

STATUS OF WILD BIRDS IN BULGARIAN ZOOS WITH REGARD TO ORTHO AND PARAMYXOVIRUS TYPE 1 INFECTIONS

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INTRODUCTION

Life in captivity, especially in zoos, eliminates the behavioral mechanisms of protection the wild birds have developed in the course of evolution. This creates prerequisites for accumulation of infectious pressure. In addition, the living conditions of these birds contribute to the potential emergence of infections, including ND and AI. Such factors are for example the high population density in small areas; flow of imported birds into the establishment; common feeders and watering devices; accumulation of excreta on the floor of premises or cages; free flow of air between cages; common personnel for several or all birds, using the same tools; multiple visitors, that could have been in contact with the pathogens; access of free-living wild birds to the facilities that are not well restricted; stress (4).

Reports about previous occurrences of NDV and AIV in Bulgarian zoos were not available. Bulgaria is on two major migration routes – Via Pontica and Via Aristotelis. This predetermines the risk of introduction of permanently circulating NDV and AIV to the synanthropic wild birds in Bulgaria and particularly among birds kept in captivity, because of the environment they inhabit.

All these factors, together with the persistence of ND infection among domestic birds during the recent years, the reports for isolation of NDV and Influenza virus A strains from wild birds in Bulgaria, directed us to obtain more information on this topic.

The purpose of the study was to use serological and molecular methods to investigate the status of wild birds inhabiting Bulgarian zoos with regard to APMV-1 and AIV infections.

MATERIAL AND METHODS

In August 2008, we obtained 88 blood samples from 38 wild bird species inhabiting four Bulgarian zoos (in Sofia, Varna, Stara Zagora and Lovech). From the same zoos, 127 cloacal swabs and faeces from 57 bird species were collected in tubes with viral transport medium (Table 1). Samples were transported to the laboratory at 0-4 °C within 4 hours.

Processed sera were tested in a hemagglutination inhibition test (HI). Known positive antigens were used at a strength of 4 hemagglutination units (4 HAU) and the standard OIE procedure was followed. For the detection of antibodies against NDV, APMV-1 Ulster 2C strain was used. H₁N₁(A/ck/lt/22A/98) and H₁N₁(A/ty/lt/9289/v02) antigens were used in tests for detection of AIV antibodies. The samples positive for APMV-1 were tested once again with APMV-3 antigen (Neophema/Holland/449/75). The employed commercial antigens were produced by Istituto Zooprofilattico Sperimentale delle Venezie, Italy.

The suspensions from treated swabs and faeces were tested using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). The extractions were performed with QIAamp[®] Viral RNA Mini Kit, and one-tube reactions were used for reverse transcription and amplification (Qiagen[®] One-Step RT-PCR Kit). The primers and probes used for detecting NDV and AIV nucleic acid were described by Wise et al. (9), and Spackman et al. (7) respectively.



Acyppius monachus

RESULTS

Out of 88 sera tested, 13 were positive for antibodies against APMV-1. The observed titers varied between 1/16 and 1/512 (from 2¹ to 2⁹ or from log₄ to log₉ when expressed as the reciprocal). Samples with antibody levels equal to or lower than 1/8 (2⁰ or log₃) were considered as negative or the result of a non-specific reaction. The 13 samples with positive results against APMV-1 were tested again in HI against APMV-3 antigen. There were no antibodies against APMV-3 in any of these samples. All tests for presence of antibodies against H₁ and H₂ were negative to both antigens. The performed rRT-PCR did not detect any NDV and AIV nucleic acids.

Ten of the positive sera were from representatives of the family Phasianidae, order Galliformes (76.9%), 2 of the family Numididae, order Galliformes (15.4%) and one of the family Columbidae, order Columbiformes (7.7%) (Table 1). Seven of these birds in whose sera antibodies were detected (5 from the family Phasianidae and 2 from the family Numididae), were vaccinated with a live vaccine 6 months prior to blood sampling. For the other birds there was no vaccination history (5 representatives of Phasianidae and 1 of Columbidae families). The latter are from one zoo, where the birds have not been vaccinated.

DISCUSSION

In this work, we investigated the presence of NDV and AIV and their antibodies in healthy zoo birds in four Bulgarian zoos.

