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SEROLOGICAL AND MICROBIAL SURVEY OF *MYCOPLASMA GALLISEPTICUM* IN WILD TURKEYS (*MELEAGRIS GALLOPAVO*) FROM SIX WESTERN STATES

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ABSTRACT: From 1986 to 1989, sera from wild turkeys (*Meleagris gallopavo*), including three subspecies (*M. gallopavo intermedia, M. gallopavo merriami* and *M. gallopavo mexicana*) trapped in six western states were tested for antibody to *Mycoplasma gallisepticum* (MG) (n = 724), *M. synoviae* (MS) (n = 461) and *M. meleagridis* (MM) (n = 354) using the rapid plate agglutination (RPA) assay. Subsamples of these sera were also evaluated using the hemagglutination inhibition (HI) assay for antibody to MG (n = 664) and MS (n = 403). Attempts were made to isolate mycoplasmas by swabbing the trachea and cloaca of 190 live wild turkeys and from various tissues (sinus, nasal turbinates, trachea, lung, ovaries and oviduct) from 76 turkeys at necropsy. Isolates were identified using an immunobinding assay. Seroprevalence of MG, MS and MM in the RPA test was highly variable among years and geographic sites, ranging from 0 to 85%, 0 to 87%, and 0 to 83%, respectively, for each mycoplasma species. Of the 724 wild turkey sera tested, 200 (28%) were positive using the RPA assay, while only 20 (3%) of 664 sera tested using the HI assay were positive (at a titer >/= 1:80) for antibody to MG. Of the 461 sera tested 178 (39%) were RPA positive for MS, whereas none of the 403 samples tested by HI were positive for MS. Antibody to MM was detected in 72 (20%) of 354 turkey sera tested by RPA. Mycoplasmas were cultured from 81 (30%) of 266 wild turkeys, including 48 that were sampled live and 33 that were examined by necropsy. Mycoplasmas were isolated from every population in which culture was attempted. *M. gallopavonis* (MGP) was isolated from 37 (46%) of 81 birds which yielded mycoplasma, representing seven of 12 populations sampled. MG was isolated from lower respiratory tissues of one Rio Grande wild turkey trapped in Texas. *M. synoviae* was isolated from five of 16 Merriam's wild turkeys trapped in Arizona. Sera of birds from which MG or MS was isolated were positive to the respective antigen in the RPA test, but were negative by the HI assay. The RPA test was effective in identifying MG and MS infected turkeys despite lack of confirmation by the HI test. These data suggest that apparently healthy wild turkeys can carry pathogenic mycoplasmas and the currently used field test (RPA) can identify culture positive wild turkeys. Serological screening using the RPA test should be conducted on all wild turkeys prior to relocation.

Key words: Wild turkeys, *Meleagris gallopavo*, mycoplasmas, *Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma gallopavonis, Mycoplasma meleagridis*, serosurvey, hemagglutination inhibition assay.

INTRODUCTION

The potential for apparently healthy wild turkeys to carry and disseminate MG concerns many state wildlife agencies and has restricted wild turkey relocation programs (Amundson, 1985). Transmission of pathogenic mycoplasmas to uninfected wild turkey populations or domestic poultry operations could occur by inadvertent relocation of MG-carrier wild turkeys. Domestic turkeys are more susceptible to the pathogenic effects of MG than are chickens (Jordan, 1979). A consistent sign of MG infection in domestic turkeys is inflammation and swelling of the infraorbital sinuses, a condition known as "infectious sinusitis" which may lead to partial or complete closure of the eye (Yoder, 1984). Even when respiratory disease is mild in affected flocks, infection persists and reproductive performance (egg production) declines (Yoder, 1984). In wild and game farm turkeys experimentally infected with MG, Rocke and Yuill (1988) and Rocke et al. (1988) reported significant morbidity and a prolonged MG carrier state in Rio Grande wild turkeys and decreased reproduction and fertility in captive reared wild turkeys.

