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EXPERIMENTAL *MYCOPLASMA GALLISEPTICUM* INFECTIONS IN CAPTIVE-REARED WILD TURKEYS

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ABSTRACT: The effects of *Mycoplasma gallisepticum* (MG) infections on egg production, fertility, and hatchability were studied in captive-reared wild turkeys (*Meleagris gallopavo*). Three groups of adult birds, each consisting of four hens and two toms, were exposed to MG by the respiratory route at the beginning of their breeding season. Fourteen control birds received sterile growth medium. Although no mortality of infected or control birds occurred, egg production during the first breeding season after infection was reduced. The mean number of eggs/hen/day produced by infected groups the first breeding season postexposure (PE) was significantly lower than the control value. The mean number of eggs produced daily by the same hens 1 yr later was unaffected by MG infection. The percentage of fertile eggs produced by infected groups was slightly reduced in both the first and second breeding seasons PE. Hatchability of fertile eggs from infected hens was significantly lower than eggs from control hens. Productivity may be impaired if MG infections occur in free-ranging wild turkey populations.

Key words: *Mycoplasma gallisepticum*, wild turkeys, *Meleagris gallopavo*, egg production, fertility, hatchability, experimental infections.

INTRODUCTION

Mycoplasma gallisepticum (MG) was recognized only recently as a potential pathogen for wild turkeys (*Meleagris gallopavo*), and little is known about its prevalence in, or effects on, wild populations. In domestic turkeys, MG causes infectious sinusitis, a disease producing lesions in the respiratory tract that lead to sinusitis and airsacculitis. In addition, MG infections in domestic poultry may result in decreased egg production, skeletal abnormalities and poor juvenile growth (Yoder, 1978). Respiratory afflictions have been reported in free-ranging wild turkeys naturally infected with MG (Davidson et al., 1982; Jessup et al., 1983), but effects of MG infection on reproductive parameters, such as egg production and hatchability, have not been assessed. Disease-induced alterations in productivity can be subtle and are not easily detected in wild populations. If egg production and hatchability or juvenile growth and survival are

compromised by MG infection, recruitment to wild populations could be affected. We examined the effects of MG infections in captive-reared wild turkeys on egg production, fertility and hatchability.

MATERIALS AND METHODS

Captive-reared wild turkeys (Touble Game Farm, Beloit, Wisconsin 53511, USA) were housed in isolation facilities and provided unlimited food and water. Broth cultures of MG-strain R were obtained from the U.S. Department of Agriculture Poultry Research Laboratory (Athens, Georgia 30605, USA), passed twice in fresh Frey's media (Frey et al., 1968), and incubated at 37 C for 18 hr. Three groups of birds were formed, each consisting of four 1-yr-old hens and two 2-yr-old toms. Each bird was infected with MG early in the breeding season of 1983 by dropping 1.0 ml of the inoculum (1×10^8 color-changing units/ml) into the trachea and nasal passages. Fourteen uninfected control birds (two groups of five 1-yr-old hens and two 2-yr-old toms each) received sterile broth. The turkeys bred freely, and eggs were collected daily for approximately 10 wk and incubated artificially until hatching. The total

number of eggs, the number of fertile eggs and the number of fertile eggs hatched were recorded for each treatment and control group at 3 to 5-day intervals.

Periodically, attempts were made to isolate MG from tracheal and cloacal swabbings collected in Frey's broth from birds in both the infected and control groups. Swabbings of the yolk sac of infertile, dead-in-shell, and pipped eggs also were cultured in Frey's broth for MG. These cultures were incubated at 37 C for 7 days or until noticeable growth occurred, at which time they were plated on Frey's agar. Agar plates were examined within 4 days. If no growth was evident, the plates were returned to the incubator for ≤ 28 days before being discarded. Mycoplasma isolates were identified using fluorescent antibodies obtained from S. Kleven (Poultry Disease Research Center, Athens, Georgia 30605, USA; Talkington and Kleven, 1983).

