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EXAMINATION OF PRESENCE OF SPECIFIC ANTIBODIES AGAINST AVIAN INFLUENZA VIRUS IN SOME SPECIES OF WILD BIRDS

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Infections caused by the avian influenza virus have been known for a long time and they are present, to a smaller or greater extent, in both extensive and intensive poultry production in many parts of the world. Epidemiological investigations have established a definite significance of the population of wild birds in maintaining and spreading this infection. Avian influenza is a zoonosis, and the virus has a great potential for causing mortality in humans, in particular its subtypes H5 and H7, which is why it has lately been provoking much attention among scientists and experts, as well as the general public.

The objective of the work was to catch a certain number of wild birds in several locations in the Republic of Serbia, to identify them, and to collect samples of their blood serum for the determination of specific antibodies against the avian influenza virus. Birds were caught in ten locations in a manner that was safe for the birds themselves, as well as for the staff that did the catching. The birds were caught in especially produced nets, and in some cases in special traps. The caught wild birds were identified using the methods described in reference literature. All the names of the wild birds were coordinated with the valid Serbian nomenclature of European birds, prepared by prominent ornithologist and bird lover Milan Ružić. Following catching and identification, blood samples were taken from the birds from the wing vein (in bigger birds) or from the leg vein (in smaller birds). In taking blood samples from wild birds, all the principles of asepsis and antisepsis were followed in order to prevent any possibility of infection. After that, the birds were returned to their natural habitat, to the same locations in which they were caught. Serums were isolated from the taken blood samples and they were stored at -20^{o}C until the final examinations. A total of 46 species of wild birds were identified among a total of 259 birds from which 259 samples of blood serum were isolated. The following were used for the detection of the presence of specific antibodies against the avian influenza virus in blood serum samples of wild birds: agar gel precipitation (AGP), the

hemagglutination inhibition test (HI) for subtypes H5 and H7, the cELISA test with antigen for the A type avian infleunza virus, and the cELISA test with antigen for subtype H5 of the avian influenza virus. Due to the fact that about 360 different species of wild birds live in the Republic of Serbia, the number of 46 identified species covered by these investigations account for 12.77% of the total number of bird species present in Serbia, which is considered a good sample. Specific antibodies against the A type avian influenza virus were established in serum samples of only 9 of the 259 birds covered by examinations using the cELISA test. Of the 46 identified wild bird species, 6 belonged to birds that live exclusively in water habitats and are considered a reservoir of the avian influenza virus (white stork, mallard, mute swan, common pochard, common goldeneye, and Eurasian coot). Among the listed species, particular attention was drawn to 4 species of wild birds of the order Anseriformes and the family Anatidae (mallard, mute swan, common pochard, common goldeneye) of which there were 30 birds among the total of 259 examined. In the 30 blood serum samples of the listed bird species, specific antibodies against the A type avian influenza virus were established in 9 (30%) serum samples using cELISA. Specific antibodies against the avian inluenza virus subtype H5 were established in 3 serum samples of mute swans (one serum sample originated from a mute swan which was tagged in Poland) and in one blood serum sample of a common pochard, or a total of 4 (13.33%) serum samples, using the hemagglutination inhibition test. Specific antibodies against the avian inluenza virus subtype H7 were established in 3 (10%) blood serum samples, in two serum samples from mallards and one sample from a mute swan, using the hemagglutination inhibition test. Specific antibodies against the avian inluenza virus type A were not established in any examined bird species using the AGP test. In the population of wild bird species in the Republic of Serbia covered by these investigations, specific antibodies against the avian influenza virus were established only in serum samples of birds of the family Anatidae. Specific antibodies against the avian inluenza virus type A established in 3 (6.52%) species of wild birds, and against subtypes H5 and H7 in 2 (4.34%) of the total of 46 examined species. The sensitivity of the cELISA test for the avian inluenza virus subtype H5 and the hemagglutination inhibition test for subtype H5 amounted to 100%.

Key words: avian influenza virus, wild birds, AGP, HI, ELISA

INTRODUCTION

The name influenza originates from an old Latin word for a disease appearing suddenly in humans and which has been interpreted as being the

result of the astrological influence of the stars and mystical forces of destiny (Russell *et al*., 1988). The first officially recorded occurrence of avian influenza was in Italy in 1878 and it was described by Perroncito (Saif *et al*., 2003). Centanni and Savonuzzi proved in 1901 that the cause of the disease is a filtrable agent, but the cause was not identified or classified as an influenza virus up until 1955 (Schafer, 1955). In 1918, the notorious Spanish influenza acquired the proportions of a pandemic when the avian influenza virus subtype H1N1 was passed to humans and killed fifty million people. Describing the appearance of this pandemic, Oxford *et al*. (2004) listed certain characteristics of a camp for young soldiers in France, where the disease appeared in the winter of 1917/18. The authors believe all the necessary conditions had been met in the training camp in Etaples for the outbreak of an influenza pandemic, including the presence of daily rotating 100 000 young soldiers, pig farms in the immediate proximity, a large market of live geese, ducks, chicken, and the presence of horses. In addition to all the above, the authors also list the fact that 24 types of gas were stored in the camp, in quantities of over 100 tons, and that some of them were mutagenic. This presented an additional factor which significantly contributed to the spreading of the infection, because certain types of gas were used for treating the army and surrounding area in the fight against fleas and other insects, while others were used as poison gas in combat. The return of several million soldiers to their homes all over the world in the autumn of 1918 marked the beginning of the pandemic caused by the avian influenza virus. At the beginning of the last century, the disease was present in Switzerland, Russia, Romania, Hungary and many other countries (Krohn, 1925). Due to the proximity of the listed states, it is highly likely that the disease was present in Serbia as well, but there are no official records about that. In Scotland, the avian influenza virus of subtype H5N9 was isolated in 1959, and the avian influenza virus of subtype H5N3 was isolated in swallows in South Africa in 1961, which then led to the forming of the scientific premise that all subtypes of the H5 avian influenza virus are highly pathogenic (Swayne and Suarez, 2000). From the year 1955 until the beginning of the 21st century, there were only some twenty outbreaks of a highly pathogenic avian influenza virus (VPVAI) in poultry and they were all caused by subtypes H5 and H7. In addition to the highly pathogenic avian influenza virus subtype H5N3 which was isolated from the swallow, high mortality among wild birds was caused by only one other virus subtype H5N1 (Ellis *et al*., 2004).