Serological surveys have indicated that

MG exposure was detectable in free-ranging and semi-domesticated wild turkey populations (Hensley and Cain, 1979; Schmitt and Cooley, 1981; Davidson et al., 1982; Jessup et al., 1983; Adrian, 1984; Amundson, 1985; Rocke and Yuill, 1987; Luttrell, 1989). However, microbial isolation of MG from wild turkeys has been reported on only three occasions (Davidson et al., 1982; Jessup et al., 1983; Adrian, 1984), each associated with signs of infectious sinusitis or a history of population decline. These findings led to the advancement of disease monitoring guidelines (Nettles and Thorne, 1982; Nettles, 1984; Amundson, 1985) and prompted state wildlife agencies to initiate testing of wild turkeys trapped for relocation. Consequently, several state agencies have since detected RPA reactions to MG antigen from apparently healthy wild turkeys (Amundson, 1985). The significance and validity of positive RPA results have been questioned for three reasons: (1) subclinical or latent infection in domestic turkeys had not been demonstrated until recently (Ley et al., 1990) and was therefore thought unlikely in wild turkeys (Luttrell, 1989); (2) attempts to confirm the presence of specific antibody using the HI assay failed (Rocke and Yuill, 1987; Luttrell, 1989); and (3) microbial isolation of MG from symptomless free-ranging reactor birds has never been reported. We report on a serologic and microbial survey of wild turkey populations purposefully selected to include flocks from geographic locations with prior serologic evidence or suspicion of mycoplasmosis. Our objectives were to determine the seroprevalence to MG, MS and MM antigens and to ascertain whether microbial isolation of these pathogens was possible from healthy, free-ranging wild turkeys.

MATERIAL AND METHODS

Study area and population history

Two subspecies of wild turkeys, Rio Grande (*Meleagris gallopavo intermedia*) and Merriam's (*M. gallopavo merriami*), were trapped between 1986 to 1989 in cooperation with state wildlife agency programs. Twelve sites were chosen in five southwestern states of the USA, based on one or more of the following criteria: (1) previous serologic evidence of exposure to mycoplasmas and specifically MG, (2) previous history of disease associated with avian mycoplasmas, or (3) census information indicating decline in wild turkey populations. Turkeys were trapped in the winter using rocket or drop nets. Merriam's turkeys were trapped in Arizona, Colorado and New Mexico. Rio Grande turkeys were trapped in Oklahoma and Texas (Fig. 1). Serologic samples were also obtained in 1986 and 1987 from hunter killed Merriam's turkeys and Merriam's turkeys captured at four sites in 1988–89 by North Dakota Game and Fish personnel. Samples obtained from Merriam's turkeys captured in 1989 in Arizona and Colorado were also submitted by Game and Fish personnel. In 1988, personnel from the New Mexico Game and Fish submitted tracheal and cloacal swabs from Gould's wild turkeys (*M. gallopavo mexicana*) for microbial testing.

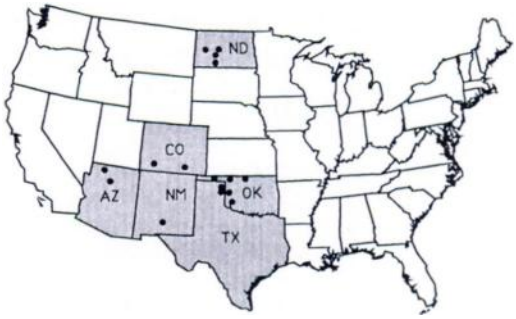

FIGURE 1. Geographic locations from which microbial or serologic samples were obtained from wild turkeys: Arizona, Kaibab (36°32'N, 112°32'W) and Chevalon (34°24'N, 110°44'W); Colorado, Trinidad (37°05'N, 104°37'W) and Durango (37°33'N, 107°57'W); North Dakota, Flocks A–D were trapped in Morton (46°36'N, 100°46'W), Dunn (47°20'N, 103°05'W), Oliver (47°10'N, 100°59'W), and Grant (46°35'N, 101°31'W) counties respectively; New Mexico, Karr Canyon (32°56'N, 105°50'W); Oklahoma, Salt Plain Wildlife Refuge (36°47'N, 98°11'W), Mangum (34°55'N, 99°31'W), Erick (35°17'N, 99°57'W), Beaver (36°50'N, 100°42'W), and Buffalo (36°50'N, 99°31'W); Texas, Wheeler (35°25'N, 100°07'W) and Shamrock (35°14'N, 100°08'W).

Sample collection and testing

Blood was collected from wild turkeys by jugular venipuncture into heparin rinsed syringes, and plasma was separated by centrifugation. Antibody to MG and MS was detected using two assays, the rapid plate agglutination (RPA) test

and the hemagglutination inhibition (HI) test. Antibody to MM was detected by the RPA test only. Where possible the RPA test was conducted in the field on fresh plasma. Hemagglutination inhibition assays were conducted in the laboratory, on aliquots of serum or plasma which had been frozen (20 C), as were RPA assays on samples submitted from wildlife agencies.

