MYCOPLASMAS IN WILD TURKEYS LIVING IN ASSOCIATION WITH DOMESTIC FOWL

Authors: Hoffman, Richard W., Page Luttrell, M., Davidson, William R., and Ley, David H.

Source: Journal of Wildlife Diseases, 33(3) : 526-535

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-33.3.526

Journal of Wildlife Diseases, 33(3), 1997, pp. 526–535

MYCOPLASMAS IN WILD TURKEYS LIVING IN ASSOCIATION WITH DOMESTIC FOWL

Richard W. Hoffman,[1] M. Page Luttrell,[2] William R. Davidson,[2,3] and David H. Ley[4]

[1] Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, Colorado 80526, USA

[2] Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA

[3] D. B. Warnell School of Forest Resources, The University of Georgia, Athens, Georgia 30602, USA

[4] Department of Food Animal and Equine Medicine, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606, USA

ABSTRACT: One hundred and nineteen Merriam's wild turkeys (*Meleagris gallopavo merriami*) and 31 domestic chickens coexisting on a ranch in west-central Colorado (USA) were surveyed for mycoplasmosis by serologic and cultural methods. Although no clinical signs were apparent in any wild turkeys tested, 51 (43%) had positive rapid plate agglutination (RPA) reactions for *M. gallisepticum* (MG) and/or *M. synoviae* (MS); 37% of 56 adults and 48% of 63 subadults were classified as positive reactors to MG and/or MS. No turkeys tested in 1992 (n = 61) and 17 (29%) of 58 turkeys tested in 1993 were RPA-positive for *M. meleagridis* (MM). Hemagglutination inhibition (HI) test results were negative for MG, MS and MM as were most enzyme-linked immunosorbent assay (ELISA) test reactions (MG = 99%, MS = 93%, MM = 87%). Immunoblotting showed mild to moderate reactivity to MG proteins in 49% of 41 samples tested. Most chickens were strongly positive for MS by RPA (81%), HI (58%) and ELISA (87%); 48% also were positive for MG by RPA but all were MG-negative by HI and ELISA. No pathogenic mycoplasmas were isolated from either group of birds. *Mycoplasma gallopavonis* was commonly identified from the wild turkeys, and *M. gallinaceum* was isolated from both the chickens and wild turkeys. In a transmission study conducted in 1994, disease-free domestic turkeys failed to seroconvert when co-housed with wild turkeys from this population that were RPA-positive for MG. Collectively, the results of this study were inconclusive regarding the status of pathogenic mycoplasmas within this wild turkey population.

Key words: Domestic fowl, isolation, Merriam's wild turkey, *Meleagris gallopavo merriami*, *Mycoplasma* spp., serologic survey.

INTRODUCTION

Mycoplasma gallisepticum (MG), *M. synoviae* (MS) and *M. meleagridis* (MM) are acknowledged avian pathogens that can cause significant losses of domestic poultry. *Mycoplasma gallisepticum* causes sinusitis in turkeys and airsacculitis in chickens (Yoder, 1991); MS produces synovitis and respiratory disease in chickens and turkeys (Kleven et al., 1991); and MM causes respiratory disease and poor growth in young turkeys (Yamamoto, 1991). Although antibodies to MG, MS and MM have been detected in wild turkeys (Hensley and Cain, 1979; Amundson, 1985; Rocke and Yuill, 1987; Fritz et al. 1992), few cases of disease or successful isolation have been reported (Davidson et al., 1982; Jessup et al., 1983; Adrian, 1984; Fritz et al., 1992). In all cases, the turkeys were in contact with domestic fowl.

Serological surveys indicate that the prevalence of birds seropositive for pathogenic mycoplasmas is greatest in Rio Grande wild turkeys (*Meleagris gallopavo intermedia*), somewhat less in Merriam's wild turkeys (*M. gallopavo merriami*) and rare in eastern wild turkeys (*M. gallopavo silvestris*) (Rocke and Yuill, 1987; Davidson et al., 1988; Fritz et al., 1992). Merriam's and Rio Grande wild turkeys commonly winter in large flocks around ranches where they take advantage of artificial food sources. Such flocks tend to be relatively tame and easy to trap. Therefore, they are often targeted as a source of birds for relocation efforts. Wild turkeys venturing into ranches frequently come in contact with domestic fowl and may be exposed to infectious diseases through this association. Various wildlife agencies have adopted guidelines which recommend

screening wild turkeys for pathogenic mycoplasmas before moving them to minimize disease dissemination into new or existing populations (Nettles and Thorne, 1982; Nettles, 1984). The objectives of this study were to evaluate the status of *Mycoplasma* spp. infection in a population of Merriam's wild turkeys with a long history of association with domestic fowl and to examine the risks of using these birds as transplant stock.

MATERIALS AND METHODS

Trapping and sampling were conducted on the Hittle Ranch located 6 km northeast of Collbran, Mesa County, Colorado (USA) (39°16'N, 107°55'W). Turkeys first appeared at this site about 25 yr ago. In recent years, between 120 to 160 wild turkeys have wintered annually at the ranch. Wild turkeys were attracted to the ranch because of the availability of oat hay, alfalfa hay, corn, beef feed and poultry feed provided for the domestic animals. From November through March, the wild turkeys were in daily contact with domestic chickens, which roamed freely about the ranch. Guinea fowl and domestic turkeys also were present in 1992 and 1994, respectively.

