

APPARENT IMMUNITY OF GOLDFISH TO *PLEISTOPHORA OVARIAE*

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Examination of histological sections of young-of-the-year and adult goldfish, *Carassius auratus*, exposed to *Pleistophora ovariae*, a microsporidan parasite that occurs in the ovaries of golden shiners (*Notemigonus crysoleucas*), revealed no infections. Although the parasite is nearly ubiquitous in golden shiners and has recently been found in the fathead minnow (*Pimephales promelas*), we could not find it in samples of goldfish from a commercial source with known infections of shiners. Intraperitoneal and intramuscular injection of goldfish with 1.9 to 3.9×10^4 *P. ovariae* spores did not result in transmission of the parasite.

INTRODUCTION

The microsporidan *Pleistophora ovariae* is an obligate intracellular parasite which undergoes schizogony and sporogony in the developing oocytes of the golden shiner (1, 2). Infection in the golden shiner (*Notemigonus crysoleucas*) is progressive with age, often causing sterility in shiners that have spawned three or more times. Host specificity of *P. ovariae* is not known, but it has recently been observed in the ovaries of the fathead minnow (*Pimephales promelas*), another commonly cultured bait minnow. Although goldfish (*Carassius auratus*) are reared by some hatcheries that produce golden shiners, and are often stocked in ponds previously used for shiners, *P. ovariae* has not been reported in goldfish. A systematic search has not been reported; however, if *P. ovariae* were as common in goldfish as it is in the golden shiner, spawning failures would have brought attention to its presence.

The present study was undertaken to determine if *P. ovariae* occurred in goldfish that had been exposed to the parasite under culture conditions, and to determine if the parasite can be transmitted by transplantation, through intraperitoneal (i.p.) or intramuscular (i.m.) injection of spores from infected shiners. Although an effective host defense mechanism may have prohibited peroral transmission of the parasite from golden shiners to goldfish, transplantation might succeed by passing normal defense mechanisms.

METHODS

We prepared slides of goldfish ovaries according to standard histological procedures, using Mallory's triple stain (4). Ovaries of 280 goldfish were examined from three groups of fish obtained from Ozark Fisheries, Stoutland, Missouri, between 1972 and 1974. Although goldfish and golden shiners were not reared in the same pond, there was potential exposure of goldfish to infective units of this parasite in ponds previously used for golden shiners, since the same ponds are alternately used for both species. Spores might also be transmitted from pond to pond by contaminated nets, hauling tanks, or animals such as birds, snakes, or frogs.

One group (Group A) of 49 slides we examined were obtained from a previous study (5) where young-of-the-year fish had been held in tanks in our laboratory and sacrificed at intervals over the winter. Ovaries of Group A fish contained oocytes of stages 3-7 according to the numerical system of Braekevelt and McMillan (6), where 7 is ripe. Ovaries collected early winter were in earlier stages than those collected later, yet all were capable of containing schizonts or sporonts according to findings by Warner (7) on the relation between parasitism and the seasonal cycle of oogenesis. Some fish in this group spawned in the tanks.

Group B consisted of 33 4-year-old brood fish that had spawned three times previ-

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ously. Ten were killed and examined in November 1974 when they were first acquired (Group B-1); 11 were given i.m. injections of 3.9×10^4 spores (described below) and placed in a 0.1-hectare (ha) experimental pond (Group B-2); and 12 uninjected fish were placed in another pond (Group B-3). The ponds were drained and the surviving fish (five of Group B-2 and six of Group B-3) were collected in June 1975. Six female young from Group B-2 were also collected (Group B-2Y).

Group C consisted of about 1000 young-of-the-year (YOY) fish obtained in October 1974 for the study on experimental transmission. About 500 fish were injected with 1.9 to 3.9×10^4 spores — one-half intraperitoneally (C-1, i.p.) and the rest intramuscularly (C-1, i.m.). Spores were obtained from infected golden shiner ovaries, which were macerated in a mortar and pestle and strained through cheesecloth. Spore numbers were estimated by counting with a hemocytometer. About 500 fish were used as uninjected controls (C-2). All Group C fish were maintained in the laboratory in 600-liter tanks, with a constant inflow of 1.9 l/min. (4.5 water changes per day), a temperature of 21 C, and a 12-hour photoperiod. The fish were fed nearly every day for 4 months. Thirty-seven to 40 of the injected females (C-1) and 10 of the control (C-2) fish were killed at intervals of 31, 61, 86, and 115 days. The experiment was terminated 12 February because gonadal maturation was progressing poorly; however, there were an abundance of oocytes in stages suitable for schizonts — stages 3-4, which are the first oocyte stages invaded by the parasite. Sporogony does not occur unless oogenesis proceeds to secondary oocytes (stage 6). The survivors, numbering about 60 of the C-1 fish, and 120 of the C-2 fish, were held in two ponds from 14 February until November. Only eight of the C-2 group (none of the C-1 group) were recovered.

RESULTS

In total we examined ovaries of 286 goldfish (Table 1) without finding a schizont or sporont. Although appropriate oocyte stages were present for at least schizont stages, none of the goldfish examined were infected with *P. ovariae*. No fish of Group B (the 33 4-year-old brood fish), which were the most likely to be infected because they had been exposed for 4 years at the Stoutland, Missouri, hatchery, was infected. None of the six young from the B-2 group (Group B-2Y) collected in June were infected.

Some of the 49 fish in Group A were mature; some had spawned; but none were infected. If they had a latent infection, it never became apparent. None of the 147 Group C-1 fish, given either i.p. or i.m. injections and held in the laboratory, had an infection. Although these fish had few oocyte stages mature enough to show sporonts, they could have exhibited schizonts. None of the 8 survivors of the combined C-1 and C-2 groups held in ponds for nine months with infected golden shiners were infected.

TABLE 1. Description of groups of goldfish examined for *Pleistophora ovariae*. None were infected.

Group	Age ^a	Site	Injected (no. spores)	No. examined	Range in maturity ^b
A	YOY	Tanks	—	49	3-4 (few 5-7)
B-1	4+ yr	Ponds	—	10	3-5
B-2	5+ yr	Ponds	i.m. (3.9×10^4)	5	3-7
B-3	5+ yr	Ponds	—	6	3-7
B-2Y ^c	YOY	Ponds	—	6	3
C-1	YOY	Tanks	i.p. ($1.9-3.9 \times 10^4$)	78	3-4
C-1	YOY	Tanks	i.m. ($1.9-3.9 \times 10^4$)	79	3-4
C-2	YOY	Tanks	—	45	3-4
C-2	YOY	Ponds	i.p. + i.m.	8	3-5

^a YOY = young-of-the-year fish.

^b Oocyte stage numbers, determined by a procedure described by Braekvelt and McMillan (6).

^c Offspring of the B-2 group.

DISCUSSION

The absence of natural infections of *P. ovariae* in this hatchery stock (all but six of the fish were hatched in Stoutland, Missouri) is surprising, since *P. ovariae* is not entirely host specific. Fathead minnows cultured with golden shiners are infected by *P. ovariae* (3). Although goldfish are similarly exposed and are in the same family (Cyprinidae) as golden shiners and fathead minnows, both of which are parasitized by *P. ovariae*, goldfish are refractory to natural infection. Lom (8) infected goldfish with *Pleistophora hyphessobryconis* by feeding the fish spores collected from an infected *Hyphessobrycon inessa*, the normal host, and by injecting spores intramuscularly; the normal host of *P. hyphessobryconis* is not a cyprinid fish. However, neither prolonged natural exposure nor artificial exposure by injection results in infection of goldfish with *P. ovariae* in spite of close family relation.

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