The RPA assay was conducted using methods and controls recommended by the manufacturer (Salsbury Laboratories, Inc., Charles City, Iowa 50616, USA). RPA reactions were scored using the following criteria: samples with no agglutination were scored (−), samples with slight agglutination were scored (+/−), and samples with clearly distinguishable agglutination were scored (+). According to the National Poultry Improvement Plan (NPIP), only samples scored (+) are positive and those scored (+/−) are considered negative or represent nonspecific agglutination reactions (USDA, 1984).

Microtiter HI tests (Williams, 1980) were performed with antigens obtained from the National Veterinary Services Laboratory (Ames, Iowa 50010, USA). An HI titer of >/= 1:80 is considered positive, and samples with a titer of 1:40 or 1:20 are considered suspect according to NPIP criteria (USDA, 1984).

Media and isolation methods changed over the course of study. Three different media were used for isolation of mycoplasmas from field material: (1) an Edward-type medium (EPJ), and (2) a modified SP4 medium (SP4-PS), have been described (Fritz et al., 1991) and (3) Frey's medium (Frey et al., 1968). The EPJ medium was used for isolation and passage of field samples. The majority of samples from the populations sampled in Arizona, Oklahoma, and Trinidad, Colorado in 1988 also were cultured in SP4-PS medium. Frey's medium was used briefly for replicate culture of samples from two populations (Kaibab, Arizona and Trinidad, Colorado in 1988).

Mycoplasma isolation was attempted from live turkeys as well as from specimens collected at postmortem examination. Sampling for isolation from live turkeys consisted of swabbing the trachea and cloaca of each bird using sterile dacron swabs and inoculating mycoplasma support media. At necropsy, swab samples of the epithelial surfaces of the cloaca, nasal turbinates, infraorbital sinuses, trachea and air sacs were obtained for microbial examination after aseptic exposure of these surfaces. Additionally, lung and reproductive tissues were obtained aseptically for microbiological examination. These samples were packed in sterile plastic bags and maintained on ice in transit to the laboratory. Lung and reproductive tissues were processed within 48 hr of collection. One lung was placed in a sterile petri dish containing 10 ml of sterile phosphate buffered saline (PBS). A section of lung extending from the second to the fifth rib groove was chosen. Individual samples were taken at the 2–3, 3–4, and 4–5 costal grooves. Pieces approximately 1 cm by 2 cm were removed and pooled in a fresh petri dish containing 3 ml of sterile mycoplasma medium. The tissues were minced with sterile scissors to approximately 2 mm by 2 mm in size. Duplicate tubes containing 7 ml of each medium (EPJ and SP4-PS or Frey's) were inoculated with 0.5 ml of tissue suspension. Ovaries and oviduct were processed similarly to lung tissue, except the preliminary wash of tissue was eliminated. Pieces of ovarian and oviduct tissue were placed in 3 ml of sterile mycoplasma media, minced and inoculated into selected media.

Broth cultures were incubated at 37 C for up to 4 wk. If growth was evident, based on pH change or turbidity, cultures were passaged onto solid medium. Inoculated plates were incubated at 37 C, in ambient atmosphere, in plastic bags containing damp paper towels. Plates were examined microscopically at low power (4X), at weekly intervals to 28 days.

Identification of isolates

The direct immunofluorescence (FA) assay (Talkington and Kleven, 1983) was used initially for mycoplasma identification, but due to colony dislodgment when using EPJ agar medium, this method was discontinued. Instead, a dot immunobinding assay (Sharp et al., 1991), capable of detecting 250 ng of homologous mycoplasma protein, was used with slight modification to determine the species of each mycoplasma isolate. Briefly, the assay was conducted on a 0.1 μm pore-size, low protein-binding polyvinylidene difluoride membrane filter (Durapore, Millipore Corp., Bedford, Massachusetts 01730, USA) placed in a 96-well microsample filtration manifold (Schleicher and Schuell, Inc., Keene, New Hampshire 03431, USA). Control and unknown antigens (50 μl), concentrated by centrifugation from 1 to 3 ml of broth medium, were deposited and fixed on the membrane by application of vacuum followed by 10% formalin for 10 min. After washing, the manifold was disassembled, and the membrane was placed in a plastic dish. Endogenous peroxidase was blocked with 20–25 ml of 0.3% H_2O_2 for 10 min followed by washing. Blocking solution (20 to 30 ml of Tris-buffered saline (TBS) and 0.02% Tween 80 (TBST) containing 10% horse serum) was added for 30 min and then removed. Next, blots were incubated for 30 min with 20 to 30 ml of polyclonal rabbit antiserum specific to the desired mycoplasma species (see below). The

