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Isolation of *Mycoplasma gallopavonis* from Free-ranging Wild Turkeys in Coastal North Carolina Seropositive and Culture-negative for *Mycoplasma gallisepticum*

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ABSTRACT: Serum samples and choanal cleft swabs were collected from livetrapped and hunter killed wild turkeys (*Meleagris gallopavo*) from Martin and Bertie counties, North Carolina (USA). Sera were tested for antibodies to *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma meleagridis* by hemagglutination inhibition (HI). Sera from 33% (five of 15) of livetrapped turkeys were positive for antibodies to *M. gallisepticum* by HI, and all were negative for antibodies to *M. synoviae* and *M. meleagridis*. Choanal cleft swabs from 22 livetrapped and five hunter killed wild turkeys cultured in Frey's broth medium resulted in 23 mycoplasma isolations. Using direct immunofluorescence, 74% (17/23) were *M. gallopavonis*, and 26% (six of 23) were unidentified; no isolate was identified as *M. gallisepticum*, *M. synoviae* or *M. meleagridis*.

Key words: Wild turkey, *Meleagris gallopavo*, *Mycoplasma gallisepticum*, *Mycoplasma gallopavonis*, mycoplasmosis, serology, culture, survey.

Mycoplasma gallisepticum (MG), *M. synoviae* (MS), and *M. meleagridis* (MM) are recognized pathogens in domestic turkeys (Yoder, 1991; Kleven et al., 1991; Yamamoto, 1991). MG is considered the most pathogenic of avian *Mycoplasma* spp., and is capable of causing sinusitis and airsacculitis in domestic turkeys (Yoder, 1991), and in free-ranging and captive-reared wild turkeys (Davidson et al., 1982; Jessup et al., 1983; Rocke and Yuill, 1988; Rocke et al., 1988). The epizootiology of MG and its role as a population determinant in free-ranging wild turkeys is unknown. However, following experimental MG exposure, captive-reared wild turkeys (*Meleagris gallopavo*) experienced moderate respiratory distress, poor egg production, and lowered fertility and hatchability (Rocke et al., 1988) and Rio Grande turkeys (*Meleagris gallopavo intermedia*) developed persistent subclinical infections (Rocke and Yuill, 1988). In an effort to minimize the risk of MG transmission to and from wild turkeys, the United States Animal Health Association recommends serologic testing of wild turkeys captured for translocation purposes (Nettles and Thorne, 1982). Although MS and MM are important pathogens in domestic turkeys, causing respiratory disease and lameness (Kleven et al., 1991; Yamamoto, 1991), tests for antibodies to MS and MM have rarely been included in serological monitoring of wild turkeys (Rocke and Yuill, 1987; Davidson et al., 1988). As part of a study on the dynamics of wild turkey populations along the Roanoke River, North Carolina (Cobb, 1990), sera were collected from livetrapped turkeys and tested for antibodies to MG, MS, and MM; and choanal cleft swabs were collected from livetrapped and hunter killed turkeys for mycoplasma culture.

The study was conducted on lands owned by Union Corporation, Georgia-Pacific Corporation, the North Carolina Wildlife Resources Commission, and private individuals. These tracts total 5,660 ha (13,980.2 ac) in area and are located near Hamilton, North Carolina (35°57′N, 77°12′W). The tracts parallel approximately 19.2 km of the Roanoke River. The vegetative characteristics of the area have been described in detail (Cobb, 1990). Generally, it is transitional between mixed alluvial hardwood and gum (*Nyssa aquatica*)/cypress (*Taxodium distichum*) backswamp communities. In suitable areas, wild turkeys were trapped at baited

sites with a 17.5 m × 10.0 m skirted rocket net. Otherwise, 2-2-2 tribromoethanol was used as an oral anesthetic (Austin et al., 1972). Samples from hunter killed wild turkeys were collected at check stations in the study area.

Blood was collected from livetrapped wild turkeys, the serum separated then stored at −5 C until tested for antibodies to MG, MS, and MM by hemagglutination inhibition (HI). HI antigens and control sera were obtained from the National Veterinary Services Laboratory (Ames, Iowa 50010, USA). In accordance with the National Poultry Improvement Plan (Anonymous, 1985), HI titers of ≥ 1:80 were considered positive, 1:40 were strongly suspicious, and less than 1:20 were negative.

Choanal clefts of livetrapped and hunter killed wild turkeys were swabbed and cultured for mycoplasmas as previously described (Kleven and Yoder, 1989) with minor modifications. Briefly, specimens were collected onto sterile cotton- or dacron-tipped (Calgi-Swab, Spectrum Laboratories, Inc., Houston, Texas 77073, USA) swabs and immediately inoculated to Frey's broth medium (Frey, 1968) supplemented with nicotinamide adenine dinucleotide-cysteine and 12% swine serum (FMS). At the study site, inoculated broth cultures were stored at −5 C during each of two survey periods (April 1987, January to May 1988), transported to North Carolina State University College of Veterinary Medicine (Raleigh, North Carolina 27606, USA) and incubated at 37 C in humidified air. Broth cultures were examined daily for mycoplasma growth, indicated by color change of the medium or visible swirl of turbidity upon agitation. Cultures indicating mycoplasma growth were inoculated onto plates of FMS agar (Kleven and Yoder, 1989), incubated as above, and examined daily for mycoplasma colonies. At 7 day intervals, 0.2 ml of each remaining culture was passed to 1.8 ml of fresh FMS broth and incubated as above. Samples were considered negative for mycoplasma isolation if no colonies appeared on agar plates inoculated from each of three successive broth passages.

