UDC 619:616.98:578.832.1:636.5:639.127:616-036.22

DOI 10.29326/2304-196X-2019-1-28-34-38

EPIDEMIOLOGICAL MONITORING
OF AVIAN INFLUENZA IN THE REPUBLIC OF CRIMEA

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SUMMARY

The paper presents the results of monitoring tests performed in the FGBI "ARRIAH" Branch in 2018 to detect AIV circulation in the Republic of Crimea. As AIVs are most frequently detected in aquatic and semiaquatic birds, waterfowl present the greatest threat of the disease introduction and spread. That's why the paper gives special attention to the Azov Sea and Sivash water area and describes natural biotopes of the Central and Eastern Sivash. During expeditions biological material samples were collected, bird species were identified and bird fauna was estimated in waterfowl aggregation sites. Laboratory PCR tests of the biological material collected during the expedition as well from killed birds and poultry revealed no AIV type A. As the number of wildfowl in their usual aggregation sites decreased due to the fact that fresh water supply through the North-Crimean Canal ceased it can be assumed that the Crimean Peninsula will manage to maintain HPAI freedom despite the fact that the disease is being reported close to the peninsula. Nevertheless, the threat of the virus introduction to the commercial and backyard farm stocks still exists as the peninsula is located in the bird migration routes and AI epidemical monitoring is a topical task.

Key words: wild waterfowl, avian influenza, Sivash, migration, bird fauna, epidemiological monitoring.

INTRODUCTION

Flyways of migrating birds cross the Crimean territory. The Azov-Black sea basin, especially Sivash flooded areas and the Azov seashore, are the resting and wintering sites for such birds, which can be natural reservoirs of influenza viruses [3, 8, 9]. The epidemiological monitoring makes it possible to trace and control avian influenza (AI) virus spread and to take effective preventive measures in a timely manner [2, 3, 5, 7].

One of the epidemiological peculiarities of AI outbreaks, reported in the Crimea in 2005–2008, included the seasonality, as the disease occurred mainly in winter. Sharp drop in environmental temperature made wild birds to migrate to the nearest settlements and sometimes travel longer distances in search of food. In the course of such migrations wild birds contacted poultry, kept in backyards [1, 3, 4, 8, 10]. 62% of cases were reported in the regions bordering the Sivash lagoon (eastern part); 23% of cases occurred in the territories, washed by the Black Sea (Feodosia Municipal District, Chernomorsky and Krasnoperekopsky Raions) and 15% of cases were registered in the Central Crimea (Simferopolsky, Belogorsky and Krasnogvardeisky Raions) [3, 5, 8].

The aim of the work was to monitor the AI epidemic situation in the regions being at risk due to landscape and geographical peculiarities.

MATERIALS AND METHODS

Biological material from wild birds was collected during expeditions to the Azov seashore and Sivash lagoon together with the specialists of the Rosselkhoznadzor Administration for the Republic of Crimea and Sevastopol city and GBU RK "Yalta Mountain-Forest Natural Reserve". Samples of biological material from wild birds, shot in different Crimean hunting areas, from poultry, kept on commercial farms and in backyards and samples of animal products from other regions (turkey meat) were also submitted to the FGBI "ARRIAH" Branch laboratory for testing.

When taking samples, wild birds were identified using the bird guides. In the course of the expeditions the condition of Central and Eastern Sivash biotopes were studied,