Blood was collected with heparin-rinsed syringes from all birds prior to the experiment and at 1, 2, 3, 5, 12 and 18 mo postexposure (PE). Rapid plate agglutination (RPA) tests for MG antibody were conducted with a commercially prepared antigen according to the manufacturer's protocol (Salsbury Laboratories, Inc., Charles City, Iowa 50616, USA). Microtiter hemagglutination inhibition (HI) tests were performed with antigens obtained from the National Veterinary Services Laboratory (Ames, Iowa 50010, USA) using their recommended procedure. Standard positive and negative sera were used for comparison in all tests.

Four experimental and two control hens were euthanized and necropsied at 5 mo PE; MG isolation was attempted from tracheas, lungs, airsacs, oviducts and ovaries. The remaining birds (two groups of controls and two groups of MG-inoculated turkeys) were maintained until the next breeding season to determine the long-term effects of MG infection.

The number of eggs/hen, collected within the 10-wk period for each group, was analyzed using nested analysis of variance (Kirk, 1982). The proportion of fertile eggs produced and the proportion of fertile eggs hatched for each group were transformed by arcsine (Sokal and Rohlf, 1969) and analyzed in the same manner. Groups of birds were nested within treatment levels. Each analysis assessed treatment, year, and treatment-year interaction effects. Multiple comparison analyses of significant differences were conducted by *t*-tests.

RESULTS

Mortality did not occur in any of the groups, but many of the MG-infected birds

developed respiratory signs (coughing and rales) which persisted for several months. Sinusitis was observed in one hen. MG was isolated from the trachea of all but one infected bird 1 mo PE and from 50% of the infected birds 2 mo PE. Isolation attempts during the second breeding season were unsuccessful.

None of the birds reacted positively to MG prior to exposure using either the RPA or HI test. Control birds remained seronegative for MG throughout the experiment. All MG-inoculated birds became MG antibody positive by the RPA test within 1 mo PE and remained positive for 18 mo PE. The proportion of infected birds with HI positive titers (i.e., titers of $\geq 1:80$) peaked within 2 mo PE and declined to 50% within 3 mo PE. A comparison of the HI and RPA tests for serologic testing for MG infection was reported previously (Rocke et al., 1985).

The number of eggs/hen (Table 1) was found to differ significantly ($P = 0.074$, $F = 7.27$, 1 and 3 df) between treatment and control groups. Differences between years was not significant ($P = 0.417$, $F = 1.03$, 1 and 2 df), but treatment-year interaction was a significant factor ($P = 0.060$, $F = 15.10$, 1 and 2 df). Multiple comparison tests showed that the MG treatment groups produced significantly fewer eggs ($P \leq 0.05$, $t = 3.39$) than control groups in 1983, but not ($P \geq 0.10$, $t = 0.30$) in 1984.

A significant trend was noted in the fertility of eggs laid (Table 1). The percentage of fertile eggs produced by infected groups was lower than control groups ($P = 0.059$, $F = 15.47$, 1 and 2 df). No significant fertility effects were found between years ($P = 0.821$, $F = 0.07$, 1 and 2 df) or from the treatment-year interaction ($P = 0.199$, $F = 3.57$, 1 and 2 df). Fertility of eggs produced by MG-infected hens was generally lower than that of control hens in both years of the study.

Hatchability of fertile eggs produced by MG-inoculated hens (Table 1) was also significantly reduced ($P = 0.027$, $F = 34.39$,

TABLE 1. Production, fertility, and hatchability of eggs laid by groups of captive-reared wild turkeys infected with *Mycoplasma gallisepticum* (MG) and uninfected controls the first (1983) and second (1984) breeding season postexposure.

| Treatment | Year | Eggs/hen | % Fertile | % Hatch |
|-----------|------|-----------------------|-------------------------|-------------------------|
| Control 1 | 83 | 27.2 (5) ^a | 91.8 (135) ^b | 52.4 (124) ^c |
| Control 2 | 83 | 24.6 (5) | 89.4 (123) | 54.5 (110) |
| MG-1 | 83 | 11.5 (4) | 84.1 (44) | 35.1 (37) |
| MG-2 | 83 | 4.0 (4) | 86.7 (15) | 30.8 (13) |
| MG-3 | 83 | 0 (4) | — | — |
| Control 1 | 84 | 14.5 (4) | 91.4 (58) | 75.0 (44) |
| Control 2 | 84 | 22.3 (4) | 96.6 (87) | 70.9 (79) |
| MG-1 | 84 | 23.8 (4) | 73.7 (95) | 66.7 (63) |
| MG-2 | 84 | 17.3 (4) | 82.8 (64) | 77.1 (48) |

^a Number of hens.

