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SEROLOGICAL STUDIES OF WILD BIRDS OF THE ORDER PASSERIFORMES IN UKRAINE FOR THE PRESENCE OF ANTIBODIES TO THE INFLUENZA A VIRUS

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Summary. The influenza A virus is classified as a particularly dangerous infection that causes severe disease in birds, humans, and animals. Given the biological characteristics of the influenza virus, its ability to rapidly mutate, and its potential to cross the interspecies barrier, special attention is currently being paid to the study of the circulation of this pathogen among various natural hosts. Wild waterfowl are believed to be the main natural reservoir of the influenza A virus, while the role of birds of the Passeriformes order remains uncertain. Notably, wild birds of the order Passeriformes comprise 60% of the global avian population, underscoring their ecological significance. This order encompasses many species with diverse biological, ecological, and behavioral characteristics. Some Passeriformes species are stable synanthropes, able to coexist with humans and domestic animals in urban and natural habitats. Due to the peculiarities of nesting, feeding, and especially watering places, they can potentially play a role in transmission to other birds. The purpose of our research was to conduct serological monitoring in Ukraine among birds of the order Passeriformes, as they can also be potential carriers of the influenza virus, but this issue has not been sufficiently studied in Ukraine. In 2023–2024, biological samples were collected from 32 species of Passeriformes in 5 regions of Ukraine in the amount of 354 samples. Blood sera and egg yolks were tested in ELISA and hemagglutination inhibition test to H5 and H7 subtypes of influenza virus. Antibodies to the influenza A virus were found in blood samples from the blackbird (seroprevalence was 11.1%), the song thrush (20%), and the blackcap (100%). The titer of antibodies in the HIT was 1:16 to the H7 influenza virus subtype from the Blackbird. ELISA detected no antibodies to the influenza virus in the egg yolk extracts

Keywords: seroprevalence, virus circulation, ELISA, hemagglutination inhibition test

Introduction. Wild birds play an important role in the transmission of many zoonotic pathogens, especially the avian influenza virus (Malekian, Shagholian and Hosseinpour, 2021).

The influenza A virus causes severe disease in wild and domestic birds and humans, causing severe respiratory disease with a mortality rate of about 60% (Kawaoka and Neumann, 2012).

The circulation of highly pathogenic strains of the avian influenza virus in recent years has posed serious challenges to the global poultry industry and public health (Zhang and Lei, 2024), and has put food security and the livelihoods of those who depend on it at risk (Cabrera-Gaytán, 2024).

The majority of influenza virus detections have been linked to birds of the Anseriformes and Charadriiformes families, as they are considered the primary reservoirs of the virus. However, Passeriformes, which account for 60% of the global avian population (Williams et al., 2023), play a significant role in the epidemiological process due to their interaction with wetlands.

Influenza viruses belong to the family Orthomyxoviridae, which consists of the following genera: *Influenza virus A*, *Influenza virus B*, *Influenza virus C*, *Influenza virus D*, *Thogotovirus*, *Isavirus*, *Myxvirus*, *Quarantavirus*, *Sardinivirus*. *Thogotovirus*, *Quarantavirus*, and *Isavirus* have no medical significance

for humans, while influenza C virus infects humans but causes mild symptoms and is mostly asymptomatic. Influenza A and B viruses cause annual epidemics, and influenza A viruses cause pandemics at random intervals (Neumann, Treanor and Kawaoka, 2021).

The influenza D virus was described in 2011 and it was realized that it circulates among cattle and pigs worldwide, but can infect other animal species (Liu et al., 2020).

In 1918–1919, an estimated 20 to 50 million people worldwide died from the Spanish Flu, which was caused by the H1N1 influenza virus (Tumpey et al., 2005).

In 1957, the reassortment of human influenza virus H1N1 and avian influenza virus H2N2 contributed to the emergence of a human influenza virus that had avian virus segments H2 and N2. This 'new' H2N2 virus caused the 'Asian flu', which is estimated to have killed about 1 million people worldwide (Kawaoka and Neumann, 2012).

