

## VIROLOGICAL MONITORING OF ESPECIALLY DANGEROUS AVIAN PATHOGENS IN SOUTHERN UKRAINE IN 2017 DURING AFTER-EPIZOOTIC PERIOD

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**Summary.** Constant monitoring of the circulation of particularly dangerous avian diseases' causative agents (influenza and Newcastle disease) in natural reservoir is an important component of global surveillance and control of these diseases. The main objective of the research is to control the circulation of influenza viruses, Newcastle disease and other avulaviruses in poultry in Ukraine, isolation of influenza virus isolates from H5 and H7 subtypes for further study. Classical virological methods of viruses' isolation using chicken embryos, serological tests using referent blood sera as well as identification of viruses via real-time PCR were used for research. Ten influenza viruses of different subtypes (including one influenza virus of H5 subtype and two influenza viruses of H7 subtype), as well as various avulaviruses of different serotypes (including AvV-1 — Newcastle disease virus) were isolated from 1,619 wild waterfowl and water-related birds of 28 species during autumn migration in 2017 in Kherson, Odesa, and Zaporizhia regions. All the viruses are isolated from wild ducks, geese and gulls. Hemagglutinating viruses from wild birds of such ecological groups as waders, herons and land birds have not been isolated. The level of infection of wild waterfowl (wild ducks) during the autumn migration in 2017 was 1.44–1.58%, and waterfowl (gulls) — 0.42%. The data we obtained is very important for predicting the epizootic situation in Ukraine, understanding the ecological features of low-pathogenic variants of viruses, which cause particularly dangerous avian viral diseases. In addition, conducting regular virological monitoring in a natural reservoir allows us to receive new strains for improvement of laboratory diagnostics.

**Keywords:** H5 and H7 subtypes of avian influenza, Newcastle disease, wild birds, surveillance, Ukraine

**Introduction.** Avian influenza (AI) and Newcastle disease (ND) are two major diseases that are particularly dangerous for birds. They have a huge impact on the poultry industry and might lead to huge economic losses: the extinction of poultry, the imposition of quarantine and other restrictive measures, the ban on trade, additional veterinary and sanitary measures). In addition, avian influenza is one of the few infections, which poses a serious threat to humans. Influenza occupies a special place as a pathogen which is dangerous to human health and which may cause a serious pandemic. Newcastle disease does not have such an epidemic potential and does not pose a serious threat to human health (Saif, 2008).

Influenza viruses belong to the Orthomyxoviridae family (Capua and Alexander, 2009; Swayne, 2016). Wild waterfowl and water-related birds are the main natural reservoir of all influenza viruses and play a major role in the circulation of this pathogen (Swayne, 2016). With regard to Newcastle disease, this pathogen belongs to avian avulaviruses (Knipe and Howley, 2013). The main role in the natural circulation of this pathogen also belongs to wild waterfowl and water-related birds (Camenisch, Bandli and Hoop, 2008; Saif, 2008).

Due to the extraordinary importance of these two diseases, the need to control the circulation of pathogens in natural reservoir worldwide, birds are constantly monitored. These activities have been carried out in Ukraine since 2005. Research results obtained at

NSC 'IECVM' have proven a widespread natural circulation of influenza virus and avulaviruses (the former name — avian paramyxoviruses) among wild birds in Ukraine (Muzyka et al., 2012, 2016a, 2016b). Ninety-five AI viruses, belonging to 15 out of 16 known HA subtypes and up to 7 out of 9 known NA subtypes, have been isolated by the beginning of 2017. However, monitoring studies should be conducted on an ongoing basis, enabling valuable information on new variants of the virus and carry out preparatory work for the next outbreaks, improve laboratory diagnostics.

Therefore, the **main objective of our research** in 2017 was to conduct virological monitoring of wild birds in places of their mass accumulation in the southern region of Ukraine.

**Materials and methods.** Wild bird surveillance was conducted during the autumn migration 2017 in south regions of Ukraine (Kherson, Zaporizhia, and Odesa regions). In 2017 samples of biological material (fecal samples) were collected from 1,619 wild birds of 28 species of wild waterfowl birds, shore birds and some birds of other ecological groups and environmental samples (Table 1).

**Virus isolation.** Virus isolation was conducted in accordance with the OIE procedures (OIE, 2012). Fecal samples were inoculated into the allantoic cavity of 9–10-day-old chicken embryonated eggs. Every sample was passaged three times.

**Table 1** — Number of samples isolated from different species of wild birds in south regions of Ukraine in 2017

Species	Samples	Species	Samples	Species	Samples
Anseriformes		Mediterranean gull	169	Gruiformes	
Mallard	442	Ruff	1	Crane	11
Greylag goose	123	Slender-billed gull	16	Demoiselle crane	12
Mute swan	23	Spoonbill	7	Ciconiiformes	
Ruddy shelduck	30	White-winged black tern	20	Grey heron	29
Shelduck	135	Little tern	5	Great white egret	15
Shoveler	2	Yellow-legged gull	104	Little egret	5
Garganey	18	Dunlin	10	Pelecaniformes	
Red-breasted goose	1	Little gull	5	Cormorant	101
Charadriiformes		Podicipediformes		Dalmatian pelican	15
Black-headed gull	238	Great crested grebe	1	White pelican	26
Gull spp.	10			Environmental	45
				Total	16,919

The presence of hemagglutinating viruses in allantoic fluid was determined by the HA test with a 1% suspension of chicken red blood cells (OIE, 2012).

**Virus identification.** The hemagglutinin (HA) virus subtype was determined by HI tests as previously described (Capua and Alexander, 2009; OIE, 2012; Spackman, 2014).