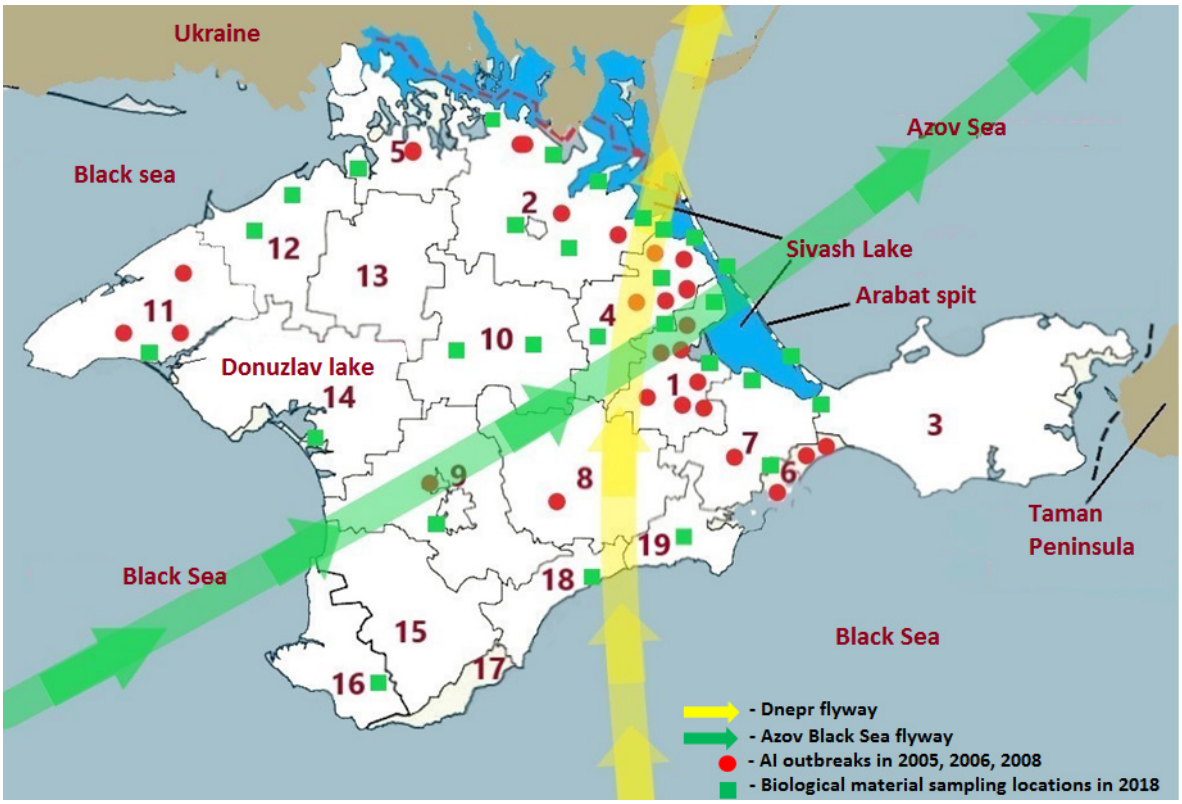

Fig. 1. Flyways over the Republic of Crimea and AI outbreaks in 2005–2008 with biological/pathological material sampling locations indicated

1 – Sovetsky Raion; 2 – Jankoysky Raion; 3 – Leninsky Raion; 4 – Nizhnegorsky Raion; 5 – Krasnoperekopsky Raion; 6 – Feodosia; 7 – Kirovsky Raion; 8 – Belogorsky Raion; 9 – Simferopolsky Raion; 10 – Krasnogvardeisky Raion; 11 –Chernomorsky Raion; 12 – Razdolnensky Raion; 13 – Pervomaisky Raion; 14 – Saksky Raion; 15 – Bakhchisaraysky Raion; 16 – Sevastopol; 17 – Yalta; 18 – Alushta; 19 – Sudak.

bird populations in those areas were estimated. Laboratory tests were performed using Russian commercial test kits pursuant to Veterinary Rules of Avian Influenza Type A Laboratory Diagnostics, approved by Order of the Ministry of Agriculture No. 105 on April 3, 2006.

The following materials were used:

1. Fecal samples and internal organs from the following migratory birds: western marsh-harriers (*Circus aeruginosus*), broad-billed sandpipers (*Limicola falcinellus*), cranes (*Grus*), common pochards (*Aythya ferina*), curlew sandpiper (*Calidris ferruginea*), curlews (*Numenius*), mallards (*Anas platyrhynchos*), waders (*Charadrii*), mute swans (*Cygnus olor*), Eurasian coots (*Fulica atra*), little terns (*Sternula albifrons*), great cormorants (*Phalacrocorax carbo*), slender-billed gull (*Chroicocephalus genei*), Kentish plovers (*Charadrius alexandrinus*), black-crowned night herons (*Nycticorax nycticorax*), common shelducks (*Tadorna tadorna*), ruffs (*Philomachus pugnax*), herons (*Ardea*), Caspian gulls (*Larus cachinnans*), gull-billed terns (*Gelochelidon nilotica*), black-necked grebes (*Podiceps nigricollis*), great crested grebes (*Podiceps cristatus*), Mediterranean gulls (*Ichthyaetus melanocephalus*), dunlins (*Calidris alpina*), lapwings (*Vanellus vanellus*), pied avocets (*Recurvirostra avosetta*).

