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SEROPREVALENCE OF AVIAN POX AND *MYCOPLASMA GALLISEPTICUM* IN RAPTORS IN CENTRAL ILLINOIS

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ABSTRACT.—We assessed prevalence of the bacterium *Mycoplasma gallisepticum* and virus *Avipoxvirus* in seven species of raptors admitted to the Illinois Raptor Center from 1 January 2014 to 1 September 2015. We used visual identification of pathology to diagnose current infections and enzyme-linked immunosorbent assays (ELISA) for avian IgY antibodies against each pathogen to determine infection history of the birds. Seroprevalence of IgY against each pathogen differed significantly among species. Species that commonly prey upon birds had a greater prevalence of antibodies against each pathogen. Our finding of infrequent physical signs of disease, but frequent antibody presence, suggests that although exposure to each of these pathogens is not a rare occurrence, these raptors are capable of mounting an effective adaptive immune response and preventing development of pathology in most cases.

KEY WORDS: *antibodies*; *Avipoxvirus*; *disease*; Mycoplasma; *raptors*.

SEROPREVALENCIA DE VIRUELA AVIAR Y DE *MYCOPLASMA GALLISEPTICUM* EN AVES RAPACES EN EL CENTRO DE ILLINOIS

RESUMEN.—Evaluamos la prevalencia de la bacteria *Mycoplasma gallisepticum* y del virus *Avipoxvirus* en siete especies de aves de presa que ingresaron al Centro de Aves Rapaces de Illinois, desde el 1 de enero de 2014 al 1 de septiembre de 2015. Identificamos de modo visual la patología para diagnosticar infecciones en curso y realizamos ensayos por inmunoabsorción ligados a enzimas (ELISA) para anticuerpos aviares IgY contra cada patógeno para determinar el historial de infección de las aves. La seroprevalencia de IgY contra cada patógeno difirió significativamente entre las especies. Las especies que comúnmente se alimentan de aves tuvieron una mayor prevalencia de anticuerpos contra cada patógeno. El hallazgo de signos físicos infrecuentes de enfermedad, pero de presencia frecuente de anticuerpos sugiere que, aunque la exposición de cada uno de estos patógenos no es un evento raro, estas aves rapaces son capaces de presentar respuestas inmunes adaptativas eficaces y de prevenir el desarrollo de patologías en la mayoría de los casos.
[Traducción del equipo editorial]

Avian diseases are important to study for many reasons. For example, diseases carried by birds have the potential to greatly influence avian population sizes and may also disrupt the ecosystems in which they are found (Friend et al. 2001). Also, some diseases harbored by birds can be transmitted to other animals. Pathogens such as West Nile virus and the influenza virus can spread from wild birds to other wildlife, livestock, and humans (Friend et al. 2001). The study of different diseases found in

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raptors is somewhat limited due to the difficulties associated with capturing raptors in the wild and the scarcity of data collected at raptor rehabilitation centers (but see Schettler et al. 2001, Sansano-Maestre et al. 2009, Millán et al. 2010). Raptors tend to be solitary birds, and little is known about the prevalence of these pathogens in raptor populations (Park 2003), although some thorough surveys have been completed at the local level (e.g., Morishita et al. 1997).

Avian pox is caused by an *Avipoxvirus* and can present itself on a bird in two distinct ways. It can appear on the skin of the bird as discrete lesions that resemble warts and it may progress to respiratory disease. Lesions are most commonly found on the parts of the body that lack feathers, such as the legs and eyelids, and in severe cases, the upper respiratory tract (Van Riper et al. 2002). Avian pox is rarely fatal, commonly only having a mild effect on the host. Birds are more likely to die from the disease if it develops on the mucous membranes or in the upper respiratory tract (Van Riper et al. 2002). Avian poxvirus has been documented as the pathogen responsible for a number of raptor and non-raptor epornitic events. Examples of these outbreaks include an epornitic in captive Eurasian Buzzards (*Buteo buteo*) in Italy (Rampin et al. 2007) and also in free-living birds, specifically Lesser Short-toed Larks (*Calandrella rufescens*) and Berthelot's Pipits (*Anthus berthelotii*) in Spain (Smits et al. 2005). Assessment of the prevalence of avian pox is uncommon among free-living raptors (but see Morishita et al. 1997). One study reported avian pox among two Eastern Screech-Owls (*Megascops asio*) and two Barred Owls (*Strix varia*) from Florida (Deem et al. 1997). Keeping captive birds in outdoor flight cages could facilitate spread of the poxvirus through direct contact in shared cages or through mosquitoes feeding on multiple, stationary birds (Deem et al. 1997). Beyond mosquito transmission, parents can spread avian pox to mates or offspring at the nest (Fitzner et al. 1985). In addition, direct contact in incidental, antagonistic, or agonistic interactions involving a bird with pox lesions could result in transmission. In rare instances, particles of the *Avipoxvirus* can spread through aerosol transmission by being carried along with dust, particularly in confined situations such as aviaries (Van Riper et al. 2002).

