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## EXPERIMENTALLY TRANSMITTED MARBLE SPLEEN DISEASE IN PEN-RAISED WILD TURKEYS\*

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Abstract: Pen-raised North American wild turkeys (Meleagris gallopavo L.) were experimentally infected with marble spleen disease (MSD) to determine their susceptibility to this disease. Gross and microscopic lesions were consistent with experimental MSD in pheasants and domestic turkeys: an enlarged mottled spleen, intranuclear inclusion bodies, and absence of pulmonary edema and hemorrhage. Detectable levels of viral antigen were not demonstrable in sera of turkeys using the agar gel precipitin test.

#### INTRODUCTION

Marble spleen disease (MSD) of ringnecked pheasants (Phasianus colchicus L.) was experimentally transmitted to pen-raised ring-necked pheasants and domestic turkeys (Meleagris gallopavo L.) by Iltis et al.<sup>3</sup> This study<sup>3</sup> proved that a filterable agent, probably a virus, was the causative agent of MSD.<sup>1,2,4,5</sup> by demonstrating infectivity with 0.20 μm filtered inoculum. However, cases of MSD transmitted in the laboratory<sup>3</sup> lacked the terminal pulmonary involvement occurring in field cases.<sup>1,2,5</sup>

Pen-raised wild turkeys bred for state repopulation programs represent an analogous situation to pen-raised ring-necked pheasants. Whereas field outbreaks of MSD occur in pen-raised pheasants none have been reported in wild turkeys raised in captivity. Domestic turkeys are susceptible to experimental MSD infection but domestic and wild turkeys must have some genetic differences; therefore, the present study was undertaken to determine the wild turkey's susceptibility.

#### MATERIALS AND METHODS

#### **Experimental Birds**

Twelve turkeys, approximately 8 weeks old, from the Pennsylvania Game Commission's Wild Turkey Farm in Williamsport, Pennsylvania were supplied by Harvey A. Roberts with the assistance of Eugene P. Nelson. Of the nine birds used for this study, five were housed in an outdoor pen off the ground and four were housed indoors in two separate units off the floor.

#### Inoculations

Spleens (kept at —60 C for 3 months) from two domestic turkeys experimentally infected with MSD and killed on postinoculation day 5 (representing the fourth passage in turkeys of the pheasant derived infectious agent in the laboratory) were prepared by adding 10 ml of sterile phosphate buffered saline (PBS), pH 7.2, then homogenizing in a Ten Broeck grinder followed by two freezethaw cycles in a dry ice-alcohol bath. One ml of this inoculum was administered orally via canula to the five turkeys

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housed outdoors and also to two turkeys housed indoors. The other two turkeys kept in the other indoor unit served as uninoculated controls. The turkeys were 12 weeks old at inoculation.

#### **Gross and Microscopic Examinations**

On postinoculation day 5 all nine turkeys were killed and viscera examined for gross lesions. Sections of spleen, liver and lung were fixed in 10% formalin, embedded in paraffin, sectioned at 5  $\mu$ m and stained with hematoxylin and eosin.

#### **Agar Gel Precipitin Tests**

Prior to inoculation, sera from the nine turkeys were tested for agar gel precipitin (AGP) antibodies to marble spleen antigen as previously described.<sup>8</sup> In addition, serum samples from each bird were taken on post-inoculation days 1 to 5 and tested for AGP antigen reactive to antibody in convalescent serum from a pheasant exposed to MSD.

### **RESULTS AND DISCUSSION**

The only gross lesions observed were enlargement of the spleen and slight splenic mottling in four of the seven experimentally infected turkeys. The two uninoculated controls had no gross or microscopic lesions. No clinical signs were observed in any of the birds.

Microscopic lesions observed in the

seven inoculated turkeys consisted primarily of intranuclear inclusion bodies (INIB) typical of MSD.<sup>1,2,5</sup> The INIB occurred in cells of spleen, liver, and lung. In addition, splenic lymphoid hyperplasia also was noted. The gross and microscopic findings observed in experimentally transmitted MSD in these seven pen-raised wild turkeys were consistent with those previously reported for the pen-raised pheasant and domestic turkey.<sup>8</sup>

No AGP antibody was detected in sera prior to experimental infection. Also, no antigen, indicative of viremia, was detected by the AGP test in sera of nine turkeys on postinoculation days 1 to 5.

Presumably, outdoor housing of turkeys and pheasants experimentally infected with MSD might better simulate field conditions, which could possibly induce typical field cases of MSD, 1,8 rather than the experimental MSD (typified by absence of terminal lung involvement and presence of INIB on postinoculation days 3, 4, and 5).3 Results of this study demonstrate that while pen-raised wild turkeys are susceptible to experimentally transmitted MSD, no change in the course of the disease between birds housed indoors and outdoors had occurred by postinoculation day 5. The possibility of field outbreaks of MSD in pen-raised wild turkeys remains unknown; however, serum samples taken from these birds in the fall and tested for AGP antibody to MSD antigen should indicate if inapparent infections occur.

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