The second case of infection with the subtype H7N7 avian influenza virus was recorded in 1959 in one man following his return from travels in Africa and Asia (Delay *et al*.,1967). The spreading of the avian influenza virus subtype H7N7 from diseased seals to humans took place in 1978, and the occurrence of conjunctivitis was recorded in humans. Conjunctivitis caused by the avian influenza virus subtype H7N7 appeared in England in 1996 in a woman who was the owner of fowl that she kept in her yard. In the course of 1997, a highly pathogenic avian influenza virus (VPVAI) subtype H5N1 caused an infection in 18 persons in Hong Kong, with resulting fatalities in 6 cases (Mounts *et al*., 1999).

Shortridge *et al*. (2003) listed numerous investigations that have demonstrated that precursors of the avian influenza virus subtype H5N1 originating from geese and ducks, together with the virus subtypes H9N2 and H6N1 isolated from quail, changed the genes of this quail, and present among bird species what pigs present among mammals, and that means they are an ideal vessel for the mixing of avian influenza viruses, because this animal species has both types of receptors for influenza (both alpha-2,3 and alpha-2,6). Fouicher *et al*. (2004) and Koopmans *et al*. (2004) described, in The Netherlands in 2003, a clinical picture of a disease caused by the highly pathogenic avian influenza virus subtype H7N7 in 86 humans who had been in contact with infected poultry, as well as in 3 members of their families. Of these 89 persons, 78 had conjunctivitis, 5 conjunctivitis with signs of an influenza-like illness, 2 had signs of an influenza-like illness, and 4 had an undetermined clinical picture. The influenza-like illness was generally mild, but fatal pneumonia was recorded in some patients in combination with acute respiratory distress syndrome. Gill *et al*. (2006) reported a serologically confirmed infection with avian influenza viruses in one duck hunter and in two researchers engaged on studies of the life of game and who had been in contact with wild birds from water habitats. Investigations using two laboratory methods established that this was an infection caused by the avian influenza virus type A and subtype H11N9 which is less present in wild ducks, but it was established in all 3 persons. Liu *et al*. (2005) reported that the appearance and the focus of the disease among migratory birds on Lake Qinghai in China in the summer of 2005, when thousands of birds died, was the first real epidemic caused by the avian influenza virus, and after which it begins to spread throughout Eurasia. Authors warn that the avian influenza virus subtype H5N1 is pathogenic and dangerous for different animal species and for humans, as well. Even today, the avian influenza virus subtype H5N1 is occasionally transmitted from diseased poultry to humans, and cases of this type of infection in humans are recorded monthly, and sometimes even weekly, confirmed with laboratory results, mostly in countries of southeast Asia where the disease is already endemic in poultry. International symposiums dedicated to avian influenza have been held on a regular basis since 1981. According to reports, the most frequent sources and reservoirs of avian influenza viuruses are free-range birds of water habitats, in particular those from the orders Anseriformes (ducks, geese, swans) and Charadriiformes (coast birds, seagulls, swallows), which together make up the main reservoir of all avian influenza viruses (Fouchier *et al*., 2004; Wallensten *et al*., 2006; Stallknecht, 2007; Jonassen and Handeland, 2007; Cattoli *et al*., 2007). In the mentioned orders of birds, the avian influenza virus usually does not cause the disease (low pathogenic avian influenza virus), with the exception of high mortality in swallows in South Africa at the time of the occurrence of a disease caused by avian influenza virus subtype H5N3. In ducks, especially mallards (Anas platyrhynchos), investigations proved the biggest percentage of the presence of avian influenza viruses, going as high as 60% in young ducks, before their migration in late summer. The avian influenza virus in nature infects numerous species of wild and domestic birds, especially those that rally around water, ponds, marshes and coastal habitats (Ellis *et al*., 2004). Avian influenza viruses

have been isolated from more than 90 species of free wild birds belonging to 13 different orders: Anseriformes (ducks, geese and swans), Charadriiformes (sea coast birds, gulls, swallows), Ciconiiformes (herons and ibises), Columbiformes (pigeons), Falconiformes (hawks), Galliformes (partridges and pheasants), Gaviiformes, Gruiformes (Eurasian coot), Passeriformes (chaffinch, sparrows, tits, and many other small birds), Pelecaniformes (cormorants), Piciformes (woodpeckers), Podicipediformes (grebes) and Procellariiformes (Estola). In ecosystems created by man (agriculture, raising pets in cages, flock as a hobby, and exhibition systems), infections have been discovered and reported in birds of the order Psittaciformes (parrots, budgies), Casuariiformes (emu), Struthioniformes (ostrich), Rheiformes (rea – an ostrich-like bird from South America) and in the most domesticated species of the orders Galliformes and Anseriformes. The last two orders include chicken, turkeys, japanese quail (Coturnix japonica), helmeted guineafowl (Numida meleagris), Bobwhite quail (Colinus virginianus), pheasant (various species), quail (Alectoris chukar), geese and ducks (mallard) (Makarova *et al*., 2003). On entering the organism, the virus replicates in the respiratory, digestive and reproductive systems and is secreted through the nose, mouth, conjunctive and cloaca of infected birds into the outer environment**.** Influenza viruses are classified into types on the grounds of the internal proteins: nucleoproteins (NP) and matrix proteins (M1). In order to investigate and determine the type of influenza virus, the agar gel precipitation test is used. (Swayne *et al.*, 2000). Influenza A type viruses can further be subtyped on the grounds of two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). To date, 16 subtypes of hemagglutinin and 9 subtypes of neuraminidase have been identified. Serological subtyping of hemagglutinin is performed using the hemagglutination inhibition test (HI), and the subtyping of neuraminidase using the neuraminidase inhibition test (NI). Since 1980, the processes of determing types and subtypes have been standardized for all types and subtypes of influenza viruses in birds, pigs, horses, and humans, as well as their nomeclature.

Of the 21 registered infections caused by the avian influenza virus subtype H5N1 in Asia, nine occurred most probably through poultry, and three of 21 infections most probably resulted from the cause being brought with the migration of wild birds. However, in 20 countries of the 23 European countries in which the illness was registered, the avian influenza virus was most probably brought by migratory wild birds, as reported by Kilpatrick *et al*. (2006).

The passing of the avian influenza virus originating from wild birds to poultry flocks, in particular of migratory birds connected with water habitats, was confirmed by Halvorson *et al*. (1985) and Campitelli *et al*. (2004) on the grounds of investigations of the philogenetic relations of the avian influenza virus subtype H7N3 isolated from wild geese and the virus of the same subtype isolated from poultry in the same area, when they established they were 99.8% homologous. That the foci of infection caused by the avian influenza virus subtype H5N1 are located precisely on the routes of migratory birds is claimed by Ducatez *et al*. (2006). The passing of the avian influenza virus to poultry most frequently occurs through the feces of infected wild ducks which are in direct contact with poultry or

indirectly through contaminated feed or water (Alexander, 2006). Examinations of the migrations of wild birds of the family Anatidae and the spread of the highly pathogenic avian influenza virus subtype H5N1 were performed by Marius Gilbert *et al*. (2006). Specific antibodies against the avian influenza virus subtype H5 were proven in a very small number of examined blood samples of wild birds, while the presence of the virus was discovered on a regular basis in dead birds found in endemic and contaminated areas, which indicates that they were themselves victims of the infection (Boyce, 2007).

