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AN INVESTIGATION OF THE PERSISTENCE OF *MYCOPLASMA GALLISEPTICUM* IN AN EASTERN POPULATION OF WILD TURKEYS

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ABSTRACT: *Mycoplasma gallisepticum* infection had been confirmed by culture and serology among wild turkeys (*Meleagris gallopavo*) in close association with domestic fowl on Cumberland Island, Georgia (USA) in 1980. In 1988, wild turkeys were surveyed by serologic and cultural methods for evidence of *M. gallisepticum*. Chickens (*Gallus gallus*) and guinea fowl (*Numida meleagris*) from the site where the disease was originally detected also were tested by serologic and cultural methods for *M. gallisepticum* infections. There was no conclusive evidence that *M. gallisepticum* was present in wild turkeys or guinea fowl. In contrast, most chickens were strongly seropositive for *M. gallisepticum*, suggesting that they had been infected, although the organism was not recovered by cultural or bioassay methods. Other species of *Mycoplasma* isolated were *M. gallopavonis* from wild turkeys, *M. gallinaceum* and *M. pullorum* from chickens, and *M. gallinaceum* from guinea fowl. It appears that *M. gallisepticum* has not persisted or spread in the wild turkey population on Cumberland Island, despite continued contact by some wild turkeys with suspected carrier chickens.

Key words: *Mycoplasma* spp., *Mycoplasma gallisepticum*, mycoplasmosis, wild turkey, *Meleagris gallopavo*, domestic fowl, survey.

INTRODUCTION

Mycoplasma gallisepticum (MG), an important cause of respiratory disease in commercial poultry, recently received consideration as a potential health hazard to free-ranging wild turkeys (*Meleagris gallopavo*). During the late 1970's, reports of wild turkeys seropositive for *M. gallisepticum* appeared (Hensley and Cain, 1979; Amundson, 1985), and, in the early 1980's, *M. gallisepticum* was isolated from wild turkeys in Georgia, California, and Colorado (Davidson et al., 1982; Jessup et al., 1983; Adrian, 1984). The relocation of wild turkeys between states was of particular concern (Amundson, 1985; Davidson, 1987) since the commercial poultry industry, in cooperation with state and federal agricultural agencies, has been attempting to eliminate mycoplasmosis from domestic poultry (Lancaster and Fabricant, 1988).

The effects of experimental infections

of *M. gallisepticum* on reproduction and egg fertility and the persistence of MG in captive wild turkeys have been investigated (Rocke and Yuill, 1988; Rocke et al., 1988). However, except for the circumstantial evidence from wild turkey populations in Colorado (Adrian, 1984), little is known about the dynamics of the disease when it occurs in free-ranging wild populations.

The detection of MG in wild turkeys on Cumberland Island, Georgia provided an opportunity to assess its persistence in an isolated population of wild turkeys over an extended period of time. The original infection occurred in 1980 on private property where a mixed backyard flock of domestic chickens and exotic fowl was maintained (Davidson et al., 1982). Objectives of the present study were to determine the distribution of MG in the wild turkey population on Cumberland Island after 8 yr and to investigate the potential

carrier status of any birds seropositive for mycoplasmosis.

MATERIALS AND METHODS

Study site

Cumberland Island is the largest and southernmost barrier island off the coast of Georgia (30°52'N, 81°27'W) and measures approximately 28.2 km long and 4.8 km wide at its broadest point. This island became a National Seashore in 1972 under the management of the National Park Service (NPS) (Hillestad et al., 1975), but private inholdings still exist. A detailed description of the island is contained in Hillestad et al. (1975).

The current population of wild turkeys is thought to be largely derived from pen-raised wild stock released to replace extirpated native birds. On the private inholding where the original *M. gallisepticum* infection occurred, about 40 to 50 domestic chickens and 15 guinea fowl (*Numida meleagris*) currently range freely in the residence yard and the nearby woodlands. Exotic fowl housed in nearby pens include peacocks (*Pavo cristatus*) and Reeve's pheasants (*Syrnaticus reevesi*). At the time of this study, approximately 15 to 20 free-ranging wild turkeys roosted nearby and fed daily with the domestic fowl.