Turkeys were baited with oat hay and corn and live-trapped with cannon nets from January through March of 1992 and 1993. Captured birds were weighed, classified to age and sex and marked with serially numbered aluminum leg bands and Allflex livestock tags (Allflex Tag Company, Culver City, California, USA) attached to the patagium. Ages were recorded as subadult (8 to 10 mo) or adult (>18 mo). Birds were examined for clinical signs of mycoplasmosis (Kleven et al., 1991; Yamamoto, 1991; Yoder, 1991), and their general physical condition was noted. Blood (8 cc) was collected from 119 wild turkeys by jugular venipuncture and placed into Vacutainer collection tubes with no additives (Becton-Dickson, Rutherford, New Jersey, USA). Serum was separated by centrifugation, pipetted into separate containers and refrigerated. Sampling (n = 56) for *Mycoplasma* spp. isolation consisted of swabbing the trachea with a sterile dacron swab that was immediately inoculated into Frey's media with 12% swine serum (FMS; Kleven and Yoder, 1989); FMS with antisera to *M. gallopavonis* was used to culture tracheas of 20 turkeys. Samples were mailed overnight express on cold paks to the Southeastern Cooperative Wildlife Disease Study (SCWDS) (College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA).

Thirty-one free-ranging chickens present on the ranch were captured with a hoop net. Blood (n = 31) and tracheal cultures (n = 15) were collected and handled in the same manner as described for the wild turkeys.

Serum samples from wild turkeys were screened for antibodies to MG, MS and MM with the rapid plate agglutination (RPA) test (Kleven and Yoder, 1989). Samples from the chickens were tested only for MG and MS by RPA. The RPA tests were performed on unfrozen serum within 48 hr of collection. Two antigen preparations were used for MG and MS, commercial antigens made by Salsbury Laboratories, Inc. (Charles City, Iowa, USA) and laboratory-prepared antigens made by the Poultry Disease Research Center (PDRC) (College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA). Turkeys were screened for MM using a laboratory-prepared antigen from PDRC in 1992 and a commercial antigen made by Intervet, Inc. (Millsboro, Delaware, USA) in 1993. Agglutination was scored on a scale of zero (no reaction) to four (strong reaction). Any reaction ≥ two was considered positive. When two antigen preparations produced different agglutination scores, the result from the antigen yielding the highest score was used. Appropriate control sera were used at each sampling period.

Serum samples from turkeys also were tested for MG, MS and MM with the hemagglutination inhibition (HI) test using laboratory-prepared antigens from PDRC. Samples from the chickens were tested for MG and MS by the HI test. All HI tests were performed according to Kleven and Yoder (1989). Titers ≥ 1:80 were considered positive, 1:40 as suspicious and ≤ 1:20 as negative. Serum samples from 86 wild turkeys and 15 chickens additionally were tested for MS and MG with commercial immunosorbent assay (ELISA) kits (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Maryland, USA).

Selected serum samples from wild turkeys representing a range of reactivity to MG and MS antigens by RPA were tested by immunoblotting as described by Avakian et al. (1992) with some modifications. Briefly, MG strains S6 or R, MS strain WVU-1853, and *M. gallopavonis* (serotype F, American Type Culture Collection, Rockville, Maryland, USA) proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose (NC). To block unoccupied protein binding sites, the NC was treated overnight at 4 C with either 1% calf serum plus 1% swine serum in tris buffered

saline (TBS) or 1.5% non-fat dry milk. Positive (National Veterinary Service Laboratory, Ames, Iowa, USA), negative (in-house mycoplasma-free turkey serum) and test sera were diluted 1:100 in TBS containing 0.75% non-fat dry milk. The NC was incubated with diluted sera for 1.5 hr at room temperature and washed three times with TBS containing 0.05% TWEEN-20 (Bio-Rad, Hercules, California, USA). The NC was then incubated at room temperature with anti-turkey horseradish peroxidase conjugate (Kirkegaard and Perry Laboratories, Inc.) for 1.5 hr, washed as above, and developed with TMB Membrane Peroxidase Substrate (Kirkegaard and Perry Laboratories, Inc.). In 1992, 29 wild turkey samples were tested by immunoblotting against MG antigens; of those samples, 18 also were tested against MS antigens and 6 against *M. gallopavonis*. In 1993, 12 wild turkey samples were tested by immunoblotting against both MG and MS antigens.