Serological tests are used to prove specific antibodies against the avian influenza virus with which they can be detected 7 days following the moment of infection. There are several techniques that can be used for serological monitoring and the diagnostics of avian influenza. In programmes of serological monitoring, the agar gel immunodiffusion test for the detection of anti-nucleoprotein antibodies is often used, primarily for the detection of antibodies specific to all avian influenza A type viruses. The test is reliable, it is not expensive and it can easily be carried out even in laboratories which do not have the most up-to-date equipment. ELISA tests have also been developed for the detection of specific antibodies against avian influenza A type viruses (Rowe *et al*., 1999; Sala *et al*., 2003; Beck and Swayne, 2003). Once the presence of specific antibodies is established using the AGID or ELISA tests, the hemagglutination inhibition test (HI) is used for further detection and identification of the avian influenza virus subtype. The pheasant is listed as a specific mediator and carrier of the virus, which originates from birds and from which people can also become ill, which has been experimentally proved (Makarova *et al*., 2003). There are many papers that indicate a wide diversity in the pathogeneity of an avian influenza virus, even in birds of the same order, in a way that it can cause 100% fatalities in one specie and yet have no visible signs of the disease in another specie (Perkins *et al*., 2003). Examinations of 8500 blood serum samples from wild birds, mostly ducks, geese and coast birds, and 1500 serum samples from other species in northern Europe established specific antibodies against the avian influenza virus in 1% serum samples (Slemons *et al*., 2003).

In recent times, epidemics and epizooties of avian influenza have indicated that our civilization is threatened and vulnerable at a global level, and stressed the importance of the role of veterinary services in the supervision, prevention, detection, diagnosis, and research activities. This problem has led to the creation of new and more efficient joint activities by the veterinary and medical services. The policies of both one and the other service must be based on common foundations and principles, because the expected results should also be the same, and that is primarily a more efficient protection of human health. The avian influenza virus shows an expressed tendency toward a constant process of adapting to the host organism. The passing of the cause itself among animals of the same species takes place swiftly and easily, but the transmission of the infection to other bird species also takes place, and to other classes of animals, to certain mammals, for example. It has been established that new influenza epidemics in the human population mostly occur in areas where birds bound to water habitats, pigs and humans are in close contact. Due to its geographical

position on the Balkan corridor of migrations of numerous bird species, as well as the presence of suitable habitats for their breeding and living, it is justified to assume that Serbia could also present a reservoir in which wild birds, and in particular migratory birds, can be the factors that transmit numerous causes of infectious diseases both to humans and to domestic fowl. No investigations have been carried out in Serbia so far of the health status of wild birds, and consequently none covering avian influenza either.

MATERIAL AND METHODS

The material for the investigations were blood serum samples from wild birds caught in 10 different localities, or biotypes (rivers, mountains, lakes, swamps, marshes, forests, etc.). In the course of the investigation, numerous tools and materials for catching and handling wild birds were used for blood sampling, transportation, isolation of the serum and storage. Wild birds were caught in a number of ways, depending on their size and habitat. Small wild land birds were caught using standard nets for catching birds (Thailand) and big water birds were caught from boats or from the coast using nets, and in certain cases also using special traps.

Methods described in the literature were used for the identification of the caught wild birds: the illustrated handbook for the identification of animal species entitled "Fauna Europas" by Garms and Borm (2007), the illustrated book "Our Birds" by Guggisberg *et al*. (1981) and the illustrated book "Canaries, songbirds of the forest and parrots" ("Kanarinci šumske pevačice i papagaji") by Bora Vasić (1973). As additional help in the identification, the biggest data base on birds existing on the Internet was used, which contains a registry of over 10 000 species of wild birds and almost 22 000 subspecies. All the names of the wild birds have been coordinated with the valid Serbian nomenclature of the birds of Europe prepared by the prominent ornithologist and bird lover, Milan Ružić. It was especially important, for the birds themselves as for the persons that did their catching, that the birds were caught in a safe manner, that the blood sample was taken one hour after that time at the latest, and care was taken of the manner in which the blood was taken and of the amount of blood that may be drawn. Blood was taken from bigger birds from the wing vein, and from small birds from the leg vein, and after this procedure the birds were returned to the same natural locality. Furthermore, in taking the blood samples from wild birds, all the principles of asepsis and antisepsis were followed by in order to prevent any possibility of infection. A total of 259 different wild birds was caught, and they were then identified according to species. Following the identification, 259 blood serum samples were separated. The following were used for investigations of the presence of specific antibodies against the avian influenza virus in blood serum samples: the agar-gel precipitation (AGP) test obtained from a specialized laboratory in Doorn, The Netherlands (Poultry Health Centre Doorn - Animal health service, VLDIAO15 - AGA – INF INF AGP / GDT ANTIGEN), the hemagglutination inhibition test for detecting specific antibodies against the avian influenza virus subtypes H5 and H7, obtained from the OIE and FAO reference

laboratory for avian influenza, Weybridge, UK. The same laboratory was the source of positive control serums used in these investigations. For the detection of specific antibodies against the avian influenza virus, two competitive ELISA tests were used, obtained as a diagnostics kit under the name "Anigen AIV Ab cELISA", ANIGEN Animal Genetics Inc., Kyonggi-do, Korea and cELISA for the detection of specific antibodies against the avian influenza virus subtype H5 under the name "AniGen H5 AIV Ab cELISA" of the same manufacturer.

The agar gel precipitation (AGP) test for the detection of specific antibodies against the avian influenza virus was performed in accordance to the procedure described in the OIE handbook. The results were read in a dark room with a strong light source, (Illuminator Leica, model 13410312). Inhibition of hemagglutination for the detection of specific antibodies against the avian influenza virus type A and subtypes H5 and H7 was performed in accordance with the procedure described in the OIE handbook. The competitive ELISA tests for the detection of specific antibodies against the avian influenza virus type A and subtype H5 were performed in keeping with the instructions of the manufacturer of the diagnostic kits (ANIGEN Animal Genetics Inc., Kyonggi-do, Korea).

