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## SEROLOGIC RESPONSE OF RIO GRANDE WILD TURKEYS TO EXPERIMENTAL INFECTIONS OF *MYCOPLASMA GALLISEPTICUM*

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**ABSTRACT:** The serologic response of Rio Grande wild turkeys (*Meleagris gallopavo intermedia*) to *Mycoplasma gallisepticum* (MG) was determined. Free-ranging turkeys were caught in southern Texas, shipped to the University of Wisconsin, Madison, and housed in isolation facilities. Fourteen birds were exposed to MG, by intratracheal and intranasal inoculation. Eight birds received sterile broth only. Two wk prior to the end of the experiment, MG exposed turkeys were stressed by challenge with a serologically unrelated mycoplasma. Serum from all exposed birds reacted positively for MG antibody by the rapid plate agglutination (RPA) procedure within 2 mo postexposure (PE) and all but one remained positive for 14 mo PE. Less than one half of the exposed birds developed positive MG antibody titers detectable by the hemagglutination inhibition (HI) test within 2 mo PE, and by 10 mo PE, none had positive titers. Antibody was detected by the HI test in two of 11 infected turkeys, 14 mo PE, and titers increased significantly within 2 wk. MG was isolated from tracheal swabs from two infected birds 2 mo PE, but attempts thereafter failed. However, at the termination of the experiment 15 mo later, MG was isolated from lung tissue of three of 11 exposed turkeys and from a blood clot found in the lower trachea of one bird.

**Key words:** *Mycoplasma gallisepticum*, wild turkeys, serology, experimental infections, carriers.

### INTRODUCTION

There have only been a few case reports of mycoplasmosis in free-ranging turkeys (Davidson et al., 1982; Jessup et al., 1983), but serologic evidence of exposure to *Mycoplasma gallisepticum* (MG) in wild turkey populations is increasing. In domestic turkeys, MG infections frequently produce a disease called "infectious sinusitis" with typical swelling of the sinuses, tracheal rales, and labored breathing (Yoder, 1978). The infection persists for long periods of time and is often complicated by the presence of other pathogens. Although mortality of infected adults is rare, serious economic losses result from downgrading of carcasses, lowered egg production, suboptimal hatching and poor poult quality.

Little is known about the pathogenicity and epizootiology of MG in wild turkeys. Sinusitis and airsacculitis were evident in naturally infected free-ranging turkeys (Davidson et al., 1982; Jessup et al., 1983), and experimental infections of MG in cap-

tive-reared wild turkeys cause moderate respiratory distress, poor egg production, lowered fertility and hatchability (Rocke et al., 1988). It is not known if MG persists for long periods of time in apparently healthy individuals, and if it is transmitted commonly among wild turkeys or to and from domestic poultry.

Since initial reports of the disease in wild turkeys, many state agencies have tried to reduce the risk of introduction and dissemination of MG by testing relocated wild turkeys for antibody to MG prior to release. However, the significance of antibody to MG in free-ranging birds without obvious signs of mycoplasmosis has been questioned. In previous studies on captive-reared wild turkeys, we determined that the rapid plate agglutination (RPA) procedure is a highly specific and a more sensitive test for MG antibody than the hemagglutination inhibition (HI) test (Rocke et al., 1985). In this study, wild turkeys of the Rio Grande subspecies were experimentally infected with MG to further eval-

uate the use of serologic tests for detecting persistently infected wild turkeys.

#### MATERIALS AND METHODS

Wild turkeys captured by cannon netting near LaPryor, Texas (28°30' to 28°50'N, 99°50' to 100°10'W) were shipped to isolation facilities at the University of Wisconsin (Madison, Wisconsin 53711, USA). The birds were maintained in enclosed rooms with straw bedding which was changed weekly. Sawhorses were used for roost sites, and food and water were provided for ad libitum consumption.

Broth cultures of *Mycoplasma gallisepticum* (MG), strain R, were obtained from the USDA poultry research laboratory (Athens, Georgia 30605, USA) and passed twice in fresh Frey's media (Frey et al., 1968). Log phase cultures of MG were used to prepare an inoculum containing  $1 \times 10^7$  color changing units/ml. Twelve hens and two toms were infected with MG by dripping 0.5 ml of the inoculum into the trachea and 0.5 ml into the nasal passages. Six hens and two toms, held in a separate building to serve as unexposed controls, similarly received sterile broth.

All birds were bled for serologic evaluation and swabbed for isolation attempts at the time of capture in Texas, just prior to experimental inoculation in Wisconsin, and periodically thereafter. Blood was collected with heparin rinsed syringes. The RPA tests for MG antibody were conducted with a commercially prepared antigen according to the protocol suggested by the producer (Salsbury Laboratories, Inc., Charles City, Iowa 50616, USA). Microtiter HI tests (Williams, 1980) were performed with antigens obtained from the National Veterinary Services Laboratory (Ames, Iowa 50010, USA) according to their recommended protocol. The antigens were diluted to provide four hemagglutination units, and standard positive and negative sera were used for comparison.

Tracheal and cloacal swabs were collected in mycoplasma growth media (Jordan, 1983) for isolation attempts. Swab samples were incubated at 37 C for 7 days or until noticeable growth occurred, at which time they were plated on agar prepared from the same liquid base and incubated. Agar plates were examined for colonies within 4 days. If no growth had occurred, the plates were returned to the incubator for up to 28 days. Direct immunofluorescence was used for identification of mycoplasmas (Talkington and Kleven, 1983).

