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Research Note—

Serologic Evidence of Exposure of Raptors to Influenza A Virus

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SUMMARY. Serum or plasma samples from raptors that prey or scavenge upon aquatic birds were tested by a commercially available blocking enzyme-linked immunosorbent assay for the evidence of antibodies to influenza A virus. Samples were taken from birds ($n = 616$) admitted to two rehabilitation centers in the United States. In addition, samples from 472 migrating peregrine falcons (*Falco peregrinus*) trapped on autumnal and vernal migrations for banding purposes were also tested. Only bald eagles were notably seropositive (22/406). One each of peregrine falcon, great horned owl (*Bubo virginianus*), and Cooper's hawk (*Accipiter cooperi*) from a total of 472, 81, and 100, respectively, were also positive. None of the turkey vultures ($n = 21$) or black vultures ($n = 8$) was positive. No clinical signs referable to avian influenza were seen in any bird at the time of capture. These data indicate that, among raptors, bald eagles do have exposure to influenza A viruses.

RESUMEN. *Nota de Investigación*—La evidencia serológica de exposición de aves rapaces al virus de la influenza.

Las muestras de suero o de plasma de aves rapaces o de rapiña que se alimentan de aves acuáticas vivas o muertas y de aves fueron analizadas mediante un ensayo de inmunoabsorción con enzimas ligadas de tipo competitivo para detectar la presencia de anticuerpos contra el virus de la influenza A. Se tomaron muestras de las aves ($n = 616$) que fueron ingresadas a dos centros de rehabilitación en los Estados Unidos. Además, también se analizaron las muestras de 472 halcones peregrinos (*Falco peregrinus*) migratorios que fueron atrapados durante las migraciones de otoño y de primavera con el propósito de identificación mediante la colocación de bandas. Sólo las águilas calvas fueron notablemente seropositivas (22/406). También se encontró un individuo positivo de los halcones peregrinos, de los búhos cornudos (*Bubo virginianus*) y de los gavilanes de Cooper (*Accipiter cooperi*) de un total de 472, 81, y 100 aves, respectivamente. Ninguno de los buitres de cabeza roja ($n = 21$) o de los buitres negros ($n = 8$) fueron positivos. No se observaron signos clínicos atribuibles a la influenza aviar en todas las aves al momento de la captura. Estos datos indican que de entre las aves rapaces, las águilas calvas pueden ser infectadas por virus de la influenza A.

Key words: eagles, b-ELISA, influenza A virus, peregrine falcon, raptors, serology, surveillance

Abbreviations: AGID = agar gel immunodiffusion; AI = avian influenza; AIV = avian influenza virus; b-ELISA = blocking enzyme-linked immunosorbent assay; c-ELISA = competitive ELISA; HI = hemagglutination-inhibition; HPAI = high pathogenicity avian influenza; LPAI = low pathogenicity avian influenza

Aquatic birds, notably dabbling ducks and shorebirds, are regarded as the natural reservoirs for all known avian influenza (AI) viruses (12,14,20). Such birds may have played a key role in the pathogenesis and spread of the high pathogenicity avian influenza (HPAI) H5N1 during the period of 2004–06 (7,24). The aquatic birds are prey to avian predators, mostly raptors, although scavenging species may consume residues of dead waterfowl. Of the extant species of raptors in North America, five species—the bald eagle (*Haliaeetus leucocephalus*), peregrine falcon (*Falco peregrinus*), Cooper's hawk (*Accipiter cooperi*), great horned owl (*Bubo virginianus*), and turkey and black vultures (*Cathartes aura*, *Coragyps atratus*)—are known to scavenge or capture aquatic birds as a significant portion of their diet. The peregrine falcon subspecies known as the tundra falcon (*Falco peregrinus tundrius*) is an obligatory predator of small- and medium-sized birds and follows the migration of shorebirds from their nesting grounds in the Arctic to the wintering grounds in the southern hemisphere. Many species of shorebirds have been observed to be captured by these falcons (23).

Prior to reports of HPAI H5N1 in raptors during the epidemic of 2006–07 (13,21), the raptors were not included among the species of concern with regard to influenza virus (20). Because of their predator-prey association, we hypothesized that certain species of raptors would have exposure to avian influenza virus (AIV). Consequently, they may develop antibodies to AIV and thereby serve as integrators over time and space for the presence of various AI viruses in the environment.