2. Internal organs from the following wild birds, shot in hunting areas: greater white-fronted geese (*Anser albifrons*), woodcocks (*Scolopax rusticola*), common moorhens (*Gallinula chloropus*), common pigeons (*Columba livia*), common pochards (*Aythya ferina*), mallards (*Anas platyrhynchos*), waders (*Charadrii*), Euirasian coots (*Fulica atra*), Eurasian wigeons (*Anas penelope*), gray partridges (*Perdix perdix*), common pheasants (*Phasianus colchicus*), common teals (*Anas crecca*), Northern pintails (*Anas acuta*).

3. Cloacal and/or tracheal swabs from poultry: hens, turkeys, geese and ducks.

Laboratory tests were performed by polymerase chain reaction (PCR). GRIPP-test kit by Federal Budget Institution of Science "Central Research Institute of Epidemiology" of the Federal Service for Customers Rights Protection and Human Well-Being was used. The test kit contains sets of reagents for RNA extraction "AmpliPrime RYBO-sorb", for reverse transcription reaction "RevertaL"; reagents for influenza virus A cDNA amplification with real-time fluorescence *in situ* hybridization.

RESULTS AND DISCUSSION

Avian influenza was reported in the territory of the Republic of Crimea in 2005 for the first time in the settlement of Nekrasovka (Sovetsky Raion), located 1 kilometer from the Sivash lagoon, where big populations of wild birds were observed. The epidemic survey of the territory showed that poultry was kept in the backyards under free-ranging conditions and had a free access to paddy fields and the coastal area [8]. During the feeding poultry had direct contacts with both synanthropic (pigeons, crows, sparrows) and wild birds (geese, ducks, gulls) and drank from open containers located outdoors. Most likely, it resulted in avian influenza occurrence and its fast spread

in the Crimea. When testing biological material from poultry, died in the settlement of Nekrasovka on November 30, 2005, it was established that the deaths had been caused by highly pathogenic avian influenza (HPAI), type A, subtype H5N1. On December 3, 2005 poultry deaths were already reported in three settlements of the Sovetsky, Jankoysky and Leninsky Raions [4, 5, 8]. By the end of 2005, HPAI spread to the Chernomorsky and Krasnoperekopsky Raions. In the beginning of 2006, the disease was reported on a poultry farm, located in the municipal district of Feodosia and in the backyards of Orjonikidze settlement (Feodosia city). By the end of January 2006 the epizootic involved the Kirovsky, Belogorsky and Simferopolsky Raions. From March 2006 to 2008 no HPAI cases were reported. New H5N1 outbreaks were reported in January – February 2008 on a poultry farm of the Krasnogvardeysky Raion and in a backyard of the Chernomorsky Raion. Sporadic deaths of wild birds were also registered, including in the territory of Sevastopol city. In total 265,110 birds died and were killed in the Crimea in 2005–2008 [1, 5, 6, 8, 11].

Major flyways cross the Crimean territory: the Dnepr flyway (geese, ducks) and the Azov-Black Sea flyway (geese, ducks, sandpipers, swans, rooks). They intersect over the Sivash lagoon, which has always been the feeding, resting and wintering site for many resident and migratory wild birds. Exactly in the Sivash lagoon coastal regions the first AI cases were reported in 2005. Then the disease spread farther in the peninsula (Fig. 1).

AI viruses are isolated from many free living wild birds (more than 75 species belonging to 10 different orders), but mostly in waterfowl and semiaquatic birds. About 60% of H5N1 infected species are associated with wetlands [8, 11], and exactly among such species the majority of deaths have been reported. This suggests that waterfowl, including those residing in the Sivash basin, presents the biggest threat of avian influenza introduction and spread in the Crimea.

The Sivash is a shallow lagoon, separated from the Azov Sea by the narrow Arabat Spit. The lagoon is connected with the Azov Sea by two narrow straits (150 and 200 m). In the west, the Sivash is separated from the Karkinit Bay of the Black Sea by the isthmus of Perekop. The entire area spreads over about 2,640 km^2 and is characterized by a rather complicated configuration of the coastline, the length of which is 2,969–3,184 km depending on the water level. Low sandy, salty and slumpy shores with an overall length of 2,276 km are very attractive for wild birds. These creates a maximum biological diversity in the area and in combination with feed resources characterize the Sivash as a large wetland harbouring 132 bird species, including 97 semiaquatic species and 35 co-residing species.

The colonies of *Ciconiiformes* are the biggest in Europe; they have been created through the input of birds from the Kuban, Dniester and Dnepr habitations. One of the key roles in the creation of local bird fauna was played by the desalination in 1963 of the Eastern Sivash due to inflows of fresh waters through the North-Crimean Channel.

