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A SURVEY OF INFECTIOUS DISEASES IN WILD TURKEYS (*MELEAGRIDIS GALLOPAVO SILVESTRIS*) FROM ARKANSAS

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ABSTRACT: Wild turkeys (*Meleagris gallopavo silvestris*) trapped as part of a relocation program by the Arkansas Game and Fish Commission were tested for selected infectious diseases and parasites. The 45 birds were trapped at four locations in Pope, Scott, and Montgomery counties (Arkansas, USA). Forty-four blood samples for serology, 27 blood smears and 12 fecal samples were collected. Of the serum samples tested, 20 of 44 (45%) were positive for *Pasteurella multocida* by enzyme-linked immunosorbent assay (ELISA), 42 of 44 (95%) were positive for *Bordetella avium* by ELISA, and 15 of 44 (34%) were positive for Newcastle disease virus antibody by the hemagglutination inhibition test. All serum samples were negative for *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, avian paramyxovirus 3, avian influenza, hemorrhagic enteritis, Marek's disease, avian encephalomyelitis, laryngotracheitis, *Salmonella pullorum* and *Salmonella gallinarum*. *Haemoproteus meleagridis* was found in eight of 27 (30%) and *Leucocytozoon smithi* in nine of 27 (33%) blood smears; all smears were negative for *Plasmodium hermani*. Enteric parasites included *Ascaridia dissimilis*, *Heterakis gallinarum*, *Eimeria dispersa* and *Railletina* spp. This study was an attempt to document the health status and disease exposure of wild turkeys in Arkansas to aid in managing and preventing the spread of disease agents to wild turkeys and other species of birds.

Key words: Bordetellosis, helminths, hematozoa, mycoplasmosis, *Meleagris gallopavo silvestris*, parasite survey, pasteurellosis, serosurvey, wild turkey.

INTRODUCTION

Eastern wild turkeys (*Meleagris gallopavo silvestris*) are abundant in certain Arkansas counties (USA), but the species is not well established in others. Relocation projects are being conducted by the Arkansas Game and Fish Commission to increase wild turkey populations in the latter counties. Relocation efforts risk introducing or disseminating disease into domestic poultry and wild bird species. Knowing the disease status of the relocated birds is important for managing the spread of disease among wild turkey populations as well as preventing them from becoming a disease reservoir, since domestic and wild turkeys are uniformly susceptible to several diseases (Davidson et al., 1982; Amundson, 1985); epidemiologic investigation of a disease outbreak in either species would be aided by current data concerning the disease status of wild turkeys. The potential crossover of diseases is most likely where open range flocks of domestic turkeys are

located near wild turkey habitats. The intention of this study was to provide data concerning diseases to be used prophylactically to help manage wild turkey populations, to determine if diseases of domestic turkeys were also present in wild turkeys, and to present documentation to protect wildlife agencies against liability for disease outbreaks in a restoration area.

MATERIALS AND METHODS

Wild turkeys were trapped in January through March 1986 by Arkansas Game and Fish Commission (Little Rock, Arkansas 72201, USA) personnel using cannon nets. Trapping locations were Holla Bend Refuge (Pope County; 35°8' to 35°10'N, 93°2' to 93°3'W), Bayou Ranger District (Pope County; 35°35' to 35°36'N, 93°4' to 93°6'W), Womble Ranger District (Montgomery County; 34°21' to 34°22'N, 93°38' to 93°39'W), and Scott County (34°51' to 34°52'N, 94°21' to 94°23'W). We examined the birds for external parasites, collected blood and feces samples, and collected swabs for bacterial culture and virus isolation near the release locations in the afternoon of the day of capture. Game

and Fish personnel estimated age, determined sex and applied leg-bands to each bird.