Standard testing methods for the detection of AIV antibody have included agar gel immunodiffusion (AGID) and hemagglutination-inhibition (HI) tests optimized for use in gallinaceous birds (19). The screening of diverse species for the presence of anti-influenza A antibodies was markedly enhanced by the development of a blocking enzyme-linked immunosorbent assay (b-ELISA) (17) that became commercially available in 2008 (FlockChek* MultiS-Screen Ab Test, IDEXX, Westbrook, ME). After validation with sera from experimentally infected avian species (2,6), this test was subsequently used for detecting antibodies against influenza A virus in a variety of birds (1).

Our objective in this study was to serologically screen large numbers of raptors admitted to wildlife rehabilitation centers in Minnesota and Virginia. In addition, tundra peregrine trapped for banding purposes on autumnal and vernal migration, on Assateague Island and Padre Island, respectively, were also evaluated (11,22). A second objective was to further assess the utility of the commercially available b-ELISA for detecting anti-influenza A antibodies in raptors.

MATERIALS AND METHODS

Source of serum samples. Serum samples were obtained from raptor species admitted as casualty birds to wildlife rehabilitation centers in

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Table 1. Prevalence of influenza A antibodies among various species of raptors.

Species	No. tested	No. positive	Percent positive
Bald eagles	406	22	5.1
Peregrine falcons	472	1	0.2
Great horned owls	81	1	1.2
Cooper's hawks	100	1	1.0
Turkey vultures	21	0	0.0
Black vultures	8	0	0.0
Total	1088	25	3.5

Minnesota and Virginia although, in most cases, the recovery location of the birds was only generally known. A total of 547 raptors were sampled at The Raptor Center, St. Paul, MN and 69 were sampled at the Wildlife Center of Virginia, Weyers Cave, VA during the period of 2006–10 (Table 1). In all cases, blood samples were collected immediately as each casualty bird was admitted to the facility, thereby eliminating the possibility of detection of antibodies resulting from exposure to AIV while in captivity. Additionally, plasma samples ($n = 472$) from migrating tundra peregrine falcons were obtained from Earthspan, Inc. (Baltimore, MD), whose biologists annually trap these birds on Assateague Island (off the coast of Maryland) in the fall and on Padre Island (off the Texas coast) in the spring. Samples from tundra falcons were collected from the basilic or jugular vein, using heparinized syringes, at the time of capture and banding. The samples were centrifuged and plasma was drawn off and stored at −20 C until accessioned to the laboratory.

Blocking ELISA. A blocking ELISA (FlockChek MultiS-Screen Ab Test, IDEXX Lab) was used in accordance with the manufacturer's instructions. All collections were done under the auspices of an approved institutional animal care and use protocol by the Minnesota Center of Excellence for Influenza Research and Surveillance and via permits from the United States Fish and Wildlife Service. Positive sera were sent to the National Veterinary Services Laboratory in Ames, IA for sub-typing by HI testing.

RESULTS AND DISCUSSION

There are several recent reports on the use of b-ELISA or competitive ELISA (c-ELISA) in a variety of avian species to detect influenza A antibodies. These reports have involved controlled infection studies (2,6,15), response to vaccination (5,18), and serologic screening of large groups of wild birds (1) including birds admitted to rehabilitation centers (15). Congruence between concurrent AGID studies and known epidemiology of AI with the b-ELISA results has been taken as *de facto* validation (1). In all situations, the b-ELISA was reported to be reliable and of greater sensitivity than were AGID or HI and, therefore, an enhancement to the diagnostic tools available for wild bird surveillance. Thus, the results obtained in the present study are in keeping with findings of others as to the utility of this test in raptors.

A total of 1088 samples from raptors were tested in this study and 25 were found positive for influenza A antibody. Antibodies were found in bald eagles (22/406), peregrine falcons (1/472), great horned owls (1/81), and Cooper's hawks (1/100). Samples from turkey vultures ($n = 21$) and black vultures ($n = 8$) were antibody negative (Table 1). The greatest consistency in finding influenza antibodies occurred in bald eagles, of which 5.15% were positive (Table 1). All eagles tested came from eastern and northern Minnesota, western Wisconsin, and eastern Iowa. The greatest number of positives (20/262) occurred among adult birds (distinguished by their completely white heads) compared to 2/144 of other age classes. Attempts to serologically sub-type the antibodies in b-ELISA–positive samples were not fruitful owing to low HI titers of sera (<1:8).

Table 2. Prevalence of influenza A antibody in bald eagles by month.