RESULTS

Wild birds were caught for the planned investigations during the period from 2006 until 2008. During that period, 259 birds were caught and 46 species of wild birds were identified among them: (Coccothraustes coccothraustes, Carduelis chloris, Fringilla montifringilla, Pyrrhula pyrrhula, Carduelis carduelis, Fringilla coelebs, Emberiza citronella, Carduelis spinalis, Dendrocopos medius, Picus viridis, Oriolus oriolus, Falco subbuteo, Anas platyrhynchos, Ciconia ciconia, Delichon urbica, Cyanistes caeruleus, Poecile montanus, Hirundo rustica, Luscinia megarhynchos, Turdus merula, Sylvia atricapilla, Lullula arborea, Erithacus rubecula, Turdus philomelos, Sturnus vulgaris, Passer domesticus, Phasianus colchicus, Columba livia, Streptopelia decaocto, Motacilla alba, Pica pica, Galerida cristata, Emberiza schoeniclus, Carduelis cannabina, Parus major, Phylloscopus trochilus, Locustella luscinioides, Acrocephalus schoenobaenus, Phylloscopus sibilatrix, Asio otus, Tyto alba, Athene noctua, Cygnus olor, Aythya ferina, Bucephala clangula and Fulica atra) (Table 1). About 360 different species of wild birds live on the territory of the Republic of Serbia and the 46 identified species covered by these investigations presented 12.77% of the totally registered bird species in Serbia, which is considered a good sample. As these wild birds originated from different habitats, or biotypes (rivers, mountains, lakes, swamps, marshes, forests, etc.) such a large diversity of species is justified.

The identified 46 species of wild birds included a group of 6 species (white stork, mallard, mute swan, common pochard, common goldeneye, and Eurasian coot) which live exclusively in water habitats, and they accounted for 12.35% of all the caught wild birds. Of the listed species, 4 species of wild birds of the order Anseriformes and the family Anatidae (mallard, mute swan, common pochard, common goldeneye) attracted particular attention, as there were 30 of these birds among the totally examined 259 birds. In the 30 examined birds of these bird

species, specific antibodies against the avian influenza type A virus were established in 9 (30%) blood serum samples from birds of this family using cELISA. Specific antibodies against the avian influenza virus subtype H5 were established in 3 serum samples from mute swans (one serum sample originated from a mute swan tagged in Poland) and in one blood serum sample of a common pochard, or in 4 (13.33%) samples of the totally examined 30 serums using the hemagglutination inhibition test. Specific antibodies against the avian influenza virus subtype H7 were established in 3 (10%) blood serum samples, in two serums from mallards and in one serum sample from a mute swan using the hemagglutination inhibition test. Specific antibodies against the avian influenza A type virus were not established in any of the examined bird species using the AGP test (Table 2, 3, 4 and Figure 1).

Table 1. Number of caught wild birds in certain localities in the Republic of Serbia and identified bird species according to the existing nomenclature

Numb er	Order	Family	Species	English name of bird	Number of birds
1	Passeriformes	Fringillidae	Coccothraustes coccothraustes	Finch	9
2	Passeriformes	Fringillidae	Carduelis chloris	Greenfinch	10
3	Passeriformes	Fringillidae	Fringilla montifringilla	Brambling	6
4	Passeriformes	Fringillidae	Pyrrhula pyrrhula	Bullfinch	9
5	Passeriformes	Fringillidae	Carduelis carduelis	Goldfinch	18
6	Passeriformes	Fringillidae	Fringilla coelebs	Chaffinch	7
7	Passeriformes	Emberizidae	Emberiza citrinella	Yellowhammer	9
8	Passeriformes	Fringillidae	Carduelis spinalis	Siskin	14
9	Piciformes	Picidae	Dendrocopos medius	Middle spotted woodpecker	2
10	Piciformes	Picidae	Picus viridis	Green woodpecker	2
11	Passeriformes	Oriolidae	Oriolus oriolus	Golden oriole	1
12	Falconiformes	Falconidae	Falco subbuteo	Hobby	1
13	Anseriformes	Anatidae	Anas platyrhynchos	Mallard	12
14	Ciconiiformes	Ciconiidae	Ciconia ciconia	White stork	1
15	Passeriformes	Hirundinidae	Delichon urbica	House martin	5
16	Passeriformes	Paridae	Cyanistes caeruleus	Blue tit	6
17	Passeriformes	Paridae	Poecile montanus	Willow tit	3
18	Passeriformes	Hirundinidae	Hirundo rustica	Barn swallow	8
19	Passeriformes	Muscicapidae	Luscinia megarhynchos	Nightingale	1

Cont. Table 1.

20	Passeriformes	Turdidae	Turdus merula	Common blackbird	7
21	Passeriformes	Sylviidae	Sylvia atricapilla	Blackcap	6
22	Passeriformes	Alaudidae	Lullula arborea	Woodlark	1
23	Passeriformes	Muscicapidae	Erithacus rubecula	Robin	3
24	Passeriformes	Turdidae	Turdus philomelos	Song thrush	1
25	Passeriformes	Sturnidae	Sturnus vulgaris	Starling	9
26	Passeriformes	Passeridae	Passer domesticus	House sparrow	32
27	Galliformes	Phasianidae	Phasianus colchicus	Pheasant	5
28	Columbiformes	Columbidae	Columba livia	Pigeon	14
29	Columbiformes	Columbidae	Streptopelia decaocto	Collared dove	4
30	Passeriformes	Motacillidae	Motacilla alba	White wagtail	1
31	Passeriformes	Corvidae	Pica pica	Common magpie	3
32	Passeriformes	Alaudidae	Galerida cristata	Crested lark	3
33	Passeriformes	Emberizidae	Emberiza schoeniclus	Reed bunting	3
34	Passeriformes	Fringillidae	Carduelis cannabina	Linnet	5
35	Passeriformes	Paridae	Parus major	Great tit	4
36	Passeriformes	Sylviidae	Phylloscopus trochilus	Willow warbler	3
37	Passeriformes	Sylviidae	Locustella luscinioides	Savi's warbler	3
38	Passeriformes	Sylviidae	Acrocephalus schoenobaenus	Sedge warbler	2
39	Passeriformes	Sylviidae	Phylloscopus sibilatrix	Wood warbler	1
40	Strigiformes	Strigidae	Asio otus	Eastern screech owl	2
41	Strigiformes	Tytonidae	Tyto alba	Barn owl	1
42	Strigiformes	Strigidae	Athene noctua	Little owl	3
43	Anseriformes	Anatidae	Cygnus olor	Mute swan	16
44	Anseriformes	Anatidae	Aythya ferina	Common pochard	1
45	Anseriformes	Anatidae	Bucephala clangula	Common goldeneye	1
46	Gruiformes	Rallidae	Fulica atra	Eurasian coot	1
Total	9 orders	22 families	46	46	259 birds

Table 2. Results obtained by examinations of blood serum samples of some wild bird species of the family Anatidae in which specific antibodies against the avian influenza virus type A were established using the AGP and cELISA methods