typing antiserum was prepared by dilution in blocking solution, using a predetermined optimal antiserum concentration (usually 1:500 or 1:1,000). After washing, 30 ml of horseradish peroxidase conjugated goat antirabbit IgG (Cappel Division, Organon Teknika Inc., Malvern, Pennsylvania 19355, USA), diluted to 1:1,000 in blocking solution, was added and incubated for 30 min. This was followed by 2 washes using TBST and a third wash in a fresh dish using TBS without Tween 80. Next, 20 to 30 ml of a freshly prepared developing solution containing 30 mg 4-chloro-1-napthol (Sigma Chemical Co., St. Louis, Missouri 63178, USA) in 10 ml methanol, 50 ml TBS and 2 ml 0.3% H_2O_2 was added. Color development was terminated by washing in distilled water, and the membrane filter was dried. All steps were carried out at room temperature. Positive and negative control antigens were included in every blot conducted. Rabbit antiserum deletion, conjugate deletion and antigen deletion controls were included in every blot to detect nonspecific binding of conjugate and serum. Species identity was determined by visual comparison of the color intensity of the unknowns with that of reference antigens.

Typing antisera

The methods employed for production of hyperimmune rabbit anti-mycoplasma antisera have been described (Sharp et al., 1991). Strains of mycoplasmas used as immunogen in rabbits were (1) the F strain of MG (obtained from S. Branton, USDA, South Central Poultry Research Laboratory, Mississippi State, Mississippi 39762, USA), (2) *Mycoplasma gallopavonis* (MGP) (ATCC #33551), and (3) MS (ATCC #25204) obtained from the American Type Culture Collection (Rockville, Maryland 20852, USA). These typing antisera were shown to be specific for the immunogen species, and do not cross-react with each other in the dot immunobinding, or with MM or *Mycoplasma iowae* in western blot assays.

Statistical analysis

Negative predictive value (NPV) was estimated for the RPA test using HI (titers 1:80) as the standard for comparison. Agreement between the RPA and HI results for MG and MS antigens obtained on the same sera was tested using McNemar's chi square for correlated proportions. A "kappa" statistic which measures the relative excess agreement above that expected by chance was also calculated (Fleiss, 1973). The kappa statistic yields a value of zero when agreement between two tests is no greater than expected by chance; a value of 1 indicates perfect agreement. Chi square analysis was used when testing for association. Significance was defined as $P < 0.05$.

RESULTS

Serology

Between 1986 and 1989, 724 wild turkeys from six western states were captured and tested serologically for exposure to mycoplasmas. The number of turkeys sampled, year sampled, geographic sites, and antibody prevalence for MG, MS, and MM are presented in Tables 1 to 3.

Antibody to MG was detected using the RPA test in 200 (28%) of 724 birds sampled. Seroprevalence of MG varied greatly among the wild turkey populations tested, ranging from 0–85% (Table 1). Of 19 populations sampled, 16 had birds with (+) agglutination reactions to MG antigen. An additional 79 turkeys yielded sera with suspicious (+/−) RPA agglutination reactions, all were distributed among the 16 populations with RPA (+) reactions. Only populations of Merriam's turkeys from Arizona and Colorado were MG nonreactors. All populations with samples which had been frozen prior to RPA testing demonstrated RPA reactors to MG.

Antibody to MG (titers >/= 1:80) was detected using the HI test in 20 (3%) of 664 turkey sera tested. Seroprevalence of MG antibody by HI ranged from 0–14% between populations. Samples from 75 (11%) turkeys had suspicious HI titers of 1:40. When the 414 paired MG antibody results from unfrozen sera obtained in the RPA and HI assays were cross-classified (Table 4), agreement was low (kappa = 0.01, $P > 0.6$). However, agreement in determination of the population's MG reactor status was better. Fifteen of the 16 populations that were MG reactors in the RPA test were also tested by HI, of which 7 (47%) had at least one HI titer >/= 1:80, and 14 (93%) had at least one titer >/= 1:40.

Antibody to MS was detected by RPA assay in 178 (39%) of 461 wild turkeys tested. Prevalence of antibody to MS was highly variable between the 12 popula-

TABLE 1. Seroprevalence of antibody to *Mycoplasma gallisepticum* among wild turkeys by site and year as determined in the rapid plate agglutination (RPA) and hemagglutination inhibition (HI) tests.