In 1968, the pandemic H3N2 strain emerged, which had genes from the H3 avian influenza virus and human influenza virus genes. The number of deaths in the United States reached 33 thousand people, but fewer people died compared to previous pandemics (Kawaoka and Neumann, 2012).

In February 2009, an increase in the number of influenza-like diseases was reported in Mexico. After

that, a new outbreak of the H1N1 influenza virus was reported. Genetic data indicate that this influenza virus originated from pigs, but no outbreaks of the influenza virus in pigs were detected or reported in the affected areas during the outbreak. The new virus had 6 segments from avian influenza virus, human influenza virus, and swine influenza virus, which has been circulating in North American swine populations for more than several decades. The outbreak spread rapidly and reached pandemic status in 2009 (Kawaoka and Neumann, 2012).

Antigenic drift, i.e., the accumulation of point mutations in antigenic sites, creates variants that cause annual epidemics. Epidemics typically affect 10–20% of the population, i.e., an estimated 500,000 deaths worldwide are attributed to the influenza virus per year (Kawaoka and Neumann, 2012).

For a considerable period, the study of influenza A virus ecology has identified waterfowl as the primary reservoir, with control studies predominantly focusing on waterfowl, with a few exceptions. An analysis of diverse bird species in Southeast Asia, where avian influenza viruses are a significant concern, revealed that land birds also carry the influenza A virus (Peterson et al., 2008). Influenza A virus has been isolated from more than 100 species of birds belonging to 26 families (Slusher et al., 2014).

Highly pathogenic avian influenza viruses of the H5N1 subtype have been distributed in most parts of Asia, Africa, and Europe, and numerous outbreaks have been reported in poultry and wild birds.

Recent studies have demonstrated a significant link between the spread of the influenza virus and bird migration (Fujimoto et al., 2015).

In a study conducted in the People's Republic of China, researchers analyzed samples from wild birds belonging to the Passeriformes order. The findings indicated that birds that exhibit seasonal migration tested positive for the influenza virus at a rate of 4.8%, compared to a positivity rate of just 1.8% among non-migratory birds. Additionally, bird species residing in open areas showed a higher prevalence of infection, with a rate of 2.9%, compared to 2.4% for species that inhabit forested regions. Research indicates that migratory bird species are more susceptible to influenza A virus infection, and the use of different habitats can also affect the transmission of the virus (Peterson et al., 2008). A study of starlings (*Sturnus vulgaris*) evaluated the possibility of transmission of the H4N6 influenza virus from a flock of passerines to quail (*Colinus virginianus*) through shared food and water resources. Of the three flocks of starlings tested, 30, 20, and 10 birds, respectively, successfully transmitted the virus to all or most of the *Colinus virginianus* in each birdhouse, as confirmed by seropositivity or the presence of influenza viral RNA. This study demonstrated that starlings, even in small flocks, can collectively release influenza virus to more susceptible bird species (Root, Ellis and Shriner, 2022).

However, there have been documented cases of interspecies transmission of influenza A viruses, including the 'Spanish flu' and the pandemic swine-origin H1N1 virus. Another notable example is the H5N1 avian influenza virus, which is currently endemic in many poultry populations and has caused outbreaks on three continents. Recent cases of highly pathogenic avian influenza virus infection in cows in the United States, marine mammals, bats, and carnivores expose humanity to a new danger, as the influenza virus has begun to cross the interspecies barrier, which may play an important role in the evolution and ecology of influenza viruses (Fereidouni et al., 2016; Horimoto et al., 2016; Lee et al., 2017; Marinova-Petkova et al., 2017; Blachere et al., 2018; Flynn et al., 2018; Hatta et al., 2018; Wasik, Voorhees and Parrish, 2021; Abdelwhab and Mettenleiter, 2023; Leibler et al., 2023; Puryear et al., 2023; Thorsson et al., 2023).

The **aim of the study** was to conduct serological monitoring of influenza A virus circulation in wild birds of the order Passeriformes in Ukraine and to determine the prevalence of infection in different regions of Ukraine.

