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ACQUIRED RESISTANCE IN DUCKS TO INFECTION WITH THE PSILOSTOME TREMATODE *Sphaeridiotrema globulus* (RUDOLPHI, 1814).

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Abstract: Half of the young Pekin ducks used in four experiments were given 250 cysts of *Sphaeridiotrema globulus* each. Sixteen days later all of the birds were given 1000 cysts each. When the birds were killed or died after 4 days, 11 of the 18 birds with initial infections were free of trematodes while all 17 of those without initial infections were parasitized. The control ducks harbored 6911 flukes, 17 times the number found in the previously infected birds.

INTRODUCTION

Because of its significance as a cause of mortality of wild and domestic ducks and because it is a tissue-invading trematode, acquired resistance to *Sphaeridiotrema globulus* was investigated.

This trematode was reported from Maryland by Price[11] who found it in scaups, *Marila affinis,* which appeared to have died from the infections. The parasite also has been found by a number of investigators to be associated with heavy mortality of ducks in the North Central States as indicated by McDonald.[9] The same parasite was reported from Oregon ducks by Dikmans.[3] Macy and Ford[7] found the developmental stages in *Flumenicola virens* (Lea), a common stream snail in Western Oregon. Burns[2] described the life cycle of *Sphaeridiotrema spinoacetabulum* which the writer[6] considers to be synonymous with *S. globulus,* after independently investigating the cycle. In vitro excystation, structure of the cyst, and host specificity were studied by Macy et al.[8] In vitro cultivation from metacercaria to egg-producing adults was carried out by Berntzen and Macy.[1]

Acquired immunity to digenetic trematodes has been studied by others in a limited number of species, mainly those of economic or medical significance. The subject has been reviewed by Jackson et al.[4] According to Smithers and Terry[12] a single exposure of the rhesus monkey to as few as 25 cercariae of *Schistosoma mansoni* conferred a high degree of resistance to later infection. Thus worms of challenge infections did not mature but were destroyed. Pantelouris[10] indicated that field studies of *Fasciola hepatica* infections in sheep and goats have failed to yield evidence of acquired immunity in these hosts, and experiments attempting to produce such immunity have not been successful. However, Lang and co-workers[5] reported that when peritoneal exudate cells from infected mice were injected into normal mice, significant immunity to later challenge infections was produced. In the case of *Clonorchis sinensis,* attempts to produce protective immunity have not been successful.[13]

MATERIALS AND METHODS

The encysted metacercariae of *S. globulus* used in this study were from naturally infected snails, *Flumenicola virens* (Lea), collected from the Tillamook River, Tillamook County, Oregon. The snails were scraped from the surface of the mud bottom of the river with a Needham scraper net, and transported in plastic containers aerated with O-tab oxygen tablets sold by Pemble Laboratories, River Falls, Wisconsin. They were maintained in the laboratory in shallow pans at 5 C until used in the experiments.

Pekin ducklings, *Anas p. platyrhynchos,* were maintained in the laboratory on a

nonmedicated, balanced chicken mash produced by Nu-lay Feeds, Portland, Oregon, and given adequate water.

Groups of snails were crushed in small amounts of saline in watch glasses and the cysts were transferred by Pasteur pipettes into clean saline where they were counted. Two hundred and fifty cysts were placed in each of a series of gelatine capsules and these were put down the throats of ducklings for the preliminary infections. A small amount of water was placed in the open bill of each bird to aid in washing down the capsule.

A method for obtaining large numbers of encysted metacercariae involved digestion of snail tissue, screening, differential centrifugation, and sugar flotation as described by Macy *et al.*[8]

Cysts so obtained were resuspended in saline and stirred at high speed on a magnetic stirrer. With a volumetric pipette, 0.1 ml of suspension was removed to a watch glass and the cysts counted. From the average number of cysts in five such samples, the amount of suspension required to yield approximately 1000 cysts was calculated and this volume in a gelatin capsule was given by mouth to each bird. The numbers of cysts obtained in one experiment was 376, 323, 380, 366, and 376, giving a mean number of 364.

INFECTION EXPERIMENTS

In each of the four experiments several ducklings were given 250 cysts each and another group was kept as controls. Sixteen days later all of the ducklings of both groups were given 1000 cysts each. After 4 days all of the living birds were killed and the intestines, including the ceca, of all birds were examined for adult *S. globulus*. It is remarkable that all 17 of the controls became infected whereas 11 of the experimental birds had no flukes at the termination of the experiments (Table 1).

TABLE 1. Summary of results of infecting young ducklings with **Sphaeridiotrema globulus** 16 days after giving each bird 1000 cysts. Summary of 4 experiments.

No. of Ducks	Immunizing Dose	Challenge Dose	Infection After Challenge	Mortality	Adult Flukes Recovered Mean (Range)
18	250 cysts	1000 cysts	7/18	0/18	22 (0-207)
17	0 cysts	1000 cysts	17/17	5/17	407 (52-938)

The 18 experimental birds had a total of only 402 adult flukes compared to a total of 6911 flukes in the controls. Thus the previously uninfected ducklings had more than 17 times as many flukes as those birds given an initial dose of 250 cysts each. Furthermore, four of the controls died and all had hemorrhagic ceca, a lesion not seen in those with previous infection. Earlier experience with experimental infections with *S. globulus* suggests that all or most of the control birds would have died a short time later.

Two of the experimental birds had 155 and 207 flukes respectively, which suggests the possibility that these birds might have regurgitated part of the 250 cysts given initially or that these worms lived longer than the 16 days previously established as the maximum life span *in vivo*.

The experiments reported here suggest that a high degree of resistance is conferred by a small, initial infection of *Sphaeridiotrema globulus*. These findings suggest that acquired resistance to this trematode may be an important factor in wild duck populations.

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