State	Site	Year	RPA(+)		HI (1:80)	
			Rate	%	Rate	%
Arizona	Kaibab	1988	0/16	0	0/16	0
Arizona*	Chevalon	1989	12/40	29	0/40	0
Colorado	Durango	1988	0/14	0	0/14	0
Colorado	Trinidad	1988	0/56	0	0/56	0
Colorado*	Trinidad	1989	29/34	85	0/36	0
New Mexico	K. Canyon	1986	6/19	32	0/19	0
North Dakota*	hunter	1986	5/56	9	—	—
North Dakota*	hunter	1987	5/40	13	1/40	3
North Dakota*	A	1989	17/29	59	4/29	14
North Dakota*	B	1989	21/29	72	0/29	0
North Dakota*	C	1989	4/32	13	1/32	3
North Dakota*	D	1989	1/28	4	1/28	4
Texas	Wheeler	1987	2/14	14	0/14	0
Texas	Shamrock	1987	19/68	28	3/68	4
Oklahoma	Mangum	1989	6/49	12	3/49	6
Oklahoma	Salt Plains	1989	18/78	23	7/72	10
Oklahoma	Erick	1989	33/58	57	0/58	0
Oklahoma	Buffalo	1989	19/58	33	0/58	0
Oklahoma	Beaver	1989	3/6	50	0/6	0
Total			200/724	28	20/664	3

* Samples which were submitted frozen.

tions tested, ranging from 0–87%. Birds from 11 populations had (+) agglutination reactions (Table 2). The number of turkeys classified as positive increased to 228 (49%) when (+/−) RPA reactions were included. However, the one RPA negative population from Durango, Colorado, remained negative. Antibody to MS (titer >/= 1:80) was not detected in the HI assay from any of the 403 samples tested. Only two birds had titers of 1:40. Both were from a sample of 49 birds trapped at Mangum, Oklahoma.

Seventy-two (20%) of 354 turkeys tested had antibody to MM in the RPA assay. Inclusion of (+/−) RPA results as positive increased the total to 119 (34%). Prevalence varied between populations tested. Birds from eight of 11 populations had (+) agglutination reactions (Table 3).

The paired results recorded for RPA reactions to MG and MS antigens of 461 wild turkeys were cross-classified according to NPIP criteria and tested for association under the assumption of independence (Table 5A). Positive agglutination to one antigen was significantly ($P < 0.001$) associated with positive agglutination to the other antigen. By inspection, however, there was little relationship between seroprevalence rates to MG and MS antigen in the same population (Tables 1 and 2). This paradox was explained by reexamination of the data according to whether the samples had been frozen prior to testing in the RPA. Of the 64 sera that reacted to both antigens (Table 5A), 33 were from frozen sera. Association between RPA reactions to the two antigens was not significant ($P = 0.15$) in unfrozen sera (Table 5B).

Sex, age (adult versus subadult) and subspecies (Rio Grande versus Merriam) were examined to determine whether these factors were associated with serologic reactivity to MG or MS antigen. No association was noted between sex and serologic reac-

TABLE 2. Seroprevalence of antibody to *Mycoplasma synoviae* among wild turkeys by site and year as determined in the rapid plate agglutination (RPA) and hemagglutination inhibition (HI) tests.

State	Site	Year	RPA(+)		HI (1:80)	
			Rate	%	Rate	%
Arizona	Kaibab	1988	14/16	87	0/16	0
Arizona[a]	Chevalon	1989	6/27	22	0/40	0
Colorado	Durango	1988	0/14	0	0/6	0
Colorado	Trinidad	1988	27/55	49	0/56	0
Colorado[a]	Trinidad	1989	29/34	85	0/36	0
New Mexico	K. Canyon	1986	15/19	79	0/19	0
North Dakota[a]	hunter	1986	2/56	4	—	—
North Dakota[a]	hunter	1987	—	—	—	—
North Dakota[a]	A	1989	—	—	—	—
North Dakota[a]	B	1989	—	—	—	—
North Dakota[a]	C	1989	—	—	—	—
North Dakota[a]	D	1989	—	—	—	—
Texas	Wheeler	1987	11/14	79	—	—
Texas	Shamrock	1987	2/35	6	—	—
Oklahoma	Mangum	1989	28/49	57	0/49	0
Oklahoma	Salt Plains	1989	33/78	42	0/70	0
Oklahoma	Erick	1989	—	—	0/48	0
Oklahoma	Buffalo	1989	8/58	14	0/58	0
Oklahoma	Beaver	1989	3/6	50	0/5	0
Total			178/461	39	0/403	0

[a] Samples which were submitted frozen.

tivity to MG or MS in RPA or HI assays. Neither was there any association between age and serologic reactivity to MG or MS antigen in the RPA assay with unfrozen sera. Age was associated ($P = 0.003$) with serologic reaction to MG in the HI assay. Only 3 (1%) of 249 subadults, compared to 17 (6%) of 275 adults had serum titers >/= 1:80 to MG in the HI assay.

