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Evaluation of Serologic Tests for *Mycoplasma gallisepticum* in Wild Turkeys

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Recent isolations of *Mycoplasma gallisepticum* (MG) from free-ranging turkeys (*Meleagris gallopavo*) in California (Jessup et al., 1983, J. Am. Vet. Med. Assoc. 183: 1245–1247), Georgia (Davidson et al., 1982, Avian Dis. 26: 402–405), and Colorado (Adrian, pers. comm.) and serologic evidence of infections in wild turkeys from Texas (Hensley and Cain, 1979, Avian Dis. 23: 62–69; Rocke and Yuill, unpubl. data), Missouri and Wisconsin (Amundson, unpubl. data) has raised concern about the potential spread of MG and other mycoplasma species through wild turkey translocation and reintroduction programs. MG infections produce sinusitis, respiratory signs (coughing, sneezing, rales and nasal discharge), and airsacculitis (Yoder, 1978, *In* Diseases of Poultry, 7th Ed., Hofstad et al. (eds.), Iowa State University Press, Ames, Iowa, pp. 236–250) and have been shown to reduce egg production and hatchability in both domestic poultry (Yoder, 1978, op. cit.; Lin and Kleven, 1982, Avian Dis. 26: 487–495) and wild turkeys (Rocke and Yuill, unpubl. data). The infection is prolonged and often results in persistent carriers. MG alone costs the table-egg industry an estimated $97 million per year, and many states are cooperating under the National Poultry Improvement Plan to eradicate the disease (Johnson, 1983, Avian Dis. 27: 342–343). In an effort to minimize the risk of MG transmission to and from wild turkeys, the United States Animal Health Association has called for routine MG testing of all wild turkeys caught for translocation purposes (Nettles and Thorne, 1982, Proc. Annu. Meet. U.S. Anim. Health Assoc. 86: 64–65).

Several states such as Wisconsin, Michigan, Wyoming and California have been monitoring translocated wild turkeys for antibodies to mycoplasmas using two serologic tests routinely conducted by personnel in the poultry industry. The rapid plate agglutination (RPA) test is an inexpensive procedure that can be easily conducted in the field, while the hemagglutination inhibition (HI) test requires more time and materials. Most poultry diagnosticians agree that MG positive reactions detected by the RPA test must be confirmed by HI titers of at least 1:80 to be considered true positives and therefore diagnostic of the infection in domestic turkeys and chickens (Yoder, 1980, *In* Isolation and Identification of Avian Pathogens, 2nd Ed., Hitchner et al. (eds.), Am. Assoc. Avian Pathol., College Station, Texas, pp. 40–42).

Both of these tests have been applied to sera from wild turkeys, but the interpretation of results obtained has been controversial. Doubts have been raised about the reliability of the RPA procedure as a field test and the merits of MG control programs for wild turkeys. For example, the Wisconsin Department of Natural Resources has implemented a program to prevent the introduction and dissemination of MG by translocated wild turkeys

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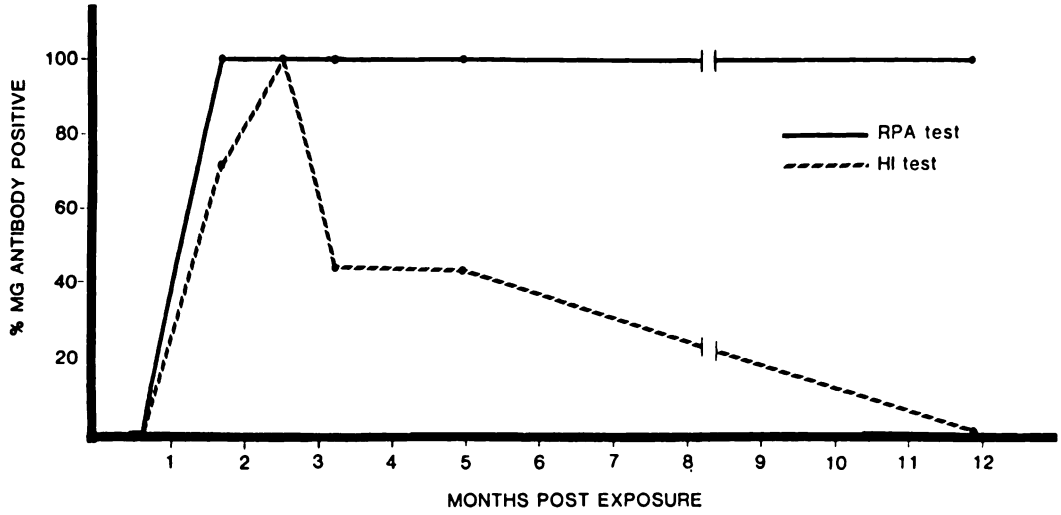