Materials and methods. Research site. The study was conducted in 2023–2024 in different geographical regions of Ukraine (Eastern, Central, Southern, and Western): Kharkiv, Poltava, Odesa, Kyiv, and Khmelnytskyi regions. A total of 347 birds of 32 species were captured (Table 1).

Sampling and sample preparation. Blood (approximately 0.1 to 0.5 ml, depending on the species) was collected from the subcutaneous vein of captured birds and centrifuged at 10,000 rpm for 10 min to obtain blood serum. A capillary blood collection system — microtubes containing Microvette CB 300 coagulation activator — was used.

Prepared egg yolks were also used for serologic studies. The study of egg yolk extracts was prepared according to the method developed at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Stehnii and Muzyka, 2004).

Serological studies. The commercial test kit 'ID Screen® Influenza A Antibody Competition Multi-species' (Innovative Diagnostics, France) was used for serological studies of blood sera and egg yolk extract. The test was performed and recorded according to the manufacturer's instructions.

Antibodies to the influenza virus in egg yolks were detected in the hemagglutination inhibition test (HIT) using a test system for the detection of antibodies to avian influenza virus of subtypes H5 and H7, manufactured by Scientific Research Enterprise 'Veterinary Medicine' LLC (Kharkiv, Ukraine) according to the generally accepted method (Williams et al., 2016; Spackman, 2020).

Results. The results of the study of the presence of antibodies to influenza virus in sera and egg yolk extracts from wild birds of the order Passeriformes are shown in Table 2.

Table 1 — Number of captured birds of the order Passeriformes

| Bird species | Location | Year | Samples, total |
|---|--|------|----------------|
| Yellowhammer, <i>Emberiza citronella</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 1 |
| | | 2024 | 3 |
| Golden oriole, <i>Oriolus oriolus</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 4 |
| Reed bunting, <i>Emberiza schoeniclus</i> | Odesa Region, Lyman village | 2024 | 1 |
| Robin, <i>Erithacus rubecula</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2024 | 6 |
| | Kharkiv Region, Kharkiv District, Haidary village | 2023 | 1 |
| Tree sparrow, <i>Passer montanus</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 14 |
| | | 2024 | 2 |
| | Odesa Region, Lyman village | 2024 | 1 |
| | Odesa Region, Trapivka-2 village | 2024 | 5 |
| House sparrow, <i>Passer domesticus</i> | Kharkiv Region, Kharkiv District, Pershotravneve village | 2023 | 8 |
| | | 2024 | 1 |
| Spanish sparrow, <i>Passer hispaniolensis</i> | Odesa Region, Trapivka village | 2024 | 3 |
| | Odesa Region, Trapivka-2 village | 2024 | 3 |
| Song thrush, <i>Turdus philomelos</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 10 |
| | | 2024 | 21 |
| | Kharkiv Region, Kharkiv District, Haidary village | 2023 | 7 |
| | Khmelnyskyi Region, Zavallia village, National Nature Park 'Podilski Tovtry' | 2024 | 1 |
| | Kharkiv Region, Kharkiv District, Pershotravneve village | 2024 | 5 |
| Blackbird, <i>Turdus merula</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 9 |
| | | 2024 | 14 |
| | Kyiv, Ecological Research Station 'Hlyboki Balyky' | 2023 | 2 |
| | Kharkiv Region, Kharkiv District, Haidary village | 2023 | 7 |
| | Khmelnyskyi Region, Zavallia village, National Nature Park 'Podilski Tovtry' | 2024 | 1 |
| | Kharkiv Region, Kharkiv District, Pershotravneve village | 2024 | 9 |
| Greenfinch, <i>Chloris chloris</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 33 |
| | | 2024 | 9 |
| | Kharkiv Region, Kharkiv District, Haidary village | 2023 | 1 |
| Chaffinch, <i>Fringilla coelebs</i> | Kharkiv Region, Kharkiv District, Haidary village | 2023 | 3 |
| | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2024 | 6 |
| | Kharkiv Region, Kharkiv District, Pershotravneve village | 2024 | 6 |
| Hawfinch, <i>Coccothraustes coccothraustes</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 3 |
| | | 2024 | 5 |
| | Kharkiv Region, Kharkiv District, Haidary village | 2023 | 8 |
| | Kharkiv Region, Kharkiv District, Pershotravneve village | 2024 | 1 |
| Barred warbler, <i>Sylvia nisoria</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 2 |
| Whitethroat, <i>Sylvia communis</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2023 | 2 |
| | Odesa Region, Lyman village | 2024 | 2 |
| | Odesa Region, Trapivka village | 2024 | 3 |
| | Odesa Region, Trapivka-2 village | 2024 | 1 |
| Blackcap, <i>Sylvia atricapilla</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklanskiy' | 2024 | 2 |
| | Odesa Region, Lyman village | 2024 | 1 |
| | Odesa Region, Trapivka village | 2024 | 1 |

