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Infectious Disease Survey of Rio Grande Wild Turkeys in the Edwards Plateau of Texas

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ABSTRACT: State wildlife agencies have translocated thousands of wild turkeys (*Meleagris gallopavo*) since the 1930s to reestablish this species. Because of threats to the domestic poultry industry and wild birds, screening for selected infectious agents has become routine since the early 1980s. One of the principal sources for Rio Grande wild turkeys (*M. gallopavo intermedia*) for translocation purposes was the Edwards Plateau of Texas (USA). Unfortunately, turkey abundance has declined in the southern Edwards Plateau since the late 1970s. Surprisingly few studies have addressed wild turkeys in this region, perhaps reflecting its status as the heart of Rio Grande turkey range. We surveyed 70 free-living Rio Grande wild turkeys from Bandera and Kerr counties, Texas, for evidence of exposure to *Salmonella typhimurium, S. pullorum, Mycoplasma gallisepticum, M. meleagridis, M. synoviae, Chlamydophila psittaci,* and the avian influenza, Newcastle disease, turkey corona, and reticuloendotheliosis viruses. Of these, 80% (56) were seropositive for both *M. gallisepticum* and *M. synoviae* on the serum plate antigen test. Ten of these individuals (14% of total) were positive for *M. synoviae* by hemagglutination inhibition testing. All other serologic tests were negative. Two adult females sampled in Kerr County, whose body mass was significantly less than that of other adult females trapped in the area, tested positive for reticuloendotheliosis virus (REV) proviral DNA on polymerase chain reaction. Reticuloendotheliosis virus was isolated from one of these individuals. The pathogenesis, transmission, and/or population-level influences of *M. gallisepticum, M. synoviae,* and REV in Rio Grande wild turkeys deserves further study.

Key words: infectious disease, *Meleagris gallopavo intermedia, Mycoplasma gallisepticum, Mycoplasma synoviae,* reticuloendotheliosis virus, Rio Grande wild turkey, serologic survey, Texas, wild turkey.

By the early 1900s, wild turkeys (*Meleagris gallopavo*) were extirpated from most of their historic range, primarily by unregulated hunting and habitat conversion and degradation (Kennamer et al., 1992). Although Rio Grande wild turkey (*M. gallopavo intermedia*) populations also were negatively influenced by these same anthropogenic factors, they probably were less seriously impacted than most other races of wild turkey. For example, although Rio Grande turkeys apparently were extirpated from New Mexico, Oklahoma, and Kansas (USA) by the early 1900s (Lee, 1959; Schorger, 1966; Hlavachick and Miller, 1997), approximately 96,000 still remained in Texas (USA) in 1928 (Texas Game, Fish and Oyster Commission, 1929; Gore, 1969), primarily in the Edwards Plateau and secondarily the South Texas Plains physiographic regions (Gould, 1962). Nearly all Rio Grande wild turkeys in the United States ultimately originated one way or another from these regions.

Because of rapidly accelerating wild turkey translocation among states, biologists became interested, during the mid-1970s, in determining whether these birds harbored infectious agents that could cause significant economic losses to the poultry industry or be transmitted to birds at release sites. Hensley and Cain (1979) tested Texas wild turkeys captured for translocation purposes during 1976–77 and found serologic evidence of exposure to *Mycoplasma gallisepticum, Salmonella pullorum,* and *S. typhimurium.* Similarly, evidence of exposure to *M. gallisepticum* and *M. meleagridis* was found for wild turkeys being translocated from Missouri to Wisconsin (USA) in 1980 (Amundson, 1985). Further, *M. gallisepticum* was isolated from sick wild turkeys in Georgia (USA;

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Davidson et al., 1982), California (USA; Jessup et al., 1983), and Colorado (USA; Adrian, 1984). Lastly, *S. typhimurium* was isolated from a clinically ill wild turkey in Alabama during 1982 (Howerth, 1985) and, along with several other *Salmonella* serotypes, from apparently healthy wild turkeys in Florida (USA; White et al., 1981).

