PANCREATIC INFECTIONS OF Myxobolus osburni HERRICK (MYXOZOA: MYXOSPOREA) IN THE PUMPKINSEED, Lepomis gibbosus (LINNAEUS) IN IOWA

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PANCREATIC INFECTIONS OF *Myxobolus osburni* HERRICK (MYXOZOA: MYXOSPOREA) IN THE PUMPKINSEED, *Lepomis gibbosus* (LINNAEUS) IN IOWA

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Abstract: Cysts of *Myxobolus osburni* occurred throughout the pancreas in 119 of 150 (79.3%) pumpkinseed *(Lepomis gibbosus)* from West Lake Okoboji, northwest Iowa during the summer, 1980. None of 341 bluegill *(Lepomis macrochirus)* were infected. Lobate cysts (up to 3 mm diameter) adjacent to the gall bladder contained sporulating plasmodia and mature spore masses. Inflammatory responses in the infected pancreas included engorged blood vessels, endocrine cell nuclei pyknosis, leukocyte (mostly lymphocyte) infiltration, fibroblast proliferation, dark pigment deposition and fibrosis. Host reaction was most pronounced in pancreas containing unencapsulated spore aggregates. Spore variability and plasmodial structure are also described.

INTRODUCTION

The myxozoan genus *Myxobolus* consists of numerous species infecting a wide variety of organs and tissues in many families of fishes.[1,5,8-12] *Myxobolus osburni* Herrick was described originally from the mesenteries and peritoneum of the smallmouth bass, *Micropterus dolomieui* (Lacépede), and the pumpkinseed, *Lepomis gibbosus* (Linnaeus) from Lake Erie.[5] This species also was reported from the gall bladder of the bluegill, *Lepomis macrochirus* (Rafinesque), and the black crappie, *Pomoxis nigromaculatus* (Le Seur), from the Lake Okoboji area in northwest Iowa.[11] Cone and Anderson[1] described *M. osburni* infections "in or on" endocrine islets and within diffuse exocrine pancreatic tissue in the pumpkinseed. This is the first report of developmental stages of plasmodia and the first report of histopathologic changes associated with pancreatic infections of *M. osburni*. Spore variability and summer prevalence also are reported.

MATERIALS AND METHODS

Fish were collected by fyke net and wire funnel traps in Little Miller's Bay, West Lake Okoboji, in northwest Iowa from June through September, 1980. Viscera from seven infected pumpkinseed were removed; five were preserved in Bouin's and two in Stieve's fixative. Tissue was paraffin embedded, serially sectioned at 6 to 7 μm, and stained with hematoxylin and eosin. Spores were examined in smears of 12 individual plasmodia in 0.6% NaCl. All measurements, recorded in micrometers, are of unstained spores.

RESULTS AND DISCUSSION

One hundred nineteen of 150 (79.3%) pumpkinseed were infected with *Myxobolus osburni*. Both sexually mature and immature fish (std. length 7.5 - 13.0 cm) were infected. Prevalence was highest in sexually mature fish, and was the same in females (74 infected/89 examined; 83%) and males (35/42; 83%). These are the highest prevalence values yet reported for *M. osburni*.[1,5] Prevalence was considerably lower in sexually immature pumpkinseed (10 infected/19 examined; 53%). These results are generally similar to those of Cone and Anderson[2] who found prevalence of *M. osburni* to increase with age of hosts. Previous estimates of prevalence are probably low. In sections we have observed microscopic cysts in the pancreas that appeared uninfected in routine examination.

We did not find cysts in 341 bluegills *(Lepomis macrochirus)* examined macroscopically during the study period. Microscopic examination of the viscera of 10 bluegills did not reveal *M. osburni* infections.

The gall bladder and bile ducts contained a few scattered spores. The report of Otto and Jahn[11] of microscopic *M. osburni* trophozoites floating free in bile in one bluegill seems questionable for this histozoic myxozoan.

Spores. Spores (Fig. 1) closely resembled those described by Herrick.[5] Seven to 10 sutural folds were distinct in most spores. Two cnidocyst discharge canals appeared as distinct anterior opacities. The angle of filament coiling was approximately 45° as drawn by Herrick,[5] although some spores showed filaments coiled at about 90° to the long axis of the spores. Dimensions of the Iowa population were: length 9-11 (mean=10, n=650); breadth 10-13 (mean=11.5, n=650); thickness 6-8 (mean=7, n=79). The majority of spores were elliptical in front view (slightly broader than long) as drawn by Herrick.