Approximately 3 to 5 ml of blood was obtained from each turkey via the brachial vein using a 5 ml syringe and a 3.8 cm 22 gauge needle (Monoject, Sherwood Medical, a Brunswick Company, St. Louis, Missouri 63103, USA). Blood was placed into sterile plastic test tubes with a snap cap and allowed to clot in a refrigerator overnight. The following day the clot was ringed with a wooden applicator stick then centrifuged at $20 \times g$ for 15 min. Serum was removed and placed into sterile cryotubes for freezing and storage prior to serologic testing.

The technique utilized for *Pasteurella multocida* serum antibody determination was a commercially available enzyme-linked immunosorbent assay (ELISA) (Idexx Corporation, Portland, Maine 04101, USA) developed for turkeys. The test for *Bordetella avium* antibody was also an ELISA that had been developed by the authors for use in domestic turkeys (Hopkins et al., 1988). Newcastle disease virus (NDV) and avian paramyxovirus 3 testing was performed using the hemagglutination inhibition (HI) assay described by Beard and Wilkes (1973). *Mycoplasma gallisepticum* and *Mycoplasma synoviae* testing was performed using the rapid plate agglutination on site and later confirmed using the HI procedure (Ryan, 1973; Rocke et al., 1985).

The tube agglutination technique was used for both *Salmonella pullorum* and *Salmonella gallinarum* testing. Agar gel immunodiffusion (AGID) tests were utilized for detecting the presence or absence of antibody to avian influenza virus, hemorrhagic enteritis virus, avian encephalomyelitis virus, Marek's disease virus and laryngotracheitis virus. Antigens used in the AGID procedures were obtained from either the National Veterinary Services Laboratory (Ames, Iowa 50001, USA) or from SPAFAS, Inc. (Norwich, Connecticut 06369, USA).

Smears were stained with Romanovsky-type stain (Diff-Quik Differential Stain Set, American Scientific Products, McGaw Park, Illinois 60085, USA). *Leucocytozoon smithi* and *Haemoproteus meleagridis* were speciated by the morphological characteristics of gametocytes in blood smears.

Fecal samples were obtained from cloacal swabs or whole feces. Parasite screening was performed using a modified McMaster technique (Whitlock, 1948).

The sinuses and trachea of each turkey were swabbed using Calgi swabs type II (Spectrum Laboratories, Inc., Houston, Texas 77001, USA). The swabs were placed into transport media or virus isolation media for the bacteria and virus isolation, respectively. Calgi swabs type I (Spec-

trum Laboratories, Inc.) were used to swab the cloaca, then placed into transport media. Sinus and trachea swabs were streaked onto blood and MacConkey's agar, while cloacal swabs were streaked onto brilliant green agar. The virus isolation media was passaged three times in 10-day-old specific pathogen free chick embryos.

RESULTS AND DISCUSSION

Antibodies were detected against three infectious agents. Forty-five percent (20 of 44) of the turkeys had antibody titers to *P. multocida* as determined by ELISA, 95% (42 of 44) of the turkeys had antibody titers to *B. avium* as determined by ELISA, and 34% (15 of 44) of the turkeys tested had antibody titers to NDV as determined by the HI test (Table 1). All positive sera were retested at least two times and were positive each time tested. All turkeys were serologically negative for *M. gallisepticum*, *M. synoviae*, avian paramyxovirus 3, hemorrhagic enteritis virus, Marek's disease virus, avian encephalomyelitis virus, and laryngotracheitis virus. The turkeys surveyed were negative for avian influenza antibodies, which supports the finding by Nettles et al. (1985), who found seven pen-raised wild turkeys to be negative for antibodies near an avian influenza outbreak in domestic poultry.

While *Salmonella* spp. have been a scourge to domestic poultry for decades, the first clinical case of salmonellosis in wild turkeys was reported only in 1985 (Howerth, 1985), although detection of antibodies against *Salmonella* spp. in wild turkeys had been reported previously (Roslien and Haugen, 1970; Hensley and Cain, 1979; Amundson, 1985). We did not find antibodies against *Salmonella pullorum* or *S. gallinarum*, similar to the results reported by Glazener et al. (1967) and Trainer et al. (1968).