Stanislavlev et al. (8) have performed a similar survey (not only caged birds) in New Zealand. Their results are indicative about a possible occurrence of APMV-1 among free-living and captive wild birds in the country. At the same time, Garnett and Flanagan (2) did not detect any antibodies against APMVs in the 1235 samples studied in Queensland, Australia. Results with a prevalence of 17.1%, with regard to APMV-1 were reported by Höfle U. et al. (3) in a study of captive and free-living wild birds in Spain. In a study with wild birds in the Andalusian region of Spain, antibodies to all APMV subtypes were detected, as well as a seasonal pattern in the incidence of antibodies and titers (5). In 1998, Pfitzer et al. (6) succeeded in isolating AIV (H10N9), but no NDV was detected in a survey of consisting of 262 birds in South Africa. The authors reported that only in one sample there was antibodies against H6 AIV but in 34 out of 46 sera antibodies against NDV were present.

In the present study, the fact that no antibodies against AIV have been detected in all studied samples is indicative for the absence of AIV infection in the surveyed zoos. However, some avian species do not form detectable antibodies against AIV and remain seronegative (10) even when infected and this should not be underestimated.

The results of the present study led to the assumption that in one Bulgarian zoo a previous contact with APMV-1 had taken place. The hypothesis is supported by the fact that none of positive samples contained antibodies against APMV-3, that typically exhibit high cross reactivity in the test of samples against APMV-1(1). However, the small number of samples tested, the low prevalence of 6.81% and the failure of rRT-PCR to detect any nucleic acids, raise an uncertainty. Therefore, a more detailed and prolonged investigation of the issue and the inclusion of a larger number of birds in various periods of the year is necessary.

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Buteo buteo

Table 1. Serum samples and cloacal swabs and faeces collected from wild birds in four Bulgarian zoos in August 2008. Results from rRT-PCR and HI tests performed. All cloacal swabs and faeces samples were negative to NDV and AIV on rRT-PCR.

