GRANULOMATOUS HEPATITIS ASSOCIATED WITH HEPATOZOOON SP. MERONTS IN A SOUTHERN WATER SNAKE (NERODIA FASCIATA PICTIVENTRIS)

Edward J. Wozniak, D.V.M., Ph.D., Sam R. Telford, Jr., Ph.D., Dale F. DeNardo, D.V.M., Ph.D., G. L. McLaughlin, Ph.D., and Jerry F. Butler, Ph.D.

Abstract: A wild-caught adult female southern water snake (Nerodia fasciata pictiventris) did poorly in captivity. A peripheral blood-film examination demonstrated numerous hemogregarines characterized as fusiform nondividing intraerythrocytic gametocytes. Xenodiagnostic typing in laboratory-reared mosquitoes demonstrated the parasite to be of the genus Hepatozoon. Gross and histopathologic examination of the liver demonstrated numerous granulomas centered on groups of one to six Hepatozoon sp. meronts, an unusual finding in naturally infected wild-caught snakes.

Key words: Hemogregarine, Hepatozoon, pathology, granulomatous hepatitis.

INTRODUCTION

Hemogregarines are the most common blood parasites of snakes. Three families of hemogregarines commonly parasitize reptiles: Hemogregarinidae, Hepatozoidae, and Karyolysidae.2 Accurate hemogregarine classification requires morphologic analysis of parasite developmental patterns within invertebrate vectors.13 Most snake hemogregarines are members of the genus Hepatozoon (family Hepatozoidae).11

The Hepatozoon life cycle typically involves meagony and gamogony within a vertebrate host and sporogony within an invertebrate vector.13 Numerous species of hematophagous arthropods, including mosquitoes, ticks, tsetse flies, and phlebotomine sand flies, are capable of supporting Hepatozoon sporogony.11,13,14 Congenital transmission1 and transmission through the ingestion of tissues containing infective dixo cysts (cannibalism and predation)6,7,11,12 have been demonstrated with some species. Experimental transmission studies have shown that reptilian hemogregarines have a low degree of vector and vertebrate host specificity.13 Effective transmission of some Hepatozoon species between snakes from different taxonomic families and lizards has been demonstrated experimentally.13,16 Within their natural (co-adapted) hosts, hemogregarine meronts are usually incidental findings and are not associated with inflammatory lesions.9,13,15,16

CASE REPORT

An adult 450-g female southern water snake (Nerodia fasciata pictiventris) was collected in Martin County, Florida, and maintained in captivity as part of the hemogregarine life cycle and transmission study. The snake was housed individually at a University of Florida animal facility in a 45-L glass aquarium equipped with a focal heat source, hide box, and water bowl with an ambient temperature of 27–29°C and a 12:12 hr light–dark cycle. A diet of commercially available fish was offered weekly. All animal procedures were approved by the University of Florida Animal Care and Use Committee.

The snake did poorly, frequently refused food, and lost weight and body condition for 6 wk. Samples of heparinized blood (100 μl) were collected by cardiocentesis at 2-wk intervals. Several thin blood smears were prepared from each sample, Giemsa-stained for 20 min (10% vol/vol Giemsa stain in buffered water, pH 7.2), and visually examined at 1,000 times magnification.

Evaluation of the blood smears demonstrated the snake to be infected with hemogregarines. The percentage of parasitemia was calculated by counting the number of parasitized cells per 1,000 erythrocytes. The parasites were all in the intraerythrocytic hemogregarine gametocyte stage. The gametocytes were fusiform cells with small, round, dense, central-to-subterminal nuclei and moderate amounts of finely vacuolated, pale-staining cytoplasm (Fig. 1). Gametocytes were oriented parallel to the long axis of the erythrocyte nucleus. The gametocytes mea-
sured 13.3 × 4.6 μm (± 1.6 × 0.6 μm; n = 30). Neither intraerythrocytic division nor pigment production was noted. The parasitemia remained between 8% and 10% over the 6-wk period.

For xenodiagnostic generic determination of the parasites, 25 laboratory-reared Aedes aegypti mosquitoes were fed on the parasitemic snake, collected, maintained for a 2-wk extrinsic incubation period, and dissected in insect ringer's solution.14 Eighteen of the 25 blood-fed mosquitoes had oo-
cysts containing sporozoite-filled sporocysts in their hemocoels and fat bodies. The oocysts contained up to 16 sporocysts, each of which contained 12–
16 fusiform sporozoites and a nodular body of res-
dual cytoplasm. This pattern of sporogonic de-
velopment is diagnostic for Hepatozoon.12,13

After 6 wk of captivity, the snake was euthana-
tized by intracardiac pentobarbital injection and necropsied. Specimens of liver, lung, spleen, and kidney were collected, fixed in 10% neutral buf-
ered formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin (H&E).

Grossly, the snake was thin and in fair body condition. Numerous off-white-to-tan punctate round foci were present on the capsular surface and throughout the parenchyma of the liver. Histopath-
ologic evaluation of the liver demonstrated numer-
ous randomly oriented granulomas centered on clusters of one to six Hepatozoon sp. meronts (Fig. 2). The meronts were round-to-ovoid structures that measured 18–40 μm and contained 8–20 fusiform merozoites with dense central nuclei and moderate amounts of finely vacuolated eosinophilic cyto-
plasm (Fig. 2). The merozoites averaged 11.0 × 4.1 μm (± 2.1 × 0.6 μm; n = 40). The granulomas measured up to 800 μm in diameter and were com-
posed of a thick wall of macrophages admixed with low numbers of heterophils surrounded by an outer concentric rim of fibroblasts, lymphocytes, and plasma cells (Fig. 2). No other significant lesion or protozoal meront was demonstrated.

**DISCUSSION**

Granulomatous inflammatory lesions centered on hemorrhagic meronts in snakes are unusual and have not been reported in wild naturally infected snakes.11 The lack of central degenerate heterophil aggregates suggests the lesions were histiocyctic rather than heterophilic granulomas.8 The finding of meronts in clusters exclusively within hepatic gran-
ulomas suggests that the response may have pre-
vented the merozoites from spreading to other tissues.

Hemogregarine infections are usually of little pathologic consequence to wild naturally infected reptiles.\textsuperscript{3,13,15} \textit{Hepatozoon} meront-associated inflammatory lesions resulting in clinical disease in reptiles have been reported only in experimentally infected unnatural host species and rarely in captive animals housed in multispecies collections.\textsuperscript{3,5,14} Hemogregarine meronts in the livers of several other wild-captured \textit{N. fasciata pictiventris} from an adjacent county in South Florida were not infiltrated by inflammatory cells (Telford and Wozniak, unpubl). It is likely that the water snake from Martin County was an unnatural host for the \textit{Hepatozoon} species or isolate involved.\textsuperscript{14,16}

When multiple reptile species are housed in close proximity to one another, accidental transmission of hemogregarines between species is likely. Because there is currently no effective treatment for hemogregarine infections, routine measures to minimize the risk of transmission within animal facilities, including vector control, food animal quality assurance, and maintenance of proper sanitation, are recommended.

LITERATURE CITED


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