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SEROLOGIC SURVEY OF WILD TURKEYS (*MELEAGRIS GALLOPAVO*) AND EVIDENCE OF EXPOSURE TO AVIAN ENCEPHALOMYELITIS VIRUS IN GEORGIA AND FLORIDA, USA

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ABSTRACT: Wild Turkeys (*Meleagris gallopavo*) are susceptible to many of the same diseases as domestic turkeys. Before 2005, most Wild Turkeys in southern Georgia, US, had little or no exposure to commercial poultry operations. As part of a pathogen survey examining the effects of commercial poultry on Wild Turkeys, samples were collected from Wild Turkeys from March 2005 through May 2008. The turkeys were collected from 13 counties in southern Georgia and Madison County, Florida, and tested for antibodies to various pathogens of poultry. Three (13%) of the turkeys were positive for antibodies to *Salmonella*. Thirteen turkeys (54%) were positive for Newcastle disease virus antibodies, and 15 turkeys (63%) were positive for antibodies to reticuloendotheliosis virus. One turkey (4%) from Madison County was positive for avian encephalomyelitis virus antibodies.

Key words: Antibodies, avian encephalomyelitis, Georgia, *Meleagris gallopavo*, reticuloendotheliosis, *Salmonella*.

INTRODUCTION

The Eastern Wild Turkey (*Meleagris gallopavo silvestris*) is abundant in many parts of Georgia, US, but populations in many areas of southern Georgia are only recently becoming established (Tapley et al. 2000). Wild Turkeys are susceptible to many of the same diseases as their domestic counterparts. Many of these diseases have been studied extensively in Wild Turkeys (Davidson et al. 1988; Hopkins et al. 1990; Charlton 2000), but several, such as avian encephalomyelitis, have received little or no attention. Before 2005, Wild Turkeys in southern Georgia had little or no exposure to commercial poultry operations. That year, a new processing plant and broiler houses were installed throughout the area. Wild turkeys could potentially experience indirect exposure to diseases through wind, dust, feathers, and litter scattered on fields. As part of a pathogen survey examining the effects of commercial poultry on Wild Turkeys, samples were collected from turkeys March 2005 through May 2008.

MATERIALS AND METHODS

Turkeys were collected from 13 counties in southern Georgia and Madison County, Florida (Fig. 1). The Florida samples were used as potential exposure controls because commercial poultry houses had been in operation there for at least 10 yr. Samples were obtained mainly through local turkey hunters and were also collected through cooperation with the Georgia Department of Natural Resources (DNR) at Chickasawhatchee Wildlife Management Area (WMA) in Baker, Calhoun, and Dougherty counties and River Creek WMA in Thomas County. Hunters and landowners were asked to submit dead or sick birds or to report them to the local DNR biologist. Georgia has a spring hunting season that normally runs from late March to mid-May. Hunters are allowed to harvest three gobblers. Therefore, this study mainly reflects the adult male turkey population during the spring hunting season. Samples were submitted to the University of Georgia Veterinary Diagnostic and Investigational Laboratory (VDIL) in Tifton, Georgia, for diagnostic analyses.

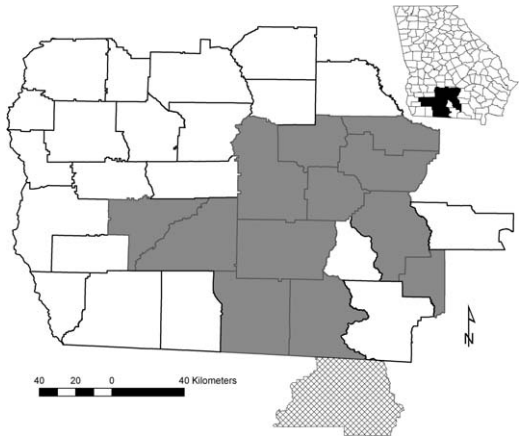

FIGURE 1. Counties included in study area of Wild Turkeys (*Meleagris gallopavo*) in southern Georgia, USA (shaded), and control area in Madison County, Florida, USA (hatched).

TABLE 1. Type and number of Wild Turkey (*Meleagris gallopavo*) tissues from Georgia and Florida, USA, tested for poultry pathogens, 2005–08.

