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SEROLOGICAL MONITORING
FOR AVIAN INFLUENZA IN THE RUSSIAN FEDERATION IN 2017–2018

M. A. Volkova[1], Ir. A. Chvala[2], P. S. Yaroslavtseva[3], V. Yu. Sosipatorova[4], O. S. Osipova[5], I. A. Chvala[6]

[1] Leading Researcher, Candidate of Science (Biology), FGBI "ARRIAH", Vladimir, Russia, *e-mail: volkovama@arriah.ru; ORCID ID 0000-0002-7674-639X*

[2] Leading Researcher, Candidate of Science (Biology), FGBI "ARRIAH", Vladimir, Russia, *e-mail: chvala_ia@arriah.ru; ORCID ID 0000-0001-5846-1262*

[3] Researcher, Candidate of Science (Biology), FGBI "ARRIAH", Vladimir, Russia, *e-mail: yaroslavtseva@arriah.ru; ORCID ID 0000-0003-0383-9912*

[4] Junior Researcher, FGBI "ARRIAH", Vladimir, Russia, *e-mail: sosipatorova@arriah.ru; ORCID ID 0000-0001-9376-4728*

[5] Veterinarian, FGBI "ARRIAH", Vladimir, Russia, *e-mail: osipova@arriah.ru; ORCID ID 0000-0002-3176-157X*

[6] Deputy Head, Candidate of Science (Veterinary Medicine), FGBI "ARRIAH", Vladimir, Russia, *e-mail: chvala@arriah.ru; ORCID ID 0000-0002-1659-3256*

SUMMARY

Avian influenza is a highly contagious avian viral disease notifiable to the World Organization for Animal Health that causes huge economic losses and poses a great threat to poultry farms worldwide. A total of 60,697 serum samples from poultry and 581 serum samples from wild and synanthropic birds were submitted to the Reference Laboratory for Avian Viral Diseases of the FGBI "ARRIAH" (Vladimir) from 34 regions in 2017–2018 in the framework of monitoring carried out by the Federal Service for Veterinary and Phytosanitary Surveillance of the Russian Federation. Antibodies to type A avian influenza virus were detected with laboratory tests in backyard poultry in the Smolensk Oblast and Republic of Crimea, antibodies to A/H5 AIV were found in backyard poultry in the Altai Krai, Rostov and Kaliningrad Oblasts, antibodies to A/H9 AIV were found in backyard poultry in the Primorsky Krai. Antibodies against A/H9 AIV were also detected in non-vaccinated chickens kept at two poultry establishments located in the Primorsky Krai in 2018. The immunity in backyard poultry in the Russian Federation Subjects covered by scheduled preventive vaccination against A/H5 AIV was found to be insufficient. Obtained test results indicated that the virus circulation in bird populations in the Russian Federation and persistent risk of avian influenza spread to poultry establishments and backyard poultry.

Key words: avian influenza, epidemiology, monitoring, poultry, wild birds.

INTRODUCTION

Avian influenza is a highly contagious viral disease of poultry and wild birds characterized with primarily respiratory and alimentary tract lesions [2]. The disease is caused by type A avian influenza virus (AIV) belonging to *Influenzavirus* genus of *Orthomyxoviridae* family. Highly pathogenic AI virus strains are responsible for systemic disease with high mortality (up to 100%) characterized by hemorrhages and inflammations in internal organs, skin and its derivatives. Highly pathogenic and low pathogenic avian influenza in poultry caused by H5 and H7 is notifiable to the World Organization for Animal Health [2, 3]. Economic losses caused by avian influenza are huge since all susceptible poultry in the disease outbreak area shall be stamped out. Initially, A/H5N1 highly pathogenic avian influenza virus was isolated from domestic geese kept on a farm located in Guangdong province, South China, in 1996. Far Eastern countries including South-Eastern Asian countries have remained HPAI-affected for many years. Recently, H5 viruses containing neuraminidases of different subtypes have tended to widespread across various European, Asian and African countries [1, 4–7]. Russia is connected with wild bird migration routes both with European and Asian countries so the risk of highly and low pathogenic avian influenza virus introduction to the Russian Federation territory remains very high [1, 8]. According to immediate notifications provided by the Veterinary Services of the RF Subjects in 2017, H5 highly pathogenic avian influenza (more than 30 cases) was detected in poultry in 9 regions: Rostov, Moscow, Nizhny Novgorod, Kostroma and Samara Oblasts, Republics of Tatarstan, Mariy-El, Udmurtia as well as in Chechen Republic. AI cases were reported in wild migratory birds in the Krasnodar Krai and Kaliningrad Oblast and in the zoo in the Voronezh Oblast. In 2018, AIV infection was reported in 15 regions. H5 avian influenza virus was detected in poultry in the Kursk, Oryol, Voronezh, Kostroma, Smolensk, Saratov, Samara, Ulyanovsk, Penza, Nizhny Novgorod, Rostov Oblasts, Republics of Mariy-El, Udmurtia, Chuvashia and Tatarstan (more than 80 cases).