Broth cultures were incubated at 37 C and inoculated to FMS agar after 7 and 14 da or when a color change was evident. After incubation at 37 C for 5 da, plates were examined for mycoplasma growth, and colonies were identified with a direct fluorescent antibody (FA) technique (Talkington and Kleven, 1983). All mycoplasma isolates from wild turkeys were tested for MG and a subsample for MS and MM by FA. All isolates from the chickens were tested for MG and MS by FA. If isolates from the chickens or turkeys were negative for these organisms, further attempts were made to identify them using conjugates for *Acholeplasma laidlawii* and the following *Mycoplasma* spp.: *M. gallinarum*, *M. pullorum*, *M. gallinaceum*, *M. gallopavonis*, *M. iners*, *M. iowae*, *M. cloacale*, *M. lipofaciens*, *M. glycophilum*, *M. columbinum*, *M. columborale* and *M. columbinasale*.

Differences in the proportion of positive and negative RPA-reactors between years, age classes and types of antigens were tested with 2×2 contingency tables unless some values were $<$ five, in which case the data were compared using Fisher's Exact Test (Siegel and Castellan, 1988).

A contact transmission study was conducted for 12 wk during February-April 1994 to determine if MG- and MS-free domestic turkeys would become infected when co-housed with wild turkeys that were RPA-positive for MG. Twenty-eight wild turkeys trapped at the Hittle Ranch were tested for MG and MS by RPA, and five negative and five positive birds were selected for the study. Samples were then mailed overnight express to SCWDS for HI and ELISA testing. Seven-wk-old domestic turkeys obtained from Longmont Foods Hatchery (Longmont, Colorado, USA) were divided into two groups of 10 and housed separately in isolation facilities at a private animal research center near Fort Collins, Colorado (USA). Blood samples were collected and tested for MG, MS and MM by RPA, HI and ELISA. One group (treatment) was housed with the five MG seropositive wild turkeys, and the other group (control) was housed with the five MG seronegative wild turkeys. All turkeys were bled and weighed at 2, 6 and 12 wk post-contact (PC), and tracheal cultures were taken at 2 and 6 wk PC in FMS with and without antisera to *M. gallopavonis*. Samples were shipped to SCWDS for testing as described before. Three individual and 9 pooled samples each containing serum from two or three birds were tested by immunoblotting for antibodies to MG and MS whole-cell proteins at 0 (wild turkeys) and 12 wk (wild and domestic turkeys) PC. Samples were pooled as follows: MG-seropositive wild turkeys, MG-seronegative wild turkeys, treatment domestic turkeys and control domestic turkeys. At 12 wk PC, all birds were necropsied and examined for gross lesions. Air sacs were examined for lesions and cultured in FMS. Tissue samples were collected from the lungs, trachea and reproductive organs, frozen at −70 C, and shipped on dry ice to SCWDS for culture (Kleven and Yoder, 1989).

RESULTS

Blood samples were obtained from 119 female wild turkeys trapped in 1992 (n = 26 adults and 35 subadults) and 1993 (n = 30 adults and 28 subadults). All birds examined appeared to be in good physical condition with no apparent clinical signs of mycoplasmosis.

Based on RPA tests, the prevalence of antibodies to pathogenic mycoplasmas increased from 1992 to 1993 (Table 1). More positive RPA reactions were obtained with commercial antigens than with laboratory-prepared antigens for MS ($P < 0.01$) but not MG ($P = 0.36$) (Table 1). When the strongest reaction was used regardless of the antigen yielding that reaction, seroprevalence rates for MG and MS were 20 and 39%, respectively (Table 2). Fifty-one (43%) birds had positive RPA reactions to MG and/or MS; 5 reacted to MG only, 27 to MS only and 19 to both MG and MS. The proportion of MG/MS positive reac-

TABLE 1. Comparison of annual prevalence of antibodies to *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS) and *M. meleagridis* (MM) in wild turkeys from Colorado tested by rapid plate agglutination (RPA) using laboratory-prepared (1) and commercial (2) antigens.

RPA Antigen	1992	1993	Combined 1992–1993
MG-1	1/61[a]	14/58	15/119
MG-2	3/61	17/58	20/119
MS-1	13/61	5/58	18/119
MS-2	14/61	27/58	41/119
MM-1	0/61	—	—
MM-2	—	17/58	—

[a] Data expressed as number positive/number tested.

TABLE 2. Seroprevalence of *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS) and *M. meleagridis* (MM) in wild turkeys from Colorado by rapid plate agglutination (RPA), hemagglutination inhibition (HI), and enzyme-linked immunosorbent (ELISA) tests, 1992–1993.

Pathogen	RPA			HI	ELISA
	Adult	Subadult	Total		
MG	12/56[a]	12/63	24/119	0/119	0/86
MS	19/56	27/63	46/119	0/119	0/86
MM	12/56	5/63	17/119	0/119	0/86

[a] Data expressed as number positive/number tested.

tors did not differ between years (P = 0.09) or age class (P = 0.19); 37% of the adults and 48% of the subadults were classified as positive reactors to MG and/or MS. The seroprevalence rate for MM was 14% (Table 2). No turkeys tested positive for MM in 1992 when the laboratory antigen was used. In 1993, 17 (12 adults and 5 subadults) positive reactions for MM were recorded when tests were performed using the commercial antigen (Table 1).