Tissue type	No. tested
Lung	57
Liver	64
Spleen	64
Kidney	55
Heart	61
Intestine	64
Esophagus	64
Trachea	59
Proventriculus	64
Ventriculus	64

Whole carcasses or internal organs were obtained from 64 turkeys, and blood was collected from the jugular vein or heart whenever possible. Twenty-four serum samples were collected over the 4-yr period. Blood samples were centrifuged at 3,200 × G for 10 min to separate the serum, and the sera were frozen until needed. Serum samples were sent to the Poultry Diagnostic and Research Center in Athens, Georgia, to test for antibodies to the following pathogens as sample volume allowed: hemorrhagic enteritis virus (HEV) by agar gel precipitation test (AGP; Charles River Laboratories, Wilmington, Massachusetts, USA); *Salmonella* Pullorum and *Salmonella* Gallinarum by tube agglutination test (USDA-APHIS 2011); avian encephalomyelitis virus (AEV), reticuloendotheliosis virus (REV), and Newcastle disease virus (NDV) by enzyme-linked immunosorbent assay (ELISA; IDEXX Laboratories, Westbrook, Maine, USA); and *Mycoplasma gallisepticum*, *Mycoplasma meleagridis*, and *Mycoplasma synoviae* by hemagglutination inhibition (HI) test (USDA-APHIS 2011). The HI test for *Mycoplasma* was considered positive for titers ≥40. Any remaining serum was tested at VDIL for group-specific antibodies to Type A influenza virus using an AGP test (National Veterinary Services Laboratory, Ames, Iowa). Routine aerobic and anaerobic cultures were performed on all available tissues (Table 1). Trachea, lung, and esophagus were also cultured for mycoplasma on mycoplasma media (University of California-Davis Veterinary Medical Biological Media Services, Davis, California, USA) and were held at 37 C for 5–7 d. Hektoen enteric agar (Remel, Lenexa, Kansas, USA) and tetrathionate broth (VDIL) followed by Rappaport-Vassiliadis broth for enrichment was used for *Salmonella* isolation from the intestine. RNA was extracted from 30 lung samples using the Qiagen RNeasy mini kit (Qiagen, Valencia, California, USA) and tested using real-time reverse-transcription (RT)-PCR for NDV following Wise et al. (2004). Fresh tissues including lung, kidney, heart, spleen, trachea, esophagus, and intestine were processed for virus isolation following Miller et al. (2003). Cell culture plates were examined daily for viral cytopathic effect (CPE) for 2 wk. The assay was considered negative if no CPE was observed within the 2-wk period. Samples of tissues from a Wild Turkey found dead in Thomas County were also preserved in formalin, processed, and stained with H&E for histopathology.

Positive and negative results were assigned a number score (1 or 0) to perform statistical analysis of the data using single-factor analysis of variance (Rosner 2006). Differences were considered significant at $P \leq 0.05$. Comparisons were made between years and between control and study groups for each test performed. Wild Turkeys were also grouped according to proximity to commercial chicken operations. All Wild Turkeys from within a 12-km radius of commercial poultry operations were grouped and compared with Wild Turkeys outside that range.

Results for serologic tests from 2005 to 2008 are provided in Table 2.

RESULTS

All 24 serum samples were negative for antibodies to *M. gallisepticum, M. melea-*

TABLE 2. Results of enzyme-linked immunosorbent assays (ELISA) for antibodies to *Salmonella*, reticuloendotheliosis virus (REV), encephalomyelitis virus (AEV), and Newcastle disease virus (NDV) for individual Wild Turkeys (*Meleagris gallopavo*) from southern Georgia, USA, and Madison County, Florida, 2005–08.[a]

Animal No./Year	*Salmonella*	REV ELISA	AEV ELISA	NDV ELISA
1/2005	Neg	Neg	Neg	Neg
2/2005[b]	Neg	Pos	Neg	Neg
1/2006	Neg	Pos	Neg	Pos
2/2006	Neg	Pos	Neg	Neg
3/2006	Neg	Neg	Neg	Neg
4/2006	Neg	Pos	Neg	Neg
5/2006[b]	Neg	Pos	Neg	Pos
6/2006	Neg	Pos	Neg	Pos
1/2007	Neg	Neg	Neg	Neg
2/2007	Neg	Pos	Neg	Neg
3/2007	Neg	Neg	Neg	Neg
4/2007	Neg	Neg	Neg	Neg
5/2007	Neg	Pos	Neg	Pos
1/2008[b]	Neg	Neg	Neg	Pos
2/2008[b]	Neg	Pos	Neg	Neg
3/2008[b]	Neg	Pos	Pos	Pos
4/2008	Neg	Pos	Neg	Pos
5/2008	Pullorum	Neg	Neg	Pos
6/2008	Neg	Neg	Neg	Pos
7/2008	Neg	Pos	Neg	Pos
8/2008	Gallinarum	Neg	Neg	Neg
9/2008	Neg	Pos	Neg	Pos
10/2008	Gallinarum	Pos	Neg	Pos
11/2008	Neg	Pos	Neg	Pos

[a] Neg = negative; Pos = positive.