After the discovery of mycoplasmosis in wild turkeys on Cumberland Island in 1980, no turkeys were moved on or off the island, insuring a closed population, and no attempts were made to control the disease. Although the total number of wild turkeys presently on the island is unknown, subjective estimates by various biologists and researchers are in the magnitude of 200–300 turkeys.

Wild turkeys

Field work was conducted during 11 January to 17 March 1988. Using the original site of MG infection (Davidson et al., 1982) as the center, the island was divided into three zones where wild turkeys were randomly sampled as subpopulations. A minimum of 15 wild turkeys was sampled from each zone. Birds were captured with alpha-chloralose (Sigma Chemical Co., St. Louis, Missouri 63178, USA) mixed with cracked corn at 2 g per 250 ml of corn (Williams, 1966). Weight, sex, and age were recorded for each wild turkey, and birds were banded to insure against resampling. Birds were examined for clinical signs of MG infection, and their general physical condition was noted.

Five ml of blood was collected from the wing vein of each bird and placed into tubes containing ethylenediaminetetraacetic acid (EDTA)

(Becton-Dickinson Vacutainer Systems, Rutherford, New Jersey 07070, USA). Blood tubes were centrifuged at 5,000 rpm for 5 minutes, and the plasma was removed and divided into 0.5 ml aliquots. One aliquot was refrigerated for rapid plate agglutination (RPA) testing within 12 hr, and the remaining aliquots were frozen at 10 C for further serological testing.

Rapid plate agglutination tests were performed on plasma from each bird before its release. Both a commercially-prepared antigen (Salsbury Laboratories, Inc., Charles City, Iowa 50616, USA) and a laboratory-prepared antigen from the Poultry Disease Research Center (PDRC) (College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA) were used for RPA testing. When the two antigens produced different results, those from the antigen yielding the higher level of activity were utilized. Agglutination was scored on a 0 to +4 scale, with 0 being negative and +4 being complete agglutination. Standard positive and negative control sera (Salsbury Laboratories, Inc.) were used for all testing. Any bird with a strong plate reaction (+3 or +4) to MG was retained for necropsy. Birds with negative or weak (+1 or +2) reactions were released at the site where they were captured.

Subsequently, plasma samples from all birds were thawed and tested for antibodies to MG with the hemagglutination inhibition (HI) test. A laboratory-prepared antigen from PDRC was used, and the test was performed as described by the United States Department of Agriculture (USDA) National Poultry Improvement Plan (Anonymous, 1985). Only titer endpoints showing 100% inhibition of hemagglutination at $\geq 1:40$ were considered positive.

Tracheal swabs were obtained from each bird and placed into 2.5 ml of Frey's medium (GIBCO Laboratory, Grand Island, New York 14072, USA) with 12% swine serum (FMS) (Yoder, 1980). Supplemental antibiotics were added at 500,000 IU of penicillin and 10 ml of 10% thallium acetate per 500 ml of medium. Cultures were incubated at 37 C until a color change produced by a phenol-red indicator was evident and were then frozen at 0 C. To insure viability, frozen cultures were transferred to a -70 C freezer within 7 to 10 days.

Tracheal cultures were thawed and streaked onto FMS agar, and agar plates were incubated at 37 C for 7 to 10 days. All *M. gallisepticum* colonies were identified using a direct fluorescent antibody (FA) technique (Baas and Jasper, 1972). Every culture showing growth of *Mycoplasma* spp. organisms was checked for *M. gallisepticum*. Once a culture was demonstrated to be negative for *M. gallisepticum* by the FA test, attempts were made to identify the

organisms using conjugates for 10 other species of *Mycoplasma*. All turkey cultures collected on the site of the original infection were passed into FMS media with antiserum (100 μ l per 2.5 ml FMS) against nonpathogenic species of *Mycoplasma* found in those cultures to determine if *M. gallisepticum* was being overgrown.

