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AVULOVIRUS CIRCULATION AMONG WILD BIRDS IN UKRAINE IN 2017–2020

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Summary. In 2017–2020, virological monitoring of the circulation of avian avuloviruses among wild waterfowl and shorebirds of 53 species belonging to 8 families was conducted in the northern (Chernihiv) and southern (Odesa, Kherson, Zaporizhzhia, and Mykolaiv) regions of Ukraine. Since almost the entire territory of Ukraine lies within the main bird migration routes, a significant number of shorebirds nest and winter in the south of our country, and in fact, the entire south of Ukraine is at high risk of spreading pathogens that can be transmitted by birds, including avulovirus infections, throughout the year. A variety of avian avuloviruses are widely distributed among different hosts, but there is a large knowledge gap in understanding the movement of these viruses in wild populations. The results of virological monitoring showed that avuloviruses of different serotypes are actively circulating among wild birds of different ecological groups. During this period, 39 isolates of avuloviruses were isolated from wild birds. Based on the serologic identification results, it was found that most avulovirus isolates (n = 18) had cross-reactions, accounting for 46.15%. A total of 9 isolates belonged to AaV-1, accounting for 23.07%. 5 isolates belonged to AaV-4, which is 12.8%. 2 isolates belonged to AaV-9, which is 5.12%. AaV-6 and AaV-7 had two isolates each, which together accounted for 10.24%. 1 isolate belonged to AaV-3, which is 2.56%. It was also found that the highest number of isolates was isolated during wintering — 23 isolates and during autumn migration — 10 isolates, and the lowest number during nesting and spring migration, 4 and 2 isolates, respectively. The infection rate of wild birds with avuloviruses ranged from 0.13% to 11.76%. The most infected with avuloviruses were the species of common lamb (11.76%), and the least infected were the gray goose (0.13%)

Keywords: hemagglutination inhibition assay, natural reservoir, monitoring

Introduction. Avian avuloviruses (AaV), which belong to the family Paramyxoviridae, are a diverse group of zoonotic viruses. Their genome consists of a single-stranded minus-RNA molecule with 15–16 thousand nucleotide pairs and includes 20 unique serotypes (1–20). The International Committee on Taxonomy of Viruses classifies these serotypes into three different genera: *Metaavulavirus* (serotypes 2, 5–8, 10, 11, 14, 15, 20), *Orthoavulavirus* (serotypes 1, 9, 12, 13, 16–19), and *Paraavulavirus* (serotypes 3, 4) (Lefkowitz et al., 2018).

Avian avuloviruses are known for their ability to mutate and recombine, leading to the emergence of new variants and virus lines (Alexander et al., 1989). This evolutionary process results in significant antigenic and genetic diversity of avian avuloviruses in nature, affecting the pathogen's virulence, which can range from non-pathogenic to highly pathogenic. Virulence is influenced by various factors, including genetic variation and environmental factors (Miller et al., 2009). The constant process of avulovirus change can pose a challenge for controlling and managing poultry diseases. Viruses can expand their spectrum and adapt to new conditions. Therefore, continuous monitoring and research of these changes are important for developing an effective strategy for controlling and managing this pathogen.

AaV-1 (known as Newcastle disease) is the best known and most common of the avulovirus serotypes and is classified into two different classes (class I or class II) and is further characterized by either 1 genotype (class I) or 15 genotypes (class II) (Diel et al., 2012). Class I viruses are isolated exclusively from wild birds, while class II viruses cover poultry and wild populations (Diel et al., 2012; Hicks et al., 2019). This avian viral infection is characterized by pneumonia, encephalitis, digestive tract damage, and multiple hemorrhages in internal organs (Alexander, 2001). In addition to AaV-1, other avulovirus serotypes are less common in poultry and usually circulate in wild birds.