Table 1 — continuation

| Bird species | Location | Year | Samples, total |
|---|--|------|----------------|
| Blackcap, <i>Sylvia atricapilla</i> | Odesa Region, Trapivka-2 village | 2024 | 3 |
| | Khmelnyskyi Region, Zavallia village, National Nature Park 'Podilski Tovtry' | 2024 | 1 |
| House martin, <i>Delichon urbica</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 7 |
| Swallow, <i>Hirundo rustica</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 12 |
| Collared flycatcher, <i>Ficedula albicollis</i> | Odesa Region, Lyman village | 2024 | 3 |
| | Odesa Region, Trapivka-2 village | 2024 | 2 |
| Spotted flycatcher, <i>Muscicapa striata</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 2 |
| | | 2024 | 1 |
| Great reed warbler, <i>Acrocephalus arundinaceus</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 4 |
| | Odesa Region, Lyman village | 2024 | 3 |
| | Odesa Region, Trapivka village | 2024 | 1 |
| | Odesa Region, Trapivka-2 village | 2024 | 1 |
| Paddyfield warbler, <i>Acrocephalus agricola</i> | Odesa Region, Lyman village | 2024 | 2 |
| Sedge warbler, <i>Acrocephalus schoenobaenus</i> | Odesa Region, Lyman village | 2024 | 1 |
| Reed warbler, <i>Acrocephalus scirpaceus</i> | Odesa Region, Lyman village | 2024 | 3 |
| | Odesa Region, Trapivka village | 2024 | 1 |
| Pied wagtail, <i>Motacilla alba</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 1 |
| Yellow wagtail, <i>Motacilla flava</i> | Odesa Region, Trapivka village | 2024 | 1 |
| Great tit, <i>Parus major</i> | Kharkiv Region, Kharkiv District, Pershotravneve village | 2023 | 11 |
| | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 7 |
| | | 2024 | 1 |
| | Kyiv, Ecological Research Station 'Hlyboki Balyky' | 2023 | 8 |
| | Kharkiv Region, Kharkiv District, Pershotravneve village | 2024 | 15 |
| | Odesa Region, Lyman village | 2024 | 1 |
| | Odesa Region, Trapivka village | 2024 | 1 |
| Jay, <i>Garrulus glandarius</i> | Odesa Region, Trapivka-2 village | 2024 | 2 |
| | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 1 |
| | | 2024 | 2 |
| | Kyiv, Ecological Research Station 'Hlyboki Balyky' | 2023 | 2 |
| | Kharkiv Region, Kharkiv District, Pershotravneve village | 2024 | 1 |
| Thrush nightingale, <i>Luscinia luscinia</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 2 |
| | Odesa Region, Lyman village | 2024 | 1 |
| | Odesa Region, Trapivka-2 village | 2024 | 1 |
| Red-backed shrike, <i>Lanius collurio</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 5 |
| Goldfinch, <i>Carduelis carduelis</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 6 |
| | Kharkiv Region, Kharkiv District, Haidary village | 2023 | 1 |
| Starling, <i>Sturnus vulgaris</i> | Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorskliański' | 2023 | 1 |
| | Odesa Region, Borodyno village | 2024 | 1 |