Necropsies were performed on six birds that died during capture and on one with a +3 RPA reaction. Tracheas, sinuses, air sacs, and oviducts were cultured, and tissues from all major organs were collected for histopathological analysis. Lungs and ovaries were frozen and later cultured.

Domestic birds

Twenty domestic chickens and nine guinea fowl were sampled from the private inholding where MG had originally been detected in 1980. Blood samples and tracheal cultures were collected and tested by the procedures described above. These domestic birds also were examined for any clinical signs of mycoplasmosis.

An *in vivo* bioassay using domestic turkeys was conducted as a backup system to *in vitro* culture to determine whether the chickens were latent carriers of MG (Mallinson et al., 1981). One-day-old poults were obtained from a commercial source (Thaxton Turkey Hatchery, Watkinsville, Georgia 30677, USA) and housed in isolation facilities at PDRC. All poults were determined free of *M. gallisepticum* by serology and by culture.

Poults were divided into two groups of 14 each, a challenge group and a control group, and housed separately. The bioassay was initiated when the birds reached 6 weeks of age. A pooled inoculum was made of all *Mycoplasma* spp. isolates taken from chickens on Cumberland Island by thawing the original cultures and passing them into 100 ml of FMS medium. This inoculum was incubated at 37 C for 48 hr until growth was evident. The challenge group received the pooled isolates via three routes: eye-drop (2 drops), foot pad injection (0.2 ml), and aerosol (10 min) (Kleven et al., 1972). The control group received uninoculated FMS medium via the same routes. Birds were bled at 2, 3, 4, and 6 wk post-inoculation. Blood samples were allowed to clot at 25 C, and serum was tested by RPA and HI tests.

At 6 wk post-inoculation, a necropsy was performed on all birds, and serum and tracheal cultures were collected. Air sacs were examined and scored for lesions; they were cultured if lesions were present. All cultures were incubated at 37 C until fermentation was evident. Cultures that did not ferment were streaked onto agar at 7 and 14 days post-inoculation. Agar

plates were incubated at 37 C for 7 to 14 days. *Mycoplasma* spp. isolates were identified with the FA technique described previously.

RESULTS

At eight sites on the island, 88 wild turkeys were captured including 10 turkeys from the site of the original infection. All birds were in good physical condition, and none had clinical signs or gross lesions commonly associated with infectious sinusitis. With the RPA tests, six of 88 turkeys reacted to MG; however, all reactions were weak (+1 or +2), except for one +3 reaction. All turkeys were seronegative on the HI test.

Mycoplasma spp. organisms were isolated from tracheal cultures in 87 of 88 wild turkeys, but all cultures were negative for MG with the FA test. After it was determined that MG was not present in these cultures, identification of other *Mycoplasma* spp. was made from a selected subsample of one to four birds representing each capture location. *Mycoplasma gallopavonis* was identified from each of 16 turkeys tested for this organism, including at least one turkey from each capture site, and was the only *Mycoplasma* sp. identified by the FA test. Cultures that were passed into FMS media with antisera against *M. gallopavonis* tested negative for MG and remained positive for *M. gallopavonis*. Of the additional cultures of sinus, air sac, and oviduct taken from seven dead turkeys, only the sinus cultures produced *Mycoplasma* sp. isolates. These isolates tested negative for MG and positive for *M. gallopavonis*. No *Mycoplasma* sp. isolations were made from lungs (six) or oviduct (one) taken from turkeys at necropsy.