Other serotypes of avuloviruses cause respiratory and other diseases of lesser severity in various avifauna (Saif, 1997). Practical observations and experimental studies show that AaV-2, AaV-3, AaV-6, and AaV-7 are capable of causing diseases with different pathogenicity in poultry (Alexander, 2000; Nerome, et al., 1978). For example, AaV-6 strains cause a decrease in egg production in turkeys and mild respiratory diseases (Gough and Alexander, 1984). AaV-4, AaV-8, AaV-9, and AaV-10 were isolated from waterfowl and other wild bird species that did not show any clinical signs of disease (Stanislawek et al., 2002; Chang et al., 2001). AaV-4 was mainly isolated from wild birds of the genus *Plateolus*. Experimental infection of chickens with AaV-4 and AaV-6 caused mild respiratory pathologies (Miller and Afonso, 2009). As for the other avuloviruses (11^{th}–21^{st} serotypes), there is currently no clear information on their role in the occurrence of diseases in poultry, wild and domestic, as well as humans.

Due to its geographical location, Ukraine plays a crucial role in global migration processes of wild waterfowl and shorebirds of various species. The massive

gatherings and close interspecific contact of birds from different continents contribute to the spread and transfer of numerous pathogens, including avuloviruses (Rahman et al., 2018; Kinde et al., 2005; Reeves et al., 2016). Pathogen exchange or bird infection occurs during feeding or resting. In case of violating veterinary and sanitary standards of keeping, poultry can be infected by wild birds through direct contact in open water or walking areas, or by using water or feed contaminated with the droppings of wild or synanthropic birds. Outbreaks of AaV-1 in poultry occur worldwide. Therefore, studying subtypes and pathotypes of AaV-1 can contribute to a better understanding of the distribution and evolution of avuloviruses worldwide. Additionally, it is important to study the ecology and circulation of other avuloviruses in their natural reservoirs. Understanding the mechanisms of pathogen circulation will allow for more effective control of these infections. The surveillance, isolation, and identification of different avulovirus subtypes are crucial for the agricultural industry's well-being in Ukraine and Europe.

Therefore, our research **aimed** to continuously monitor avian avulovirus circulation in Ukraine's natural reservoir. This scientific work is a continuation of many years of research by scientists of the Department of Avian Diseases of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' on monitoring and studying the biological characteristics of epizootically relevant avulovirus isolates circulating among wild birds in Ukraine (Stegniy et al., 2012; Muzyka et al., 2014).

Materials and methods. ***Sample collection.*** Biological material was collected from wild birds during seasonal migrations (spring and autumn), nesting, and wintering in the northern and southern regions of Ukraine between 2017 and 2020. A total of 13,710 fecal samples were collected in the Azov-Black Sea region of Southern Ukraine, including Kherson, Odesa, Mykolaiv, and Zaporizhzhia regions. This area is one of the most important regions in Eastern Europe for the migration of wild birds of different ecological groups. In the north of Ukraine (Chernihiv Region), 1,203 samples of wild bird feces were collected. The locations of mass bird gatherings were identified by ornithologists from various organizations, including the Azov-Black Sea Ornithological Station in Zaporizhzhia Region, Askania-Nova Biosphere Reserve, Black Sea Biosphere Reserve in Kherson Region, and Danube Biosphere Reserve in Odesa Region, and leading ornithologists from Odesa and Chernihiv. A total of 14,913 fecal samples were collected from 53 species of wild birds using standard methods (Capua and Alexander, 2009; WOAH, 2023; Spackman, 2020). Samples were collected in cryotubes containing 1.0 cm^3 of viral transport medium (BHIV, brain heart infusion broth, Sigma-Aldrich, # 53286-100G) with antibiotics (penicillin 10,000 U/ml, streptomycin 10 mg/ml, gentamicin 250 μg/ml, and nystatin 5,000 U/ml) (Williams et al., 2016). Samples were stored in liquid nitrogen in the field and at –70 °C in the laboratory.