Bird species	Number of examined blood serum samples	Number of positive serum samples for avian influenza virus type A	
		AGP	cELISA
Mute swan	16	0	4
Mallard	12	0	4
Common pochard	1	0	1
Common goldeneye	1	0	0
Total (number and percent)	30	0 (0%)	9 (30%)

Table 3. Results obtained by examinations of blood serum samples of certain species of wild birds of the family Anatidae in which specific antibodies against the avian influenza virus subtype H5 were established using the hemagglutination inhibition and cELISA methods

Bird species	Number of examined blood serum samples	Number of positive serum samples for subtype H5	
		HI	cELISA
Mute swan	16	3	3
Mallard	12	0	0
Common pochard	1	1	1
Common goldeneye	1	0	0
Total (number and percent)	30	4 (13.33%)	4 (13.33%)

Table 4. Results obtained by examinations of blood serum samples of certain wild bird species of the family Anatidae in which specific antibodies against the avian influenza virus subtype H7 were established using the hemagglutination inhibition method

Bird species	Number of examined blood serum samples	Number of positive serum samples for subtype H7
		HI
Mute swan	16	1
Mallard	12	2
Common pochard	1	0
Common goldeneye	1	0
Total (number and percent)	30	3 (10%)

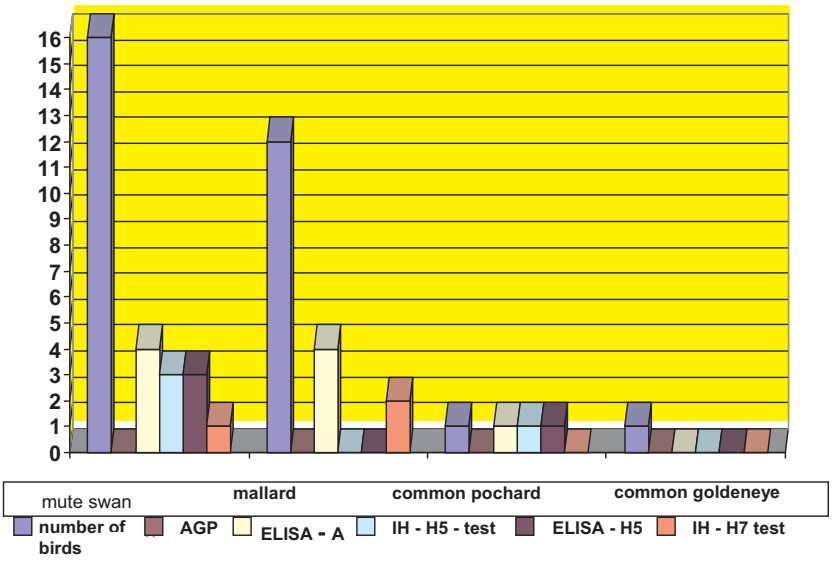

Figure 1. Number of blood serum samples of wild birds of the family Anatidae, according to species, in which specific antibodies against the avian influenza virus type A and subtype H5 or H7 were established using the tests AGP, HI and ELISA

Upon analysis of the number of blood serum samples with specific antibodies against the avian influenza A type virus comparing the species of examined wild birds, using cELISA, it can be seen that specific antibodies were established in 25% serum samples from mute swans and 33.33% serum samples from mallards. Using the methods of hemagglutination inhibition and cELISA for the avian influenza virus subtype H5, antibodies against this virus subtype were established in 18.75% serum samples from mute swans in comparison with the total number of examined serum samples from mute swans. In mute swans, specific antibodies against the avian influenza virus subtype H7 were established in 6.25% blood serum samples using the hemagglutination inhibition test. In blood serum samples from mallards, no specific antibodies against subtype H5 were found using the hemagglutination inhibition test and cELISA. In one blood serum sample from a common pochard, specific antibodies against the avian influenza virus subtype H5 were established using the HI test and cELISA for the mentioned subtype. Specific antibodies against the avian influenza virus subtype H7 were established in 16.66% blood serum samples using hemagglutination inhibition. Of the 259 blood serum samples examined using the ELISA test, specific antibodies against the avian influenza type A virus were established in 3.47%, against the total number of examined birds, while 1.54% serum samples with specific antibodies against the avian influenza virus subtype H5 were established using cELISA and HI with antigens for subtype H5. Examination of 259 blood serum samples of wild birds to detect the presence of specific antibodies against the avian influenza virus subtype H7 established their

presence in 1.16% samples using the HI test with antigen against subtype H7. In the 46 species of wild birds that were examined, specific antibodies against the avian influenza virus were discovered in 3 species, or in 6.52%.

The titer of specific antibodies against the avian influenza virus subtype H5 was from 1:16 to 1:32 in mute swans, and the titer in the common pochard was 1:64, with examinations performed using the hemagglutination inhibition test. Specific antibodies against the avian influenza virus subtypes H5 and H7 were established in 2 (4.34%) bird species of the total number of examined species. The titer of specific antibodies against the avian influenza virus subtype H7 was from 1:16 to 1:32, when the hemagglutination inhibition test was used. Specific antibodies against the avian influenza virus type A were not detected in 250 (96.53%) blood serum samples of the 259 birds covered by these investigations when the cELISA test was used. During examinations of 259 blood serum samples of wild birds using the hemagglutinaton inhibition test with subtype H5, specific antibodies against the avian influenza virus subtype H5 were established in 4 (1.54%) samples, and against the subtype H7 in 3 (1.16%) blood serum samples. The sensitivity of the cELISA test for the avian influenza virus subtype H5 against the hemagglutination inhibition test for subtype H5 amounted to 100%. In examinations of 259 blood serum samples of the 46 identified wild bird species, specific antibodies against the avian influenza virus type A were not established using AGP. In blood serum samples of wild birds, specific antibodies against the avian influenza virus type A and subtypes H5 and H7 were established only in bird species of the family Anatidae, using IH and cELISA.

