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Experimental Infections of Waterfowl with *Sphaeridiotrema globulus* (Digenea)

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ABSTRACT: *Sphaeridiotrema globulus* in experimentally infected mute swans (*Cygnus olor*), mallard ducks (*Anas platyrhynchos*) and Canada geese (*Branta canadensis*) induced ulcerative hemorrhagic enteritis. Sites of infection include the jejunum and ileum. The digeneans ulcerated the intestine. The inflammatory response was primarily lymphocytic with some eosinophils. Severe hemorrhage from damaged submucosal capillaries provided a blood meal for the parasite and caused anemia in the host. Extra-medullary hematopoiesis occurred in the liver, and an erythroid hyperplasia occurred in the bone marrow of infected birds. Infected birds exhibited muscular weakness and died from shock associated with severe blood loss. Mallards and Canada geese were less susceptible to fatal infection than the mute swan as evidenced by survivors in the higher dose groups.

Key words: *Sphaeridiotrema globulus*, mute swan, *Cygnus olor*, mallard, *Anas platyrhynchos*, Canada goose, *Branta canadensis*, pathology, hemorrhagic enteritis, digenean.

Approximately 25 mute swans have died each year since 1970 on Lake Musconetcong (Netcong, New Jersey, USA; 40°45′N, 74°42′W) from infection with *Sphaeridiotrema globulus* (Roscoe and Huffman, 1980). The intermediate host for the parasite at the lake is the pleurocerid gastropod *Goniobasis virginica* (Huffman and Fried, 1983). *Sphaeridiotrema globulus* has been implicated in a fatal enteritis of several species of waterfowl (Price, 1934; Cowan and Cornwell, 1963; Trainer and Fischer, 1963; Campbell and Jackson, 1977; Roscoe and Huffman, 1983). Macy (1973) investigated acquired resistance to *S. globulus* in Pekin ducklings (*Anas boschas*). In the present study mute swans (*Cygnus olor*), mallards (*Anas platyrhynchos*) and Canada geese (*Branta canadensis*) were experimentally infected with *S. globulus* to study the pathogenesis of the disease.

In order to prevent spontaneous infections with digeneans, eggs of mute swans, mallards and Canada geese were collected from nests in Newport (Rhode Island 02840, USA) and Hackettstown (New Jersey 07840, USA) and hatched in incubators in May and June (Rockport Game Farm, Hackettstown, New Jersey 07840, USA). Young birds were housed together on a concrete floor in an unheated building for 1 mo before transfer to an outdoor pen. Water and food (Complete Grower, Agway Inc., Syracuse, New York 13221, USA) was provided ad libitum throughout the study. The birds were conditioned to the diet and pen for 4 mo prior to experimentation.

The mute swans and mallards were divided into four groups of three birds each. Groups A, B and C were administered approximately 2,000, 500 and 100 metacercarial cysts, respectively, via an esophageal catheter. The cysts were obtained from naturally infected *Goniobasis virginica* snails at Lake Musconetcong (Huffman and Fried, 1983). Controls in group D did not receive any metacercaria. The Canada geese were divided into three groups of four birds each. Groups A and B were administered approximately 1,000 and 500 metacercarial cysts, respectively. Controls in group C did not receive any metacercaria. Controls were administered distilled water via an esophageal catheter not used for metacercarial infection procedures.

Heparinized blood samples (0.75 to 1.00 ml) were collected by brachial venopuncture from all experimental animals prior to infection, day 3 postinfection (PI), and at 1 day intervals until the experimental animal succumbed or until 21 days had elapsed. The duration of the study and sampling schedule were based on the reported lifespan of 16 days for *S. globulus* (Berntzen and Macy, 1969).

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Standard procedures were used to determine erythrocyte counts, packed cell volumes and hemoglobin concentrations for each sample on the day of collection (Wintrobe et al., 1975). Air dried blood smears were fixed and stained using the Harleco Diff Quick (American Scientific Products, Edison, New Jersey 08818, USA) system and examined microscopically. The birds were examined daily for clinical signs of infection and feces were examined for blood.

Birds that died during the course of the study were necropsied as soon as possible after death. Controls and the remaining experimental animals were killed by decapitation and necropsied 21 days PI. The intensity of infection was determined by the number of parasites recovered. Gross lesions were recorded and tissues collected for histology. Tissues were fixed in 10% neutral buffered formalin (NBF), sectioned at 6 μm and stained with hematoxylin and eosin.

All infected swans and mallards from groups A, B and C and Canada geese from group A displayed a watery blood-stained diarrhea, weakness, unsteady gait and disorientation by day 5 of the study. Moribund birds had pronounced anemia characterized by erythroblasts containing mitotic figures or multiple nuclei in peripheral blood smears. Birds died as the result of hypovolemic shock.