Documentation of economically important poultry diseases in free-roaming wild turkeys, including those that might be ecologically significant to wild turkey populations, led the Wildlife Disease Association to develop and publish a health monitoring protocol for wild turkeys being considered for translocation (Amundson, 1985). Although some aspects of this protocol received little attention, several comprehensive surveys of *Mycoplasma* spp. and/or *Salmonella* spp. prevalence were conducted (Rocke and Yuill, 1987; Davidson et al., 1988; Luttrell et al., 1991; Fritz et al., 1992; Veatch et al., 1998; Charlton, 2000). A few serologic surveys of avian influenza and Newcastle disease virus also were completed (Rocke and Yuill, 1987; Davidson et al., 1988; Charlton, 2000).

Although the Edwards Plateau of Texas traditionally supported high densities of Rio Grande wild turkeys, turkey abundance in certain southern portions of the plateau declined dramatically since the late 1970s but did not exhibit this trend elsewhere in this physiographic region (Texas Parks and Wildlife Department, unpubl. data). Few ecological studies of Rio Grande wild turkeys have been conducted in the Edwards Plateau—perhaps reflecting this area's status as the heart of Rio Grande turkey range—and few comprehensive ecological studies were completed anywhere within historical Rio Grande wild turkey range since about 1980 (Peterson, 1998). Causes of declining wild turkey abundance in the southern Edwards Plateau remain unclear, although Texas Parks and Wildlife Department biologists suspect landscape-scale phenomena, such as gradual changes in habitat suitability and infectious agents. Few studies addressing the infectious agents of wild turkeys have been conducted in the Edwards Plateau. Histomoniasis, avian pox, and various species of mites were found for four wild turkeys from this region (Hightower et al., 1953; Thomas, 1964), three cestode species and *Heterakis gallinarum* were identified from Rio Grande turkeys in the northern extreme of the plateau (Pence and Bickel, 1977), and zero of 27, one of 28, and zero of 28 Rio Grande wild turkeys were serologically positive for *S. pullorum, M. gallisepticum,* and *Chlamydophila psittaci,* respectively, in the eastern extreme of this region (Hensley and Cain, 1979).

We surveyed free-living Rio Grande wild turkeys from Bandera and Kerr counties, Texas, for evidence of exposure to 10 microparasitic agents known to cause disease in galliforms. There was no commercial poultry industry near our study sites, although some ranch managers might have maintained poultry for their own use in the past.

Rio Grande wild turkeys were captured using walk-in traps (Davis, 1994) baited with milo in the southern Edwards Plateau (Gould, 1962; Bandera and Kerr counties, 99°25′N, 29°52′W and 99°32′N, 30°02′W, respectively), during spring (February–March), 2001. We banded each captured turkey with a numbered, blue anodized aluminum leg band, attached a radio transmitter, and recorded sex, body mass, and age as either juvenile or adult using Ammann's (1944) outer primary technique. A 3 ml blood sample was taken via jugular venipuncture from each wild turkey. Approximately 0.25 ml of each sample was immediately placed in a heparinized tube (Capiject; Terumo Medical Corporation, Elkton, Maryland, USA) and the remainder in a heparinized vacuum tube. The vacuum tubes were centrifuged and plasma placed in sterile vials (≤3 hr). Both plasma and whole blood samples were held at approximately −20 C pending analysis (≤10 days).