[b] Turkeys from Madison County control group.

gridis, *M. synoviae*, and HEV. Three (13%) of the serum samples collected in 2008 were positive for antibodies to *Salmonella*. Two of the three turkeys were positive for antibodies to *Salmonella* Gallinarum, and one was positive for *Salmonella* Pullorum antibodies. Two of the *Salmonella* antibody-positive turkeys were within a 12-km radius of a commercial poultry operation. Thirteen (54%) turkeys were positive for NDV antibodies. Three of those were from the Madison County control group, and five were within the 12-km radius. Fifteen (63%) turkeys were positive for antibodies to REV. Four were from the Madison County control group and five were within the 12-km radius of a commercial poultry operation. One (4%) turkey from the control group was positive for AEV antibodies. Nineteen samples were also tested for avian influenza antibodies (Type A), and all were negative.

Two (3%) turkeys that were found dead in 2006 were culture positive for *Salmonella* spp. One isolate was a Group E *Salmonella*. The second isolate was not typed. No serum was available from either turkey. The trachea from one turkey was culture positive for *Mycoplasma gallopavonis*. All turkeys were negative for NDV by RT-PCR. No viruses were isolated. There were no significant pathologic findings in the turkeys found dead.

Significant differences in antibody prevalence were seen between years for NDV ($P=0.038$) and between the control group (Madison County) and the test group for AEV ($P=0.048$). No other significant differences were seen when comparing

control and study groups or proximity to commercial poultry operations.

DISCUSSION

All 24 turkeys were negative for antibodies to *M. gallisepticum, M. meleagridis*, and *M. synoviae*. All are important pathogens in the commercial turkey industry. Investigators have documented evidence of exposure of Wild Turkeys in Georgia to *M. gallisepticum* (Luttrell et al. 1991), *M. meleagridis* (Davidson et al. 1988), and *M. gallopavonis* (Luttrell et al. 1992). *Mycoplasma gallisepticum* infections in Wild Turkeys appear to be rare and are almost always related to a domestic source, especially free-ranging poultry (Rocke and Yuill 1987; Luttrell et al. 1991). One turkey in our study was culture positive for *M. gallopavonis*. *Mycoplasma gallopavonis* was first reported in Wild Turkeys in Texas in 1987 (Rocke and Yuill 1987), and Luttrell et al. (1991) reported a high prevalence of *M. gallopavonis* on Cumberland Island, Georgia. Investigators working in North and South Carolina (Luttrell et al. 1992) concluded that *M. gallopavonis* is nonpathogenic and widespread in Wild Turkey populations, but isolates have been shown to be lethal to domestic chicken and turkey embryos (Rocke and Yuill 1987). The low prevalence of *M. gallopavonis* in our South Georgia population compared with other populations could be a result of a lower population density.

Three turkeys were positive for antibodies to *Salmonella*, and two were culture positive. Various *Salmonella* spp. have been isolated from Wild Turkeys (White et al. 1981). *Salmonella* Gallinarum is now rare in commercial operations but can be found in noncommercial flocks of various species (McMullin 2004). The three turkeys found positive for antibodies to *Salmonella* were collected in 2008, and the two culture-positive birds were from 2006. One of those birds was found dead in a field that had been fertilized with chicken litter. Lack of mucosal lesions in the intestinal sections suggested ulcerative enteritis was not severe enough to be the cause of death. Pullorum disease and fowl typhoid have not been reported in Wild Turkeys, but antibodies to *Salmonella* Pullorum have been reported (Crupper and Applegate 2002). To our knowledge this is the first report of antibodies to *Salmonella* Gallinarum in Wild Turkeys.

Domestic turkeys are considered one of the most permissive domestic hosts of avian influenza viruses (AIVs; Webby et al. 2007), but there is only one record of a Wild Turkey with antibodies to AIV (Charlton 2000). There was no evidence of antibodies to AIV in more than 600 serum samples collected from Wild Turkeys in several states (Davidson et al. 1988), and Nettles et al. (1985) failed to isolate AIV from Wild Turkeys in Virginia during an outbreak in domestic poultry. Webby et al. (2007) suggests that this may be a function of the limited number and timing of studies of influenza in Wild Turkeys and that the possibility exists that Wild Turkeys, like the domestic turkeys, are highly susceptible to transient epizootics of influenza. We also found no turkeys positive for AIV antibodies, but with the emergence of new, more virulent strains of avian influenza (AI), the possibility of AI causing mortality in Wild Turkeys should not be ruled out.