Table 2 — Study of the presence of antibodies to influenza virus in blood sera and egg yolk extracts from wild birds of the order Passeriformes

| Bird species | Year | Samp- les, total | Yolk ext- ract | Results | | Seropre- valence, % |
|--------------------|------|------------------------|----------------------|---------------|---------------|---------------------------|
| | | | | nega- tive | posi- tive | |
| Kharkiv Region | | | | | | |
| Blackbird | 2023 | 7 | — | 7 | 0 | 0 |
| Chaffinch | | 3 | — | 3 | 0 | 0 |
| Goldfinch | | 1 | — | 1 | 0 | 0 |
| Great tit | | 11 | — | 11 | 0 | 0 |
| Greenfinch | | 1 | — | 1 | 0 | 0 |
| Hawfinch | | 8 | — | 8 | 0 | 0 |
| House sparrow | | 8 | — | 8 | 0 | 0 |
| Robin | | 1 | — | 1 | 0 | 0 |
| Song thrush | | 7 | — | 7 | 0 | 0 |
| Total | | 47 | — | 47 | 0 | 0 |
| House sparrow | 2024 | 1 | — | 1 | 0 | 0 |
| Song thrush | | 5 | — | 4 | 1 | 20 |
| Blackbird | | 9 | — | 9 | 0 | 0 |
| Chaffinch | | 6 | — | 6 | 0 | 0 |
| Hawfinch | | 1 | — | 1 | 0 | 0 |
| Great tit | | 15 | — | 15 | 0 | 0 |
| Jay | | 1 | — | 1 | 0 | 0 |
| Total | | 38 | — | 37 | 1 | 2.6 |
| Poltava Region | | | | | | |
| Barred warbler | 2023 | 2 | — | 2 | 0 | 0 |
| Blackbird | | 10 | — | 9 | 1 | 11.1 |
| Golden oriole | | 4 | — | 4 | 0 | 0 |
| Goldfinch | | 6 | — | 6 | 0 | 0 |
| Great reed warbler | | 4 | — | 4 | 0 | 0 |
| Great tit | | 7 | 1 | 8 | 0 | 0 |
| Greenfinch | | 33 | 3 | 36 | 0 | 0 |
| Hawfinch | | 3 | — | 3 | 0 | 0 |
| House martin | | 7 | — | 7 | 0 | 0 |
| Jay | | 1 | — | 1 | 0 | 0 |
| Pied wagtail | | 1 | — | 1 | 0 | 0 |
| Red-backed shrike | | 5 | — | 5 | 0 | 0 |
| Song thrush | | 10 | — | 10 | 0 | 0 |
| Spotted flycatcher | | 2 | — | 2 | 0 | 0 |
| Starling | | 1 | — | 1 | 0 | 0 |
| Swallow | | 12 | — | 12 | 0 | 0 |
| Thrush nightingale | | 2 | — | 2 | 0 | 0 |
| Tree sparrow | | 14 | — | 14 | 0 | 0 |
| Whitethroat | | 2 | — | 2 | 0 | 0 |
| Yellow-hammer | | 1 | — | 1 | 0 | 0 |
| Total | | 126 | 4 | 129 | 1 | 0.79 |
| Blackbird | 2024 | 14 | — | 14 | 0 | 0 |
| Blackcap | | 2 | — | 2 | 0 | 0 |
| Chaffinch | | 6 | — | 6 | 0 | 0 |
| Great tit | | 1 | — | 1 | 0 | 0 |