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Samples were submitted to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL; College Station, Texas) for serologic and genetic testing. Samples were tested for specific antibody to *S. typhimurium* and *S. pullorum* using tube agglutination tests (Veterinary Services, 2000) with antigens obtained from the University of Minnesota (St. Paul, Minnesota, USA). Samples were screened for antibodies to *M. gallisepticum*, *M. meleagridis*, and *M. synoviae* using serum plate agglutination tests (SPA; Veterinary Services, 2000) with antigens from Intervet America (Millsboro, Delaware, USA). Samples agglutinating within 2 min were considered positive and subsequently tested by the microhemagglutination inhibition test (HI; Veterinary Services, 2000) with antigens obtained from the National Veterinary Services Laboratory (Ames, Iowa, USA). Because our purpose was to evaluate whether free-roaming wild turkeys had been exposed to *Mycoplasma* spp., rather than differentiate recent natural infections from background vaccine titers, we considered titers ≥1:10 positive evidence of *Mycoplasma* sp.-specific antibody. We tested for *C. psittaci*-specific antibody (IgM) using an elementary body agglutination test (Grimes et al., 1994). Antigen was prepared by the TVMDL following Grimes et al. (1994), and antibody titers ≥1:20 were considered positive. An agar gel immunodiffusion assay (Veterinary Services, 2000), utilizing antigen and specific antisera obtained from the National Veterinary Services Laboratory, was used for screening samples for avian influenza virus specific antibody. A HI test was used to test for antibodies against Newcastle disease virus (Beard and Wilkes, 1973). Antigen was obtained from the National Veterinary Services Laboratory and antibody titers ≥1:10 considered positive. Samples were tested for turkey coronavirus antibodies using an indirect fluorescent antibody technique (Patel et al., 1975). Whole blood samples were screened for reticuloendotheliosis virus (REV) proviral DNA using a polymerase chain reaction (PCR; Aly et al., 1993; Davidson et al., 1995), and virus isolations (Fadly and Witter, 1998) were attempted for all PCR-positive samples.

Blood samples were obtained from 25 and 48 Rio Grande wild turkeys in Bandera and Kerr counties, respectively (Table 1). All individuals appeared healthy when captured. On the SPA test, 80% (56 of 70) were seropositive for both *M. gallisepticum* and *M. synoviae;* none were positive for *M. meleagridis* (Table 1). Of the 56 positive birds, 10 were seropositive for *M. synoviae* by HI, while none were seropositive for *M. gallisepticum*. All other serologic tests were negative (Table 1). Two adult female Rio Grande wild turkeys sampled in Kerr County were positive for REV proviral DNA by PCR (Table 1), and REV was isolated from one of these birds. These two birds also were seropositive for *M. gallisepticum* and *M. synoviae* by SPA, one of which was seropositive for *M. synoviae* by HI (1:10). The body masses of the PCR-positive individuals (3,570 and 3,450 g) were significantly less than the mean body mass of the 33 adult females found REV negative by PCR from the same study area (mean=4,086 g; 99% CI: 3,968–4,250 g). Both REV positive birds, and seven of the 10 individuals found seropositive for *M. synoviae* by HI, were captured at a single trap site at a ranch headquarters in Kerr County (Table 1). The other two positive birds from this study area were captured ≤1.4 km away, and were commonly found at the ranch headquarters using radio telemetry.

Our demonstration of high *M. gallisepticum* and *M. synoviae* seroprevalence based on SPA testing, yet much lower seroprevalence for only *M. synoviae* based on HI, is not unusual. For example, surveys of free-roaming Rio Grande and other western wild turkey subspecies also found much higher prevalences of *M. gallisepticum* and *M. synoviae* using the SPA versus HI tests (Rocke and Yuill, 1987; Fritz et al., 1992). Similar results were obtained for Merriam's wild turkeys (*M. gal-*

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TABLE 1. Number of Rio Grande wild turkeys found positive (*n* tested) for 1) specific antibody to *Salmonella typhimurium* (*S. typ.*), *S. pullorum* (*S. pul.*), *Mycoplasma gallisepticum* (*M. gal.*), *M. meleagridis* (*M. mel.*), *M. synoviae* (*M. syn.*), *Chlamydophila psittaci* (*Chlam.*), and avian influenza (AIV), Newcastle disease (NDV), and turkey corona (TCV) viruses; and 2) reticuloendotheliosis virus (REV) proviral DNA by PCR and REV isolation (Isol.).