For these studies, the following antisera were used: H1N1, H2N3, H3N8, H4N6, H5N1, H6N8, H7N1, H8N4, H9N2, H10N7, H10N9, H11N6, H12N5, H13N6, H14N6, H15N9, H16N3, AvV-1, AvV-2, AvV-3, AvV-4, AvV-6, AvV-7, AvV-8, AvV-9 produced by Veterinary Laboratories Agency (Weybridge, UK); and the antisera H1N1, H2N3, H3N8, H4N8, H5N3, H6N2, H7N3, H8N4,

H9N7, H10N1, H11N9, H12N5, H13N6, H14N5, H15N9, H16N3 produced by the Instituto Zooprofilattico Sperimentale delle Venezie (Padova, Italy).

**Results.** According to the results of virological studies using chicken embryos, sixteen hemagglutinating isolates with activity from 1:32 to 1:2048 were obtained from wild birds' fecal samples. These isolates were obtained from the mallards, shelducks, gray geese, gulls as well as from environmental samples in Kherson and Odesa Regions. No positive specimen was found in the Zaporizhia Region. After isolation, all hemagglutinating isolates were identified using HI test with reference sera. The results of virological research and identification are shown in Table 2.

**Table 2** — Results of virological studies of wild birds' fecal samples in 2017

Place	Species	Total amount	Result of virological study			HI identification
			Negative	Positive	Name of isolates	
Kherson Region	Mallard	250	242	8	Mallard/Drugelubovka-3/6-10/6-08/17	H4
					Mallard/Drugelubovka-3/1-5/6-08/17	H3
					Mallard/Drugelubovka/1-3/5-09/17	AvV-4
					Mallard/Syvashivka/1-4/4-09/17	H5/AvV-6
					Mallard/Khorly/16-20/29-11/17	H7/H9
					Mallard/Chongar/13-16/3-09/17	H5
					Mallard/Mytrofanivka/1-4/4-09/17	AvV-1
	Mallard/Oleksiivka-2/33-36/5-09/17	H4				
	Black-headed gull	48	47	1	B.h.Gull/Drugelubovka-3/1-3/6-08/17	H11/H13
Greylag goose	21	20	1	Greylag Goose/Mytrofanivka/1-4/4-09/17	AvV-1	
Shelduck	46	45	1	Shelduck/Sergiivka/11-15/6-02/17	AvV-1	
Environmental	45	44	1	Envir./Novodmytrivka/41-45/7-08/17	AvV-6	
Odesa Region	Mallard	192	190	2	Mallard/Zhovtyi Yar/1-5/10-08/17	H4
					Mallard/Ermakov/41-50/17	H3
	Shelduck	89	87	2	Shelduck/Zhovtyi Yar /1-5/27-10/17	H7/H3
					Shelduck/Bazivka/13-16/28-10/17	H2/H5/H7

Thus, 10 influenza viruses of different subtypes (H2, H3, H4, H5, H7, H9, H11, H13), as well as five avulaviruses and one mix of isolates were obtained during the autumn migration from wild waterfowl. Based on the results of virological studies, we calculated the infection level of wild birds. It has been found that 1.44–1.58% of wild waterfowl (wild ducks) during the autumn migration in 2017 were infected with the influenza virus, while wild shore birds (gulls) were only 0.42% infected. According to the results of serological identification, some viral isolates showed cross-reactions with referent sera to different subtypes of hemagglutinin H7/H9, H11/H13, H2/H5/H7. In this regard, they were studied in real-time PCR.

According to the results of real-time PCR, it was found that all viral isolates that had cross-reactions were A virus viruses, the Mallard/Chongar/13-16/3-09/17 viral isolate belongs to the influenza virus of the subtype H5 and 3 viral isolates (Shelduck/Bazivka/13-16/28-10/17, Shelduck/Zhovtyi Yar/1-5/27-10/17 and Mallard/Khorly/16-20/29-11/17) belong to the H7 subtype influenza virus. Thus, the circulation of influenza viruses has been confirmed to be the most dangerous for poultry wildlife subtypes in 2017.

**Discussion.** It should be noted that the study of wild birds in 2017 was carried out in places where outbreaks of H5N1, H5N8 subtypes of highly pathogenic avian influenza were recorded in 2006, 2016–2017. In addition, in previous years, for the first time in Ukraine, the circulation of low-pathogenic influenza viruses in these regions was detected in 15 of 16 known hemagglutinin subtypes and 7 of the 9 known neuraminidase subtypes. There is also a large genetic diversity of these viruses, and

their connection with other geographic regions (Europe, Southeast Asia, Western Siberia) (Muzyka et al., 2012, 2016a, 2016b). That is to say that this territory is the place of traditional circulation of influenza viruses. The results we obtained confirm this assertion. These days, the circulation of avian influenza viruses of different subtypes continues in this region. According to many scientists, today a large global circulation of low-pathogenic influenza viruses in a natural reservoir has been proved, and our data coincides with these studies. It is also important to note that in the post-epizootic period (after the outbreaks of highly pathogenic H5N8 avian influenza in Ukraine in 2017) in wild birds, we obtained field isolates of H5 and H7 influenza virus. This may also indicate an independent circulation of highly pathogenic and low pathogenic avian influenza viruses in wild birds. As in our previous studies, all influenza viruses were isolated only from waterfowl and water-related birds, mainly from ducks.

Concerning avulaviruses, including Newcastle disease virus, we can also confirm that the circulation of these pathogens in wild birds continues, and this should be taken into account when planning of preventive measures in poultry farming.

**Conclusion.** The data we obtained is very important for predicting the epizootic situation in Ukraine, understanding the ecological features of low-pathogenic variants of pathogens, which cause particularly dangerous viral diseases of poultry. In addition, conducting regular virological monitoring in a natural reservoir allows us to receive new strains to improve laboratory diagnostics.

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