DISCUSSION

Examining 310 blood serum samples of wild birds from water habitats in Finland, Erika Lindh *et al* (2008) established specific antibodies against the avian influenza virus in only 5 samples (4 serum samples from mallards - Anas plathyrhynchos and in one serum sample from the Eurasian teal – Anas crecca), and in our investigations of 259 serum samples of wild birds we established antibodies in 9 samples (in 4 serum samples from mallards – Anas plathyrhynchos, in 4 serum samples from mute swans – Cygnus olor and in one serum sample from a common pochard – Aythya ferina). In the investigations of the mentioned authors, as well as in our own, two species of wild ducks had the biggest number of samples with specific antibodies against the avian influenza virus type A, with emphasis that in both investigations mallard was the species with the biggest number of serum samples (4 samples) with specific antibodies against the avian influenza virus. Wu *et al*. (2007) compared ELISA, AGP and the hemagglutination inhibition test during investigations of 263 blood serum samples, and in our investigations the same methods were used for 259 blood serum samples. The authors established greater sensitivity of the ELISA test than the AGP and HI test, which was also the case in our investigations. None of the examined serum samples showed specific antibodies when the AGP was used, which means that its sensitivity was 0%. The ELISA test established the presence of specific antibodies against the avian influenza virus type A in 9 (3.47%) of the

259 examined serum samples. When the same 259 serum samples were examined using the hemagglutination inhibition test for the avian influenza virus subtype H5 specific antibodies against this subtype were established in 4 serum samples, and using the HI test for subtype H7 they were established in 3 serum samples, which presents 7 blood serum samples, or 2 samples less than in the examinations using the cELISA test for the avian influenza virus type A. Two serum samples that were positive using the cELISA test belonged to some other avian influenza virus subtype, which differed from subtype H5 or H7. The conclusion of the mentioned authors, with whom we also fully agree, is that the ELISA test presents a good choice in serological diagnostics in monitoring programmes for avian influenza for different bird species which can carry several avian influenza virus types and are considered to be their reservoirs, as this test makes it possible for the serum examination to be carried out using one analysis, while this is not possible with the HI test, and the AGP test has been shown to be non-sensitive. De Marco *et al*. (2005) established 52.2% seroprevalence in ducks, and in our investigations using the ELISA test, seroprevalence in the same bird species was 33.33%. Terregino *et al*. (2007) concluded on the grounds of obtained results that birds from water habitats of the order Anseriformes present the main reservoir of the avian influenza virus subtypes H5 and H7, which is fully in agreement with our investigations, since only wild birds of the order Anseriformes were positive to the presence of specific antibodies against the avian influenza virus subtypes H5 and H7. Schnebel *et al*. (2005) examined 543 blood serum samples of wild birds of the family Passeridae for the presence of specific antibodies against the avian influenza virus type A and subtypes H5 and H7. The mentioned authors did not find specific antibodies against the avian influenza virus subtypes H5 and H7 in a single blood serum sample, which is in accord with our results. Arenas *et al*. (1990) used the ELISA and hemagglutination inhibition test and established specific antibodies in 40% blood serum samples of birds of the family Anatidae, while we examined blood serum samples of wild birds of the family Anatidae and established specific antibodies in 30% of the examined samples also using the ELISA test. In samples from the house sparrow (Passer domesticus) the authors established specific antibodies against the avian influenza virus in 31% of the examined blood serum samples. In our investigations, specific antibodies against the avian influenza virus were not established in blood serum samples from house sparrows with the used tests (ELISA, AGP, HI). Obon *et al*. (2007) performed serological examinations of 443 blood serum samples originating from 38 species of wild birds in the United Arab Emirates, and we identified 46 species in 259 wild birds. Serum samples from the birds were examined using the hemagglutination inhibition test for subtype H5N1 according to the OIE diagnostics handbook, which we also used. On that occasion we established specific antibodies against the avian influenza virus subtype H5 in 4 (1.54%) blood serum samples. The above authors established, in a total of 58 (13%) blood serum samples, specific antibodies against subtype H5N2 of which the titer of specific antibodies ranged from 1:8 to 1:128, with the highest titer of specific antibodies being established in the blood serum of a hawk. In the results that we obtained, the titer of specific antibodies against the avian influenza virus subtype H5 had a slightly smaller

range, from 1:16 to 1:32 in the mute swan, and 1:64 in the common pochard. The same authors also analyzed results obtained according to the bird orders, and they presented these results as follows: Anseriformes 15 of 76 or 20%, Charadriformes 1 of 34 or 3%, Ciconiiformes 18 of 60 or 30%, Columbiformes 1 of 4 or 25%, Falconiformes 10 of 130 or 7.7%, Galiiformes 10 of 76 or 13.5% and Gruiformes 3 of 63 or 4.7%. Our results indicated only blood serum samples with specific antibodies against the avian influenza virus subtype H5, only in birds of the order Anseriformes: in 4 of 30 (13.33%), while blood serum samples of birds from the following 8 orders were negative: Passeriformes, Gruiformes, Strigiformes, Columbifomes, Galliformes, Ciconifromes, Falconifromes and Piciformes.

Pittman *et al*. (2007) stated that specific antibodies against the avian influenza virus subtype H5 were discovered in 62.8% swans and in 16.3% ducks. In our investigations, the percentage of positive blood serum samples with specific antibodies against the avian influenza virus subtype H5 was also most represented in swans and amounted to 75% of the total number of positive seruum samples. Newman *et al*. (2007) carried out epidemiological investigations of infections caused by the avian influenza virus subtype H5N1 in wild birds in the period June-July 2007 in the Czech Republic, Germany and France and discovered them most frequently in the mute swan, as we did in our investigations. Khawaja *et al*. (2005) used the hemagglutination inhibition test to examine the presence of specific antibodies against the avian influenza virus subtype H7N3 in wild birds, including ducks and turtle-doves, which were also covered by our investigations. The serum samples originating from turtle-doves were negative in both investigations, but the mentioned authors did not establish specific antibodies in duck serum samples while we detected specific antibodies against the avian influenza virus subtype H7 in 2 serum samples from mallards in a total of 12 (16.66%). Aleksejuniene Ilona *et al*. (2006) used the ELISA test to examine the presence of specific antibodies against the avian influenza virus in a population of wild birds (mallards, geese, white-fronted goose, pigeons and sparrows). Our investigations using the same test covered blood serum samples of mallards, pigeons and sparrows, and the results coincide when pigeons and sparrows are concerned, but contrary to the mentioned authors, we detected specific antibodies against the avian influenza virus in the serum of mallards.