There were significant differences in the RPA seroprevalence to MG and MS between the turkey subspecies. Using NPIP criteria, 6 (6%) of 104 Merriam's and 56 (23%) of 240 Rio Grande turkeys demonstrated (+) agglutination reactions to MG with unfrozen serum. These frequencies were significantly different ($P = 0.0002$). Of 104 Merriam's turkeys, 56 (54%) were recorded as (+) in the RPA with MS antigen, while 85 (35%) of 240 Rio Grande turkeys tested yielded (+) agglutination reaction. These frequencies also were significantly different ($P = 0.002$). Seroprevalence to MG in the HI assay did not differ significantly by subspecies.

Isolations

Mycoplasmas were isolated from 81 (30%) of 266 wild turkeys sampled, using both live and postmortem isolation techniques (Table 6). Mycoplasmas were isolated from 48 (25%) of 190 live turkeys, and 33 (45%) of 76 turkeys sampled postmortem. All mycoplasma isolates produced an acid pH shift in broth indicating fermentation of glucose. Species identification was attempted using antisera to MG, MS and MGP, which all ferment glucose. Only one isolate, from lung tissue of a turkey trapped in Shamrock, Texas, was identified as MG in the immunobinding assay. Isolates from necropsy specimens from five of 16 wild turkeys trapped at Kaibab, Arizona were identified as MS in the immunobinding assay. Sera from these turkeys produced strong positive agglutination

TABLE 3. Seroprevalence of antibody to *Mycoplasma meleagridis* among wild turkeys by site and year as determined in the rapid plate agglutination (RPA) test.

State	Site	Year	RPA(+) Rate	RPA(+) %
Arizona	Kaibab	1988	0/16	0
Arizona*	Chevalon	1989	—	—
Colorado	Durango	1988	0/14	0
Colorado	Trinidad	1988	1/36	3
Colorado*	Trinidad	1989	—	—
New Mexico	K. Canyon	1986	14/19	74
North Dakota*	hunter	1986	4/56	7
North Dakota*	hunter	1987	—	—
North Dakota*	A	1989	—	—
North Dakota*	B	1989	—	—
North Dakota*	C	1989	—	—
North Dakota*	D	1989	—	—
Texas	Wheeler	1987	11/14	79
Texas	Shamrock	1987	0/34	0
Oklahoma	Mangum	1989	12/49	25
Oklahoma	Salt Plains	1989	22/52	42
Oklahoma	Erick	1989	—	—
Oklahoma	Buffalo	1989	3/58	5
Oklahoma	Beaver	1989	5/6	83
Total			72/354	20

* Samples which were submitted frozen.

reactions with MS antigen in the RPA test; however, no antibody reactivity was detected using the HI test. *Mycoplasma gallopavonis* was identified from 37 of 81 (46%) turkeys yielding mycoplasma isolates. *Mycoplasma gallopavonis* positive isolates were cultured from lung tissue, trachea, nasal turbinates, sinus and cloaca of different wild turkeys. The species of mycoplasma was not determined for isolates from 38 (47%) of the birds.

TABLE 4. Paired results showing lack of correspondence between rapid plate agglutination (RPA) and hemagglutination inhibition (HI) assays of antibody to *Mycoplasma gallisepticum* in 414 unfrozen wild turkey sera.

		HI (+)	HI (−)	Total
RPA	(+)	4	100	104
	(−)	9	301	310
	Total	13	401	414

The negative predictive value (NPV) was calculated using HI results (titer >/= 1:80) as the standard. NPV = 301/310 = 97%; 95% C.I. (95.5, 98.5). Kappa = 0.01, $P > 0.6$.

TABLE 5. Assocation of paired results of rapid plate agglutination (RPA) assays of antibody to *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in 461 wild turkey sera: A, data from all paired sera. B, data from unfrozen paired sera.