Conjunctivitis is a disease in birds that can cause an array of pathological conditions. Birds with a mild case of conjunctivitis display a slight pink discoloration of the conjunctiva of the eye. However, in more severe cases, the bird's conjunctiva can become red and swollen. The bird can also suffer from feather loss around the eye, fluid discharge from the eye, respiratory disease, and potentially blindness (Davis 2010). Conjunctivitis can result from a variety of sources in birds, some of which are infectious, and some of which are not infectious. In addition, conjunctivitis can be acute or chronic. The predominant pathogen responsible for conjunctivitis in a wide range of wild birds is the pathogenic bacterium, *Mycoplasma gallisepticum* (Dhondt et al. 2014). Others have documented non-*Mycoplasma* conjunctivitis in birds, including raptors (Silvanose et al. 2001), but infectious conjunctivitis caused by *M. gallisepticum* has not been reported in raptors.

In a study of free-ranging, healthy wild raptor nestlings and raptors from rehabilitation centers in Germany, *Mycoplasma* spp. DNA was present in all the tracheal swabs from the healthy, free-living raptor nestlings as well as the birds at the rehabilitation center (Lierz et al. 2008). An overall lack of clinical signs of disease among tested birds suggested that *Mycoplasma* spp. in many raptors may not typically be pathogenic, but commensal (Lierz et al. 2008). In contrast, respiratory disease and conjunctivitis have been reported in buzzards, falcons, and vultures in Spain associated with various species of *Mycoplasma*, including *M. anatis, M. columborale, M. gallisepticum, M. gallinaceum,* and *M. gallinarum* (Poveda et al. 1990a, 1990b).

Our objective in this study was to assess the seroprevalence of *Avipoxvirus* and *M. gallisepticum* in raptors upon admission to the Illinois Raptor Center (IRC) in Decatur, IL. Determination of the seroprevalence of immunoglobulins, or antibodies, can reveal infection history on a broad temporal scale. Immunoglobulin Y (IgY) is the most abundant antibody in avian blood and is also the longest-lasting antibody class against a pathogen. Surveying the birds for IgY can increase our knowledge of the proportion of raptors that have encountered these pathogens in the wild.

METHODS

We collected data from free-living raptors upon admission to the Illinois Raptor Center (IRC) for rehabilitation from 1 January 2014 to 1 September 2015. We included birds of all age classes (juvenile, immature, and adult) admitted for any reason (i.e.,

because they were injured, weak and starving, or orphaned). Over this time, 220 raptors were admitted to the center, and we studied the seven most commonly admitted species, for a final sample size of 142 birds. All birds admitted to the IRC were inspected by JTN or JS, using a standardized protocol, within one day of arrival at the center. In order to detect physical signs of *Mycoplasma*, we examined the eyelids for inflammation and the eyes for chemosis and ocular discharge. We also examined the inside of the mouth, and the upper respiratory tract for redness and inflammation. For the detection of physical signs of *Avipoxvirus*, we examined the feet, legs, and bills of birds for pox lesions.

We then collected blood samples into heparinized microhematocrit capillary tubes following puncture of the brachial vein with a 22-gauge needle. We collected approximately 140 µl of blood from each bird. We separated the plasma portion of the blood from the cellular portion of the blood via centrifugation (5 min at 12,000 rpm) less than 10 min after collecting the blood sample. The plasma portion was stored at −20°C in screw-top cryovials until later use in enzyme-linked immunosorbent assay (ELISA).

The first 66 blood samples collected from the birds in this study were used in a polymerase chain reaction (PCR) survey for *Avipoxvirus*. From the PCR analysis, we confirmed the presence of *Avipoxvirus* DNA in the blood of an American Kestrel (*Falco sparverius*) admitted to the IRC with pox lesions. We also confirmed the presence of *Avipoxvirus* DNA in a songbird (Blue Jay [*Cyanocitta cristata*]) with pox lesions captured at Friends Creek Conservation Area near Decatur, IL. We used the plasma samples from each of these birds as positive controls in an ELISA for immunoglobulin Y antibodies (IgY) against *Avipoxvirus*. We also used negative controls (again confirmed via PCR) in the ELISA.