FIGURE 1. Serologic response of captive-reared wild turkeys to *Mycoplasma gallisepticum* (MG) using the rapid plate agglutination (RPA) test and the hemagglutination inhibition (HI) test.

based on serologic field testing prior to release. Any MG antibody positive birds detected by the RPA test are not released and are held in captivity for further study or are killed and necropsied. However, inconsistent RPA results have been obtained from different laboratories and frequently RPA positive sera are negative by the HI test. Improper storage and handling of samples has been suggested as the cause of nonspecific agglutination or false positive readings with the RPA procedure, but not verified. Comparisons of RPA and HI responses of wild turkeys following MG infection have not been reported previously.

While conducting experiments on the effects of MG infections in captive-reared wild turkeys (Touble Game Farm, Beloit, Wisconsin 53511, USA), we measured their serologic response to MG and evaluated the RPA procedure under different conditions. Eighteen adult turkeys were exposed to MG by intubating 1.0 ml of an 18 hr culture (10^8 color changing units/ml) intratracheally and intranasally. Fifteen uninfected controls received sterile broth only. Each bird was bled prior to treatment and at periodic intervals thereafter. Blood was collected with heparin-rinsed syringes to avoid serum clots except when serum rather than plasma was desired.

RPA tests were conducted for MG with a commercially prepared antigen according to the protocol suggested by the producer (Salsbury Laboratories, Inc., Charles City, Iowa 50616, USA). Standard positive and negative sera were used for comparison. Microtiter HI tests were performed with antigens obtained from the National Veterinary Services Laboratory, Ames, Iowa 50010, USA.

None of the turkeys reacted positively to MG with either test prior to infection. Likewise, none of the uninfected controls developed MG antibody during the course of the experiment. All of the infected birds became MG antibody positive by the RPA test within 1 mo post exposure (PE) and remained positive to MG through the last time of testing 1 yr later (Fig. 1). The number of turkeys with HI positive reactions (titers of at least 1:80) reached 100% by 2 mo PE, but within 3 mo PE had dropped to less than 50%. One yr PE, none

of the exposed birds had positive HI titers to MG.

The transient nature of MG HI antibody compared to RPA antibody is very evident from this experiment. Snell and Cullen (1978, Br. Vet. J. 134: 198–204) reported similar results (intermittent HI antibody, persistent RPA reactions) in domestic turkey poults experimentally inoculated with MG. Other investigators have demonstrated that the immunoglobulin class responsible for the HI response to mycoplasma antigens is IgG, whereas the RPA test primarily measures early antibody or IgM and is relatively insensitive to IgG (Kleven and Pomeroy, 1971, Avian Dis. 15: 291–298; Roberts, 1969, J. Appl. Bacteriol. 32: 395–401). The fact that the tests measure two different antibody classes may explain the discrepancies reported between these tests when applied to wild turkey sera.

Results of MG RPA and HI tests on wild turkey sera agree for only a short interval after exposure. The antibody class responsible for producing hemagglutination reactions declines, yet the agglutinating antibodies remain. In the domestic poultry industry, flocks can be sampled repeatedly and infections detected early when both types of antibodies are present. Thus, the HI test would be expected to confirm RPA results. Wild turkeys, on the other hand, are caught and tested only once. According to our experiments, an MG RPA positive bird may be negative by the HI test if exposure occurred earlier than 3 mo prior to sampling. The RPA test can accurately diagnose MG infected wild turkeys for at least one year PE, whereas the HI test is accurate for only a few mo PE. Interestingly, MG infections have been confirmed in certain domestic flocks with recurrent, weak positive RPA reactions and negative HI titers (Mallinson, 1983, Avian Dis. 27: 330–331).