Enzyme-linked immunosorbent assay (ELISA) test kits for detection of type A AIV-specific antibodies are used for high-throughput screening for type A AIV infection. In case of positive results, subtyping shall be performed with hemagglutination inhibition (HI) test. The said methods are used for determination of poultry postvaccinal immune status. Anti-A/H5N1 AIV vaccination was carried out in Asia and in the Middle East [9, 10]. Recently, anti-A/H5N1 AIV vaccination of outdoor poultry and zoo birds has been permitted in some EU Member States [3]. In Russia, vaccination against type A avian influenza is performed in accordance with Rules for avian influenza control as amended by the Order of the RF Ministry of Agriculture No. 195 of July 6, 2006.

Permanent control and surveillance of infectious animal diseases including avian influenza is required for maintaining sustainable epidemic freedom of the country.

Results of AI serological monitoring carried out in the Russian Federation in 2017–2018 within the framework of the Rosselkhoznadzor measures for highly dangerous animal disease diagnosis and prevention taken for the RF territory protection from animal disease introduction and spread are presented in the paper.

MATERIALS AND METHODS

Biological materials (sera from birds) were submitted for testing by the Rosselkhoznadzor Territorial Administrations. Tests were carried out with commercial test-kits

Table 1
Results of HI and ELISA tests of sera from poultry kept at poultry establishments for antibodies against avian influenza virus

Federal Okrug of the Russian Federation	RF Subject	Number of tested samples		Number of positives	
		2017	2018	2017	2018
Central	Vladimir Oblast	1,714 (5)[1]	1,132 (5)	0	0
	Ivanovo Oblast	2,417 (3)	1,004 (1)	0	0
	Kostroma Oblast	2,014 (6)	1,073 (5)	0	0
Far-East	Primorsky Krai	925 (5)	1,370 (4)	174 (2)[2]	176 (2)[2] 411 (2)[3]
	Amur Oblast	920 (5)	1,660 (1)	0	404 (1)[3]
	Khabarovsk Krai	380 (2)	50 (2)	0	0
	Sakhalin Oblast	n/t	750 (1)	n/t	0
Volga	Nizhny Novgorod Oblast	2,310 (7)	2,025 (7)	0	0
	Saratov Oblast	609 (5)	500 (1)	0	0
	Samara Oblast	n/t	180 (3)	n/t	0
	Republic of Mariy El	250 (1)	920 (6)	0	0
	Kirov Oblast	n/t	120 (2)	n/t	0
	Republic of Bashkortostan	n/t	612 (5)	n/t	0
	Republic of Tatarstan	n/t	810 (5)	n/t	0
	Republic of Udmurtia	n/t	600 (5)	n/t	0
North-Caucasian	Stavropol Krai	1,940 (6)	2,388 (6)	0	0
Siberian	Altai Krai and Republic of Altai	955 (5)	500 (4)	0	0
	Krasnoyarsk Krai	1,385 (7)	1,016 (7)	0	0
	Republic of Tyva	n/t	300 (1)	n/t	0
North-West	Kaliningrad Oblast	580 (3)	400 (2)	0	0
South	Astrakhan Oblast	861 (2)	772 (4)	0	0
	Krasnodar Krai	1,836 (9)	1,855 (6)	0	0
	Rostov Obalst	107 (3)	340 (4)	0	0
	Volgograd Oblast	1,072 (3)	3,022 (2)	0	0
TOTAL		**20,275 (77)**	**23,399 (89)**	**174[2]**	**176[2]** **815[3]**

[1] Number of establishments is given in brackets;
[2] post-infection antibodies;
[3] postvaccinal antibodies;
n/t – not tested.

manufactured by the FGBI "ARRIAH" and used according to the manufacturer's instruction as well as with diagnostica (antigens and sera) manufactured by GD (Netherlands) and IZSVe (Italy) using standard methods [3]. HI test results were considered positive when serum titre was 4.0 $\log_2$ or higher. ELISA test-kits were used for testing chicken sera; HI test-kits were used for testing poultry sera (chickens, turkeys, ducks, geese, quails, guinea fowl), wild and synanthropic bird sera. Submitted sera were inactivated at temperature of 56 °C for 30 min.

RESULTS AND DISCUSSION

Type A AI serological monitoring tests in poultry are aimed at following: control of non-vaccinated flocks for infection, detection of circulation of the field viruses distinct from vaccine virus strains as well as control of vaccinated poultry for postvaccinal immunity.