Thirteen turkeys (54%) had antibodies to NDV by ELISA, indicating that they had been exposed to the virus. Antibodies to NDV have been found in Wild Turkeys, but clinical disease has not been reported (Hopkins et al. 1990; Charlton 2000). Most wild birds are considered susceptible to infection with NDV, with cases of high mortality occasionally recorded. These cases are most often associated with exposure to domestic birds (Alexander 1995). A significant difference was seen between years for NDV serology ($P=0.038$; Fig. 2). Monitoring of both wild and domestic populations may provide insight as to the origin of the exposure in the Wild Turkeys.

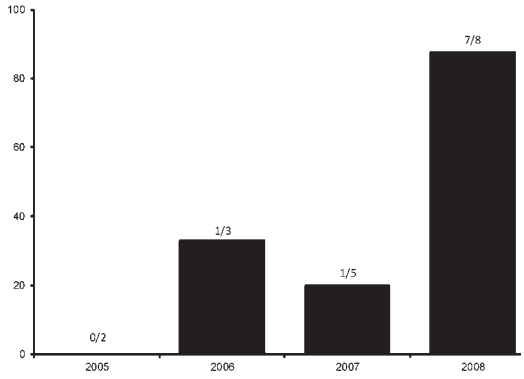

FIGURE 2. Results by year for enzyme-linked immunosorbent assay for antibody to Newcastle disease virus in Wild Turkeys (*Meleagris gallopavo*) in southern Georgia, USA (Madison County, Florida birds not included). Number turkeys positive/number tested indicated above bars.

We tested 24 turkeys for HEV antibodies and all were negative. Hemorrhagic enteritis is caused by turkey adenovirus I and, along with associated secondary bacterial infections, can cause significant loss to commercial turkey operations (Pierson and Fitzgerald 2008). Domermuth et al. (1977) and Hopkins et al. (1990) surveyed more than 200 Wild Turkeys in Arkansas, Florida, and Texas and failed to find antibodies to HEV. Our study supports the reports that HEV is not a concern in free-ranging Wild Turkeys.

Fifteen of the 24 turkeys we tested were positive for antibodies to REV, showing that they had been exposed to the virus, but we did not see a significant change in the number of positive turkeys during the course of this study or across the study area. Reticuloendotheliosis virus can be found as a contaminant in poultry vaccines, and infection can cause economic loss due to runting syndrome and chronic neoplasia (Fadly et al. 2008). Lymphoproliferative diseases have been reported from Wild Turkeys in the southeastern US, and in a few of these, REV was established as the causative agent (Hayes et al. 1992). Other serologic surveys have indicated that REV is widespread in a variety of avian species, especially in the southeastern US, but is not always associated with clinical disease (Hayes et al. 1992). Further studies should be done to determine whether REV is established in the population and, if so, whether significant health issues are developing.

In 2008, one of the turkeys we tested from Madison County, Florida, was positive for antibodies to AEV. Avian encephalomyelitis virus is widespread in chickens, pheasants, and domestic or pen-raised turkeys (Van Steenis 1971) but has not been reported in Wild Turkeys (Hopkins et al. 1990; Neimanis and Leighton 2004). Infection with AEV can result in reduced egg production and hatchability and ataxia and tremors in young birds. Mortality occurs most often in young birds (Van Steenis 1971). The turkeys in Madison County have been exposed to commercial poultry for at least 10 yr, and it is possible that the turkeys were exposed to AEV through domestic birds. Continued and increased monitoring is recommended to determine whether this is an isolated event or whether AEV is becoming widespread in the Madison County Wild Turkey population.

Given our findings of AEV and *Salmonella* Gallinarum and increasing NDV antibody prevalence, we recommend continued and more intense investigation of Wild Turkey populations associated with domestic poultry flocks. Both AEV and *Salmonella* Gallinarum are generally associated with domestic birds; however, *Salmonella* Gallinarum is considered rare in commercial flocks and is currently eradicated in the US. The relationship of NDV to domestic birds remains unclear, and contact with other species of wild birds could be a source of NDV exposure for Wild Turkeys. Our study only represents the initial years post-establishment of the domestic poultry industry in southern Georgia. Continued monitoring of Wild Turkey populations will help determine whether they are being affected by the domestic poultry industry.

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