Nonspecific agglutination results have been attributed to improper mixing of antigen and sera, delayed reading of the test, contaminated serum (Adler and Wiggins, 1973, World's Poult. Sci. J. 29: 345–353), storage of serum at 4 C, and freezing and thawing (Roberts, 1969, op. cit.). To test the reliability of the RPA test, MG positive and negative serum samples and plasma from blood collected with heparin were stored either at room temperature, refrigerated or frozen for several wk, and retested periodically. Heparinizing blood samples did not alter the results of the test. The sensitivity of the RPA test (number positive reactors/number true positives) was quite high. There was one false negative reaction after storage of the samples at room temperature for 2 wk. Likewise, specificity of this test was very good. There was one false positive reaction after 1 wk of refrigeration. When this antiserum–antigen mixture was examined with a microscope, it was obviously not a true agglutination reaction, but due to particulate matter in the sample which could be removed by centrifugation. Although storage of samples did not appreciably interfere with the RPA procedure, we and others (Hammar et al., 1958, Avian Dis. 2: 213–226) have observed that repeated freeze thawing and long term refrigeration can result in precipitates that may be confused with positive reactions.

The RPA test works best with fresh, clear serum or plasma and is easier to read if conducted as soon after collection as possible. When the agglutination reaction is weak, slow, or otherwise questionable, retests on serum dilutions may be helpful. According to provisions of the National Poultry Improvement Plan, RPA reactions at 1:10 or greater are considered positive (Anonymous, 1982, USDA APHIS-91-40).

We agree with Snell and Cullen's conclusion (1978, op. cit.) that more reliance could be placed in the MG RPA test for detecting MG reactors. Until a better diagnostic method is developed, this is particularly true for flocks of wild turkeys. If conducted according to protocol with ap-

propriate positive and negative controls, the MG RPA is a reliable, effective field test. The presence of HI antibody is not a diagnostic prerequisite, but would indicate the occurrence of a very recent exposure.

Unfortunately, the relationship between detectable antibody and the carrier state of mycoplasma in wild turkeys is unknown. Until more research is conducted, it can only be assumed that an MG reactor is a carrier of the disease and may be infectious for other wild and domestic birds. Control programs for MG in translocated turkeys, similar to those in Wisconsin, Michigan, Wyoming, and California should be instituted as a necessary precaution.

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Infection of *Exophiala salmonis* in Atlantic Salmon (*Salmo salar* L.)

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There have been three previous reports of infections of *Exophiala salmonis* in salmonids in fresh and saltwater (Carmichael, 1966, Sabouraudia 5: 120–123; Ajello, 1975, Pan Am. Health Organ. Sci. Publ. 304: 126–130; Richards et al., 1978, J. Fish Dis. 1: 357–368), one in error (Ajello, pers. comm.). This note describes the first report of infection by *E. salmonis* in the United States.

Over a 4-mo-period (April–June 1982), three adult Atlantic salmon (University of Rhode Island Accession No. M112, M151, and M657) with similar lesions were necropsied at the Comparative Aquatic Pathology (CAP) Laboratory, University of Rhode Island. Originally obtained from hatcheries in East Orland, Maine and New Brunswick, Canada, these fish had been held at the University Aquaculture Center in a partial reuse system for up to 20 mo prior to death. City water was maintained at 12 to 18 ppt salinity by the addition of rock salt. Fish were fed raw calf's liver supplemented with vitamins.

Tissues from fish were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned at 6 μm and stained with hematoxylin and eosin. Special stains included periodic-acid Schiff (PAS) and Grocott's silver stain (Luna, 1968, Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, Washington, D.C., 258 pp.).

Accession No. M112 had a reddish discoloration 3.0 mm in diameter on the surface of the liver which extended into the parenchyma. Postmortem examination of M151 revealed petechial hemorrhages throughout the viscera. The pyloric cecae contained approximately 50 adult cestodes (*Eubothrium* sp.). The posterior kidney capsule was thickened and the parenchyma mottled gray. Accession No. M657 was cachexic. An ulceration (1.0 cm

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