In 2017, 20,275 sera submitted from 77 poultry establishments located in 17 RF Subjects were tested with ELISA and HI (Table 1).

Anti-A/H9 AIV antibodies were detected in 174 samples collected from chickens vaccinated against avian influenza (antibodies against homologous virus subtype) at two establishments located in the Primorsky Krai. The majority out of all poultry species covered by monitoring tests in 2017 were chickens (99.3%). The poultry of other species were also tested: turkeys, geese, ducks and quails.

In 2018, the testing coverage was slightly expanded: 23,399 serum samples from poultry (chickens, turkeys, ducks, geese) from 89 poultry establishments located in 24 RF Subjects were tested. Anti-A/H9 AIV antibodies were detected in 176 serum samples collected from non-vaccinated chickens kept at two establishments located in the Primorsky Krai and in 815 serum samples collected from vaccinated chickens kept in the Amur Oblast (1 poultry establishment) and Primorsky Krai (2 poultry establishments) (antibodies against homologous vaccine virus strain).

In 2017–2018, monitoring tests were carried out in backyard poultry and on small poultry farms (Table 2). More than 92% of tested samples were collected from chickens, 4–5% – from ducks and the rest samples – from other poultry species (geese, turkeys, quails and guinea fowl).

In 2017, tests of 9,908 serum samples from poultry kept in 16 regions of the Russian Federation were carried out.

Table 2
Results of ELISA and HI tests of serum samples from backyard poultry and poultry kept on small poultry farms for antibodies against AI virus

Federal Okrug of the Russian Federation	RF Subject	Number of tested samples		Number of positives	
		2017	2018	2017	2018
Central	Vladimir Oblast	142	342	0	0
	Smolensk Oblast	n/t	6	n/t	1[1]
Volga	Republic of Tatarstan	n/t	200	n/t	0
	Nizhny Novgorod Oblast	90	n/t	0	n/t
	Samara Oblast	n/t	384	n/t	0
	Republic of Chuvashia	n/t	22	n/t	0
North-West	Kaliningrad Oblast	805	110	5[1]	0
Siberian	Altai Krai	45	500	0	22[1]
Far East	Zabaikalsky Krai	1,066	388	0	0
	Primorsky Krai	100	3	0	2[1]
	Khabarovsk Krai	620	n/t	0	n/t
North-Caucasian	Republic of Dagestan	291	500	19[2]	0
	Karachay-Cherkess Republic	120	n/t	0	n/t
	Republic of Ingushetia	225	500	2[2]	0
	Chechen Republic	1,139	500	138[2]	248[2]
South	Astrakhan Oblast	1,471	1,324	118[2]	124[2]
	Republic of Adygheya	176	n/t	2[2]	n/t
	Rostov Oblast	2,341	2,636	14[1] 25[2]	177[2]
	Volgograd Oblast	1,000	n/t	0	n/t
	Republic of Crimea	277	n/t	7[1]	n/t
TOTAL		**9,908**	**7,415**	**26[1] 304[2]**	**25[1] 550[2]**

[1] post-infection antibodies;
[2] postvaccinal antibodies;
n/t – not tested.

Anti-A/H9 AIV antibodies were detected in 304 samples from AI-vaccinated chickens kept in the Astrakhan and Rostov Oblasts, Chechen Republic, Republics of Dagestan, Ingushetia and Adygheya. Anti-A/H5 AIV antibodies were detected in samples collected from non-vaccinated backyard chickens in the Rostov and Kaliningrad Oblasts (in 14 and 5 samples, respectively). Seven samples submitted from the Republic of Crimea were ELISA tested positive for type AI.

In 2018, 7,415 serum samples collected form chickens, turkeys, ducks, geese, quails and guinea fowls kept in 14 RF Subjects were tested (Table 2). Specific antibodies were detected in 550 samples collected from AI-vaccinated poultry (chickens, quails, guinea fowl) in 3 RF Subjects (Astrakhan and Rostov Oblasts, Chechen Republic) (against subtype H5). Post-infection antibodies were detected by HI test in non-vaccinated chickens in the Altai and Primorsky Krais (against subtype H5 and H9, respectively) and by ELISA in one sample from a chicken in the Smolensk Oblast. Type A AIV-positive samples submitted from the Republic of Crimea and Smolensk Oblast were HI tested, the HI test did not confirm presence of anti-H5 and H9 AIV antibodies. When anti-H5 и H9 AIV antibodies were detected in non-vaccinated birds, the test results were forwarded to the relevant Rosselkhoznadzor Territorial Administrations for taking appropriate measures. No A/H5 AIV-specific antibodies were detected in commercial and backyard ducks and geese during monitoring period.