Eighteen of 20 chickens showed activity on the RPA tests for *M. gallisepticum* with the majority having strong (+3 and +4) reactions. Four had HI titers of 1:20, and another four had titers of 1:40. Guinea fowl were uniformly negative with both the RPA and HI tests. *Mycoplasma* spp. were isolated from all chickens and three of nine

guinea fowl, but all cultures were negative for *M. gallisepticum*. *Mycoplasma gallinaceum* and *M. pullorum* were identified in three of 20 cultures tested from chickens, and *M. gallinaceum* was identified in one of three cultures tested from guinea fowl. Cultures that were passed into FMS media with antisera against *M. gallinaceum* produced negative FA results for MG but remained positive for *M. gallinaceum*.

In the bioassay, all turkeys remained negative for antibodies to *M. gallisepticum* throughout the trial, and culture attempts for this organism likewise were negative.

DISCUSSION

The discovery of MG infections among wild turkeys on Cumberland Island was the first, and currently the only, reported occurrence of infectious sinusitis in free-ranging wild turkeys in the eastern United States (Davidson et al., 1982). Although some details remain unknown regarding this case, observations made during the present study suggest that favorable conditions existed for disease transmission from domestic to wild birds at this location. A mixture of domestic chickens and exotic fowl were being housed together and were allowed to range freely on the premises. There had been a long history of gamebird and exotic fowl production on this property, including intermittent propagation of pen-raised wild turkeys. Reportedly, pen-raised turkeys had been released periodically to supplement the wild population. Wild turkeys had free access to the property, and they often fed and intermingled with the backyard domestic fowl. Similar interactions between wild turkeys and domestic birds have continued since the initial detection of *M. gallisepticum* infection in 1980.

Interpretations of serologic tests for *Mycoplasma* are based on procedures and criteria developed for commercial poultry. Lower ranges of reactivity on RPA and HI tests are considered insignificant, and pos-

itive tests should be confirmed by isolation of the causative agent (Glisson et al., 1984). An important factor to consider is the possible occurrence of nonspecific reactions, especially with the RPA test. Causes of nonspecific reactions pertinent to testing of wild birds include presence of other microorganisms such as *Staphylococcus* sp., *Streptococcus* sp., and other *Mycoplasma* spp. (Roberts and Olesiuk, 1967; Thornton, 1973; Kleven, 1975), variations in antigen production, and improperly stored or contaminated serum (Kleven, 1975).

Although RPA and HI tests have not been critically evaluated for their reliability for use with wild turkeys, studies suggest that results from these tests for wild turkeys are not substantively different from those in domestic poultry. The RPA test is considered highly sensitive in detecting antibodies to *M. gallisepticum* in wild turkeys over long periods of time (Rocke et al., 1985; Rocke and Yuill, 1988). Antibody levels measured by the HI test in experimentally infected wild turkeys dropped to low or negative titers a few months after initial exposure (Rocke and Yuill, 1988), a pattern which also has been recognized in commercial poultry (Kleven, 1975, 1985). Wild turkeys chronically infected following experimental inoculation with MG retained strong RPA activity and low HI activity (Rocke and Yuill, 1988).

Application of standard criteria for interpretation of serologic data to the present study would classify three of six turkeys with RPA reactions to *M. gallisepticum* as potentially seropositive. Two of these were weak reactions (+2) and one was a moderately strong reaction of +3. The single bird with a +3 reaction was examined by necropsy, but no further evidence of *M. gallisepticum* infection was found by culture or serologic or histopathologic tests. Although the presence of nonpathogenic *M. gallopavonis* in the wild turkeys could have hampered the cultural recovery of the more slowly growing MG, other testing procedures did not support the RPA results. Because of the lack of confirmation

by HI or by culture and the lack of clinical signs of *M. gallisepticum*, we consider the few generally weak RPA reactions in the wild turkeys in this study to be false positive or nonspecific reactions.