Results of the HI and ELISA tests did not agree with the RPA test results (Table 2). The HI tests were uniformly negative for MG, MS and MM, with the exception of one suspicious HI titer of 1:40 for MM. None of 86 samples tested positive by ELISA for MG, MS or MM, and only 16 samples produced reactions considered suspicious (MG = 1, MS = 4, MM = 9, MS and MM = 2).

Wild turkey serum samples tested by immunoblotting in 1992 showed mild to moderate reactivity to MG, moderate reactivity to *M. gallopavonis* and mild or no reactivity to MS. Half of 12 wild turkey sera collected in 1993 were moderately reactive to MG and mildly reactive to MS proteins (Fig. 1).

Fifty isolates of *Mycoplasma* spp. were made from 56 tracheal cultures. All isolates were tested for MG and found to be negative, including those isolates grown from cultures initially inoculated into FMS containing antisera to *M. gallopavonis*. Twenty-eight isolates also were tested for MS, MM, *Acholeplasma laidlawii* and 12 other *Mycoplasma* spp.; 12 were identified as *M. gallopavonis*, six as *M. gallinaceum* and 10 could not be identified.

Twenty-five of 31 chickens were strongly

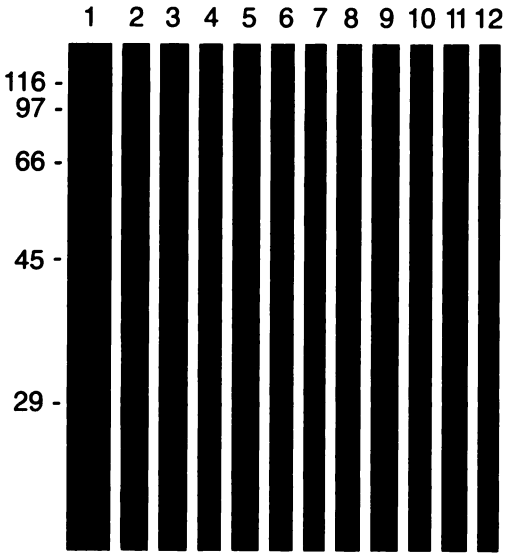

FIGURE 1. Immunoblots to MS strain WVU-1853 using wild turkey sera from birds captured in Mesa County, Colorado (USA) in 1993: lane 1 = molecular weight standard in kDa; lane 2 = NVSL positive control domestic turkey anti-MS polyclonal serum; lane 3 = in-house negative control domestic turkey serum; lanes 4-12 = individual serum samples from captured wild turkeys. These results are representative of those seen for wild turkey sera immunoblots to MS, showing little or no antibody reactivity. The protein band between 97 and 66 kDa was seen for all sera including the MS negative control; therefore, it is considered to be a non-specific reaction.

TABLE 3. Rapid plate agglutination (RPA) results for wild and domestic turkeys tested for antibodies to *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS), and *M. meleagridis* (MM) in a contact transmission study conducted in 1994. Wild turkeys were selected based on initial MG-RPA results.

Weeks post-contact (PC)	Wild turkeys			Domestic turkeys		
	MG	MS	MM	MG	MS	MM
Seronegative Wild Turkey Group						
0	0/5[a]	0/5	3/5	0/10	0/10	0/10
2	1/5	1/5	1/5	0/10	0/10	0/10
6	0/5	0/5	0/5	0/10	0/10	0/10
12	1/5	0/5	0/5	0/10	0/10	0/10
Seropositive Wild Turkey Group[b]						
0	5/5	0/5[c]	3/5	0/10	0/10	0/10
2	0/3	0/3	0/3	1/10	0/10	0/10
6	0/3	0/3	0/3	0/10	0/10	0/10
12	2/3[d]	0/3	0/3	0/9	0/9[c]	0/9

[a] Data expressed as number positive/number tested.
[b] Two wild turkeys in the seropositive group escaped at 12 da PC and one domestic turkey died of an unknown cause at 9 wk PC.
[c] One bird positive for MS by ELISA.
[d] One bird positive for MG by ELISA.

positive (RPA score ≥ 3) for MS, and 15 were positive for MG. The HI and ELISA tests supported the RPA tests for MS, but not MG. Eighteen of 31 samples had HI titers ≥ 1:80 for MS, whereas no samples had positive HI titers for MG. Of 15 samples tested by ELISA, 12 were positive for MS and none for MG. *Mycoplasma* spp. were isolated from all 15 tracheal cultures collected from the chickens, but no pathogenic mycoplasmas were identified. Upon further testing two were identified as *M. gallinaceum*, one as *M. pullorum* and one as *M. gallinarum*.

Throughout the contact transmission study, domestic turkeys were seronegative for MG, MS and MM by all tests, with two exceptions (Table 3). One bird tested positive on the MG-RPA test at 2 wk PC and another tested positive for MS by ELISA at 12 wk PC. Both these birds were housed with the MG-seropositive wild turkeys.

Wild turkeys had inconsistent RPA test results during the transmission study (Table 3). Seropositive wild turkeys tested negative for MG one or more times during the trial, and subsequent positive reactions were weaker than the initial field test reactions. In addition, two wild turkeys that initially tested negative were positive when sampled during the trial. Only one bird had a positive reaction to MS, whereas several birds in both groups initially had reactions to MM. All birds were negative by HI for MG, MS and MM. Most birds also were negative by ELISA except for one bird that was positive for MS at 0 wk PC and another that was positive for MG at 12 wk PC.

