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Short Report: Antibody Prevalence of Select Arboviruses in Mute Swans (*Cygnus olor*) in the Great Lakes Region and Atlantic Coast of the United States

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Abstract. Mute swans (*Cygnus olor*) are an invasive species in the United States. The dramatic increase in their populations in localized areas has led to various problems, among them competition with native species and attacks on humans by aggressive swans. However, very little is known about the ability of these swans to transmit pathogens to humans, domestic birds, or wildlife or participate in enzootic maintenance. To learn more about select pathogens that mute swans may harbor, a survey was conducted from April of 2011 to August of 2012 in the Great Lakes region and localized areas of the Atlantic coast, which revealed serologic evidence of arbovirus exposure in mute swans. Of 497 mute swans tested, antibodies were detected for eastern equine encephalitis (4.8%), St. Louis encephalitis (1.4%), West Nile (1.2%), and Turlock (0.6%) viruses. Samples were also tested for evidence of antibodies to La Crosse virus, but none were positive.

INTRODUCTION

The rapid unprecedented spread of West Nile virus (WNV) across the United States after its introduction into the country in 1999[1] led to heightened awareness about the efficiency at which birds can move pathogens long distances,[2] and it highlighted the ease with which an exotic pathogen can become established. This led to state-specific surveillance programs that monitored the geographic and temporal spread of WNV[3] and an increased interest in the role of wild birds in the transmission and spread cycles of arboviruses.

In addition to WNV, a number of other arboviruses in the United States have raised concerns for public and animal health. For example, eastern equine encephalitis virus (EEEV), La Crosse virus (LACV), and St. Louis encephalitis virus (SLEV) can produce severe clinical signs in people, frequently resulting in central nervous system disease and mortality.[4] Another arbovirus, Turlock virus (TURV; *Bunyaviridae*: *Orthobunyavirus*), is regularly found in North and South America but receives less recognition; it is only known to infect birds as opposed to humans or domestic animals, and its effects are species-dependent.[5]

Mute swans (*Cygnus olor*) are an invasive species to the United States, and in recent years, they have reached nuisance levels in Michigan and other states within the Great Lakes region as well as localized areas of the Atlantic coast. This has resulted in increased conflicts with humans, including mute swan attacks on people. In addition, they often displace native waterfowl by eating large amounts of vegetation,[6] damaging aquatic habitats,[7] and usurping nesting habitat.[8] Their ability to transmit pathogens or participate in their enzootic maintenance is also a concern, especially because it is well-documented that wild birds can serve as disseminators of various microorganisms.[9] The US Department of Agriculture (USDA), Animal and Plant Health Inspection Service, Wildlife Services (WS) program removes mute swans in heavily infested areas to minimize damage, such as that described above. To better understand the risk of pathogen transmission related to mute swans in mosquito-abundant areas where human activities overlap, we examined serum from mute swans for EEEV, SLEV, WNV, TURV, and LACV antibodies.

MATERIALS AND METHODS

Sample collection. From April of 2011 to August of 2012, mute swans were lethally removed by USDA personnel for wildlife damage management purposes. Of these swans, 497 swans were opportunistically sampled post-mortem in Michigan, New Jersey, Rhode Island, New York, and Indiana. During the first year of collection, 100 samples from swans collected in areas that were considered enzootic for EEEV were selectively submitted for testing. During the second year, samples were submitted for testing from all mute swans collected to more fully assess any patterns of occurrence of exposure.

Blood was collected from the jugular vein within 2 hours post-mortem. Serum samples were shipped within 3 days of collection to the National Wildlife Disease Program in Fort Collins, Colorado, where they were stored at –80°C.

Testing. All sera were tested at the University of Texas Medical Branch in Galveston, Texas. Samples submitted for EEEV, SLEV, and WNV were screened initially with goose red blood cells (Lampire Biological Laboratories, Inc., Pipersville, PA) using the hemagglutination inhibition (HI) assay as described previously.[4] Titers ≥ 20 were considered positive and confirmed by the ability of sera to neutralize EEEV, SLEV, WNV, and TURV using an 80% plaque reduction neutralization test ($PRNT_{80}$) as previously described.[4] Samples with $PRNT_{80}$ titers ≥ 20 were considered positive. An enzyme-linked immunosorbent immunoglobulin G (IgG) assay[4] was used to test samples for LACV using sucking mouse brain antigens and California group-specific capture monoclonal antibody 10G5.4 provided by the Centers for Disease Control and Prevention (CDC) in Fort Collins, Colorado. Conjugated horseradish peroxidase (HRP) bird IgG heavy- and

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TABLE 1

Apparent antibody prevalence based on the results of $PRNT_{80}$ of EEEV, SLEV, WNV, and TURV by state (%) with 95% confidence intervals

State	EEEV	SLEV	WNV	TURV
Indiana ($N = 7$)	0	0	0	0
Michigan ($N = 323$)	5.0 (2.9–7.9)	0.9 (0.19–2.7)	0	0.6 (0.5–1.6)
New Jersey ($N = 99$)	3.0 (0.6–8.6)	3.0 (0.6–8.6)	5.0 (1.7–11.4)	1.0 (0.9–4.5)
New York ($N = 14$)	0	0	0	0
Rhode Island ($N = 54$)	9.3 (3.1–20.3)	1.9 (0.04–9.9)	1.9 (0.04–9.9)	0

All samples were screened initially by HI assays, and HI-positive samples were confirmed with $PRNT_{80}$.

light-chain antibodies were purchased from Bethyl Laboratories, Inc. (Montgomery, TX).