In 2017, a total of 4,790 environmental fecal samples were collected in places of mass gatherings of wild birds. The sampling was combined with ornithological surveys of bird populations in the area. Fecal samples were collected from 42 species of wild birds from seven different families: Anseriformes, Charadriiformes, Podicipediformes, Gruiformes, Ciconiiformes, Pelecaniformes, and Falconiiformes. We collected 92 samples in Zaporizhzhia Region, 10 samples in Mykolaiv Region, 1,904 samples in Odesa Region, 2,209 samples in Kherson Region, and 475 samples in Chernihiv Region. Samples were collected during the periods of bird migration in the Azov-Black Sea region of Ukraine, as well as in the north during autumn migration (August–October), wintering (November–February), spring migration (March–May), and during localized movements in June–July, which usually occur after the nesting period. In 2018, a total of 6,120 samples were collected from 44 species of wild shorebirds and waterbirds belonging to 6 families. Of these, 5,721 were collected in southern Ukraine (Odesa, Kherson, Zaporizhzhya, and Mykolaiv regions) and 399 in northern Ukraine (Chernihiv region). In 2019, field expeditions were conducted to cover both shorebirds and waterbirds at migratory stopover sites, resulting in the collection of 3,056 fecal samples. Of these, 329 were collected in Chernihiv Region and 2,727 were collected in the south from 32 bird species belonging to five families.

Samples from background bird species were collected at sentinel sites during 2020 in southern Ukraine, near the coasts of the Azov and Black seas, close to the sources of major rivers (the Danube and the Dnipro). A total of 947 fecal samples were collected from 12 bird species belonging to two families. Table 1 presents a summary of the wild bird sampling data for 2017–2020.

Virological research. Virus isolation from fecal samples was performed according to standard OIE procedures (WOAH, 2023; Spackman, 2020). To infect chicken embryos, a fecal suspension was prepared in phosphate-buffered saline (PBS) pH (7.2 ± 0.1) with antibiotics, infection was performed in the allantois cavity at a dose of 0.2 cm^3, and three passages were performed. The embryos were incubated for 4–5 days at 37 °C and ovoscoped twice a day; embryo death during the first day was considered nonspecific. After preliminary cooling at 4 °C, embryos were dissected. The presence of hemagglutinating viruses in the allantois fluid was determined by a hemagglutination assay in polystyrene plates with a V-shaped bottom with a 1% suspension of rooster erythrocytes (Capua and Alexander, 2009; WOAH, 2023).