Leucocytozoon smithi and *Haemoproteus meleagridis* were found in 33 (9 of 27) and 30 (8 of 27)% of the turkeys, respectively (Table 1). A moderate leukocytosis was noted in the *Leucocytozoon* sp. infected smears while *Haemoproteus* sp.

TABLE 1. Serologic results and blood parasites from different ages and sexes of wild turkeys relocated in Arkansas.

Organism	All hosts	Adults	Juveniles	Males	Females
<i>Pasteurella multocida</i>	20/44 ^a (45) ^b	18/33 (55)	2/11 (18)	2/9 (22)	18/35 (51)
<i>Bordetella avium</i>	42/44 (95)	32/33 (97)	10/11 (91)	7/9 (78)	35/35 (100)
NDV ^c	15/44 (34)	9/33 (27)	6/11 (55)	1/9 (11)	14/35 (45)
<i>Leucocytozoon smithi</i>	9/27 (33)	9/18 (50)	0/9 (0)	3/9 (33)	6/18 (33)
<i>Haemoproteus meleagridis</i>	8/27 (30)	4/18 (22)	4/9 (44)	2/9 (22)	6/18 (33)

^a Number positive/number tested.

^b % Prevalence.

^c Newcastle disease virus.

infected smears contained numerous immature erythrocytes. A dual infection was found in one turkey. The prevalence of turkeys infected with these species was lower than reported previously (Kozicky, 1948; Cook et al., 1966; Roslien and Haugen, 1970; Eve et al., 1972a, b; Forrester et al., 1974; Nobelet and Moore, 1975; Atkinson et al., 1983). An explanation for this might be the fact that we made no attempt to identify the trophozoite stage. There was a distinct difference in the prevalence of these blood parasites among counties (Table 2), which may be an indication of the geographic distribution of vectors in the respective counties (Nobelet and Moore IV, 1975; Atkinson et al., 1983).

Leucocytozoon smithi was not detected in juvenile birds, and *Haemoproteus meleagridis* was found in greater numbers in the juveniles than adults (Table 1). A higher prevalence of *Haemoproteus* sp. in juvenile turkeys has been reported by other researchers (Cook et al., 1966; Roslien and Haugen, 1970; Forrester et al., 1974). Roslien and Haugen (1970) proposed an age

factor for susceptibility to *Haemoproteus* sp. involving undetermined factors of host-vector and/or host-parasite interaction that is supported by findings in the present study. *Plasmodium* sp. was not detected in the blood smears, probably because sub-inoculations of blood into susceptible turkeys must be done to accurately determine the prevalence of *Plasmodium* sp. (Forrester et al., 1974; Christensen et al., 1983).

Other important results in our study include a higher prevalence of NDV antibodies among juveniles versus adult birds. Also, female turkeys seemed to have a higher prevalence of parasitism than males, which may be a result of closer contact of greater numbers of females versus males, genetic differences regarding sex, or both.

Examination of feces revealed the presence of *Ascaridia dissimilis*, *Heterakis gallinarum*, *Eimeria dispersa*, and *Railletina* spp. Blackhead, or histomoniasis, has been reported in wild turkeys (Kozicky, 1948; Thomas, 1964; Amundson, 1985; Davidson et al., 1985) and may be transmitted by *H. gallinarum*. *Heterakis*

TABLE 2. Serologic results and blood parasites in wild turkeys from different localities in Arkansas.

Disease or parasite	Montgomery	Pope ^c	Pope ^d	Stott
<i>Pasteurella multocida</i>	4/8 (50) ^a	15/30 (50)	0/3 (0)	1/3 (33)
<i>Bordetella avium</i>	8/8 (100)	30/30 (100)	2/3 (67)	2/3 (67)
NDV ^b	3/8 (38)	12/30 (40)	0/3 (0)	0/3 (0)
<i>Leucocytozoon smithi</i>	7/7 (100)	2/14 (14)	0/3 (0)	0/3 (0)
<i>Haemoproteus meleagridis</i>	0/7 (0)	7/14 (50)	1/3 (33)	0/3 (0)

^a Number positive/number tested (% prevalence).