The inability to isolate *M. gallisepticum* from the tracheas of strongly seropositive chickens by in vitro and in vivo procedures was not unexpected. *Mycoplasma gallisepticum* is known to decline and eventually disappear from the tracheas of experimentally infected chickens a short period of time after inoculation (Kleven, 1985), and its isolation can be confounded by the presence of nonpathogenic *Mycoplasma* spp. In backyard chickens, a lack of clinical disease, low rate of spread, low virulence, and high rates of RPA reactivity to *M. gallisepticum* coupled with negative or low HI titers are characteristic of a carrier state (S. H. Kleven, unpubl. data). These features also have been observed in commercial chickens (Bencina and Dorrer, 1984; Yoder, 1986) and infrequently in turkeys (Ley et al., 1988). In the chickens on Cumberland Island, the organism may have become localized in lungs or reproductive organs from which it is not readily transmitted to other birds. The main mode of perpetuation is probably via the transovarian route (Yoder, 1986). In this particular instance, serological results could not be confirmed by necropsy.

The negative serology and cultures of the guinea fowl indicate no significant role of these birds in the occurrence of MG infection in wild turkeys on the island. This is supported by the lack of any published reports of MG in guinea fowl.

Collectively, the results of all tests indicate that MG has not spread in the wild turkey population on Cumberland Island. Although we did not test the entire population for this disease, negative results from 88 birds provide a good statistical inference (Steel and Torrie, 1980) for this conclusion. Assuming a base population of 200 birds, apparently negative tests from 88 consecutive birds provided a 95% con-

fidence level that the maximum potential prevalence was <0.5% or 1 of 200 birds.

The epidemiology of MG on Cumberland Island at the time of the original diagnosis and during the intervening years is unknown. A cycle of infection involving domestic chickens may have occurred with the disease eventually spilling into free-ranging birds. Alternatively, the periodic release of pen-raised wild turkeys on Cumberland Island may have played a role in the occurrence of this disease in the wild turkeys. At some time after the discovery of MG, the release of pen-raised wild turkeys was halted. Despite the lack of disease control procedures and the continued association between wild turkeys and domestic fowl on the island, the occurrence of *M. gallisepticum* in wild turkeys apparently was transitory and self-limiting.

This case further emphasizes the role of domestic or pen-raised stock in the transmission of MG to wild birds. The isolation of *M. gallisepticum* from wild turkeys has been relatively rare, but a domestic source of infection has always been implied. On a California farm, *M. gallisepticum* was isolated from several wild-type turkeys that had been feeding with a domestic flock of broad-breasted bronze turkeys (Jessup et al., 1983). One of the domestic turkeys was also culture positive, and turkeys on a neighboring farm were seropositive. The decline of a population of wild turkeys in Colorado has been associated with mycoplasmosis (Adrian, 1984). A possible source of *M. gallisepticum* was artificial feeding sites located on farms with commercial turkey operations. In addition, a recent survey of pen-raised wild turkeys confirmed that *M. gallisepticum* infection occurs among some of these birds and that it may cause infectious sinusitis in them as well (Schorr et al., 1988). To minimize the threat of mycoplasmosis to wild turkeys, interactions of wild turkeys with domestic poultry or pen-reared wild turkeys should be avoided, and the release of pen-raised wild stock should be discouraged.

Mycoplasma gallopavonis infection ap-

pears to have been ubiquitous among the wild turkeys on Cumberland Island since cultures representing all capture sites tested positive for this organism. In all probability, it was the same organism isolated from the tracheas of all 87 culture-positive turkeys. *Mycoplasma gallopavonis* has been isolated from wild turkeys in Texas (Rocke and Yuill, 1987) and in South Carolina (S. H. Kleven, unpubl. data). Isolates of *M. gallopavonis* have been shown to be lethal to embryos of domestic turkeys and chickens (Rocke and Yuill, 1987), but the pathogenicity of *M. gallopavonis* in wild turkeys is unknown. Additional studies of this apparently frequent organism in wild turkeys are warranted.

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