There was no reactivity to MS among 12 samples consisting of individual and pooled sera from wild (n = 7) and domestic turkeys (n = 5) that were tested by immunoblotting. Likewise, none of the samples from domestic turkeys showed reactivity to MG, whereas all of the samples from wild turkeys showed mild to moderate reactivity to MG (Fig. 2).

Pathogenic mycoplasmas were not isolated from wild or domestic turkeys at anytime during the transmission study, nor did any exhibit clinical signs of mycoplasmosis. *Mycoplasma gallopavonis* was isolated from every turkey at 2 wk PC. Therefore, antisera to *M. gallopavonis* was added to the culture media during subsequent sampling periods. At 6 wk PC, six of eight cultures from wild turkeys and 12 of 20 cultures from domestic turkeys had mycoplasmal growth. Of the 6 isolates from wild turkeys, two were identified as *M. gallopavonis*, two as *M. cloacale* and one as *M. iners*; one isolate remained unidentified. Seven isolates from the domestic birds were identified as *M. gallopavonis*, and the remaining five could not be identified. No cultures collected at 12 wk PC produced mycoplasmal growth.

DISCUSSION

Collectively, results from all testing methods were inconclusive with regard to the status of MG, MS and MM within this population of Merriam's wild turkeys. The

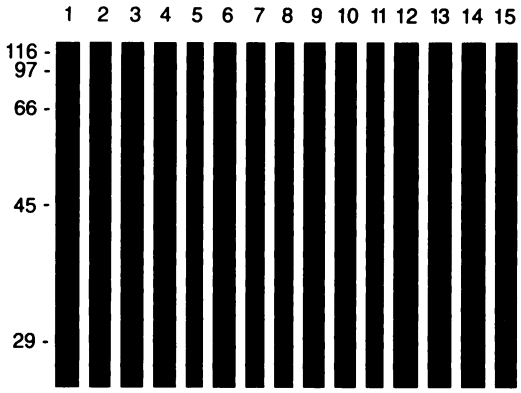

FIGURE 2. Immunoblots to MG strain R: lane 1 = molecular weight standard in kDa; lane 2 = NVSL positive control domestic turkey anti-MG polyclonal serum; lane 3 = in-house negative control domestic turkey serum; lane 4 = individual domestic turkey serum, control group (12 wk PC); lanes 5 and 7 = pooled domestic turkey sera, control group (12 wk PC); lanes 6 and 8 = pooled domestic turkey sera, treatment group (12 wk PC); lane 9 = individual wild turkey serum, treatment group (12 wk PC); lane 10 = individual wild turkey serum, control group (12 wk PC); lanes 11 and 12 = pooled wild turkey sera, control group (12 wk PC); lane 13 = pooled wild turkey sera, treatment group (12 wk PC); lane 14 = pooled wild turkey sera, control group (0 wk PC); lane 15 = pooled wild turkey sera, treatment group (0 wk PC). Domestic turkey sera show mild or no reactivity to MG proteins. Wild turkey sera show mild to moderate reactivity to MG proteins.

four serologic tests used (RPA, HI, ELISA and immunoblotting) produced mixed and inconsistent results, and attempts to confirm infection through culture or contact transmission were unsuccessful. These results were not unexpected since several other studies have either failed to confirm or had difficulty confirming infection among populations of wild turkeys with RPA reactions to MG, MS or MM. Rocke and Yuill (1987) reported numerous RPA reactors to MG, MS and MM among 480 Rio Grande wild turkeys sampled from five locations in Texas (USA). However, HI tests were uniformly negative, and no pathogenic mycoplasmas were identified among 215 isolates. Fritz et al. (1992) reported many RPA reactors to MG, MS and MM among 724 Merriam's and Rio Grande wild turkeys surveyed from six states, but found few (20 of 664 samples tested) positive HI titers for MG and none for MS; HI testing was not done for MM. Their data indicated a lack of correspondence between MG-RPA and HI results for 414 unfrozen samples. Of 81 *Mycoplasma* spp. isolates identified by Fritz et al. (1992), one MG isolate was obtained from a Rio Grande turkey in Texas and five MS isolates were obtained from a single population of Merriam's wild turkeys in Arizona (USA). In contrast to Merriam's and Rio Grande turkeys, serologic testing of 882 clinically normal eastern wild turkeys from eight states disclosed few seroreactors to MG, MS or MM, nor were any pathogenic mycoplasmas identified among 511 of 572 isolates obtained from culturing these birds (Davidson et al., 1988; Luttrell et al., 1991, 1992; Cobb et al., 1992).