Month	No. tested	No. positive	Percent positive
1	31	1	3.2
2	11	1	9.1
3	42	2	4.8
4	33	1	3.0
5	25	3	12.0
6	33	2	6.1
7	34	1	2.9
8	29	0	0.0
9	38	0	0.0
10	54	5	9.3
11	36	3	8.3
12	40	3	7.5
Total	406	22	

In a previous study on free-ranging nestling white-tailed sea eagles (*Haliaeetus albicilla*) and nestling peregrine falcons (9), no antibody to AI was detected using a c-ELISA protocol. This is not surprising, given the very young age and general lack of exposure opportunities for eagles in that subset. The present study, on the other hand, involved samples taken from fledged and, in most instances, fully adult free-ranging birds. Among eagles, the distribution of antibody-positive samples was strongly biased toward the adult birds. This differential rate of seropositivity may simply reflect a longer period of time for exposure to occur. As the duration of influenza A antibodies in eagles is unknown, it is not possible to further explain this difference.

Sixty-one per cent of all eagles admitted were traumatically injured (vehicle collision, projectile, trap, power line collision, and unidentified trauma), with the largest category being unidentified trauma, of which 3.8% were seropositive. Toxicity in the form of lead poisoning constituted the largest discreetly identifiable cause of admission (23%) of which six (6.5%) were influenza A positive.

Eagles were admitted in all months of the year (Table 2) and, except for February ($n = 11$), the number averaged 35 birds (range 27–42) per month. The greatest percentage of positive samples occurred during October–December (7.5% to 9.3%) and a second peak occurred in May–June (6.1% to 12%; Table 2). Given the small percent of birds positive, no conclusions can be drawn as to the seasonality of infection or to the extent these variations reflect prevalence of type A influenza virus in its host reservoir.

Among other raptors, the peregrine falcon (1/472), great horned owl (1/81), and Cooper's hawk (1/100) showed evidence of exposure to AIV. The peregrine was caught on southward fall migration on Padre Island off the coast of Texas on October 17, 2007. Autumnal migration is a time when influenza in prey species (shorebirds) is relatively low compared to the northward spring migration (20). A total of 67 peregrines were trapped on Padre Island on the vernal migration and none were positive. There was no opportunity to trap east-coast migrating peregrines in the spring. The general lack of seroconversion in vultures, peregrines, Cooper's hawks, and vultures may be a reflection of lack of exposure in the food items they select, presence of innate resistance derived from their aviphagic habits such that they do not seroconvert upon exposure, or an incorrect assumption on our part about the presence of influenza viruses in their prey. This latter point may be particularly pertinent to the great horned owls.

Earlier reports of AIV in raptors (13,21) clearly showed that, upon encountering influenza A viruses, raptors were susceptible to infection, expressed either as morbidity-mortality or seroconversion. Several reports attest to the susceptibility of a variety of raptors to HPAI including Thai crested hawk eagles (*Spizaetus nipalensis*)

smuggled into Belgium (21), peregrine falcon in Hong Kong (4), Saker falcons (*Falcon cherrug*) in the Middle East (16), buzzards (*Buteo buteo*) and eagle owls (*Bubo bubo*) in Europe in 2006 (5), and kestrels (*Falco sparverius*) in experimental settings (10). Little is known on the occurrence of low pathogenicity avian influenza (LPAI) in raptors, the only reported instance being an LPAI H7 virus isolated from an owl at a rehabilitation center in Italy (3). There is a recent report of the isolation of mixed influenza A viruses from a bald eagle (8). This paucity of information may simply be a reflection of a general lack of surveillance among free-living raptors due possibly, in part, to the relative difficulty and expense in conducting virus isolation, especially among species that are not seen as major elements of influenza dynamics. The b-ELISA used in this study provides an economical way to conduct preliminary screening in these birds.

North American bald eagles are frequent scavengers and predators on the waterfowl species that are part of the reservoir system for AI viruses. The serologic information provided in this study demonstrates an approximate 5% prevalence of antibodies in a population of casualty eagles submitted to rehabilitation facilities. Lacking information from concurrent virus isolation and sub-typing in these sampled birds, we cannot say whether this provides evidence of infection, or simply exposure in free-living eagles, or that eagles are playing a role in the dynamics of influenza viruses. However, we do suggest that ongoing serologic surveillance of eagles may provide evidence that contributes information to our knowledge about influenza viruses. The results reported herein would indicate that, of the raptor species examined, evidence of exposure to type A influenza is negligible in all but the bald eagle.

The b-ELISA used in this study appeared to generate meaningful results that compared favorably to the experience of other investigators in other species and suggested that bald eagles have a modest rate of exposure to influenza viruses. This finding also suggests that, should there be a high pathogenicity type A influenza virus in waterfowl populations, bald eagles would likely be affected.

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