| Bird species | Year | Samp- les, total | Yolk ext- ract | Results | | Seropre- valence, % |
|---------------------|------|------------------------|----------------------|---------------|---------------|---------------------------|
| | | | | nega- tive | posi- tive | |
| Greenfinch | 2024 | 9 | — | 9 | 0 | 0 |
| Hawfinch | | 5 | — | 5 | 0 | 0 |
| Jay | | 2 | — | 2 | 0 | 0 |
| Robin | | 6 | — | 6 | 0 | 0 |
| Song thrush | | 21 | — | 21 | 0 | 0 |
| Spotted flycatcher | | 1 | — | 1 | 0 | 0 |
| Tree sparrow | | 2 | — | 2 | 0 | 0 |
| Yellow-hammer | | 3 | — | 3 | 0 | 0 |
| Total | | 72 | — | 72 | 0 | 0 |
| Kyiv Region | | | | | | |
| Blackbird | 2023 | 2 | 1 | 3 | 0 | 0 |
| Great tit | | 8 | — | 8 | 0 | 0 |
| Jay | | 2 | — | 2 | 0 | 0 |
| Total | | 12 | 1 | 13 | 0 | 0 |
| Odesa Region | | | | | | |
| Blackcap | 2024 | 5 | | 5 | 0 | 0 |
| Collared flycatcher | | 5 | | 5 | 0 | 0 |
| Great reed warbler | | 3 | — | 3 | 0 | 0 |
| Great tit | | 4 | — | 4 | 0 | 0 |
| Paddyfield warbler | | 2 | — | 2 | 0 | 0 |
| Reed warbler | | 4 | — | 4 | 0 | 0 |
| Sedge warbler | | 1 | — | 1 | 0 | 0 |
| Spanish sparrow | | 6 | — | 6 | 0 | 0 |
| Thrush nightingale | | 2 | — | 2 | 0 | 0 |
| Tree sparrow | | 6 | — | 6 | 0 | 0 |
| Whitethroat | | 5 | — | 5 | 0 | 0 |
| Yellow wagtail | | 1 | — | 1 | 0 | 0 |
| Total | | 46 | — | 46 | 0 | 0 |
| Khmelnyskyi Region | | | | | | |
| Blackbird | 2023 | — | 1 | 1 | 0 | 0 |
| Song thrush | | — | 2 | 2 | 0 | 0 |
| Chaffinch | | — | 1 | 1 | 0 | 0 |
| Total | | — | 4 | 4 | 0 | 0 |
| Song thrush | 2024 | 1 | — | 1 | 0 | 0 |
| Blackcap | | 1 | — | 0 | 1 | 100 |
| Blackbird | | 1 | — | 1 | 0 | 0 |
| Total | | 3 | — | 2 | 1 | 33.3 |

According to the results of the study, the presence of antibodies to influenza virus in the yolk extract was not detected in ELISA, but in the study of blood sera of wild birds, antibodies to influenza virus were detected in the following species: the blackbird (captured in Poltava Region during spring migration in 2023), the song thrush (captured in Kharkiv Region during autumn migration in 2024), the blackcap (captured in Khmelnytskyi Region during summer migration in 2024).

In Ukraine, the blackbird (*Turdus merula*) is usually a nesting, migratory and rarely wintering species. The main wintering grounds are European countries. According to the nature and type of behavior during nesting and migration, it usually uses places associated with water bodies. Main habitats: forests, gardens. Feeds mainly on moist ground near water bodies.

The song thrush (*Turdus philomelos*). Nesting species, distributed almost all over Ukraine, rarely wintering. The main habitats are also forests and gardens, including nearby water bodies.

The blackcap (*Sylvia atricapilla*). Nesting and migratory species, nests in most of the territory of Ukraine, except for the steppe. Habitats: deciduous and mixed forests, shrubs, parks and gardens. During migration, birds often visit forest watering places where they may come into contact with other species.

As an additional serological study, egg yolks from 5 species of wild birds of the Passeriformes were examined for the presence of antibodies to influenza virus subtypes H5 and H7. The study was conducted by HIT. Bird species studied: the great tit (*Parus major*), the greenfinch (*Chloris chloris*), the blackbird (*Turdus merula*), the song thrush (*Turdus philomelos*), and the chaffinch (*Fringilla coelebs*) (Table 3).