			Serology											REV	
					M. gal.		*M. mel.*	*M. syn.*							
Texas county	Sex[a]	Age[b]	*S. typ.*	*S. pul.*	SPA[c]	HI[d]	SPA[c]	SPA[c]	HI[d]	*Chlam.*	AIV	NDV	TCV	PCR	Isol.
Bandera	F	A	0 (21)	0 (21)	14 (21)	0 (14)	0 (21)	14 (21)	1[c] (14)	0 (21)	0 (21)	0 (19)	0 (11)	0 (19)	
	M	J	0 (2)	0 (2)	1 (2)	0 (1)	0 (2)	1 (2)	0 (1)	0 (2)	0 (2)	0 (1)	0 (2)	0 (2)	
	M	A	0 (1)	0 (1)	1 (1)	0 (1)	0 (1)	1 (1)	0 (1)	0 (1)	0 (1)	0 (1)		0 (1)	
Kerr	F	J	0 (3)	0 (3)	3 (3)	0 (3)	0 (3)	3 (3)	1[f] (3)	0 (3)	0 (3)	0 (3)	0 (2)	0 (3)	
	F	A	0 (33)	0 (33)	32 (33)	0 (32)	0 (33)	32 (33)	2[e],3[f], 2[g] (32)	0 (33)	0 (33)	0 (33)	0 (23)	2 (35)	1 (2)
	M	J	0 (7)	0 (7)	3 (7)	0 (3)	0 (7)	3 (7)	1[e] (3)	0 (7)	0 (6)	0 (5)	0 (6)	0 (7)	
	M	A	0 (3)	0 (3)	2 (3)	0 (2)	0 (3)	2 (3)	0 (2)	0 (3)	0 (3)	0 (2)	0 (1)	0 (3)	
Total			0 (70)	0 (70)	56 (70)	0 (56)	0 (70)	56 (70)	4[e], 4[f], 2[g] (56)	0 (70)	0 (70)	0 (64)	0 (45)	2 (70)	1 (2)

[a] F = female, M = male.
[b] J = juvenile, A = adult.
[c] Serum plate agglutination.
[d] Hemagglutination inhibition.
[e] Titer = 1:10.
[f] Titer = 1:40.
[g] Titer = 1:160.

lopavo merriami) living in close association with domestic galliforms (Hoffman et al., 1997). This commonly observed lack of agreement between the SPA and HI tests probably arises for several reasons. For example, experimental infection of wild turkeys with *M. gallisepticum* demonstrated that the SPA test detected antibody sooner and in a higher proportion of exposed birds than did HI (Rocke et al., 1985; Rocke and Yuill, 1988). Probably more important, the antibody detected by the SPA test also persisted longer than did that detected by the HI, and *M. gallisepticum* was isolated from experimentally infected individuals that were HI negative, yet SPA positive (Rocke and Yuill, 1988). Further, it is possible that variant strains of *Mycoplasma* sp. exist among wild turkeys that cause agglutination on the SPA test but not HI, or that SPA-positive samples might result from nonspecific reactions caused by other microorganisms (Fritz et al., 1992; Hoffman et al., 1997). Those managing wild turkey populations, therefore, are faced with the unsettling fact that while positive SPA reactions, by themselves, do not necessarily imply current infection, SPA-positive, yet HI-negative individuals might well be infectious.

Because our plasma samples were briefly frozen, then thawed prior to SPA testing, nonspecific SPA agglutination reactions may have been increased (Bradbury and Jordan, 1973; Kleven, 1975). This is likely for the *M. gallisepticum* reactions; all sera positive by SPA for *M. gallisepticum* also were positive for *M. synoviae,* 10 of these were HI positive for *M. synoviae,* yet none were HI positive for *M. gallisepticum* (Table 1). Similarly, Fritz et al. (1992) demonstrated an association between positive SPA reactions for *M. gallisepticum* and *M. synoviae* when frozen sera were used. Thus, it is likely that many, if not most, of the samples we found positive for *M. gallisepticum* by SPA, actually represented cross reactions caused by exposure to *M. synoviae* (Bradbury and Jordan, 1973; Kleven, 1975), or possibly a nonpathogenic *Mycoplasma* sp. that cross-reacts with *M. synoviae* on HI testing (Hoffman et al., 1997).