Because previous studies typically had been unable to confirm MG, MS or MM infection among RPA-positive wild turkeys through HI testing or culture, we incorporated ELISA and immunoblotting as additional confirmatory serologic tests in our diagnostic protocol. The ELISA test, considered more sensitive than the HI when applied to domestic poultry (Avakian et al., 1988), also failed to confirm RPA results. Immunoblotting is generally regarded as a sensitive and highly specific adjunct to other serological tests, because it is capable of showing antibody reactivity to a range of proteins possessed by infectious agents, some of which may be considered species specific. For example, p64 and p56 have been described as immunodominant MG-specific proteins (Avakian et al., 1992). Sera that react with MG proteins in immunoblots, especially to p64 and p56, would most likely have originated from hosts currently or previously infected with MG. In the present study, each set of serum samples from wild turkeys tested by immunoblotting showed mild to moderate reactivity to MG proteins (Fig. 2). This pattern of reactivity was not strong, but it clearly and consistently indicated that sera from the wild turkeys contained antibodies

to MG proteins. This antibody reactivity to MG proteins also was reflected in positive RPA tests. The RPA test is generally regarded as more sensitive but less specific than HI and ELISA tests (Avakian et al., 1988). Therefore, antibody reactivity to MG proteins as seen in the immunoblots would more likely be detected by RPA than HI or ELISA tests. Additionally, the wild turkey MG immunoblot patterns were similar to those seen in domestic turkeys following experimental infections with variant strains of MG, which reacted with less intensity and to fewer proteins than turkeys infected with a reference pathogenic strain (Avakian et al., 1992).

The aggregate of MG serologic results obtained for wild turkeys in this study suggests two possibilities: (1) wild turkeys were infected with MG and the serologic response was detected only by immunoblotting and RPA tests, or (2) wild turkeys were not infected with MG and serologic reactivity shown by immunoblotting and RPA tests was non-specific or cross reactive (i.e., not indicative of exposure to MG). Unfortunately, there is not sufficient data to eliminate either possibility. The ideal standard for MG diagnosis is culture and isolation of the organism. However, failure to culture MG, especially from seropositive birds, does not exclude the possibility of previous or even current infection. This is particularly true for birds that may be chronically infected, and/or infected with other *Mycoplasma* spp., as in the present case. *Mycoplasma gallopavonis* commonly was isolated from the wild turkeys in this study, and a mild to moderate antibody response to *M. gallopavonis* proteins was seen on immunoblots. Therefore, antisera to *M. gallopavonis* was included in some media to inhibit its growth and improve the opportunity to culture MG from wild turkeys. Although this measure did not result in any MG isolates, it does not exclude the possibility of MG exposure. It also is possible that antibodies to *M. gallopavonis* (or some other *Mycoplasma* spp. or other microorganism) could cross react with MG proteins resulting in "false positive" immunoblot and RPA tests. Additional research is necessary to definitively explore this possibility. However, in a pilot study, hyperimmunized rabbit antiserum to *M. gallopavonis* showed very little reactivity to MG proteins on immunoblots, suggesting that serologic cross reactivity between these two *Mycoplasma* spp. is not likely.

All serologic data from domestic chickens indicated they were infected with MS, but we could not isolate this organism from tracheal cultures. Luttrell et al. (1991) was unable to isolate MG from the tracheas of strongly seropositive chickens on Cumberland Island, Georgia (USA). They suggested the chickens were in a carrier state, having previously been infected with MG, and that the organism had become localized in the lungs or reproductive tissue where it could not be readily transmitted to other birds or detected using tracheal swabs. In addition, the presence of other fast-growing *Mycoplasma* spp., such as *M. gallinaceum*, makes isolation of MG or MS more difficult.

Whenever pathogenic mycoplasmas have been isolated from wild turkeys, a domestic source of infection has been implicated (Davidson et al., 1982; Jessup et al., 1983; Adrian, 1984). For free-ranging wild turkey populations, association with domestic fowl does not appear to have a negative impact on population health (Rocke and Yuill, 1987; Luttrell et al., 1991). Findings from a related study on the population we surveyed supports this conclusion (Hoffman et al., 1995). In that study, body weight, nesting effort, clutch size, nesting success, fertility and hatching success did not differ between MG/MS seropositive and seronegative hens.

Another finding consistent with previous surveys of wild turkeys was the frequent isolation of *M. gallopavonis*, which has been identified in Merriam's, Rio Grande and eastern wild turkeys from widely separated geographic areas (Rocke and Yuill, 1987; Luttrell et al., 1991, 1992;

Cobb et al., 1992; Fritz et al., 1992). The common occurrence of *M. gallopavonis* in this study supports the belief that this species is a ubiquitous and presumably nonpathogenic microorganism of wild turkey populations throughout the United States (Cobb et al., 1992; Luttrell et al., 1992). Isolation of unidentified mycoplasmas also was consistent with previous surveys of wild turkeys (Rocke and Yuill, 1987; Cobb et al., 1992; Fritz et al., 1992; Luttrell et al., 1992). This report, however, presents the first confirmation of infections by *M. gallinaceum*, *M. iners* and *M. cloacale* among wild turkeys; these organisms are not known to cause disease in poultry (Dierks et al., 1967; Bradbury and Forrest, 1984).