Infected swans had hematocrits (PCV, %) ranging from 3% to 7% within 12 hr of death. Hematocrits from control birds ranged from 21% to 49% throughout the study. Hemoglobin concentrations for infected birds within 12 hr of death were <4 g/dl. Hemoglobin concentrations from controls ranged from 9 g/dl to 14 g/dl throughout the study. Erythrocyte counts in infected swans within 12 hr of death ranged from $0.20 \times 10^6/mm^3$ to $0.45 \times 10^6/mm^3$. Values from control birds ranged from $1.10 \times 10^6/mm^3$ to $2.81 \times 10^6/mm^3$ throughout the study. The blood parameters of birds that received high numbers of metacercariae (group A) decreased after day 3 until death of the birds on day 5. The parameters were affected 1 day later in the medium dose birds (group B) and dropped at a slower rate until death of the birds between days 8 and 10. Similarly, the PCV, hemoglobin concentration and erythrocyte counts of birds receiving low doses of metacercariae were affected after day 5 and declined more gradually to their lowest values between days 11 and 17. These values returned to normal in the two surviving birds by day 20.

Mallards given a high dose of metacercariae died from the infection on day 5. The PCV ranged from 9% to 10%, prior to death. Hemoglobin concentrations were 4 g/dl prior to death. The erythrocyte counts ranged from $0.45 \times 10^6/mm^3$ to $0.50 \times 10^6/mm^3$. The high dose group had a greater decline in blood parameters than the medium dose and low dose groups. The medium and low dose groups recovered from the infection.

Two of the Canada geese given a high dose of metacercariae died from the infection on day 7 and day 8. The PCV ranged from 10% to 11%, prior to death. Hemoglobin concentrations were 4 g/dl prior to death. The erythrocyte counts ranged from $0.50 \times 10^6/mm^3$ to $0.60 \times 10^6/mm^3$. The remaining experimental animals in this group recovered from the infection. The other experimental group receiving 500 cysts showed very little change in blood parameters and recovered from the infection.

Pericloacal feathers of all infected birds were stained with blood. Pallor in the skeletal muscles, myocardium and lungs was pronounced. Petechiae were present on the ventricular myocardium, which occasionally displayed white streaks running parallel to the muscle fibers. A serous hydropericardium was observed. The liver was friable, walnut brown and streaked with bile. The gallbladder was distended. Splenomegaly was observed.

Ballooning of the jejunum and ileum was accompanied by a generalized cyanotic appearance of the affected jejunum and

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ileum. Foci of hemorrhage circumscribing trematodes were visible through the bowel serosa. Congestion of intestinal veins and arterioles was grossly apparent. The jejunum and ileum when opened released large quantities of fluid blood. Casts of fibrin, erythrocytes, digeneans and desquamated epithelium remained in the lumen. Washings revealed a hyperemic mucosa with small (1 to 2 mm), white parasites nestled singly or in groups in ulcers. These ulcers were circumscribed by a halo of hemorrhage and were enumerated as "lesion sites." In the mute swan infections, the average number of lesion sites were 52 (group A), 32 (group B) and 24 (group C); for mallards and Canada geese equivalent values were 45 (group A), 4 (group B) and 1 (group C), 20 (group A), and 5 (group B), respectively. The range of fatal infections in mute swans was six to 172 parasites; in mallards and Canada geese there were 79 to 142 and 53 to 115, respectively.

Histologic lesions were evident in the intestine of infected birds. Ulceration of the mucosa occurred in the jejunum and ileum. Ulcers penetrated to the circular muscle layer. Intestinal villi appeared as mounds of loose connective tissue. Crypts were obliterated in such areas, and capillary damage resulted in hemorrhage. Erythrocytes from damaged capillaries were observed on the interface of the acetabulum and corium and in the intestine of *S. globulus*. Lymphocytic infiltration of the villi was the primary cellular response. A few neutrophils and eosinophils were also present. In fatal infections histologic lesions were evident in the spleen, liver and bone marrow as well as in the intestine. A periportal hepatitis characterized by lymphocytic infiltration was observed. Extramedullary hematopoiesis was characterized by nests of erythroblasts in sinusoids. Secondary nodules were characteristic of reactive spleens. The femoral marrow exhibited an erythroid hyperplasia. The sinusoids and veins were filled with round, basophilic, immature erythrocytes.

The clinical signs, gross pathology, histopathology and fatal course of the disease occurring in mute swans at Lake Musconetcong were reproduced in swans, mallards and Canada geese with known doses of *S. globulus*. While Huffman and Roscoe (1986) have shown that resistance in mallard ducks to *S. globulus* infections can be acquired, this study suggests mallards and Canada geese have a greater innate resistance to the infection than do swans since the seasonal average of 85% of *G. virginica* at Lake Musconetcong are infected with *S. globulus* metacercariae (Huffman and Fried, 1983) and 60% of these contain a potentially lethal dose ($\geq$100 metacercariae) for swans. Swans which ingest a single snail from the lake have a 51% chance of ingesting a lethal dose of *S. globulus* and never have an opportunity to acquire an immunity to the infection. If the birds ingest multiple snails the likelihood of any birds surviving is greatly reduced. These findings combined with possible differences in exposure due to feeding habits explain why swans are the only species dying at the lake.

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