Absence of *M. meleagridis*-specific antibody on SPA tests from the Edwards Plateau contrasts with relatively high prevalences (20–33%) seen in several studies of western wild turkey subspecies (Fritz et al., 1992; Hoffman et al., 1997; Charlton, 2000), including Rio Grande wild turkeys from elsewhere in Texas (42%; Rocke and Yuill, 1987). Veatch et al. (1998), however, also found low prevalence (1%) of *M. meleagridis* specific antibody on SPA assays conducted in Kansas (USA).

Our negative results for other serologic tests were consistent with several studies of western wild turkey populations. For example, prevalence of specific antibody to *S. pullorum, S. typhimurium,* and *C. psittaci* in Rio Grande wild turkey populations previously studied in Texas varied from 0–5%, 0–2%, and 0–2% (respectively, Trainer et al., 1968; Roslien and Haugen, 1970; Hensley and Cain, 1979). Veatch et al. (1998) and Charlton (2000) found comparable results for *S. pullorum* and *S. typhimurium* in western wild turkey subspecies elsewhere. Further, the avian influenza and Newcastle disease viruses do not appear to be endemic in wild turkey populations in Texas (Trainer et al., 1968; Roslien and Haugen, 1970; Hensley and Cain, 1979; Rocke and Yuill, 1987) or elsewhere (Davidson et al., 1988; Charlton, 2000). Because ours is apparently the first survey of free-living wild turkey exposure to turkey coronavirus, it is difficult to contextualize our results.

Reticuloendotheliosis virus previously was isolated from a moribund eastern wild turkey (*M. gallopavo silvestris*) in North Carolina (USA; Ley et al., 1989) and a severely emaciated eastern turkey exhibiting neurological signs from a Georgia barrier island (Hayes et al., 1992). Ours is the first demonstration, however, of REV proviral DNA during a survey of wild turkeys that were not terminally ill and the first isolation of this virus from Rio Grande wild

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turkeys. Although the biologists who captured the two PCR-positive individuals did not consider them to be clinically ill, their markedly decreased body mass suggested otherwise. The female from which the virus was isolated died 2 mo later and the second bird died within 8 mo. Unfortunately, because access to the private properties where these birds died could not be obtained in a timely manner, the carcasses could not be recovered in time to conduct meaningful necropsies. The avian REV is a retrovirus that can cause acute reticuloendotheliosis, immunodepression, stunting, and chronic neoplastic disease in susceptible domestic turkeys (Witter, 1997). Clinical signs in wild turkeys appear similar (Ley et al., 1989; Hayes et al., 1992).

The two REV positive and nine of 10 individuals found seropositive by HI for *M. synoviae* (including one of two REV positive birds) were trapped near a ranch headquarters on the study area in Kerr County with stable, rather than declining turkey abundance. There were no domestic poultry at the ranch headquarters, but corn and/or milo were used to feed wild turkeys and white-tailed deer (*Odocoileus virginianus*) at these and most other trap sites. No positive individuals were found elsewhere on this study area.

In light of the diseases identified in this survey, wildlife disease researchers might consider addressing the pathogenesis, transmission, and population-level influences of *M. synoviae* and REV, and perhaps other species of *Mycoplasma*, for Rio Grande wild turkeys. Some researchers have questioned whether positive SPA reactions are due to pathogenic strains of *M. gallisepticum*, *M. synoviae*, and *M. meleagridis* (e.g., Hoffman et al., 1995, 1997). Further, little is known about the pathogenesis or transmission of *M. synoviae* and *M. meleagridis* in wild turkeys or the population-level significance of any species of *Mycoplasma*. Finally, REV theoretically could limit host populations (Anderson and May, 1979, 1981; Bowers et al. 1993), so further study regarding pathogenesis, transmission, and population-level influences of REV in wild turkeys is warranted.

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