Because evidence of MG, MS or MM infection in clinically normal wild turkeys primarily has been restricted to equivocal, unconfirmed, RPA reactions (Hensley and Cain, 1979; Amundson, 1985; Rocke and Yuill, 1987; Davidson et al., 1988; Luttrell et al., 1991; Cobb et al., 1992; Fritz et al., 1992), there have been conflicting interpretations of these data. Some investigators suggest that MG, MS and MM are present in RPA-positive populations (Fritz et al., 1992) and that wild turkeys may serve as reservoirs of these mycoplasmas (Rocke and Yuill, 1987; Fritz et al., 1992). Others contend that without isolation, positive RPA reactions do not necessarily indicate current infection (Davidson, 1987; Davidson et al., 1988; Luttrell et al., 1991; Cobb et al., 1992). Regardless, all these investigators acknowledged that numerous factors may influence serologic test results or the ability to isolate MG, MS or MM from wild turkeys. Few of these factors have been experimentally investigated. Previous studies (Rocke and Yuill, 1988; Rocke et al., 1985, 1988) showed that the response of wild turkeys to experimental infection with reference strains of MG, and evaluation of the infection by standard diagnostic tests, did not differ substantially from those of domestic turkeys. However, no experimental studies have addressed the possible occurrence of variant strains of MG among wild turkeys or the possibility that positive RPA reactions may represent nonspecific reactions caused by the presence of other microorganisms.

The rationale for screening wild turkeys for pathogenic mycoplasmas is to avoid introducing disease into new or existing populations during restoration or range expansion programs and to prevent such programs, especially those involving interstate translocations, from being falsely blamed for future occurrences of mycoplasmosis among domestic poultry (Amundson, 1985; Davidson, 1987). Serologic testing of eastern wild turkeys has produced data indicating little risk of spreading MG, MS or MM through restoration programs. However, the situation regarding western subspecies remains uncertain and controversial.

With one exception (Adrian, 1984), a consistent finding has been that seropositive wild turkey populations have not experienced adverse impacts attributable to mycoplasmosis (Rocke and Yuill, 1987; Fritz et al., 1992; Hoffman et al. 1995). This suggests that the MG, MS and MM seroreactions among wild turkeys are not due to pathogenic strains. If this assumption is correct, than the RPA reactions could be due to variant, nonpathogenic strains or the reactions could be nonspecific cross-reactions caused by other microbes prevalent in wild turkeys. These two scenarios are not mutually exclusive, and false-positive RPA results due to testing protocol problems are possible in either situation.

The current equivocal data leaves wildlife agencies in a dilemma when setting disease management policy for wild turkey restoration programs. Relocation of RPA-positive turkeys could be rationalized by assuming that clinical mycoplasmosis would not result from moving turkeys harboring either variant strains of MG and MS or potential cross-reacting microbes. However, this approach entails the risk of relocating the rare turkey that may actually

harbor pathogenic strains capable of producing clinical mycoplasmosis. Furthermore, domestic poultry interests are concerned about pathogenic and nonpathogenic strains of MG, MS and MM, because both types must be investigated when antibodies are detected during surveillance programs. Until causes of seroreactions to MG and MS can be better defined, we urge that caution be exercised with restoration efforts for Merriam's and Rio Grande turkeys. We recommend that future research emphasize experimental approaches to investigate the possibility of variant strains or cross-reactions as causes of RPA reactions in wild turkeys. Utilization of improved organism detection methods such as polymerase chain reaction also should be an aid in confirming results. Application of routine diagnostic tests in additional field surveys, as in this study, offer little chance of resolving this issue.

ACKNOWLEDGMENTS

We are grateful to L. Hittle for allowing access onto his property and to S. H. Kleven at the University of Georgia for use of laboratory facilities. Numerous employees of the Colorado Division of Wildlife provided assistance including R. T. Magill, A. W. Hoag, A. Clements, R. D. Piccolo and T. A. Artiss. Early drafts of the manuscript were reviewed by C. E. Braun, W. J. Adrian and M. A. Wild. This work was supported by the Colorado Division of Wildlife through Federal Aid in Wildlife Restoration Project W-167-R.

LITERATURE CITED

ADRIAN, W. J. 1984. Investigation of disease as a limiting factor in wild turkey populations. Ph. D. Dissertation, Colorado State University, Fort Collins, Colorado, 61 pp.

AMUNDSON, T. E. 1985. Health management in wild turkey restoration programs. Proceedings National Wild Turkey Symposium 5: 285–294.

AVAKIAN, A. P., S. H. KLEVEN, AND J. R. GLISSON. 1988. Evaluation of the specificity and sensitivity of two commercial enzyme-linked immunosorbent assay kits, the serum plate agglutination test, and the hemagglutination inhibition test for antibodies formed in response to *Mycoplasma gallisepticum*. Avian Diseases 32: 262–272.

———, D. H. LEY, AND M. A. T. MCBRIDE. 1992. Humoral immune response of turkeys to strain S6 and a variant *Mycoplasma gallisepticum* studied by immunoblotting. Avian Diseases 36: 69–77.

BRADBURY, J. M., AND M. FORREST. 1984. *Mycoplasma cloacale*, a new species isolated from a turkey. International Journal of Systematic Bacteriology 34: 389–392.