Table 1 — List of samples collected from wild bird species in 2017–2020

Bird species		Period				Total
Common Name	**Scientific Name**	**2017**	**2018**	**2019**	**2020**	
Anseriformes						
White-fronted Goose	*Anser albifrons*	1,033	1,115	620	304	3,072
Red-breasted Goose	*Rufibrenta ruficollis*	23	304	–	–	327
Garganey	*Anas querquedula*	43	84	4	–	131
Mallard	*Anas platyrhynchos*	1,100	1,299	734	248	3,381
Shelduck	*Tadorna tadorna*	429	578	409	26	1,442
Ruddy shelduck	*Tadorna ferruginea*	103	301	90	–	494
Mute swan	*Cygnus olor*	49	27	2	–	78
Whooper swan	*Cygnus cygnus*	270	387	95	240	992
Greylag goose	*Anser anser*	386	176	160	15	737
Wigeon	*Anas penelope*	15	161	–	10	186
Shoveler	*Anas clypeata*	24	–	–	–	24
Teal	*Anas crecca*	61	110	–	47	218
Mixt of species (wild duck)		38	–	–	–	38
Bewick's swan	*Cygnus bewickii*	–	–	10	1	11
Pintail	*Anas acuta*	6	9	–	–	15
Gadwall	*Anas strepera*	–	25	3	–	28
Red-crested pochard	*Netta rufina*	–	15	–	–	15
White-fronted goose + Red-breasted goose	*Anser albifrons* + *Rufibrenta ruficollis*	–	70	51	–	121
Whooper swan + Bewick's swan	*Cygnus cygnus* + *Cygnus bewickii*	–	15	–	6	21
Charadriiformes						
Yellow-legged gull	*Larus cachinnans*	133	194	89	19	435
Black-headed gull	*Larus ridibundus*	262	113	162	30	567
Slender-billed gull	*Larus genei*	36	53	33	–	122
Ruff	*Phylomachus pugnax*	102	70	1	–	173
Mediterranean gull	*Larus melanocephalus*	189	131	31	–	351
Common gull	*Larus canus*	18	40	–	1	59
Great black-headed gull	*Larus ichtyaetus*	40	16	–	–	56
Dunlin	*Calidris alpina*	10	–	10	–	20
Snipe	*Gallinago gallinago*	15	1	1	–	17
Little gull	*Larus minutus*	5	30	3	–	38
Little tern	*Sterna albifrons*	5	1	–	–	6
Lapwing	*Vanellus vanellus*	–	50	2	–	52
White-winged black tern	*Chlidonias leucopterus*	20	–	–	–	20
Caspian tern	*Hydroprogne caspia*	7	–	–	–	7
Avocet	*Recurvirostra avosetta*	5	17	52	–	74
Gulls spp.		14	–	10	–	24
Gull-billed tern	*Gelochelidon nilotica*	–	52	50	–	102
Grey plover	*Pluvialis squatarola*	–	11	–	–	11
Collared pratincole	*Glareola pratincola*	–	20	–	–	20
Common tern	*Sterna hirundo*	–	16	25	–	41
Black-winged stilt	*Himantopus himantopus*	–	4	–	–	4
Greenshank	*Tringa nebularia*	–	11	–	–	11
Sandwich tern	*Thalasseus sandvicensis*	–	26	56	–	82
Wader spp.		–	7	–	–	7
Whiskered tern	*Chlidonias hybrida*	–	45	45	–	90
Black-headed gull + Mediterranean gull	*Larus ridibundus* + *Larus melanocephalus*	–	40	–	–	40

Table 1 — continuation

Bird species		Period				Total
Common Name	**Scientific Name**	**2017**	**2018**	**2019**	**2020**	
Podicipediformes						
Great crested grebe	*Podiceps cristatus*	1	–	–	–	1
Gruiformes						
Demoiselle crane	*Anthropoides virgo*	12	–	–	–	12
Crane	*Grus grus*	33	–	15	–	48
Coot	*Fulica atra*	9	59	109	–	177
Ciconiiformes						
Great white egret	*Egretta alba*	15	–	5	–	20
Little egret	*Egretta garzetta*	5	10	–	–	15
Grey heron	*Ardea cinerea*	29	7	–	–	36
Spoonbill	*Platalea leucorodia*	7	1	–	–	8
White stork	*Ciconia ciconia*	–	10	–	–	10
Pelecaniformes						
Dalmatian pelican	*Pelecanus crispus*	15	13	10	–	38
Cormorant	*Phalacrocorax carbo*	131	174	140	–	445
White pelican	*Pelecanus onocrotalus*	41	109	29	–	179
Pygmy cormorant	*Phalacrocorax pygmaeus*	5	–	–	–	5
Falconiiformes						
Marsh harrier	*Circus aeruginosus*	1	–	–	–	1
Poultry (backyards)						
Domestic geese		–	87	–	–	87
Environmental samples (domestic geese + duck)		–	26	–	–	26
Environmental		45				45
Total		**4,790**	**6,120**	**3,056**	**947**	**14,913**

Virus identification. The AaV virus serotype was determined using the hemagglutination inhibition assay (HAI) (Williams et al., 2016; WOAH, 2023; Spackman, 2020). The following antiserums were used for these studies: AaV-1, AaV-2, AaV-3, AaV-4, AaV-6, AaV-7, AaV-8, and AaV-9, manufactured by the Veterinary Laboratories Agency (Animal and Plant Health Agency, Weybridge, UK) and AaV-1, AaV-2, AaV-3, AaV-4, AaV-6, AaV-7, AaV-8, and AaV-9, manufactured by Instituto Zooprofilattio Sperimentale delle Venezie (Padua, Italy).

