An Epidemiological Investigation of Type A Influenza Infections in Avian Collections at Ohio Zoos

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Abstract

Zoos have a diverse collection of avian species native to multiple continents; such environments might provide the appropriate host for foreign viruses to become established in North America, but there is little information on the occurrence of type A influenza virus infections in avian zoological collections. Furthermore, it is unclear what risk wild birds pose to zoo collection birds. A collaborative epidemiological investigation between Ohio State University and avian health care providers at Ohio zoos was initiated in 2006. It was projected that following infections zoo birds can demonstrate a serologic antibody response to type A influenza antigen; most likely following exposure to influenza A virus contaminated environments originating from free-ranging wild birds visiting the zoo. Serology data collected during 2006 and 2007 at two zoos along with zoo records were used in the investigation. Of 607 sera samples from zoo collection birds, 101 tested positive by agar gel immunodiffusion (AGID) testing for antibodies to type A influenza antigen (16.64%). Further statistical analysis showed trends that favored influenza A virus infections in specific species (Chilean Flamingos and Rosy Billed Pochards) and dates of collection (Spring more than Fall) as well as some suggestion of a positive correlation with the amount of interaction with wild, non-resident birds and the rate of positive AGID tests. These findings have resulted in changes in zoo husbandry protocols and interest in a more in-depth, ongoing collaborative investigation to further define the dynamics and threats of influenza A virus infections in zoo birds.

Introduction

Highly pathogenic H5N1 avian influenza virus (AIV) has been documented to infect many avian species, yet the exact role that wild birds play in transmitting this virus to other avian species is unknown (1,6). In an attempt to create a more natural habitat, many zoos have open air cages where multiple wild and captive avian species interact. As a result, numerous preventive medicine practices controlling infectious diseases are compromised. Zoo birds have close contact with humans as well as migratory and indigenous birds within the zoo enclosures, thus it is important to determine and monitor their AIV infection status (7).

The route by which zoo birds acquire AIV infection is unknown; however, visiting wild birds, environmental contamination, and pre-existing AIV infection prior to importation into the United States are potential sources. Testing zoo birds for antibodies to AIV has not been routine; consequently there is currently inadequate data for assessing the prevalence of AIV infection in zoo birds. Because zoos maintain detailed records of each animal, every bird is identified and its origins, sex, species, housing location and history are tracked. This information, along with AIV serological and virus surveillance results, may provide insight into how and where AIV infections occur as well as what avian species are at greatest risk of infection. In this study, combined data from AGID testing and zoo records were used to identify preliminary insight into AIV infection. It is proposed that some zoo collection birds will have a serologic antibody response to type A influenza virus following initial exposure; by and large from a contaminated environment visited by ducks. Information from this epidemiological investigation was reported to zoos for use in improvement of husbandry protocols, to determine the necessity of surveillance for type A influenza, as well as for possible future collaborative projects.

Materials and Methods

Oroesopharangeal and cloacal swabs and sera samples were obtained from selected zoo birds in two Ohio zoos during 2006 and 2007. The samples were taken bi- yearly when the birds are moved indoors in fall and outdoors in the spring. Using zoo records, frequency distribution by age, sex, species, origin, length of zoo residency, date and location of collection, and summer and winter housing were analyzed using standard statistical methods to determine the prevalence of AGID sera positives in a given population (15). Virus isolation was attempted using samples from oroesopharangeal and cloacal swabs collected from a subset of birds at the time of sera sampling. Zoo 1 also collected environmental samples from both open air habitats (allowing access to wild and indigenous birds) and confined habitats (no access to wild and indigenous birds) that included, but weren't limited to feces, water, and ground material such as soil, leaves, and gravel.

