КФХ РФ в ИФА и РТГА в 2019 г.

Federal district	Subject of the Russian Federation	Number of tested samples			
		ND		AI	
		total	positive	total	positive
NWFD	Murmansk oblast	n/t	n/t	10	0
CFD	Vladimir oblast	444	151	397	0
	Bryansk oblast	55	24	55	0
	Kursk oblast*	70	30	70	0
	Oryol oblast	191	7	148	1
	Smolensk oblast	76	32	76	0
PFD	Republic of Tatarstan	287	85	389	0
	Republic Of Mari El	n/t	n/t	75	0
	Republic of Mordovia	36	0	36	0
	Samara oblast	50	50	50	0
UFD	Tyumen oblast	325	12	325	0
SiFD	Omsk oblast*	1	1	1	0
	Altai Republic	100	0	139	69
	Republic of Tyva	155	46	155	0
	Irkutsk oblast	31	0	31	0
FEFD	Zabaikalsky Krai*	362	50	362	0
	Amur oblast	475	1	575	0
	Khabarovsk Krai	350	41	350	0
	Primorsky Krai*	300	300	600	264
SFD	Astrakhan oblast	200	39	400	106
	Volgograd oblast	300	144	300	0
	Krasnodar Krai*	100	100	100	78
	Republic of Adygeya	173	140	173	0
	Rostov oblast	1,914	459	2,092	245
NCFD	Republic of Ingushetia	400	265	400	0
	Republic of Dagestan	200	88	200	0
	Stavropol Krai*	510	233	520	3
	Karachay-Cherkess Republic	200	128	200	0
	Chechen Republic*	583	339	470	374

n/t – not tested (пробы не исследовали);
* Regions, where ND outbreaks were reported in 2019 (according to the data received from the Veterinary Services).
* Регионы, в которых, по данным ветеринарных служб в 2019 г., были зарегистрированы вспышки ньюкаслской болезни.

Newcastle disease virus-specific antibodies were detected in three poultry species: chicken, turkey and goose. Antibodies to the Newcastle disease virus were detected in 39% of all the chicken serum samples tested in the Russian Federation. No specific antibodies were detected in unvaccinated chicken in three regions – the Republic of Mordovia, the Irkutsk oblast, and the Republic of Altai. According to the accompanying documents, chicken for backyards were purchased mainly from commercial poultry farms, where they had been vaccinated against Newcastle disease, however, after that they were not vaccinated anymore. The majority of small scale farms carried out re-vaccination of poultry against Newcastle disease. According to the accompanying documents, antibodies to the Newcastle disease virus were detected in 65% of the tested samples taken from vaccinated chicken. The low percentage of positive results may be indicative of insufficient effectiveness of the preventive vaccination against Newcastle disease carried out on small scale farms and in backyards in the studied regions.

In vaccinated turkeys, the antibodies were found in 100% of the tested samples received from the Samara oblast and in one sample from a small scale farm in the Amur oblast. No antibodies were found in serum samples from non-vaccinated turkeys received from the Rostov oblast and the Zabaikalsky Krai. Goose serum samples (133 samples) were delivered from 7 regions and 6 Federal Districts. Antibodies to the Newcastle disease virus were found in 3 samples from the Republic of Tatarstan.

Many bird species are natural reservoirs and carriers of infectious diseases, therefore, constant monitoring of epidemic situation in wild birds shall be one of the components in the system for the prediction, control and prevention of diseases, including such highly dangerous viral diseases as avian influenza and Newcastle disease.

Table 3 presents the HI test results for sera from wild and synanthropic birds collected in 2019.

No antibodies to avian influenza virus of H5 and H7 subtypes were found in the study of 236 serum samples collected from wild and synanthropic birds in 6 Subjects of the Russian Federation.

Antibodies to the Newcastle disease virus were detected in 153 out of 255 samples received from 4 Subjects of the Russian Federation, in 123 samples from synanthropic birds (crows and pigeons) and in 30 samples from wild birds. In most cases, positive samples from pigeons were collected near large poultry farms, which poses a risk of disease introduction into the poultry farms.