Cone and Anderson[1] reported longer, spherical spores: length 10-13 (mean=12, n=40); breadth 10-14 (mean=12, n=40); thickness 6-7 (mean=6.5, n=5). We have seen some spherical spores in plasmodia containing predominantly elliptical forms, indicating considerable intraspecific variation in this myxobolid. The total range of spore size recorded is 9-13 × 9.5-14 × 6-8.

Plasmodia. Creamy-white to yellowish lobate cysts (up to 3 mm in diameter) were observed in pancreatic tissue in mesenteries posteromedial to the gall bladder (Fig. 2). One (rarely two) of these large cysts was seen per infected pumpkinseed. Previous reports,[1,5] described cysts containing only mature spores anterior to the gall bladder. In our sections, single or diffuse aggregates of microscopic cysts containing mature spores in exocrine and endocrine pancreas were in the vicinity of the gall bladder and bile duct.

Lobate cysts (Figs. 2, 3) of individual sporulating plasmodia and/or fully ripened micro- and macroscopic spherical or multilocular spore masses usually were encapsulated by connective tissue.

Immature (sporulating) plasmodia showed discrete striated eosinophilic ectoplasm. The endoplasm contained an outermost region of undifferentiated

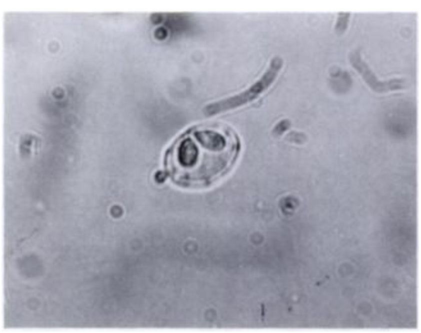

FIGURE 1. *Myxobolus osburni* spore, unstained, 830×.

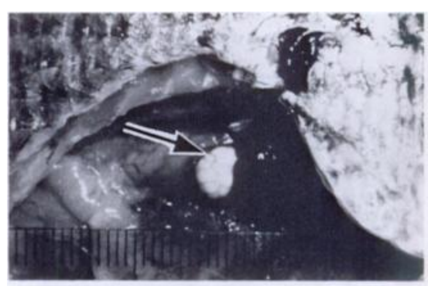

FIGURE 2. Infected pumpkinseed showing large lobate cyst behind gall bladder. Approximately life size. Arrow indicates cyst.

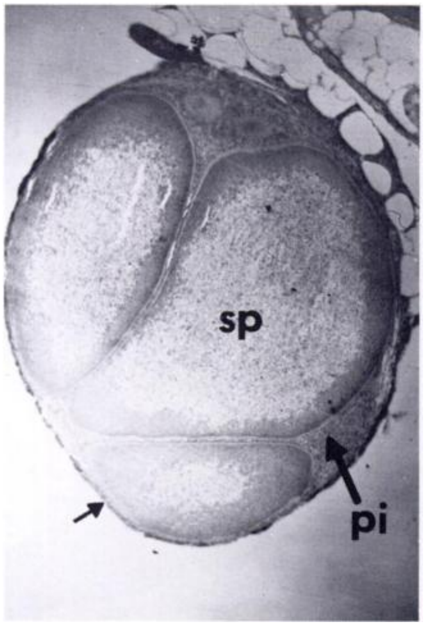

FIGURE 3. Section of lobate cyst adjacent to gall bladder showing three sporulating plasmodia with core of mature spores (sp) in pancreatic islet tissue (pi). Arrow indicates thin border of exocrine pancreatic tissue. H&E. 35×.