Results. ***Virological research.*** According to the results of virological studies of biological material from wild birds in 2017–2020, 39 isolates of avuloviruses were isolated. It should be noted that many more hemagglutinating isolates were isolated during virological studies, some of which were later identified as avian influenza viruses and were not used in this work. As a rule, avuloviruses were isolated on passages 1 and 2, with hemagglutinating activity ranging from 1:64 to 1:2,048. According to the results of serological identification of avuloviruses, it was found that 9 isolates belong to AaV-1 (Newcastle disease), 5 isolates to AaV-4, 2 isolates to AaV-9, 2 isolates to AaV-6, 2 isolates to AaV-7, 1 isolate to AaV-3, and the remaining 18 had cross-reactions, which requires further in-depth studies. The result is shown in Table 2.

As can be seen from the results presented in Table 2, 15 isolates of avulaviruses were isolated from biological material collected in Kherson Region, 11 in Odesa Region, 12 in Zaporizhzhia Region, and one in Mykolaiv Region. No isolates were isolated in Chernihiv Region. Most isolates were found in mallard (*Anas platyrhynchos*) (n = 19), white-fronted goose (*Anser albifrons*) (n = 9), common shelduck (*Tadorna tadorna*) (n = 4), ruddy shelduck (*Tadorna ferruginea*) (n = 3), teal (*Anas crecca*) (n = 1), common snipe (*Gallinago gallinago*) (n = 2), and greylag goose (*Anser anser*) (n = 1).

According to the results of the research, the infection rate of birds was determined according to species (Table 3).

Table 2 — List of identified avulovirus isolates from wild birds

No.	Isolate name	Identification result
2017		
1	Shelduck/Sergiyivka/11-15/6-08/17	AaV-1
2	Mallard/Katranka/6-10/1-12/17	AaV-1
3	Mallard/Sivashivka/1-4/4-09/17	H5/AaV-6/H3/H6
4	Mallard/Druzhelyubivka/1-3/5-09/17	AaV-4
5	Mallard/Mytrofanivka/1-4/4-09/17	AaV-1/AaV-3/AaV-4/AaV-7/AaV-9
6	Greylag goose/Mytrofanivka/1-4/4-09/17	AaV-1/AaV-4/AaV-7/AaV-9
7	Shelduck/Churyuk/1-5/2-11/17	AaV-1
8	Mallard/Novodmytrivka/11-15/4-09/17	AaV-6/AaV-2/AaV-7
9	Ruddy shelduck/Khorol/11-15/27-01/17	AaV-1/AaV-7/AaV-4
10	Shelduck/Churyuk/1-5/2-11/17pool946	AaV-9
11	Ruddy shelduck/Novodmytrivka/1-5/5-08/17	AaV-1/AaV-9
12	Mallard/Vasylivka/1-5/24-01/17	AaV-1/AaV-3/AaV-4
2018		
13	Mallard/Primorske T./41-45/7-10/18	H3/H6/AaV-4
14	White-fronted goose/Primorske K./6-10/25-02/18	AaV-1/AaV-4
15	White-fronted goose/Primorske K./21-25/25-02/2018	AaV-1
16	White-fronted goose/Primorske K./31-35/25-02/2018	AaV-1/AaV-6
17	White-fronted goose/Velyka Balka/1-5/01-04/18	AaV-6
18	White-fronted goose/Primorske K./11-15/25-02/18	AaV-1
19	Ruddy shelduck/Askania-Nova/7-11/22-01/18	AaV-1
20	Mallard/Askania-Nova/131-135/30-01/18	AaV-4/AaV-3
21	Mallard/Oleksandrivka/21-25/10-08/18	AaV-7
22	White-fronted goose/Primorske K./6-10/25-02/2018	AaV-3
2019		
23	Mallard/Askania-Nova/4-25-02/19	AaV-1/AaV-7
24	Snipe/Ermakov/23-10/19	AaV-1
25	Mallard/Askania-Nova/45-48/5-12/19	AaV-4
26	Mallard/Askania-Nova/27-23-12/19	AaV-6
27	Mallard/Askania-Nova/32-3-12/19	AaV-1/AaV-4/AaV-7/AaV-9
28	Mallard/Askania-Nova/29-32/3-12/19	AaV-1/AaV-4/AaV-7/AaV-9
29	Mallard/Askania-Nova/24-4-01/19	AaV-4
30	White-fronted goose/Primorske/71-75/14-01/19	H6/AaV-4
31	White-fronted goose/Izmail/1-25-02/19	AaV-1/AaV-4/AaV-7
32	Teal/Ermakov/24-10/19	AaV-1
33	Snipe/Koblevo/4-06/19	AaV-7
34	Shelduck/Mytrofanivka/11-15/17-06/19	AaV-1/AaV-9/AaV-3
35	White-fronted goose/Stroganivka/36-40/01-04/19	AaV-1
2020		
36	Mallard/Askania-Nova/242-16-01/20	AaV-4
37	Mallard/Askania-Nova/171-6-01/20	AaV-9
38	Mallard/Askania-Nova/292-3-02/20	AaV-4
39	Mallard/Askania-Nova/308-10-2/20	AaV-3/AaV-7/AaV-9