Starting from 2014 the delivery of fresh water to the Crimean Peninsula was ceased, leading to the rapid degradation of the area and, consequently, a decrease in the number and restricted composition of bird population in this region. During expeditions in February – June, 2018 the colonies of nesting birds, seen previously (in 2016–2017) were not observed anymore. This suggests that

Fig. 2. Eastern Sivash; a carcass of a mute swan

thanks to the decrease in the number of waterfowl, especially in the locations of their traditional accumulations, the Crimean Peninsula manages to maintain the freedom from HPAI, notwithstanding the fact that the disease is registered in the vicinity of the peninsula. For example, in 2016 in the Kherson Region (Ukraine) three HPAI outbreaks were reported, and in 2016–2018 HPAI occurred in Astrakhan, Rostov Oblasts and Krasnodar Krai. In 2017 in the Krasnodar Krai H5N8 HPAI virus was detected in mute swans [7, 11]. In June 2018 there were positive results for AI in animal products (turkey meat), imported from the Penza Oblast.

In the Russian Federation AIV, Eurasian lineage H5N8, clade 2.3.4.4 of Group B was detected in wild birds near Uvs Nuur Lake in the Republic of Tyva for the first time. Then it widely spread in Russia and many European, African and Asian countries [7, 11]. In February 2017 during one of the expeditions to the eastern shore of the Sivash lagoon a carcass of a mute swan (*Cygnus olor*) was found. H5N8 AI virus was found in the pathological materials taken from this carcass (Fig. 2).

In late 2017 – early 2018 41 samples from wild birds shot in seven Raions: Jankoisky, Alushtinsky, Sovetsky, Nizhnegorsky, Krasnogvardeisky, Razdolninsky and Kirovsky were submitted to the FGBI "ARRIAH" Branch laboratory. No AIV genome was detected by PCR testing.

In February 2018, waterfowl deaths were reported on the shore of the Donuzlav Lake near Medvedovo settlement of the Chernomorsky Raion. About 1,500 birds died, predominantly Eurasian coots (*Fulica atra*) and black-necked grebes (Podiceps nigricollis). When testing pathological material from dead birds by PCR in the FGBI "ARRIAH" Branch laboratory no AIV genome was found.

In July 2018 80 samples of biological material were taken from poultry, kept in the backyards of the Balaklavsky Raion (Sevastopol city); no AIV genome was found.

Expeditions to the waterfowl accumulation sites in Crimea (Kuchuk-Ajigol, Sasyk-Sivash, Donuzlav lakes and Sivash lagoon), organized by the FGBI "ARRIAH" Branch together with the Rosselkhoznadzor and ornithologists were continued in October – November, 2018 (Fig. 3). In the course of these expeditions, 54 samples of biological material (feces) were taken; negative results were obtained in PCR testing for avian influenza, type A.

Fig. 3. Sasyk-Sivash Lake, Yevpatoria city

In late November 2018, bird wintering did not start in Crimea. Based on the results of the incomplete recording the following birds were observed: Donuzlav lake: Eurasian coot (*Fulica atra*) – 2,000 birds, black-necked grebe (*Podiceps nigricollis*) – 100 birds, great cormorants (*Phalacrocorax carbo*) – 20 birds, black-headed gull (*Chroicocephalus ridibundus*) and Caspian gulls (*Larus cachinnans*) – 40 birds, mute swan (*Cygnus olor*) – 22 birds; Sivash (Eastern part): common shelduck (*Tadorna tadorna*) – 2,000 birds, Northern pintail (*Anas acuta*) – 1,000 birds, mallard (*Anas platyrhynchos*) – 1,000 birds. Common pochards (*Aythya ferina*) have not wintered in the Crimean territory for the last two years, possibly due to the absence of feeds. During the period of observation, about 2,000 by-passing birds of this species were recorded. When PCR-testing seven samples of biological material for AIV, type A from the following wild birds: common teals (*Anas crecca*), common pheasants (*Phasianus colchicus*), mallards (*Anas platyrhynchos*), woodcocks (*Scolopax rusticola*), shot in the hunting areas in the Belogorsky Raion, negative results were obtained.

CONCLUSION

A successful poultry production is possible only if the epidemic situation is stably favorable. Notwithstanding the fact, that since 2009 no HPAI cases or related deaths among poultry have been reported in the Crimean territory, the risk of the virus introduction into commercial or backyard flocks is still high. The reason for that is that the peninsula is located along the major flyways. That is why avian influenza monitoring is a topical and socially significant task.

***Conflict of interest*.** The authors declare no conflict of interest.

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Submitted on 13.12.18
Approved for publication on 31.01.19