Figure 3 shows the location of the RF regions where antibodies to avian influenza and Newcastle disease viruses were detected in poultry and wild birds.

Of the 50 regions represented in the monitoring studies, Newcastle disease and avian influenza viruses specific antibodies were not detected in two oblasts (the Murmansk and the Arkhangelsk oblast).

It should be noted that the serological study conducted in the Russian Federation in 2019 did not show the presence of antibodies to avian influenza virus in unvaccinated poultry, while the studies conducted in 2017–2018 showed the presence of post-infectious antibodies to influenza A/H5 virus in serum samples from birds in the Altai Krai, the Rostov and Kaliningrad oblasts, and to influenza A/H9 virus in serum samples from birds in backyards and on two poultry farms in the Primorsky Krai. Analysis of the serological monitoring data collected over the past three

years showed a continuous unstable situation regarding Newcastle disease in the Russian Federation, because, despite the high seropositivity of poultry on commercial poultry farms, inadequate level of protection on small scale farms due to the absence of mass vaccination poses a constant threat of the disease occurrence.

CONCLUSION

Thus, the results of the serological studies on Newcastle disease conducted in the framework of the State epidemic monitoring in 2019 showed a high level of positive results in birds on commercial poultry farms, associated with mass vaccination against this disease, and insufficient protection of poultry against Newcastle disease virus on small scale farms, which is confirmed by the reporting of primary disease outbreaks, caused by the virulent virus strain, specifically in poultry on small scale farms. Seroprevalence of Newcastle disease in wild and synanthropic birds, which are the most likely natural reservoir of Newcastle disease virus strains with different pathogenicity, was high in some regions of the Russian Federation.

The results of the monitoring studies on avian influenza showed, in general, the absence of avian influenza virus specific antibodies in the studied unvaccinated poultry on commercial poultry farms and in backyards and a low level of post-vaccinal immunity to avian influenza on small scale farms in certain regions of the Russian Federation.

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Table 2

Detection of antibodies to ND and AI viruses in sera collected from different poultry species in backyards and small scale farms in the Russian Federation in 2019

Таблица 2

Выявление антител к вирусу ньюкаслской болезни и гриппа птиц в сыворотках крови разных видов птиц из ЛПХ и КФХ РФ в 2019 г.

Bird species	Number of tested samples			
	ND		AI	
	total	positive	total	positive
chicken	7,020	2,711	7,572	1,140
turkey	580	51	711	0
duck	155	0	186	0
goose	133	3	212	0
quail and pheasant	n/t	n/t	18	0
Total	7,888	2,765	8,699	1,140

n/t – not tested (пробы не исследовали).

Table 3

Detection of antibodies to ND virus and AI viruses in sera from wild and synanthropic birds using HI test

Таблица 3

Результаты исследования в РТГА проб сыворотки крови диких и синантропных птиц на наличие антител к вирусам ньюкаслской болезни и гриппа птиц

Subject of the Russian Federation	Bird species	Number of tested samples / number of positive samples		
		ND	HPAI H5	HPAI H7
Yaroslavl oblast	field and forest birds (capercaillie, black grouse, woodcock)	10/10 (100%)*	10/0	10/0
	waterfowl (wild ducks and geese)	27/20 (74%)	27/0	27/0
	synanthropic birds	12/12 (100%)	12/0	12/0
Samara oblast	synanthropic birds (pigeons)	30/0	30/0	30/0
Republic of Mordovia	synanthropic birds (crows, pigeons)	112/88 (79%)	112/0	112/0
	field and forest birds (lark, starling, rook)	10/0	10/0	10/0
Republic of Tatarstan	synanthropic birds (pigeons)	14/12 (86%)	n/t	n/t
Krasnoyarsk Krai	synanthropic birds (pigeons)	30/11 (37%)	25/0	25/0
Republic of Tyva	semiaquatic birds (great crested grebe, dabchick, herring gull, kite)	10/0	10/0	10/0
Total		255/153 (60%)	236/0	236/0

n/t – not tested (не исследовали);
* Positive sample percentage of the total number of samples tested.
* В скобках указан процент положительных от общего количества исследованных проб.