A. Frozen plus unfrozen sera

		MS (+)	MS (−)	Total
MG	(+)	64	36	100
	(−)	114	247	361
	Total	178	283	461

$\chi^2_{Yates} = 33.4$, $P < 10^{-7}$

B. Unfrozen sera only

		MS (+)	MS (−)	Total
MG	(+)	31	31	62
	(−)	110	172	282
	Total	141	203	344

$\chi^2_{Yates} = 2.1$, $P = 0.15$

DISCUSSION

Cooperation with state wildlife agencies enabled microbial and serologic testing of wild turkeys captured at eleven sites and serologic testing at an additional seven sites in six western states. The results reported here summarize the largest and geographically most extensive surveillance of mycoplasmosis among wild turkeys yet conducted. The populations surveyed were purposefully selected and, therefore, inference with regard to the results must be restricted to the populations studied. The results presented show (1) subclinical infection is possible, as demonstrated by isolation of MG and MS from apparently healthy wild turkeys; (2) the RPA test was more sensitive and detected all birds from which MG or MS was isolated, whereas the less sensitive HI assay did not; (3) the RPA and HI tests agreed in determining the flock infection/exposure status to MG, but were discrepant in classification of individual birds; (4) moderate to high RPA

TABLE 6. Crude mycoplasma isolation rate and proportional rate by species of mycoplasma, among wild turkeys by state, site and year.

State	Site	Year	Crude rate[a]	Proportional rate by species[b]			
				MG	MS	MGP	Untyped
Arizona	Kaibab	1988	8/16	0/8	5/8	0/8	3/8
Colorado	Durango	1988	9/15	0/9	0/9	3/9	6/9
Colorado	Trinidad	1988	4/32	0/4	0/4	0/4	4/4
Colorado	Trinidad	1989	17/36	0/17	0/15	7/17	10/17
New Mexico	K. Canyon	1986	7/19	0/7	—	3/7	4/7
New Mexico	W. Canyon	1988	2/5	0/2	0/2	0/2	2/2
Texas	Wheeler	1987	5/10	0/5	—	0/5	5/5
Texas	Shamrock	1987	1/11	1/1	—	0/1	0/1
Oklahoma	Mangum	1989	2/4	0/2	0/2	2/2	0/2
Oklahoma	Erick	1989	9/58	0/9	0/9	9/9	0/9
Oklahoma	Buffalo	1989	14/54	0/14	0/10	11/14	3/14
Oklahoma	Beaver	1989	3/6	0/3	0/3	2/3	1/3
Total			81/266	1/81	5/62	37/81	38/81

[a] The number of wild turkeys from which mycoplasmas were isolated over the total number of birds sampled.
[b] The number of mycoplasma isolates of the indicated species over the number of mycoplasmas isolated. MG, *Mycoplasma gallisepticum*; MS, *Mycoplasma synoviae*; MGP, *Mycoplasma gallopavonis*.

seroprevalence to multiple pathogenic mycoplasma antigens may occur among wild turkey populations in the absence of overt disease; and (5) known causes of nonspecificity or immunologic cross-reactivity did not appear to explain the RPA serologic responses.

Except for the successful microbial isolation of MG and MS, and confirmation of some RPA reactions to MG antigen by the HI test, the results presented here were similar to recent studies of apparently healthy wild turkeys. Surveys of Rio Grande turkeys in Texas (Rocke and Yuill, 1987) and Eastern turkeys in Georgia (Luttrell, 1989) demonstrated MG RPA reactors, but failed to isolate MG from live turkeys. Agglutination reactions in the RPA assay with MG, MS and MM antigens were more frequent in the present study, perhaps reflecting the selection of sites with prior evidence of mycoplasmosis. Reactions to multiple antigens by the same sera or by sera from the same population tested by RPA were seen in the present and earlier studies. In both prior studies, microbial isolation attempts from necrospy specimens were limited; however, MGP, as reported here, was readily isolated from live birds. Signs of infectious sinusitis were not seen among the wild turkeys captured and sampled in the current or earlier studies (Rocke and Yuill, 1987; Luttrell, 1989).

The proportion of wild turkeys yielding MG and MS isolates (0.4% and 1.9%, respectively), were low in the present study. Jordan (1979) cited the presence of competing flora, especially fast growing mycoplasmas, and/or low colonization densities (e.g., few numbers of mycoplasma per gram of tissue) in tissues sampled as reasons for lack of culture success from field specimens. *Mycoplasma gallopavonis* was the species most frequently isolated in the present study. The type strain of this species grows vigorously in all media used in this study. Thus, it is possible that the frequent presence of MGP and untyped mycoplasma strains may have significantly obscured the ability to detect the more slowly growing MG, MS or MM.