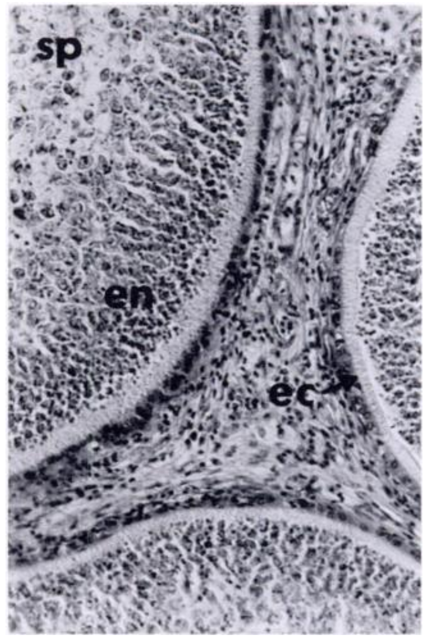

FIGURE 4. Edges of three plasmodia showing striated ectoplasm (ec), sporulating endoplasm (en), and mature spores (sp). Note diffuse leukocytic infiltration between plasmodia. H&E. 260×.

nuclei, an intermediate zone of pansporoblasts and a central core of mature spores (Fig. 4).

Histopathology. Sporulating plasmodia within lobate cysts typically were surrounded by normal pancreatic islet cells in direct contact with plasmodial ectoplasm. The edges of developing plasmodia were bordered by layers of flattened islet cells, fibroblasts, or a thin connective tissue capsule. Several plasmodia seemed to have developed within a single islet, replacing endocrine cells. Enlarged islet capillaries adjacent to plasmodia suggested some cysts had developed from intracapillary infections. Mature spore masses (Fig. 5) were usually enclosed in a connective tissue capsule (up to 10 μm thick). Isolated spores and capillaries were common in these capsules.

A diffuse inflammatory response around most plasmodia and encapsulated spore masses was manifested as engorged small blood vessels, pyknotic endocrine cell nuclei and diffuse leukocytic infiltration (Fig. 4). Granulocytes and lymphocytes were common in enlarged capillaries. Melanin macrophage involvement was indicated by occasional dark pigment deposition in cyst capsules. Pancreas containing unencapsulated spore masses had developed granulomatous areas showing infiltration of lymphocytes and macrophages, proliferation of fibroblasts and fibrosis among endocrine and exocrine cells (Fig. 6).

FIGURE 5. Encapsulated spore mass surrounded by normal exocrine pancreas; pancreatic islet (pi). H&E. 270×.

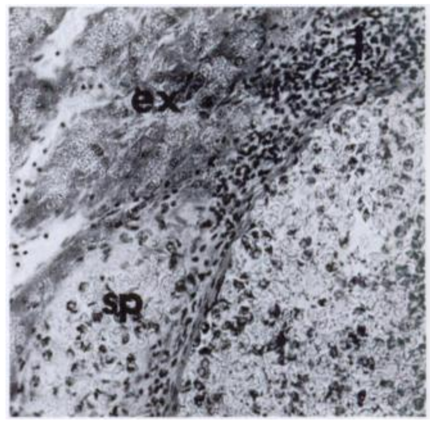

FIGURE 6. Leukocytic infiltration (l) of pancreas containing unencapsulated spore mass (sp). Exocrine pancreatic cells (ex) containing zymogen granules. H&E. 230×.

Heckmann and Jensen found unencapsulated spore masses of *Henneguya sebasta* associated with necrosis, hyperplasia, hypertrophy, and proliferation of connective tissue in smooth muscle of the bulbus and truncus arteriosus in bocaccio, *Sebastes paucispinis* (Ayres).[1] These authors also reported severe atrophy and necrosis of skeletal muscle associated with cyst development and encapsulation of spores of *Kudoa clupeidae* in bocaccio. Dykova and Lom[3] described inflammatory responses leading to cyst degradation and granuloma formation in late stages of gill-inhabiting species of *Henneguya*. Epithelioid granuloma formation has been reported surrounding spore masses of the cartilage-invading myxozoans, *Myxosoma cartilaginis* and *M. cerebralis*.[6,7] All of these studies indicate that while growing plasmodia of myxozoans may cause tissue destruction, host reactions to these parasites become most pronounced after spore maturation. Experimental studies with other *Myxobolus (=Myxosoma)* species indicate that several weeks or months may elapse after plasmodia appear in tissue before cellular reactions appear.[6,7] The occurrence of developing plasmodia and mature spore masses in granulation tissue within large lobate cysts of *M. osburni* indicates that chronic infections do not elicit host protective immunity in the pumpkinseed.

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