Thus, it was found that isolates from mallard were mostly isolated during wintering (13 isolates) and during autumn migration (6 isolates), from white-fronted goose during wintering (7 isolates) and during spring migration (2 isolates), from shelduck during nesting (2 isolates) and autumn migration (2 isolates), from ruddy shelduck during wintering (2 isolates) and breeding (1 isolate), from snipe during breeding (1 isolate) and wintering (1 isolate), from teal 1 isolate during fall migration and from greylag goose 1 isolate also during fall migration (Table 3). The highest percentage of infection was found in snipe — 11.76%, the lowest in greylag goose — 0.13%. In other poultry species, this figure ranged from 0.27% to 0.6%.

Table 3 — Infection rate of wild birds with avuloviruses of different serotypes in the period 2017–2020

Bird species	Number of samples	Number of isolates	Infection rate ,%
Mallard (*Anas platyrhynchos*)	3,381	19	0.56
White-fronted goose (*Anser albifrons*)	3,072	9	0.29
Shelduck (*Tadorna tadorna*)	1,442	4	0.27
Ruddy shelduck (*Tadorna ferruginea*)	494	3	0.60
Teal (*Anas crecca*)	218	1	0.45
Snipe (*Gallinago gallinago*)	17	2	11.76
Greylag goose (*Anser anser*)	737	1	0.13

Conclusions. The findings indicate that avulaviruses are circulating among wild birds in Ukraine and can infect various bird species in different habitats with different migratory behaviors. Therefore, the risk of continued spread of these viruses among wild birds and the threat to poultry remains high in Ukraine. Pathogen circulation is concentrated in the ecological environments of the southern regions.

The field expeditions conducted from 2017 to 2020 covered both shorebirds and water birds. These birds are the primary carriers of avuloviruses. The expeditions focused on stopover sites for migratory birds. Biological material was collected from birds of background species, particularly in southern Ukraine near the coasts of the Azov and Black seas, close to the sources of large rivers such as the Danube and the Dnipro. Additionally, this region has a large poultry population. The virus has been detected in wild birds, highlighting the emergence and risk of avian avulovirus outbreaks in these areas. However, there are still gaps in understanding the natural environment and circulation of avian avuloviruses. Therefore, it is essential to find new natural hosts and study factors that may contribute to overcoming these interspecies barriers.

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