^b Newcastle disease virus.

^c Holla Bend Refuge, Arkansas River Valley.

^d Bayou Ranger District, Ozark National Forest.

gallinarum was found in the present study but there was no evidence of *Histomonas meleagridis*. Lice (*Oxylipeurus corpulentus*) were observed on one emaciated hen.

Several different bacterial species were isolated, but they were considered to be part of the normal flora of the oral cavity. These included *Streptococcus* spp., *Staphylococcus* spp., *Corynebacterium xerosis*, *Xanthomonis*, *Pseudomonas fescularis*, and *Corynebacterium pseudodiphtheriticum* or *Corynebacterium hefmanni*.

This study is the first to report antibodies against *B. avium* in wild turkeys. While the importance of this infection is unknown in wild turkeys, *Bordetella avium* causes mortality due to rhinotracheitis in young domestic turkey poults; it is a primary invader of the trachea, usually resulting in subclinical disease, but a severe disease situation is created with secondary invaders (Simmons et al., 1979). It is possible that the level of exposure to *B. avium* and secondary invaders is low, posing no great disease threat to wild turkey poults in their natural environment.

Pasteurella multocida has been reported as a disease of wild turkeys (Glazener et al., 1967), and it is a pathogen of interest in monitoring programs (Amundson, 1985). The poor survival of *P. multocida* in the soil and water (Backstrand and Botzler, 1986) and the low flock densities of the wild turkeys in Arkansas seem to aid in preventing great losses to fowl cholera. We did not find previous reports of the prevalence of *P. multocida* antibodies or isolations among wild turkeys.

Newcastle disease is known to be a potential disease problem to wild turkeys and has been monitored by several researchers (Glazener et al., 1967; Trainer et al., 1968; Roslien and Haugen, 1970; Hensley and Cain, 1979). Although one wild turkey was suspected of dying from NDV (Hensley and Cain, 1979), each reported negative findings for isolation and antibodies. Similarly, we did not isolate NDV. However, the present study is unique in that antibodies against NDV were detected.

The significance of the serologic findings to the wild turkey population is unknown except that the birds must have been exposed to the disease agents at some time. Such a survey is biased toward relatively healthy birds, since it necessarily excludes birds unable to travel to trap sites. Thus, the full severity of disease in the population cannot be accurately determined by the methods used in this study. In Arkansas, sparse populations in mountainous forest habitat probably lower the risk of disease due to *B. avium*, NDV and other infectious agents, in contrast to more dense populations in areas with larger bodies of waters where exposure to disease vectors and migrating waterfowl is more likely. The former environment (Bayou Ranger District) and the latter (Holla Bend Refuge) are represented in Table 2. Since this report is the first to present serologic evidence of exposure to *B. avium* and NDV, further investigation is needed to determine the importance of these pathogens to the wild turkey.

Aspergillus fumigatus was isolated from several groups of turkeys and was shown to be teratogenic in chick embryos. Aspergillosis has been previously reported in wild turkey poults (Durant and Tucker, 1935) and one adult (Davidson et al., 1985). Apparently *A. fumigatus* is ubiquitous to the forest floor and could be responsible for early poult mortality and lower hatch rates if present in the eggs.

Although antibodies to three important diseases were detected, only one pathogenic organism (*Aspergillus fumigatus*) was isolated. Another pathogen, fowl pox, was not included in the present study, but since previous reports document deaths resulting from fowl pox in wild turkeys in the southeastern U.S. (Thomas, 1964; Simmons et al., 1979; Amundson, 1985; Davidson et al., 1985), future disease surveys should test for it. The level of parasitism was mild to moderate, with no important parasitic infections observed. Apparently, these turkeys were in good health and did not represent a threat to the restoration

program or to domestic flocks within this range.

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