

SCREENING OF WILD PASSERINES FOR THE AVIAN INFLUENZA VIRUS IN THE DANUBE DELTA (ROMANIA), IN 2018

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Abstract. Waterfowls are considered to be the main natural source of avian influenza. The virus spreads at local, regional, continental and intercontinental levels via bird migration. There is a lot of information regarding infections in waterfowls and domestic birds, but little regarding other birds. Still, there is evidence of sensitivity and the importance of passerines in the spread of the avian influenza virus. In various regions worldwide, passerines were found to be naturally infected with the virus. Even more evidence is provided by the laboratory infections of several species of passerines, including some present in the Romanian avifauna. It is proved that synanthropic species can infect domestic birds in different types of breeding facilities. In most cases, transmission occurs via drinking water. Poultry drinkers are also used by passerines and several studies show high quantities of virus in these drinkers. In May 2018, 203 samples from 9 passerines species were collected. All of the samples showed negative results for avian influenza RT PCR testing. We tested tracheal exudates harvested from *Acrocephalus agricola* (40 samples), *A. scirpaceus* (95), *Emberiza schoeniclus* (5), *Hirundo rustica* (30), *Motacilla alba* (14), *Panurus biarmicus* (4), *Passer domesticus* (5), *Sturnus vulgaris* (5) and *Sylvia curruca* (5 samples). The results of our research are the same as those reported in other studies, which state that passerines may play a lesser role in the transmission of the avian influenza virus compared to other domestic or wild birds.

Keywords: avian influenza virus, passerines, wetlands, Danube Delta.

Rezumat. Supravegherea virusului gripei aviare la paseriforme sălbatice, în Delta Dunării (România), în anul 2018. Păsările acvatice sunt considerate principalele surse naturale pentru virusul gripei aviare. Prin intermediul migrației păsărilor, virusul gripei aviare este răspândit la nivel local, regional, continental și intercontinental. Există foarte multe informații cu privire la infecția păsărilor acvatice sălbatice și domestice cu virusul Gripei Aviare, însă sunt foarte puține informații pentru alte categorii de păsări. Cu toate acestea, în mai multe regiuni pe glob s-a evidențiat atât sensibilitatea, cât și rolul paseriformelor în transmiterea virusului Gripei Aviare. Paseriformele au fost găsite infectate natural cu virusul Gripei Aviare în diferite zone de pe glob. Sunt furnizate dovezi de infectări în laborator la mai multe specii de paseriforme sălbatice, inclusiv unele prezente în avifauna românească. S-a demonstrat că paseriformele sălbatice sinantropice pot infecta păsările domestice din diferite tipuri de crescătorii. În cele mai multe cazuri, transmiterea virusului are loc prin intermediul apei potabile. Conform unor studii, virusul a fost găsit în cantități mari, în adăpătorile din crescătoriile de păsări care sunt, de asemenea, utilizate de paseriformele sălbatice. În luna mai 2018 au fost recoltate 203 probe, de la 9 specii de paseriforme, toate fiind negative la testarea pentru detectarea virusului Gripei Aviare (Real Time RT-PCR). Astfel, au fost testate cu rezultate negative, exudate traheale recoltate de la *Acrocephalus agricola* (40 de probe), *A. scirpaceus* (95), *Emberiza schoeniclus* (5), *Hirundo rustica* (30), *Motacilla alba* (14), *Panurus biarmicus* (4), *Passer domesticus* (5), *Sturnus vulgaris* (5) and *Sylvia curruca* (5 samples). Rezultatele cercetărilor noastre sunt similare cu alte asemenea studii din diferite zone pe glob, unde se concluzionează că paseriformele joacă un rol minor în transmiterea virusului Gripei Aviare la alte păsări domestice și sălbatice.

Cuvinte cheie: virusul gripei aviare, paseriforme, zone umede, Delta Dunării.