It can be seen in the report from the 7th meeting of national laboratories for avian influenza of EU member-states that a monitoring programme for avian influenza was realized in Spain in wild birds using the ELISA method and that no specific antibodies against this virus were found in any of the examined serum samples. The part of the report that deals with a monitoring programme for wild birds in Italy, covering 15 examined species of wild birds many of which originated from water habitats, antibodies against the avian influenza virus subtype H7N1 were detected only in pigeons and sparrows this being the virus which caused an epidemic among domestic fowl in the course of that year (Anonymous, 2001; 2006). Using the same test, we established specific antibodies in ducks, but, in pigeons and sparrows, we did not establish specific antibodies against the avian influenza virus type A or against subtypes H5 or H7. David *et al*. (2006)

underscored that, in Norway in 2005 and 2006, four bird species were the main reservoirs of the avian influenza virus type A and subtype H5: mallard, Eurasian wigeon, gulls and garganey. According to the results of our investigations, these are the mute swan and the common pochard. Račnik *et al*. (2006) examined 90 blood serum samples of wild birds (blue tit, robin, blackcap, sparrow, great tit, blackbird, sedge warbler, savi's warbler, willow warbler) which we also examined for the presence of specific antibodies against the avian influenza virus type A and against subtypes H5 and H7 using the ELISA test and the hemagglutination inhibition test. The authors did not detect specific antibodies against the avian influenza virus type A or against any subtype in the serum of the listed birds. Wallensten *et al*. (2006) carried out continuous examinations of blood serum samples of birds from water habitats (mostly mallards - Anas platyrhynchos) in southern Sweden and they established specific antibodies against the avian influenza virus on the average in 4%, but at most in 9.5% of the examined birds. In our investigations, specific antibodies against the avian influenza virus were found in mallards in 16.66% samples. Such a finding supports the opinion that mallards, both in Serbia and in Sweden, are a constant source and reservoir of different subtypes of the avian influenza virus. Chen *et al*. (2005) monitored migratory birds of water habitats in China and they serologically examined 493 blood serum samples from 15 wild bird species. The authors detected a low level of specific antibodies against the avian influenza virus subtypes H2, H9 and H10 in gulls, little egrets, black-crowned night herons, bar-tailed godwits, whimbrels, and common greenshanks. Contrary to the results obtained using the hemagglutination inhibition test, and which clearly pointed at the presence of specific antibodies against the avian influenza virus subtypes H5 and H7 in 3 bird species (mute swan, mallard and common pochard), we detected the presence of specific antibodies against the avian influenza virus type A using the ELISA test in 2 more blood serum samples – from mallards, which means that specific antibodies against the avian influenza virus type A can belong to some other subtype. Saldan *et al*. (2006) discovered specific antiobodies against the avian influenza virus subtype H5N1 in 24% of the examined blood serum samples of wild birds, while we established specific antibodies against the avian influenza virus type A using the ELISA test in 3.54% of the totally examined blood serum samples. Coven *et al*. (2005) investigated the presence of specific antibodies against the avian influenza virus in wild birds in a zoo (caged birds, ducks, wild pigeons, quail, parrots, canaries, sparrows, starlings, crows) using the hemagglutination inhibition test and agar gel precipitation (AGP). All the blood serum samples reacted negative to the presence of specific antibodies, which coincides with our results obtained using the same methods regarding samples taken from sparrows, starlings and pigeons. Lebarbenchon *et al*. (2007) examined 72 species of wild birds belonging to 10 orders. The authors concluded that the absence of infection with the avian influenza virus in birds of the order Passeriformes supports all the findings so far and the suppositions that the prevalence of this virus in land bird species is small. These findings are close to the results of our investigations, because birds of the order Passeriformes accounted for almost 75% of all our examined samples of wild birds and specific antibodies against the avian influenza virus type A and

subtype H5 were not detected in any blood serum samples. Astorga *et al*. (1994) examined the sera of wild birds from water habitats and detected specific antibodies against the avian influenza virus type A in 44 samples (6.2%) using the ELISA test. We established the presence of specific antibodies against the avian influenza virus in 9 (3.47%) serum samples. Stanislawek *et al*. (2001) carried out comprehensive serological examinations of the health status of mallards (Anas platyrhynchos) and they detected specific antibodies against the avian influenza virus in 32.5% serum samples using the ELISA method, which is similar to the results of our examinations for the same bird species, 33.33%. Hua *et al*. (2005) examined 230 blood serum samples of mallards to detect specific antibodies against the avian influenza virus subtype H5 using the hemagglutination inhibition test and detected antibodies in 2 blood serum samples, while our examinations using the same method established the presence of specific antibodies against only avian influenza virus subtype H7 in 2 serum samples. Brown *et al*. (2006) established, using serological investigations, that the hemagglutination inhibition and agar gel precipitation tests can detect specific antibodies also in ducks and swans, but that the HI test is much more sensitive than the AGP test, which is in keeping with the results of our investigations. We point our that the AGP test in our investigations of blood serum of wild birds did not detect specific antibodies to the avian influenza virus, even though they were detected for subtypes H5 and H7 using the ELISA and HI tests. Loza Rubio E *et al*. (1997) compared the agar gel precipitation test and the hemagglutination inhibition test, which is the reference test for diagnosis of avian influenza. The authors claim that the sensitivity of the AGP test is low in comparison with the HI test, and the same detected the presence of specific antibodies against the avian influenza virus only in blood serum which had an antibody titer of at least 1:320 in examinations using the HI test. Such conclusions explain why all the blood serum samples of wild birds in our examinations were negative to the presence of specific antibodies against the avian influenza virus, since the examined samples had a titer of specific antibodies ranging from 1:16 to 1:64 using the HI test.

Jin *et al*. (2004) compared results obtained using the hemagglutination inhibition test and the commercial ELISA test for avian influenza and established that the ELISA test was more sensitive, with results being identical in 82% cases. Our results for the examinations of specific antibodies against the avian influenza virus subtype H5 in serum samples obtained using the ELISA test and the HI test are fully in accord. Starick *et al*. (2006) examined the validity of the ELISA test in the detection of specific antibodies against the avian influenza virus in blood serum samples of ducks, geese and other species of wild birds. On that occasion, the authors established a high sensitivity and specificity of this test, which is in agreement with our investigations. De Boer *et al*. (1992) established that the ELISA test enables the making of a swift serological diagnosis and that it is suitable for the control and monitoring of the presence of specific antibodies against avian influenza, in particular in different animal species that can carry different subtypes of the avian influenza A type virus. Our experience with the implementation of the ELISA test also coincides with the experience of the mentioned authors. Brown *et al.* (2006) compared the sensitivity of the agar gel

precipitation test and the hemagglutination inhibition test for the avian influenza virus subtype H5 in examinations of the blood serum of artificially infected mallards and mute swans with the avian influenza virus subtype H5N1 and established that the HI test is far more sensitive for the avian influenza virus subtype H5 than the agar gel precipitation test, which is in agreement with our results, as well. Starick *et al.*, (2006) pointed out that the ELISA test is very practical and suitable for implementation in programmes of monitoring during the detection of specific antibodies against different subtypes of the avian influenza virus in different animal species, and that the subtyping can be carried out using the HI test. The presence of specific antibodies against the avian influenza virus type A in a total of 9 blood serum samples of wild birds was established using the cELISA test and against the avian influenza virus subtype H5 in 4 blood serum samples, and against the avian influenza virus subtype H7 in 3, or a total of 7 serum samples of wild birds. Two blood serum samples in which specific antibodies were detected using the cELISA test against the avian influenza virus type A probably belonged to some other subtype of the avian influenza virus and not to subtypes H5 and H7. In these examinations, the implementation of the cELISA test detected the presence of specific antibodies in more than 30.66% blood serum samples of wild birds than using the HI test. In comparison with the ELISA test used for the determination of specific antibodies against the avian influenza virus type A of which the sensitivity was 100%, the sensitivity of the hemagglutination inhibition test was 69.33%, which is in keeping with the results of similar investigations. However, the sensitivity of the cELISA test for the avian influenza virus subtype H5 and the hemagglutination inhibition test for this same subtype amounted to 100%.