Table 3 — Results of studies on the presence of antibodies to influenza virus subtypes H5 and H7 in the yolks of eggs of wild birds of the order Passeriformes (HIT)

| Bird species | Location | HIT titer | |
|--------------|--------------------|-----------|------|
| | | H5 | H7 |
| Song thrush | Khmelnyskyi Region | 0 | 0 |
| Blackbird | | 0 | 1:16 |
| Song thrush | | 0 | 0 |
| Chaffinch | | 0 | 0 |
| Blackbird | Kyiv Region | 0 | 0 |
| Great tit | Poltava Region | 0 | 0 |
| Greenfinch | | 0 | 0 |
| Greenfinch | | 0 | 0 |
| Greenfinch | | 0 | 0 |

According to the results of the HIT, a 4 log₂ antibody titer to the H7 influenza virus subtype was detected in the yolk extract from the blackbird (*Turdus merula*) originating from Khmelnytskyi Region. No antibodies to the H5 influenza virus subtype were detected in egg yolks.

Discussion. Recent studies have shown that the spread of influenza virus is very closely related to bird migration (Fujimoto et al., 2015). In 2011, a serological study was conducted in China to detect the presence of antibodies to influenza virus in the field sparrows (*Passer montanus*). A total of 800 birds were tested, most of which were captured in the wild and some of which were purchased from pet shops. The results showed that 94 birds were seropositive, indicating exposure to the

influenza virus (Han et al., 2012). A serologic study of samples from wild birds of the order Passeriformes in Ohio for the presence of antibodies to the influenza A virus revealed no positive samples (Morishita et al., 1999). During the spring and fall migration of wild birds on the island of Helgoland (North Sea) in 2001, biological material was collected from short-distance migrants such as the chaffinch (*Fringilla coelebs*) and the song thrush (*Turdus philomelos*) as well as long-distance migrants such as the garden warbler (*Sylvia borin*) and the common redstart (*Phoenicurus phoenicurus*). As a result of pathogen isolation and serologic identification, no influenza A virus was detected (Schnebel et al., 2005). However, our studies of blood sera from a long-distance migrant, the blackcap (*Sylvia atricapilla*), captured in the Khmelnytskyi Region on the territory of the National Nature Park 'Podilski Tovtry', revealed the presence of antibodies to the influenza A virus.

A study on the prevalence of antibodies to influenza virus subtypes H5N1, H7N1, and H9N2 in the white-necked ravens (*Corvus albicollis*) was conducted in the United Arab Emirates during 2003–2006. According to the results of the study, no antibodies against influenza viruses were detected in these birds (Jöstl et al., 2023). Our studies also showed that birds of the family Corvidae did not have antibodies to influenza A. In Spain, 39 serum samples from the house sparrows (*Passer domesticus*) were serologically tested for the presence of antibodies to influenza A. The results showed that 13 samples were positive by ELISA and 3 samples were positive by HIT. The authors note that ELISA is the most sensitive method for serologic diagnosis in such studies (Arenas et al., 1990). In addition, according to the results of our studies, the presence of antibodies in ELISA in song thrushes and blackbirds, and the presence of antibodies in the yolk of eggs of blackbirds was established by ELISA. Thus, wild birds of the order Passeriformes are susceptible to the influenza A virus and should become an integral part of surveillance, which will allow more effective monitoring of influenza virus circulation and reduce the risk of HPAI spread.

Conclusions. Our research results show that wild birds of the order Passeriformes living on the territory of Ukraine, which are at the same time migratory birds, have antibodies to the avian influenza virus and make up 0.86% of seropositivity. The role of Passeriformes remains an open question. We found a rather low seroprevalence, the source of which also remains unclear. During migration, the main source of the virus is the contact of birds with each other during feeding, nesting, or migration, especially in places with high concentrations of birds, such as forest water sources or water banks, where all wild birds have many contacts with waterfowl, including waterbirds, so monitoring is needed to better understand the situation and which subtypes of influenza viruses are circulating in Ukraine and whether they can pose a threat to humans. Further research should focus on virus isolation, followed by influenza virus subtype identification and sequencing.

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