INTRODUCTION

A series of factors, mainly anthropogenic, have caused vector proliferation of various pathogens. These factors include climate change, land use changes, zootechnical practices, deforestation, irrigation and hydrological planning, and much more. All of this is compounded by the increased resistance of pathogens to chemical control actions. The Danube Delta, as the breeding ground or migration area of millions of birds (ZEHTINDJIEV & LIECHTI, 2003 in DROZ et al., 2016), is the main gateway for entry of an impressive series of pathogens into the country. Subsequently, with secondary migrations of birds and local transmission cycles, outbreaks are triggered in areas further from the delta. Birds migrate in various ways, depending on the species, moving in broad fronts or in narrow corridors, many of them using certain areas for additional feeding or rest. Considering climate and land use changes in Europe (but also globally), it is expected that the vector, host and reservoir species of some zoonotic pathogens will expand their distribution areas.

The epidemiology of Avian Influenza over the past 16 years has been characterized by two major worldwide panzootic events. The first panzootic wave began in 2004 and reached its climax in 2006, with the activity of the virus progressively decreasing until 2012. Since 2013, a second panzootic wave has been observed, climaxing in 2015 and 2016. The second panzootic wave is still ongoing (***, 2018). In the case of Romanian epizootics, Tulcea Sanitary Veterinary and Food Safety Authority specialists have identified two bird species infected with the avian influenza virus (from samples collected from 6 avian influenza outbreaks in 2005): *Aythya ferina* (Common Pochard) and *Cygnus olor* (Mute Swan). During the same decade, Romania reported (to the World Health Organization ***, WHO) two other episodes, both with the highly pathogenic H5N1 subtype only in domestic poultry in Tulcea County. The first was on

November 27, 2007 and the second on March 16, 2010. Other epizootics (mostly in domestic birds) were later reported, but these were minor until 2015.

On March 25 and 26, 2015, 107 dead Dalmatian Pelicans (*Pelecanus crispus*) were collected from the Ceaplace Island (for testing and incineration) (KISS et al., 2016). Six of the individuals were tested on March 26, 2015 at the Sanitary Veterinary and Food Safety Laboratory in Tulcea and tests confirmed the avian influenza virus, H5N1 subtype (***, 2015).

Considering that the virus usually persists for several years in an area, an avian influenza epizootic was observed over the following two years. Thus, between November 2016 and March 21, 2017, 37 outbreaks of avian influenza were confirmed in Romania in domestic poultry (hens, ducks, geese, guinea fowl): Tulcea (5 outbreaks: Crișan, Periprava, C. A. Rosetti, Sulina, Pardina), Prahova (2 outbreaks), Bacău (1 outbreak), Brașov (1), Mureș (1) Constanța (5), Giurgiu (1), Argeș (2), Mehedinți (1), București (1) and Teleorman (17 outbreaks) (***, 2017). The total number of infected domestic birds was 2042. Regarding cases of avian influenza detected in wild birds during the same period, a total of 160 cases were confirmed in wild ducks, great cormorants, egrets, gulls, geese, mute and whooper swans: Constanta (50 cases), Ialomița (2), Galați (17), Tulcea (1), Neamț (55), Giurgiu (3), Iași (1), Bacău (13), Teleorman (3), Bucharest (13), Ilfov (1) and Arad (1) (***, 2017, several previous Romanian National Sanitary Veterinary and Food Safety Authority releases - ANSVSA).

In 2018, two waves of avian influenza episodes were recorded in Europe in late spring and late autumn, related to spring migration and autumn migration. Both waves were recorded in Bulgaria, Denmark, Germany, the Netherlands, and Russia, while a single wave (mainly the spring one) was recorded in Finland, Ireland, Italy, Slovakia, Switzerland and the United Kingdom. In 2018, there were several avian influenza episodes, with both weakly and highly pathogenic strains, in poultry farms (especially in Western Europe) and in natural areas. There were no reports of avian influenza in 2018 in Romania, although there were several epizootics in Bulgaria, of which one outbreak was just across the border with Romania (Dobrich) (***, 2018).

There are many bird species affected by or only involved in the transmission of the avian influenza virus. However, aquatic species are the most important, especially since infections are facilitated by aquatic environments. The vast majority of the species at risk of introducing and transmitting the avian influenza are frequent and abundant in the Danube Delta Biosphere Reserve. Their classification into risk categories is made according to several ecological and behavioural aspects (gregarism, contact with domestic species, migration to areas where the virus is endemic, etc.) (HAGEMEIJER & MUNDKUR, 2006). At the same time there is little information on other bird species. In several regions around the globe, evidence has been found of the sensitivity and role of the passerines in the transmission of avian influenza virus. In our study, we aim to provide new data on the role of the Romanian passerines in the avian influenza epidemiology.