CONCLUSION

On the grounds of the results obtained in examinations of blood serum samples of 259 wild birds within which 46 species were identified, the following conclusions have been drawn: in blood serum samples of wild birds, specific antibodies against the avian influenza virus type A and against the avian influenza virus subtypes H5 and H7 were established only in bird species of the order Anseriformes and the family Anatidae using the HI and ELISA tests, while no specific antibodies were established using the AGP test. During the determination of the presence of specific antibodies against the avian influenza virus type A using the ELISA test and the hemagglutination inhibition test, a greater sensitivity of the ELISA test was established. However, in the determination of the presence of specific antibodies against the avian influenza virus subtypes H5 and H7, the methods of hemagglutination inhibition and both cELISA tests showed approximately the same sensitivity.

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ISPITIVANJE PRISUSTVA SPECIFIČNIH ANTITELA PROTIV VIRUSA AVIJARNE INFLUENCE KOD NEKIH VRSTA DIVLJIH PTICA

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SADRŽAJ

Infekcije izazvane virusom avijarne influence, su već odavno poznate i prisutne u manjem ili većem obimu, kako u ekstenzivnoj, tako i u intenzivnoj živinarskoj proizvodnji, u mnogim delovima sveta. Epidemiološkim ispitivanjima je utvrđen nesumnjiv značaj populacije divljih ptica u očuvanju i širenju ove infekcije. Avijarna influenca je zoonoza, a virus ima veliki potencijal da izazove visoku smrtnost kod ljudi, posebno njegovi podtipovi H5 i H7, tako da u novije vreme izaziva veliku pažnju, kako naučne i stručne, tako i najšire javnosti.

Cilj ovog rada je bio da se na nekoliko lokacija u Republici Srbiji uhvati određeni broj divljih ptica, izvrši njihova identifikacija i prikupe uzorci krvnog seruma radi otkrivanja specifičnih antitela protiv virusa avijarne influence. Hvatanje ptica vršeno je na deset lokacija na bezbedan način, kako za same ptice, tako i za osoblje koje ih je hvatalo. Hvatanje ptica obavljano je posebnim za te svrhe proizvedenim mrežama, a u nekim slučajevima i posebnim zamkama (klopkama). Za identifikaciju uhvaćenih divljih ptica korišćene su metode koje su opisane u stručnoj literaturi. Svi nazivi divljih ptica usklađeni su sa važećom srpskom nomenklaturom ptica Evrope. Nakon hvatanja i identifikacije, pticama je uzimana krv iz krilne vene (kod većih ptica) ili iz nožne vene (kod malih ptica). Prilikom uzimanja krvi od divljih ptica poštovani su svi principi asepse i antisepse, kako bi se sprečila svaka mogućnost infekcije. Nakon toga, ptice su vraćane u prirodu, na iste lokacije na kojima su i uhvaćene. Od uzetih uzoraka krvi izdvojeni su serumi koji su ostavljani na - 20^{o}C i čuvani do konačnog ispitivanja. Identifikovano je 46 vrsta divljih ptica sa ukupno 259 jedinki od kojih je izdvojeno 259 uzoraka krvnog seruma. Za otkrivanje prisustva specifičnih antitela protiv virusa avijarne influence u uzorcima krvnog seruma divljih ptica korišćeni su agar gel precipitacija (AGP), test inhibicije hemaglutinacije (IH) za podtipove H5 i H7, cELISA test sa antigenom A tipa virusa avijarne influence i cELISA sa antigenom podtipa H5 virusa avijarne

influence. S obzirom na činjenicu da na teritoriji Republike Srbije živi oko 360 različitih vrsta divljih ptica, broj od 46 identifikovanih vrsta obuhvaćenih ispitivanjem, činio je 12,77% od ukupnog broja prisutnih vrsta ptica u Srbiji, što se smatra dobrim uzorkom. Specifična antitela protiv A tipa virusa avijarne influence ustanovljena su u uzorcima seruma samo 9 od 259 jedinki koje su bile obuhvaćene ispitivanjem primenom cELISA testa. U identifikovanih 46 vrsta divljih ptica 6 je pripadalo pticama koje žive isključivo u vodenim staništima i smatraju se rezervoarom virusa avijarne influence (bela roda, patka gluvara, labud grbac, riđoglava patka, patka dupljašica i liska). Od navedenih vrsta posebnu pažnju privukle su 4 vrste divljih ptica iz reda *Anseriformes* i familije *Anatidae* (patka gluvara, labud grbac, riđoglava patka, patka dupljašica) kojima je od ukupno 259 ptica pripadalo 30 jedinki. U 30 uzoraka krvnog seruma navedenih vrsta ptica, specifična antitela protiv A tipa virusa avijarne influence utvrđena su u 9 (30%) uzoraka seruma, primenom cELISA. Specifična antitela protiv podtipa H5 virusa avijarne influence su ustanovljena u 3 uzorka seruma labudova grbaca (jedan uzorak seruma je poticao od labuda grbca koji je prstenovan u Poljskoj) i u jednom uzorku krvnog seruma riđoglave patke, ili ukupno u 4 (13,33%) uzorka seruma, primenom testa inhibicije hemaglutinacije. Specifična antitela protiv podtipa H7 virusa avijarne influence utvrđena su u 3 (10%) uzorka krvnog seruma i to u dva seruma pataka gluvara i u jednom serumu labuda grbca, primenom inhibicije hemaglutinacije. Specifična antitela protiv A tipa virusa avijarne influence nisu ustanovljena ni kod jedne ispitivane vrste ptice, primenom AGP testa. U populaciji divljih vrta ptica u Republici Srbiji obuhvaćenih ovim ispitivanjem, specifična antitela protiv virusa avijarne influence ustanovljena su samo u uzorcima seruma ptica iz familije *Anatidae*. Specifična antitela protiv A tipa virusa avijarne influence su otkrivena kod 3 (6,52%) vrste divljih ptica, a protiv podtipova H5 i H7 kod 2 (4,34%) od ukupno 46 vrsta koje su ispitivane. Senzitivnost cELISA testa za podtip H5 virusa avijarne influence i testa inhibicije hemaglutinacije za isti podtip iznosila je 100%.