| Scientific name of bird | Common name of bird | No. of sera tested | No. of sera reacted in HI with antigen at serum dilution | | | | No. of cloacal swabs and faeces tested |
|---------------------------------|--------------------------------|--------------------|-------------------------------------------------------------------------------------------|-------------------|----------------|----------------|----------------------------------------|
| | | | 1/16 to 1/512 (2 ¹ to 2 ⁹ or log ₄ to log ₉) | APMV-3 | H ₁ | H ₂ | |
| Numida meleagris | Helmeted Guineafowl | 7 | 2* | - | - | - | 7 |
| Melospiza gallopavo | Wild Turkey | 1 | - | - | - | - | 1 |
| Gallus domesticus (Bantam) | Silkie Bantam | 2 | 2* | - | - | - | 2 |
| Pavo cristatus | Indian Blue Peafowl | 1 | - | - | - | - | 1 |
| Pavo cristatus var. nigripennis | Black-Winged Peacock | 1 | - | - | - | - | 1 |
| Crossoptilon murchisonium | Brown-eared Pheasant | 2 | - | - | - | - | 2 |
| Phasianus colch. mongolicus | Mongolian Ring-necked Pheasant | 1 | - | - | - | - | 1 |
| Lophura erythromera | Silver Pheasant | 3 | 1* + 2 | - | - | - | 3 |
| Lophura swinhoii | Swinhoe's Pheasant | 1 | - | - | - | - | 1 |
| Phasianus colchicus | Common Pheasant | 2 | 1* | - | - | - | 2 |
| Syrnium reevesii | Reeves's Pheasant | 1 | - | - | - | - | 1 |
| Phasianus colchicus torquatus | Coloured Pheasant | 2 | - | - | - | - | 2 |
| Phasianus versicolor | Green Pheasant | 3 | - | - | - | - | 3 |
| Lophura leucomenelos | Kali Pheasant | 2 | - | - | - | - | 2 |
| Alcedo gaeae | Black Partridge | 1 | - | - | - | - | 1 |
| Coturnix coturnix | Common Quail | 2 | - | - | - | - | 2 |
| Cairina moschata | Muscovy Duck | 7 | - | - | - | - | 7 |
| Anas platyrhynchos | Mallard | 5 | - | - | - | - | 5 |
| Anas domestica | Duck | 2 | - | - | - | - | 2 |
| Anser Plat dom arrov | Emerald Headed duck | 3 | - | - | - | - | 3 |
| Anser sparsus | Wood Duck | 4 | - | - | - | - | 4 |
| Tadorna ferruginea | Ruddy Shelduck | 1 | - | - | - | - | 1 |
| Tadorna tadorna | Common Shelduck | 1 | - | - | - | - | 1 |
| Anas indicus | Indian Duck | 1 | - | - | - | - | 1 |
| Aix galericulata | Mandarin Duck | 1 | - | - | - | - | 1 |
| Anser fabalis | Bean Goose | 1 | - | - | - | - | 1 |
| Anser albifrons | White-fronted Goose | 2 | - | - | - | - | 2 |
| Anser Anser F. domesticus | Goose | 2 | - | - | - | - | 2 |
| Anser cygnoides domesticus | Swan Goose | 2 | - | - | - | - | 2 |
| Pelecanus onocrotalus | Royal Pelican | 3 | - | - | - | - | 3 |
| Cygnus olor | Mute swan | 1 | - | - | - | - | 1 |
| Cygnus atratus | Black Swan | 1 | - | - | - | - | 1 |
| Cygnus cygnus | Whooper swan | 1 | - | - | - | - | 1 |
| Ciconia ciconia | White Stork | 5 | - | - | - | - | 5 |
| Columbidae | Pigeon | 5 | 1 | - | - | - | 5 |
| Agryppus monachus | Black Vulture | 1 | - | - | - | - | 1 |
| Bubo Bubo | Eagle Owl | 1 | - | - | - | - | 1 |
| Buteo buteo | Common Buzzard | 2 | - | - | - | - | 2 |
| Buteo buteo vulpinus | Steppe Buzzard | n/t | n/t | n/t | n/t | n/t | 2 |
| Falco peregrinus | Peregrine Falcon | n/t | n/t | n/t | n/t | n/t | 1 |
| Agelaius chrysaetos | Golden Eagle | n/t | n/t | n/t | n/t | n/t | 1 |
| Accipiter gentilis | Golden Hawk | n/t | n/t | n/t | n/t | n/t | 2 |
| Asio otus | Long-eared Owl | n/t | n/t | n/t | n/t | n/t | 2 |
| Athene noctua | Little Owl | n/t | n/t | n/t | n/t | n/t | 1 |
| Tyto alba | Barn Owl | n/t | n/t | n/t | n/t | n/t | 2 |
| Strix aluco | Tawny Owl | n/t | n/t | n/t | n/t | n/t | 2 |
| Strix uralensis | Ural Owl | n/t | n/t | n/t | n/t | n/t | 1 |
| Chrysolophus amherstiae | Lady Amherst's Pheasant | n/t | n/t | n/t | n/t | n/t | 2 |
| Crossoptilon crossoptilon | White Eared Pheasant | n/t | n/t | n/t | n/t | n/t | 2 |
| Crossoptilon murchisonium | Brown Eared Pheasant | n/t | n/t | n/t | n/t | n/t | 2 |
| Lophura edwardsi | Edwards's Pheasant | n/t | n/t | n/t | n/t | n/t | 3 |
| Alcedo sinensis | Egyptian Goose | n/t | n/t | n/t | n/t | n/t | 5 |
| Ciconia nigra | Black Stork | n/t | n/t | n/t | n/t | n/t | 1 |
| Nymphicus hollandicus | Cockatoo | n/t | n/t | n/t | n/t | n/t | 2 |
| Bonasa krameri | Rose-ringed Parakeet | n/t | n/t | n/t | n/t | n/t | 2 |
| Streptopelia turtur | Turtle Dove | n/t | n/t | n/t | n/t | n/t | 2 |
| Cathartes aura | Turkey Vulture | n/t | n/t | n/t | n/t | n/t | 2 |
| Total | | 88 | 7*(7,95%) | 16 (6,81%) | 0 | 0 | 127 |

* = samples are from vaccinated birds

n/t = not tested

- = no antibodies detected at a serum dilution 1/8 (2⁰ or log₃) or higher and no NDV or AIV nucleic acid detected



Tadorna ferruginea