MATERIAL AND METHODS

Birds were captured using mist nets (KEYES & GRUE, 1982) in May 2018 at Grindul Lupilor (Ornithological Monitoring Center Grindul Lupilor) and Enisala (Enisala Channel - Enisala Research Base) – both located in the Danube Delta Biosphere Reserve, Romania.

Tracheal exudate samples were collected by swabbing (Fig. 1) from birds living near water, most of them migratory. A small number of samples were also collected from resident or partially migratory birds.

The identification of the avian influenza virus genome, the Matrix protein, was performed using the Real Time RT-PCR method (SWAYNE & BROWN, 2018 in ****, 2018a) by the Tulcea Sanitary Veterinary and Food Safety Laboratory following a commercial order (made on May 30, 2018), in accordance with ANSVSA Order no. 96 from July 22, 2014 on the approval of tariffs applicable in the veterinary and food safety field (***, 2014).

In order to identify the viral genome, the samples were analysed as a mix (a pool of 5 samples from 5 different birds but belonging to the same species). Subsequently, if the pool was positive, the 5 samples were analysed separately and the exact positive sample was identified.

RESULTS AND DISCUSSION

Altogether, 218 samples of exudates were collected, out of which 203 samples were selected and grouped into 41 sample pools (39 pools of 5 samples and 2 pools of 4 samples) (see Table 1). The 41 pools of samples were collected from 9 bird species. Most samples were obtained from the Common Reed-warbler and Paddyfield Warbler (*Acrocephalus scirpaceus* and *A. agricola*) – Fig. 2 and Barn Swallow (*Hirundo rustica*). Each pool was formed by sampling 5 other species: *Emberiza schoeniclus*, *Panurus biarmicus*, *Passer domesticus*, *Sturnus vulgaris* and *Sylvia curruca*. Exudate samples from birds of other species were also collected (*Anthus trivialis*, *Hippolais pallida*, *Lanius collurio*, *Locustestella luscinioides*, *Oriolus oriolus*, *Phoenicurus phoenicurus*, *Sylvia borin* and *S. atricapilla*), but at least 4 or 5 were not present so that pools of these species could not be formed.

Following analyses for the detection of the avian influenza virus genome, the 203 samples analysed as pools were all identified as negative. These results have led to the premise that the role of passerines, including aquatic ones, is neutral at the start of the nesting season when the amount of virus is low.

However, in several regions around the globe, there has been evidence of the sensitivity and role of the passerines in the transmission of avian influenza. Several wild bird species (*Emberiza schoeniclus* - also tested by us, Table 1, *Hypsipetes amaurotis* and *Turdus pallidus*) have been infected in laboratory conditions with H5N1. Mortality was 100% for the first two species, while seroconversion was detected in the third species (FUJIMOTO et al., 2015). Also, several species of passerines (including *Passer domesticus* – also tested by us, Table 1) have also been infected (H7N9) in China to verify the hypothesis that these species transmit the avian influenza virus to poultry in farms (PERKINS & SWAYNE, 2003; JONES et al., 2015).

In nature, the transmission of the avian influenza virus most likely occurs via drinking water (domestic poultry drinkers are also used by synanthropic wild passerines), as it has been detected in the drinkers of these birds. It has been shown that synanthropic passerines more easily infect domestic quail than they do chickens. The transmission of the avian influenza infection is greater from wild to domestic birds, and lower the other way around (CUMMING et al., 2008; JONES et al., 2015). Along the same line, it has even been proposed that passerines are bridge species, i.e. species that function as a disease-transmitting relay. So, just as in the example above, sparrows can take the virus from infected wild birds and retransmit it to domestic birds (***, 2015a).