Results

Figure 1. 2006 and 2007 cumulative serotype data performed using AGID

	AGID Sera Positives	Total Samples	Prev-alence
Zoo 1	66	437	15.10%
Zoo 2	35	170	20.59%
TOTALS	101	607	16.64%

Zoo 1

Figure 2. Prevalence of AGID Positive Sera According to Collection Date for Zoo 1

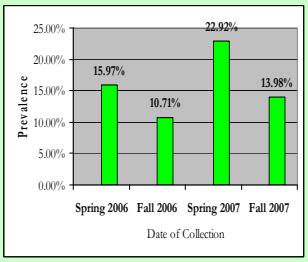
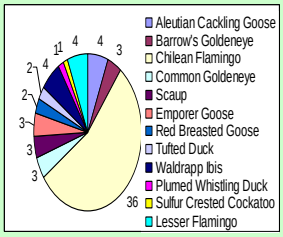


Figure 3. Total AGID Positive Sera According to Species for Zoo 1



Zoo 2

Figure 3. Prevalence of AGID Positive Sera According to Collection Date for Zoo 2

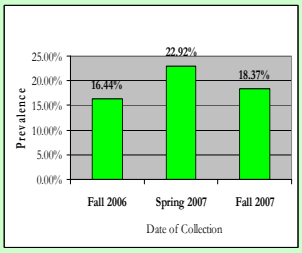
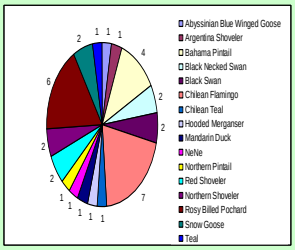


Figure 5. Total AGID Positive Sera According to Species for Zoo 2



Discussion

Through serologic testing, zoo birds were found to have evidence of AIV infections at two different Ohio zoos. Of the 607 sera samples, 16.64% possessed antibodies to a type A influenza virus protein signifying that these birds were previously infected with type A influenza. Zoo 2 birds had a slightly higher prevalence of AGID sera positive tests than zoo 1 birds ((20.59% and 15.10%, respectively) which could be attributed to the difference in the species of birds tested between zoos and/or a result of the difference in exposure to wild and indigenous birds within the zoological habitats.

of 52 environmental samples collected, two samples showed positive virus isolation results (prevalence= 3.85%). These findings prove that some active virus shedding is occurring within the bird population. Because the zoo birds and wild birds are continuously intermingling and the birds aren't constantly observed, the origin of the environmental sample can not be definitively defined. Therefore it is impossible to know if wild/indigenous birds or zoo birds were the source of virus.

A higher prevalence of AGID sera positives was detected in the spring seasons of 2006 and 2007 for birds in both zoos; however, the percent positive of each zoo were not the same. For example, zoo 1 had the same spring prevalence of positive AGID tests as zoo 2 (22.92%) in 2007, but in fall of the same year, zoo 2 had a higher percentage of AGID positives than zoo 1 (18.39% and 13.98% respectively). Because of higher densities within fall housing; there is an increased frequency of contact with birds that are shedding AIV. This may lead to the higher prevalence of AIV in the spring-- just after the birds are moved from indoor fall housing. Another theory is that this variation could be the result of cyclical seasonal shedding of AIV (3, 4).

A correlation between AIV antibody response and species was less defined, most likely as a result of the small sample size used in this study. It is likely that increased antibody prevalence in zoo species residing in or near water could be the result of more contact with wild and indigenous birds within the zoo habitat. The country of origin, genetics, or species predilection may also play a role in the ability of these birds to mount an immune response. Previous studies have shown that certain species of flamingoes possess higher antibody titers after being vaccinated for AIV suggesting that flamingos are able to wage better immunologic responses to AIV than other avian species (2, 8).

The propensity to possess AIV antibodies does not appear to be solely determined by species predilection, but rather by the birds' contact with the environment. Although these findings suggest that environment plays a large role in the transmission of AIV, it can not be assumed that it is the only contributing variable. More controlled studies encompassing careful observation of the interactions between wild and indigenous birds and zoo birds, correlating with positive AGID data, are needed to determine the true source of AIV within zoo birds.

In depth studies are needed to resolve the intricacies of AIV infections in zoo collection birds. In order to further investigate the source of AIV infections in zoo birds, comparative subtype evaluations from seropositive zoo birds and AIV positive free ranging mallards from zoo 1 are currently underway. Environmental samples collected from areas shared by zoo and wild birds in zoo 1 have tested positive for AIV. Subsequently, there could be an increased likelihood that the positive AIV status of zoo birds is the result of exposure to an AIV contaminated environment. However the significance of the birds' country of origin or species can not be discounted. This pilot study will be used for the design and implementation of future studies so that the most useful and functional data is collected and tested. In addition, preliminary results from this study will give the participating zoos a first hand glance of the potential risk factors of AIV exposure and the possibilities of prevention.

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