In addition, we used tracheal and ocular swabs collected and stored in *Mycoplasma* broth (Hardy Diagnostics) from the first 66 birds admitted during this study in a PCR survey for *M. gallisepticum*. From the PCR analyses, we identified four raptors admitted to the IRC that were PCR positive for *M. gallisepticum* (Bald Eagle [*Haliaeetus leucocephalus*], Barred Owl, Eastern Screech-Owl, and Red-tailed Hawk [*Buteo jamaicensis*]). We also confirmed the presence of *M. gallisepticum* DNA in a Blue Jay with conjunctivitis captured at Sand Creek Conservation Area near Decatur, IL. Plasma samples from these birds were used as positive controls in an indirect ELISA for IgY against *M. gallisepticum*. All samples were collected under the provisions of Illinois Scientific Permit R-15-135 and R-15-136, as well as U.S.F.W.S. Rehabilitation Permit MB783453-0, and Illinois Permit for Possession of Endangered or Threatened Species 96-1E.

***Avipoxvirus* ELISA.** We used an indirect ELISA to test for the presence of IgY to *Avipoxvirus* (Ha et al. 2013). To complete the assays, we coated a 96-well flat-bottomed microplate with avian pox antigen (purified from the Zoetis Chick-N-Pox TC Fowl Pox Vaccine, Zoetis, Inc., Kalamazoo, MI U.S.A.). The purified antigen was diluted to a final concentration of 0.2 µg/ml in coating buffer (Bethyl Laboratories, Inc., Montgomery, TX U.S.A.). For the secondary antibody, we used HRP-conjugated goat anti-bird IgY from Bethyl Laboratories. We determined the absorbance of each sample in a BioRad iMark microplate reader (BioRad Laboratories, Inc., Hercules, CA U.S.A.) at 450 nm. We divided the optical density (OD) of each sample by the average OD for the negative controls, and all samples with values greater than or equal to that of the positive control (the lowest positive/negative for a positive control = 2.21) were considered positive for the antibodies.

***Mycoplasma gallisepticum* ELISA.** We used a commercially available indirect ELISA to test for the presence of IgY to *M. gallisepticum* (AffiniTech, LTD, Bentonville, AR U.S.A.; Botus et al. 2010). We completed all procedures as described in the manufacturer's ELISA kit insert. All samples with ELISA units >5 were considered positive for antibodies to *M. gallisepticum* (per manufacturer's instructions).

Statistical Analysis. We used a chi-square test of association to determine if seroprevalence of either pathogen differed among the seven species. Results with $P < 0.05$ were considered statistically significant for each analysis.

RESULTS

Of the 142 raptors surveyed (Table 1), no birds showed physical signs of conjunctivitis. Only two raptors (one American Kestrel and one Red-tailed Hawk) showed physical signs of avian pox, each with pox lesions on their feet.

The overall seroprevalence was 41.5% for *M. gallisepticum* and 46.5% for *Avipoxvirus*. The prevalence of IgY against *Avipoxvirus* differed significantly

Table 1. Prevalence of *Mycoplasma gallisepticum* (Mg) and *Avipoxvirus* (pox) in a sample of 142 raptors admitted to the Illinois Raptor Center inferred from physical signs of pathology and immunoglobulin Y antibodies against each pathogen present in the bird's plasma. Values represent percentages of individuals of each species represented in each condition.

SPECIES	*n*	MG ONLY	POX ONLY	MG AND POX	NEITHER PATHOGEN
American Kestrel (*Falco sparverius*)	15	26.7	13.4	13.4	52.5
Bald Eagle (*Haliaeetus leucocephalus*)	10	30.0	30.0	0.0	40.0
Barred Owl (*Strix varia*)	22	22.7	31.8	9.1	36.4
Cooper's Hawk (*Accipiter cooperii*)	18	5.6	5.6	66.7	22.2
Eastern Screech-Owl (*Megascops asio*)	14	64.2	7.1	14.2	21.4
Great Horned Owl (*Bubo virginianus*)	34	14.7	44.1	20.6	20.6
Red-tailed Hawk (*Buteo jamaicensis*)	29	17.2	34.4	6.9	34.5
Overall seroprevalance	142	22.5	27.5	19.0	31.0

among species ($\chi^2 = 13.111$, $P = 0.03$; Table 1). The seroprevalence of IgY against *Mycoplasma gallisepticum* also differed significantly among species ($\chi^2 = 25.369$, $P = 0.0003$; Table 1). Specifically, Cooper's Hawks (*Accipiter cooperii*) and Great Horned Owls (*Bubo virginianus*) had the greatest seroprevalence of *Avipoxvirus*. Eastern Screech-Owls had the lowest seroprevalence of *Avipoxvirus*. Cooper's Hawks and Red-tailed Hawks had the greatest seroprevalence of *M. gallisepticum*. Great Horned Owls had the lowest seroprevalence of *M. gallisepticum*.