Fourteen mo PE, MG infected turkeys were exposed to an unclassified but serologically unrelated mycoplasma by intratracheal and intranasal inoculation. This unidentified myco-

plasma which had been isolated from the sinuses of a wild turkey, does not cross react with MG (unpubl. results), and has been designated WTM. This challenge inoculum was prepared and administered as previously described for MG. All birds were killed and necropsied 2 wk later. In addition to tracheal and cloacal swabbings, lungs and air sacs were collected and cultured for mycoplasma. Various tissue samples were preserved for histologic examination, including lung, air sac, trachea, liver, kidney, heart, spleen and ovary.

#### RESULTS

None of the birds used in this experiment were serologically positive to MG at the time of capture or prior to MG exposure 2 mo after capture. Attempts to isolate MG from tracheal and cloacal swabbings prior to exposure were unsuccessful. Within 1 wk PE, most of the exposed birds showed signs of respiratory distress, but sinusitis did not develop in any case. One hen that died 2 mo PE had severe airsacculitis. MG was isolated from swabs of lungs, air sacs and trachea and probably contributed to the death of this bird. Two other inoculated birds died later from causes unrelated to MG infection. Attempts to isolate MG from tissues from these two birds were unsuccessful.

All of the exposed birds were MG antibody positive by the RPA test within 2 mo PE (Fig. 1). However, only five of 14 birds had HI titers that would be considered positive ( $\geq 1:80$ ), and the highest HI titer recorded was only 1:160. Within 6 mo, only two of 13 infected birds had positive HI titers and none did after 10 mo PE, although all but one were MG antibody positive by the RPA test. MG was isolated from the tracheas of two birds 2 mo PE, but was not recovered from tracheal or cloacal swabbings on the next two attempts.

Approximately 14½ mo PE, one of 11 MG exposed birds became HI positive to MG. Serum from these birds was collected just prior to inoculation with the WTM mycoplasma strain. MG was not isolated from tracheal and cloacal swabbings. At

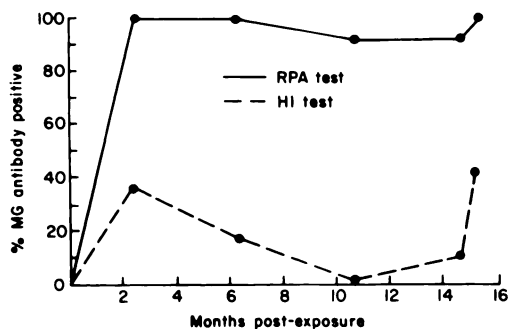


FIGURE 1. Serologic response of Rio Grande wild turkeys to *Mycoplasma gallisepticum* (MG) using the rapid plate agglutination (RPA) test and the hemagglutination inhibition (HI) test.

the termination of the experiment, 2 wk following WTM challenge, MG was isolated from the lungs of three of 11 infected birds and also from a blood clot found in the lower trachea of an additional infected bird. Again, however, MG was not recovered from tracheal and cloacal swabbings, and attempts to isolate MG from airsacs were unsuccessful. HI titers to MG increased significantly over this time period. Of 11 sera collected just prior to death, five had positive HI titers; the remainder had suspicious ( $<1:80$ ) titers.

Other mycoplasmas, not serologically related to MG, were isolated from cloacal and tracheal swabbings. Most of these were probably WTM, but antiserum for serological confirmation of this isolate was not available. No significant lesions associated with mycoplasma infection were noted upon histological examination of tissues.

#### DISCUSSION

The isolation of MG from four of 11 turkeys that had been experimentally exposed 15 mo earlier, illustrated the persistent nature of this organism in wild turkeys. MG was recovered from lungs of three birds that appeared to be normal on gross inspection. The organism was not isolated from swabbings of the upper trachea of these individuals or from any of the other infected birds. Perhaps MG localizes in the lower respiratory system of wild

turkeys and is not obtained by swabbing. Interestingly, MG antibody was consistently detected by the RPA test in serum from all three turkeys that carried MG in their lungs. However, only one of these birds had a positive HI titer to MG (1:160) 2 wk prior to necropsy, and two had positive HI titers (1:80 and 1:160) at the termination of the experiment.

The results of RPA and HI tests for MG antibody did not agree over the course of this experiment in wild turkeys. This confirmed earlier observations with captive-reared wild turkeys experimentally exposed to MG (Rocke et al., 1985). MG antibody detected by the HI test does not persist for as long as antibody detected by the RPA test. Furthermore, less than one half of the wild turkeys exposed to MG in this experiment developed HI titers considered positive ( $\geq 1:80$ ), and the highest titer recorded was only 1:160. Similar results were noted in healthy chickens that were demonstrated to be MG carriers (Bencina and Dorrer, 1984). High numbers of positive reactions to MG were detected by the RPA test 1 yr PE, yet HI antibody titers were very low, ranging from 1:32 to 1:128.

The increase in HI titers observed 14½ mo PE was unexpected. Possibly, a carrier state was reactivated and renewed replication stimulated a secondary immune response. Also, it is possible that transmission of MG between experimental birds occurred. Although no specific effects of the challenge mycoplasma (WTM) were evident, the mixed infection and the additional stress of capturing and handling may have exacerbated the infection. Social stress can also influence resistance to MG infections (Gross and Colmano, 1969). Just before the end of this experiment, the turkeys had begun breeding and laying eggs, activities which are both socially and physiologically stressful.

MG carriers are very important in the epizootiology of mycoplasmosis in domestic poultry (Yoder, 1978). It is unknown if persistent infections of MG occur in nat-

urally infected free-ranging turkeys, but transmission of disease to other wild and domestic birds is a risk to be considered before relocating wild turkeys. At present, the RPA test is the most effective method available to detect wild turkeys that have been exposed to MG and may be carrying this pathogen.

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