^b Number of eggs set.

^c Number of fertile eggs incubated until hatched.

1 and 2 df) compared to controls. Significant year effects were detected ($P = 0.02$, $F = 47.91$, 1 and 2 df), but there were no significant treatment-year interactions ($P = 0.15$, $F = 5.12$, 1 and 2 df). Improved hatchability in 1984 was probably due to adjustments made to the egg incubators the second year of the study. Attempts to recover MG from dead embryos and hatched poult were not successful.

Necropsy of four infected hens and two controls at the end of the first breeding season (5 mo PE) revealed severe airsacculitis in each infected bird examined. MG was not recovered from collected tissues. In three of the four infected birds, ovarian tissue was approximately one-third to one-half the size of that of uninfected controls.

DISCUSSION

Egg production of MG-infected captive-reared wild turkeys was depressed during the first breeding season PE, but the effect did not persist into the subsequent breeding season. This was consistent with the reported effects in domestic turkeys naturally infected with MG (Abbott et al., 1960) and in poultry experimentally infected. Lin and Kleven (1982) determined that egg production of chickens exposed to the same strain (strain R) of MG, declined significantly within 4 wk PE, then increased to values not significantly dif-

ferent from controls. Inflammation of the oviduct (salpingitis) characterized by caseous plugs has been described in both chickens (Domermuth and Gross, 1962; Pruthi and Kharole, 1981) and turkey poult (Domermuth et al., 1967) experimentally infected with MG, and the organism was recovered from these plugs for several weeks. Roberts and McDaniel (1967) reported isolation of MG from the oviducts of experimentally infected chickens for up to 15 wk PE. Because our experimental design required completion of a full breeding season, it was not feasible to collect histologic specimens early in the course of the infection to determine the pathologic effects of MG infection on ovaries or oviducts, nor to culture these tissues to determine the severity and duration of infection.

Although fertility of MG-infected turkeys was not severely reduced, the lowered percentage of viable eggs produced by these birds relative to controls and the persistence of this effect to the second breeding season PE merits some consideration. MG has adherent properties which enable it to attach to certain cells, such as spermatozoa (Clyde, 1983), and it has been isolated from semen of infected turkeys (Yamamoto and Ortmayer, 1967) and chickens (Yoder and Hofstad, 1964; Roberts and McDaniel, 1967). Yet, in our study, MG was not isolated from sperm collected

from all the infected toms in 1983, and the sperm was normal in number and motility. Other factors such as ovarian lesions or concurrent viral or bacterial infections may have contributed to the lowered fertility observed. Since the fertility of each egg was determined by candling after several days of incubations, the lowered percentage of viable eggs might have been the result of very early embryo mortality.

The total number of fertile eggs that developed and survived until hatching was generally low, presumably because of inadequacies of the egg incubator. Even so, hatchability of eggs from infected hens was significantly lower than controls, at least in the first breeding season PE. Poor egg hatchability is a documented effect of MG in naturally infected poultry. Abbott et al. (1960) reported a 40% decrease in hatchability of eggs produced by domestic turkeys with a chronic respiratory disease associated with mycoplasma. For free-ranging wild birds that lay a limited clutch of eggs, even slight reductions in the number of fertile or hatchable eggs could have a significant impact on total productivity. Our failure to isolate MG from eggs suggests transovarial transmission did not occur, or occurred infrequently. Other investigators have reported egg transmission rates of $\leq 3\%$ in chickens similarly exposed to MG by aerosol routes (Roberts and McDaniel, 1967; Lin and Kleven, 1982).

In our study, MG infections of game farm wild turkeys reduced egg production, fertility, and hatchability. The same effects may occur in free-ranging turkeys, but the effects of naturally acquired infections of MG on wild turkey populations are difficult to predict without field studies. MG-induced reductions in egg production and juvenile survival would probably have the most impact on small or newly established populations of wild turkeys. State game agencies and wildlife biologists involved in wild turkey restoration and relocation programs should be aware of the potential effects of mycoplasma infections.

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