COBB, D. T., D. H. LEY, AND P. H. DOERR. 1992. Isolation of *Mycoplasma gallopavonis* from free-ranging wild turkeys in coastal North Carolina seropositive and culture-negative for *Mycoplasma gallisepticum*. Journal of Wildlife Diseases 28: 105–109.

DAVIDSON, W. R. 1987. Disease monitoring in wild turkey restoration programs. Proceedings of the Western Association of Fish and Wildlife Agencies 67: 113–118.

———, V. F. NETTLES, C. E. COUVILLION, AND H. W. YODER, JR. 1982. Infectious sinusitis in turkeys. Avian Diseases 26: 402–405.

———, H. W. YODER, M. BRUGH, AND V. F. NETTLES. 1988. Serological monitoring of eastern wild turkeys for antibodies to *Mycoplasma* spp. and avian influenza viruses. Journal Wildlife Diseases 24: 348–351.

DIERKS, R. E., J. A. NEWMAN, AND B. S. POMEROY. 1967. Characterization of avian mycoplasma. Annals New York Academy of Science 143: 170–189.

FRITZ, B. A., C. B. THOMAS, AND T. M. YUILL. 1992. Serological and microbial survey of *Mycoplasma gallisepticum* in wild turkeys (*Meleagris gallopavo*) from six western states. Journal of Wildlife Diseases 28: 10–20.

HENSLEY, T. S., AND J. R. CAIN. 1979. Prevalence of certain antibodies to selected disease-causing agents in wild turkeys in Texas. Avian Diseases 23: 62–69.

HOFFMAN, R. W., M. P. LUTTRELL, AND W. R. DAVIDSON. 1995. Reproductive performance of Merriam's wild turkeys with suspected *Mycoplasma* infection. Proceedings National Wild Turkey Symposium 7: 145–151.

JESSUP, D. A., A. J. DAMASSA, R. LEWIS, AND K. R. JONES. 1983. *Mycoplasma gallisepticum* infection in wild-type turkeys living in close contact with domestic fowl. Journal of the American Veterinary Medical Association 183: 1245–1247.

KLEVEN, S. H., AND H. W. YODER, JR. 1989. Mycoplasmosis. *In* A laboratory manual for the isolation and identification of avian pathogens, 3rd ed., H. G. Purchase, L. H. Arp, C. H. Domermuth, and J. E. Pearson (eds.). American Association of Avian Pathologists, Kennett Square, Pennsylvania, pp. 57–62.

———, G. N. ROWLAND, AND N. O. OLSON. 1991. *Mycoplasma synoviae* infection. *In* Diseases of Poultry, 9th ed., B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr.

(eds.). Iowa State Press, Ames, Iowa, pp. 223–231.

LUTTRELL, M. P., T. H. ELEASER, AND S. H. KLEVEN. 1992. *Mycoplasma gallopavonis* in eastern wild turkeys. Journal of Wildlife Diseases 28: 288–291.

———, S. H. KLEVEN, AND W. R. DAVIDSON. 1991. An investigation of the persistence of *Mycoplasma gallisepticum* in an eastern population of wild turkeys. Journal of Wildlife Diseases 27: 74–80.

NETTLES, V. F. 1984. Report of the fish and wildlife health committee. Proceedings of the International Association of Fish and Wildlife Agencies 74: 89–101.

———, AND E. T. THORNE. 1982. Annual report of the wildlife disease committee. Proceedings of the United States Animal Health Association 86: 64–65.

ROCKE, T. E., AND T. M. YUILL. 1987. Microbial infections in a declining wild turkey population in Texas. The Journal of Wildlife Management 51: 778–782.

———, AND ———. 1988. Serologic response of Rio Grande wild turkeys to experimental infections of *Mycoplasma gallisepticum*. Journal of Wildlife Diseases 24: 668–671.

———, ———, AND T. E. AMUNDSON. 1985. Evaluation of serologic tests for *Mycoplasma gallisepticum* in wild turkeys. Journal of Wildlife Diseases 21: 58–61.

———, ———, AND ———. 1988. Experimental *Mycoplasma gallisepticum* infections in captive-reared wild turkeys. Journal of Wildlife Diseases 24: 528–532.

SIEGEL, S., AND N. J. CASTELLAN. 1988. Nonparametric statistics for the behavioral sciences, 2nd ed. McGraw-Hill Book Company, New York, New York, 399 pp.

TALKINGTON, F. D., AND S. H. KLEVEN. 1983. A classification of laboratory strains of avian mycoplasma serotypes by direct immunofluorescence. Avian Diseases 27: 422–429.

YAMAMOTO, R. 1991. *Mycoplasma meleagridis* infection. *In* Diseases of Poultry, 9th ed., B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. (eds.). Iowa State Press, Ames, Iowa, pp. 212–223.

YODER, H. S., JR. 1991. *Mycoplasma gallisepticum* infection. *In* Diseases of Poultry, 9th ed., B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. (eds.). Iowa State University Press, Ames, Iowa, pp. 198–212.

Received for publication 20 December 1996.