The Figure shows geographical location of the RF regions where anti-AIV post-infection and postvaccinal antibodies were detected in poultry.

Anti-AIV antibodies detected in commercial and backyard chickens can be accounted for circulation of low virulent virus strains that cause respiratory and intestinal disorders of various severity and are not associated with

Fig. Regions of the Russian Federation where antibodies against avian influenza were detected in poultry by monitoring tests in 2017–2018

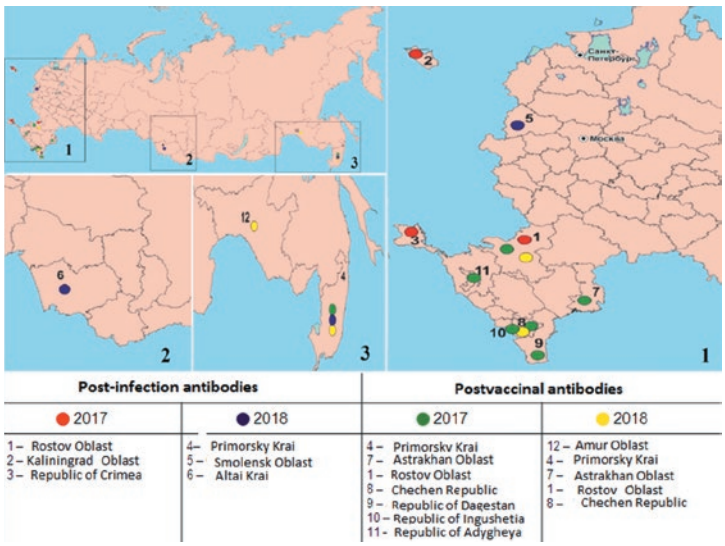

Table 3
Results of HI tests of serum samples from wild and synanthropic birds for antibodies against avian influenza virus

RF Subject	Bird species	Number of tested samples*	
		subtype H5	subtype H7
Nizhny Novgorod Oblast (2017)	Synanthropic birds (rock pigeon, rook, crow, jackdaw)	0/40	0/40
	Waterfowl (wild geese and ducks)	0/53	0/53
	Birds of field and forest (black grouse, woodcock)	0/6	0/6
Krasnoyarsk Krai (2017)	Synanthropic birds (rock pigeon)	0/130	0/130
	Waterfowl (mallard, wigeon, European teal)	0/20	0/20
	Wild birds of meadow, field, marsh (snipe, booted warbler, marsh snipe, stonechat, yellow wagtail, tree sparrow)	0/90	0/90
	Wild birds of forest (tree pipit, marsh tit, woodcock, wryneck, nuthatch, missel thrush, crested titmouse)	0/60	0/60
Republic of Tyva (2017)	Waterfowl and wetland birds (great-crested grebe, gadwall, red-crested pochard, cormorant, tern, herring gull, black-headed gull)	0/31	0/31
Samara Oblast (2018)	Wild birds (wild ducks, etc.)	0/55	0/55
	Synanthropic birds (pigeons, etc.)	0/96	0/96
TOTAL		**0/581**	**0/581**

*Number of positives/total number of samples.

mass mortality of poultry. However, circulation of such virus strains creates an enabling environment for their selection/mutation and emergence of highly virulent avian influenza virus strains.

It should be noted that postvaccinal immunity level in vaccinated backyard chickens was low. According to accompanying documents, inactivated A/H5 AIV antigen-containing vaccines were used for vaccination; however, postvaccinal antibodies were detected in less than 50% of vaccinated poultry.

Both waterfowl and land wild birds serve as a natural reservoir for avian influenza virus. Migratory wild birds in most cases can be an initial source of the infection that poses a risk of further infection introduction to poultry flocks and the disease spread to the disease-free regions.

314 and 266 serum samples from wild and synanthropic birds, respectively, from 4 RF regions were submitted for testing in 2017–2018. The obtained results were given in Table 3. Test group of synanthropic birds comprised bird species which habitats and ways of living were associated with humans and humans' dwellings. No antibodies against subtype H5 and H7 avian influenza virus were detected in 581 serum samples tested with HI.

CONCLUSION

Permanent HPAI emergence and wide spread pose a serious threat to poultry. Strict compliance with veterinary and sanitary standards and high biosecurity level can minimize avian influenza infection risk at indoor poultry establishments. In poultry industry, avian influenza is the most dangerous for backyards and small outdoor poultry farms where poultry can directly contact to wild and synanthropic birds being a source of the infection. Therefore, serological tests for avian influenza performed in the framework of official epidemiological monitoring should be continued and their number should be increased, as they are an essential component of the system for avian influenza control, prevention as well as AI occurrence prediction in the Russian Federation territory.

Conflict of interests. The authors claim no conflict of interests.

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