DISCUSSION

We found that the general rate of exposure to *M. gallisepticum* and *Avipoxvirus* was far greater than the frequency at which raptors expressed physical signs of infection from either pathogen. Although >50% of all birds surveyed possessed antibodies against at least one of these pathogens, there were some clear differences in seroprevalence among species. Raptors that eat other birds are more likely to be exposed to *Avipoxvirus* and *M. gallisepticum* from consuming infected prey. *M. gallisepticum* is a known pathogen of passerines and near-passerines at feeder sites throughout the midwestern and eastern United States (Dhondt et al. 2007, 2014), including the central Illinois region from which these raptors were sampled (Wilcoxen et al. 2015), which may partially explain why the prevalence of IgY against *M. gallisepticum* was twice as high in Cooper's Hawks and Eastern Screech-Owls as in any other species. Those two species regularly inhabit urban and suburban areas, where bird feeders are abundant, and are known to prey upon species that use backyard feeders (Artuso 2010), some of which may carry this pathogen. Dhondt et al. (2007) found that *M. gallisepticum* ingested by House Finches (*Haemorhous mexicanus*) caused less severe signs of infection than in birds intentionally inoculated in the conjunctiva. Our observation that raptors frequently demonstrated seroprevalence of antibodies against *M. gallisepticum*, but not physical signs of infection, was consistent with the findings of Dhondt et al. (2007), assuming raptors are mostly infected with *M. gallisepticum* via ingestion.

Conversely, Eastern Screech-Owls had the lowest prevalence of IgY against *Avipoxvirus*, which either suggests that they are exposed to the pathogen less frequently, or that they are less capable of mounting an adaptive immune response against that pathogen. Cooper's Hawks and Great Horned Owls had IgY against *Avipoxvirus* at a rate at least 20% greater than any other species. Along with the aforementioned bird-based diet of the Cooper's Hawk, the diet of the Great Horned Owl, though broad, also includes birds, and they, too, are known to consume species for which avian pox infections have been well documented (Manarolla et al. 2010). Although *Avipoxvirus* in different species of birds are morphologically similar, they do regularly exhibit host species specificity. There are at least two known avian pox viruses in raptors, including Falcon poxvirus and Accipiter poxvirus (Ritchie 1995). Vaccination of raptors with a fowlpox vaccine (as used as the antigen in our ELISA) has been reported to be successful (Ritchie 1995); therefore, it is plausible that, as with the ingestion of *M. gallisepticum*, birds consuming pox-infected prey could undergo seroconversion.

Despite the differences in antibody prevalence among species, and the general finding that >50% of all raptors surveyed had antibodies against these pathogens, very few showed any physical signs of these pathogens. This suggests that exposure to each pathogen is far greater than one would

anticipate based on other published studies of animals showing pathology.

In a survey of 124 raptors that were admitted to the Veterinary Medical Teaching Hospital at the University of California–Davis from 1983–1994, 22 of the 122 birds that died from infectious disease were diagnosed with avian pox (Morishita et al. 1998). A survey of 180 raptors admitted to a rehabilitation center in Brazil revealed no birds with reactive antibodies against *M. gallisepticum* using a haemagluttinin-inhibition test (de Andery et al. 2013). Other than these, there have been very few studies on how *M. gallisepticum* and *Avipoxvirus* affect raptors. In many other studies, the sample size is usually very small, only one or two raptors. For example, avian pox infection was reported for a male juvenile Golden Eagle (*Aquila chrysaetos*; Shrubsole-Cockwill et al. 2010) and a Crested Serpent-Eagle (*Spilornis cheela*; Chen et al. 2010) in Taiwan. The authors of the latter study found that this strain of *Avipoxvirus* shared many genetic similarities to another strain found in a White-tailed Eagle (*Haliaeetus albicilla*) in Japan (Chen et al. 2010).

Our conclusions regarding the prevalence of these pathogens in raptors may be biased because the raptors that were sampled throughout this study were a subset of individuals that had suffered some kind of trauma, taken from the much larger, healthy population. However, these birds were free-living just days, or sometimes hours, before samples were collected, which is unlikely to be long enough for them to have contracted either of these pathogens and develop detectable IgY responses against the pathogen in captivity. In addition, our survey included all birds admitted to the Illinois Raptor Center during this time period, and therefore, represents a combination of injured, weak and starving, and orphaned birds, as well as juvenile, immature, and adult birds for each species. Antibody data in this study are likely indicative of the rate of exposure to these pathogens in these seven species in their free-living state in central Illinois and suggest that the majority of these raptors are capable of mounting a full adaptive immune response against these pathogens, with advancement to pathological conditions being the exception to the normal course of infection, clearance of the pathogen, and sustained immunity.

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