Mycoplasma colonies on agar plates were speciated by direct immunofluorescence (Gardella et al., 1983; Talkington and Kleven, 1983) using fluorescein conjugated rabbit antisera to *M. gallisepticum*, *M. gallinarum*, *M. pullorum*, *M. gallinaceum*, *M. gallopavonis*, *M. meleagridis*, *M. iowae*, and *M. synoviae* provided by S. H. Kleven (Department of Avian Medicine, University of Georgia, Athens, Georgia 30605, USA).

Of 15 serum samples obtained from livetrapped turkeys, five were positive for MG and four had strongly suspicious HI titers. All were negative by HI for antibodies to MS and MM. Choanal cleft swabs from 22 livetrapped and five hunter killed wild turkeys cultured in Frey's broth medium resulted in 23 mycoplasma isolations. Of these isolates, 74% (17 of 23) were unmixed cultures of *M. gallopavonis*, and 26% (six of 23) were not identified; no isolate was identified as MG, MS or MM.

Despite serologic evidence of MG exposure in the Roanoke River wild turkey population, this organism was not isolated. However, culture results indicated that infection with *M. gallopavonis* was common in the population sampled. Difficulty or inability to isolate MG from seropositive wild turkeys has been previously reported (Rocke and Yuill, 1987; Rocke and Yuill, 1988; Rocke et al., 1988), and is not uncommon in commercial (Yoder, 1986; D. H. Ley, unpubl. data) and backyard (Luttrell, 1991; D. H. Ley, unpubl. data) poultry. There are several possible explanations for the occurrence of MG seropositive, culture-negative test results. MG reactors may have been serologic false-positives; however, this would not be expected with the HI test which is regarded as highly specific (Anonymous, 1985; Kleven and Yoder, 1989; Yoder, 1991). Also, previous or chronic MG infections may have resulted in detectable HI antibodies followed by clearance or sequestration of the microbe. Although the choanal cleft has proven to

be an excellent ante- and postmortem sample site for the recovery of MG from infected chickens (Branton et al., 1984) and domestic turkeys (Dingfelder et al., 1991), other methodologies such as culture or bioassay of samples taken from various tissues at necropsy may be necessary to demonstrate MG infection (Mallison et al., 1981; Rocke and Yuill, 1988). Finally, the culture or identification systems used may have been inadequate to detect MG infected birds. In poultry, it is not uncommon to suspect the presence of pathogenic *Mycoplasma* spp. from serologic evidence, but identify only nonpathogenic *Mycoplasma* spp. on culture (Bradbury and McClenaghan, 1982). Although immunofluorescence can identify *Mycoplasma* spp. in mixed cultures, it may fail to do so if one species is present in very low numbers or if a fastidious species is masked by overgrowth of a more prolific species (Bradbury and McClenaghan, 1982; Gardella et al., 1983). It is possible that *M. gallopavonis* and the unidentified mycoplasmas, which together were isolated from 85% (23 of 27) of the wild turkeys sampled in this study, masked the presence of MG.

It may be useful in future surveys of this type to use growth inhibition techniques (Bradbury and McClenaghan, 1982; Clyde, 1983) to decrease or eliminate *M. gallopavonis* from cultures, which may reveal other *Mycoplasma* spp. not detected by immunofluorescence alone. In addition, the serum plate agglutination (SPA) or rapid plate agglutination (RPA) test should be included in serologic surveys of wild turkeys for MG. In both captive-reared and livetrapped wild turkeys experimentally infected with MG, the RPA test detected MG antibodies sooner and for a longer period of time compared to HI (Rocke et al., 1985; Rocke and Yuill, 1988). However, to avoid false-positive test results, serum or plasma for use in the SPA or RPA tests must be of high quality (Kleven and Yoder, 1989): without bacterial or fungal contamination, and used fresh, not frozen, or after storage for not more than a few days at 4 C. Serum or plasma for use in the HI test can be used fresh or after storage for not more than a few days at 4 C. For longer storage, samples should be frozen at or below −20 C. Collection, handling, and storage of samples for culture should also be optimized (Leach, 1983). Briefly, specimens should be inoculated to mycoplasma culture medium at the time of collection and incubated at 37 C in humidified air as soon as possible. For transport or brief storage, mycoplasma cultures should be maintained at 4 C; for long term storage, cultures should be snap-frozen in liquid nitrogen, or at −20 C, then maintained at or below −70 C.

This report adds to the increasing evidence that *M. gallopavonis* may be geographically widespread and common in wild turkey populations. Published reports and recent surveys have found high prevalence rates of *M. gallopavonis* in wild turkey populations in Texas (Rocke and Yuill, 1987; D. H. Ley, unpubl. data), Georgia (Luttrell, 1991), Oregon, and Kansas (D. H. Ley, unpubl. data). At present there is no evidence that *M. gallopavonis* is pathogenic in wild turkeys. However, *M. gallopavonis* was reported to cause mortality in embryos of domestic turkeys and chickens (Rocke and Yuill, 1987). Therefore, its role as a population determinant in wild turkeys deserves further investigation. Additional experimental infections and careful applications of serologic and culture techniques to monitor wild turkeys in translocation and field studies are necessary to elucidate the epizootiology and impact of mycoplasma infections in wild turkey populations.

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