Factors known to increase colonization densities of MG, thereby increasing culture success, include concurrent viral infections and environmental conditions which compromise host defenses (Jordan,

1979). It is unlikely that free-ranging wild turkeys experience the level of exposure to such factors as is commonly found in the production environment, consequently, colonization densities of MG, MS and MM may remain lower in infected wild turkeys. This may explain the absence of typical signs of "infectious sinusitis" among MG seropositive wild turkeys. Additionally, in conjunction with the presence of competing mycoplasma flora, low colonization densities could account for the infrequent isolation of MG and MS from serologically positive wild turkeys as reported in this and other studies (Rocke and Yuill, 1987; Luttrell, 1989). This scenario is not exclusive of other factors which may also contribute to differences in the severity of disease and infrequent isolation of MG, such as genetic differences between wild and domestic turkeys or differences in the virulence of MG strains found in wild turkeys. Understanding the role of host, agent, and environmental factors and their interactions to explain differences in the epizootiology of MG infection in wild turkeys will require more work and a more sensitive standard to determine the presence of pathogenic mycoplasmas.

Results of experimental infection of wild turkeys with MG have shown that the RPA test detected antibody earlier and in a higher proportion of exposed birds than the HI assay and that antibody detected by the RPA test persisted longer than that detected by the HI assay (Rocke et al., 1985, 1988). Similar findings have been reported for domestic poultry (Snell and Cullen, 1978; Kleven, 1975). This difference in persistence of detectable antibody may explain the lack of statistical agreement among individual sera in their paired RPA and HI results to MG antigen (Table 4). Furthermore, it has been reported that the HI test is less sensitive in detecting MS specific antibody in turkey sera than in chicken sera (Olson, 1984), which may explain the absence of HI reactions in sera from MS infected or MS RPA reactor birds. Despite these problems, agreement between the RPA and HI tests in classifying MG exposure status of the wild turkey populations studied was good when highly suspicious HI titers of 1:40 were taken into account. This finding supports the practice of using the RPA tests to determine population infection/exposure status and not that of individual birds.

Contaminated serum, freezing and thawing of serum samples (Bradbury and Jordan, 1973; Kleven, 1975), variability of RPA antigens, presence of staphylococci, or exposure to other mycoplasmas (Roberts and Olesiuk, 1967; Kleven, 1975; Thornton, 1973) may all contribute to nonspecific RPA agglutination reactions. For domestic poultry, the frequency of nonspecific agglutination reactions is usually low (Kleven, 1975; USDA, 1984). Nonspecific RPA reactions resulting from freezing and thawing of sera were the most likely explanation for the apparent seroconversion to MG between 1988 and 1989 in the population trapped near Trinidad, Colorado, and for the highly significant association between paired MG and MS reactions of frozen sera in the RPA assay. Statistical independence between paired RPA results to MG and MS antigens in unfrozen sera (Table 5B) indicated that RPA results were immunologically specific for each mycoplasma species and did not result from cross-reactions, which have been reported between MG and MS (Avakian et al., 1988).

What was not determined in this study was the extent to which agglutinating antibody detected in the RPA test predicted the presence of persistent infection and the capacity to transmit these pathogenic mycoplasmas. Commercial flocks that demonstrate serologic reactions generally remain capable of transmitting the agent (Yoder, 1984). Findings from a related study of wild turkeys captured from two of the sites included in this study demonstrated that RPA MG-seropositive wild turkeys were capable of transmitting MG to mycoplasma-free domestic turkeys (B. A. Fritz, unpublished data). There have

been no reports of studies to determine whether RPA (−) birds selected from RPA (+) flocks will also transmit the agent. We calculated the negative predictive value of the RPA test for MG using HI results with titers of >/= 1:80 as the standard for comparison. These results indicate that for every 100 RPA (−) birds relocated from the populations studied, three would be HI positive (Table 4). However, the low sensitivity of the HI test for detecting infection suggests that many more could actually be infected with MG.

The implications for wild turkey management are that subclinical infections may place populations at risk of respiratory disease if concurrent infections or environmental stresses occur. Of greater consequence is the potential long-term effect on reproductive performance and recruitment. These results support recommendations advanced by several wildlife agencies (Nettles and Thorne, 1982; Nettles, 1984; Wildlife Disease Association, 1985; Amundson, 1985; Kennamer, 1987) to test and select RPA (−) wild turkeys for relocation from populations historically shown to be serologically negative using the RPA for MG, MS and MM.

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