Data analysis. Prevalence and 95% confidence limits (95% CLs) for each pathogen within each state were calculated using the Clopper–Pearson method for exact binomial distributions.

RESULTS

We tested 497 mute swan samples collected from five states for EEEV, SLEV, WNV, and TURV (Table 1); the seroprevalence rates across all states measured by HI assay were 8.7%, 2.6%, 1.4%, and 2.4%, respectively. HI positives were confirmed by $PRNT_{80}$ (Table 2) 56%, 54%, 86%, and 25% of the time for EEEV, SLEV, WNV, and TURV, respectively, yielding a confirmed EEEV seroprevalence of 4.8% (95% CL = 3.1–7.1) followed by SLEV (1.4%; 95% CL = 0.5–2.9), WNV (1.2%; 95% CL = 0.4–2.6), and TURV (0.6%; 95% CL = 0.4–1.2). The seroprevalence of EEEV was highest in samples collected from Rhode Island followed by those from Michigan and New Jersey, respectively (Table 1). Seroprevalence of SLEV was highest in samples collected from New Jersey followed by those from Rhode Island, and Michigan. Seroprevalence of WNV was highest in samples collected from New Jersey and Rhode Island, respectively; WNV antibodies were not detected in any samples collected in Michigan. The seroprevalence of TURV antibodies was similar in samples collected in Michigan and New Jersey (Table 1). No arbovirus antibodies were detected in any of the samples collected in Indiana or New York, perhaps because of the small sample sizes. Antibodies for multiple arboviruses were detected in five swans. LACV antibodies were not detected in any of the samples submitted for testing.

DISCUSSION

By examining sera from opportunistically collected mute swans, we detected exposure to a wide range of arboviral pathogens, including WNV, SLEV, EEEV, and TURV. To our knowledge, this is the most extensive dataset available on arboviral pathogen exposure in this invasive avian host, with populations and ranges that have been expanding. Much of the expansion of mute swan populations has occurred in close proximity to urban or suburban areas, and WNV, SLEV, and EEEV all have zoonotic potential. The data reported here only indicate arboviral exposure, and currently, there is limited information available to determine if mute swans develop viremia that is high enough to infect mosquitoes and therefore, influence viral transmission dynamics. It has been suggested with EEEV specifically that small passerine birds have a greater potential for infecting mosquitoes with EEEV than larger birds,[10] but additional data are needed before we can fully understand what role mute swans play in the transmission of the arboviruses studied here.

TABLE 2

Number of mute swans tested for EEEV, SLEV, WNV, and TURV with the HI assay and $PRNT_{80}$ by result

	HI titer								$PRNT_{80}$ titer					
Arbovirus	< 20	20	40	80	160	320	640	1,280	< 20	20	40	80	160	> 640
EEEV	454	10	19	9	4	0	1	0	19	9	6	4	2	2
SLEV	484	6	5	1	0	1	0	0	6	3	3	1	0	0
WNV	490	2	0	1	0	3	0	1	1	0	1	0	1	4
TURV	485	6	3	3	0	0	0	0	9	3	0	0	0	0

The detection of SLEV antibodies in a mute swan in Rhode Island was unexpected, because no human cases of disease have been detected over the past 50 years.[11] However, only one bird was antibody-positive. Although mute swans in the United States only move short distances based on weather,[12] it is possible that the antibody-positive bird migrated from a nearby state, where SLEV infections are more common. Because morbidity and mortality are generally limited in avian hosts,[13] it is possible that SLEV may have circulated undetected in these areas. In contrast to SLEV, WNV detection was lower than expected in areas where it is actively transmitted. This may be because of high rates of mortality in exposed swans, because mortality in geese (also of the family *Anatidae*) attributed to WNV infection has been documented.[13] Another explanation is that the larvae of mosquitoes that are most commonly involved in transmitting WNV occur in stagnant water[14] and not wetlands, where mute swans are typically found, meaning that the probability of mute swans being exposed is relatively low.

Antibody prevalence of TURV in mute swans was also higher than expected and indicates that, although TURV is fatal to some avian species, mute swans are probably unaffected or exhibit low rates of mortality. Although this is the first detection of TURV exposure in mute swans in the United States of which we are aware, it has been previously detected in mute swans in southern Moravia.[15]

The higher seroprevalence based on the HI assays of EEEV, SLEV, and WNV as opposed to the results of $PRNT_{80}$ is not surprising, because cross-reaction between arboviruses within the same family is a known property of the HI assay.[4] The $PRNT_{80}$ results are likely a more accurate representation of the true prevalence in mute swans, because this test is more specific than the HI assay, especially for the alphaviruses that do not have cross-reactivity among antigenic complexes.[16] Although cross-reactions among flaviviruses are common, especially after a second infection, most of our positive sera were reactive against only WNV or SLEV, suggesting primary infections and an accurate reflection of infection history.

Because most of our samples (85%) were collected from after hatch year birds, we were unable to identify any age-related patterns of infection. Also, both the timing and number

of samples collected varied from each state, and consequently, we were unable to ascertain any seasonal infection patterns. The duration of antibody persistence for these viruses is unknown for mute swans and would require additional studies to explore patterns of seasonal infection and whether swans might serve as good biological indicators for identifying areas of highest risk and severity for the upcoming infection cycle.

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