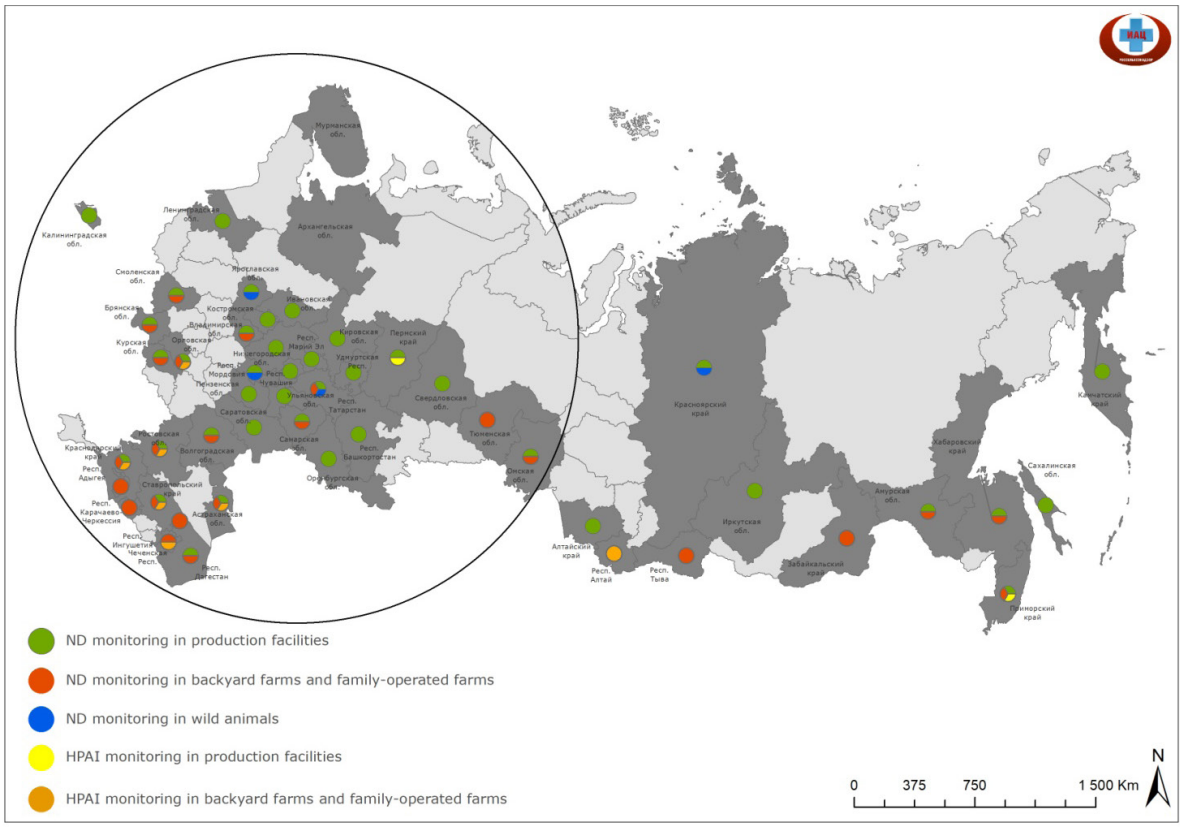

Fig. 3. RF Regions in which monitoring studies revealed antibodies to ND and AI viruses in 2019 (highlighted regions – the ones from which samples were delivered)

Рис. 3. Регионы Российской Федерации, на территории которых в ходе мониторинговых исследований у птиц было выявлено наличие антител к вирусам гриппа птиц и ньюкаслской болезни в 2019 г. (выделены регионы, из которых доставлены пробы)

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