Evidence of the role of the House Sparrow (*Passer domesticus*) in transmitting avian influenza resulted from experimental infections and the research of BROWN et al. (2009) (unlike the Rock Dove, whose role in avian influenza virus transmission is very low). In natural conditions as well, passerines have been found infected with avian influenza virus by isolating the virus or by detecting antibodies (ALEXANDER, 2000; GILBERT et al., 2006; CHANG et al., 2014; SHRINER et al., 2016). Furthermore, some studies found that the role of passerines is very important in transmission of avian influenza (for example in the USA, FULLER et al., 2010). As well, avian influenza viral infection was reported in a Common Starling (*Sturnus vulgaris*) in Israel, a species also included in our study (Table 1) (LIPKIND, et al. 1982; SHRINER et al., 2016).

However, other studies indicate that passerine species, or at least some of them, are not an important reservoir of the virus, having no role or playing only a minor role in transmitting it to wild and domestic animals (SLUSHER et al., 2014; KOU, 2009; NEMETH et al., 2009). Also, the negative results obtained in our study have shown that, for the time being, passerines are not a reservoir of the avian influenza virus in the Danube Delta. Nevertheless, they still need to be monitored, especially during periods when the disease develops in the Danube Delta area, as well as in the immediately subsequent periods, to better evaluate the passerine's role in the interspecific transmission of the virus, as well as early detection of possible new avian influenza virus reservoir species. In addition, during periods when avian influenza virus circulation is reported in poultry farms, besides testing waterfowl, it is also necessary to check both the synanthropic bird species as well as water from poultry drinkers, and also the small aquatic bodies used both by domestic and wild birds.



Figure 1. Barn Swallow (*Hirundo rustica*) sampling – Grindul Lupilor, Danube Delta Biosphere Reserve (Romania), May 25, 2018 (original).



Figure 2. Common Reed-warbler (*Acrocephalus scirpaceus*) and Paddyfield Warbler (*A. agricola*) before sampling – Grindul Lupilor, Danube Delta Biosphere Reserve (Romania), May 23, 2018 (original).

Table 1. Wild bird species from the Danube Delta from which tracheal exudate samples were harvested in May 2018 for Real Time RT-PCR testing to identify the avian influenza virus genome.

Crt. no.	Species	No. of pools created and tested	No. of positive pools	No. of samples (individuals)
1	<i>Acrocephalus agricola</i>	8	0	40
2	<i>Acrocephalus scirpaceus</i>	19	0	95
3	<i>Emberiza schoeniclus</i>	1	0	5
4	<i>Hirundo rustica</i>	6	0	30
5	<i>Motacilla alba</i>	3	0	14
6	<i>Panurus biarmicus</i>	1	0	4
7	<i>Passer domesticus</i>	1	0	5
8	<i>Sturnus vulgaris</i>	1	0	5
9	<i>Sylvia curruca</i>	1	0	5
	Total	41	0	203

CONCLUSIONS

There are many bird species affected by or somehow involved in avian influenza virus transmission, but aquatic species are the most important, especially since the infection is facilitated by the aquatic environment.

Most bird species with a major and medium risk of introducing and transmitting avian influenza are frequent and abundant in the Danube Delta Biosphere Reserve.

In order to highlight the role of the passerines in the transmission of the avian influenza virus, we analysed 203 tracheal exudate samples collected from (mostly) migratory passerines in the vicinity of water bodies. All samples were tested (Real Time RT-PCR) and all results came out negative, leading to the premise that passerines were not involved in the possible circulation of the avian influenza virus in the Danube Delta Biosphere Reserve (Romania) in the first half of 2018.

Most probably the role of passerines, including aquatic ones, is very small or even neutral when the avian influenza virus is not developing intensively in the region.

However, in several regions around the globe, there has been evidence of the sensitivity and role of the passerines in the transmission of the avian influenza virus both naturally and through experimental infections, including some of the species tested by here (*Emberiza schoeniclus*, *Passer domesticus* and *Sturnus vulgaris*).

The results of our research are the same as those reported in other studies, which state that passerines may play a lesser role in the transmission of the avian influenza virus to other domestic or wild birds. At least for the 2018 regional situation, it could not be said that the passerines were affected or could have played a role in transmitting the avian influenza virus.

In 2018, there were several avian influenza episodes in Europe, with one outbreak occurring just across the border with Romania (Dobrich, Bulgaria), but there were no reports of detection in Romania. In order to verify once again the role of the passerines, it is necessary to repeat our experiment during the high circulation of the virus, but also to test medium and large water birds for the avian influenza virus.

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