

## *Hepatozoon griseisciuri* INFECTION IN GRAY SQUIRRELS OF THE SOUTHEASTERN UNITED STATES<sup>1</sup>

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**Abstract:** Blood films and selected tissue sections from 270 gray squirrels (*Sciurus carolinensis*) from 18 sites in 11 southeastern states were examined for *Hepatozoon griseisciuri*. This parasite was found in 110 (41%) squirrels from 17 of the 18 sites. Data suggested transmission of the parasite apparently occurs throughout the year and increases from spring to late summer. The infection was more prevalent in subadults and adults than in juveniles. Schizogonic stages were found only in the lungs, suggesting this organ is a major site of schizogony. Pathologic changes associated with *H. griseisciuri* infection were thickening of alveolar walls, eosinophil infiltration, pulmonary congestion and atelectasis.

### INTRODUCTION

*Hepatozoon griseisciuri* Clark 1958 generally is considered to be the only species of *Hepatozoon* occurring in gray squirrels of North America.<sup>5,10</sup> This haemogregarine was reported from the District of Columbia, Maryland,<sup>1,6,7</sup> Rhode Island,<sup>12</sup> Wisconsin,<sup>3</sup> and Virginia.<sup>9,10</sup> Sporogonic development of *H. griseisciuri* in the natural vector, *Haemogamasus reidi*, was detailed.<sup>1,11</sup>

Schizogonic development of *H. griseisciuri* was described in the spleen, liver and bone marrow of a naturally infected 1.5-day-old squirrel<sup>1</sup> and in the liver and spleen of a naturally infected 1-day-old squirrel.<sup>10</sup> The infections were attributed to transplacental transmission of the parasites. Schizogonic stages also were described from the liver, spleen, bone marrow and lungs of a 2-week-old captive squirrel.<sup>4</sup> Recently, a detailed de-

scription of *in vitro* development of schizogonic stages in primary squirrel kidney cell cultures was presented.<sup>5</sup>

There is limited information on the location and pathogenicity of schizogonic stages in natural *H. griseisciuri* infections, and no information on variations of infection related to age or sex of the host, season or geographic distribution. This report includes information on these aspects of naturally occurring *H. griseisciuri* infections.

### MATERIALS AND METHODS

Three collection sites were established in each of the six major vegetative types of the southeastern United States:<sup>8</sup> Appalachian oak, oak-hickory, oak-hickory-pine, southern floodplain, southern mixed and mixed mesophytic. Five gray squirrels were collected by shooting within 1-month periods during the spring

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(1972), late summer (1972), and mid-winter (1973) from each of the 18 sites. Blood films were prepared immediately after death, air dried, fixed in methanol and later stained with Giemsa's stain. Postmortem examinations were made within 4 h after death, and sections of lung, liver, kidney and tongue were preserved in 10% buffered formalin. Tissues were embedded in paraffin, sectioned at 6  $\mu\text{m}$  and stained with hematoxylin and eosin.

Blood films were examined for *H. griseisciuri* by counting the number of gametocytes per 100 mononuclear leukocytes. If gametocytes were not detected by this procedure, circulating gametocytes were considered absent. Tissue samples were examined for schizogonic stages. Data were analyzed by the Chi square test.

#### RESULTS

*H. griseisciuri* was found in 110 (41%) squirrels from 17 of the 18 collection sites. The parasite was widely distributed, occurring in 11 southeastern states. Only the squirrels examined from Cumberland County, Tennessee were not infected.

Circulating gametocytes were observed in 90 (82%) infected squirrels. Gametocytes also occurred in mononuclear leukocytes and endothelial cells in the lungs of 48 (44%). Schizogonic stages were found in the lungs of 42 (38%).

Statistical analyses revealed significant differences ( $P > 0.05$ ) in the prevalence of infection between different seasons and age classes but not between different vegetative types or sexes. The prevalence of infection was significantly higher ( $P > 0.05$ ) in the winter than in the spring (Table 1). Subadults and adults had a significantly higher ( $P > 0.01$ ) prevalence of infection than juveniles (Table 2).

Circulating gametocytes occurred in mononuclear leukocytes in the peripheral blood and occasionally were free in the plasma due to rupture of host cells. Gametocytes indented the host cell nucleus and displaced the cytoplasm, but other pathologic effects were not noted.

Gametocytes were observed in mononuclear leukocytes in the alveolar capillaries and occasionally in endothelial cells in the lungs. Gametocytes in the endothelial cells frequently were surrounded by granules probably discharged from nearby eosinophils or by intact eosinophils.

Schizonts occurred in endothelial cells of the alveoli (Fig. 1). Immature schizonts were 10 (6 to 20)  $\mu\text{m}$  in diameter, circular to oblong, and contained up to 28 nuclei located around the perimeter. Mature schizonts were 17 (8 to 24)  $\mu\text{m}$  in diameter, contained 12 (6 to 19) merozoites, and were surrounded by a clear parasitophorous vacuole.

Schizonts distended the host cell and displaced the nucleus. Intense schizogonic activity resulted in thickening of alveolar walls and was associated with areas of atelectasis and pulmonary congestion. Inflammatory cells were not associated with schizogony. Schizogonic stages were not found in liver, kidney or striated muscle.

#### DISCUSSION

In an early report the *Hepatozoon* of gray squirrels in North America was tentatively designated as *H. sciuri*;<sup>2</sup> however, additional studies on the parasite from the same source locality revealed that it was a new species, *H. griseisciuri*.<sup>1</sup> Further studies on the sporogonic cycles of both *H. sciuri*<sup>2</sup> and *H. griseisciuri*<sup>11</sup> confirmed this difference. It is now generally accepted that *H. griseisciuri* is the only species in gray squirrels in North America.<sup>4, 5, 10, 11</sup>

The prevalence of *H. griseisciuri* infection during this study was comparable to all previous reports<sup>1, 5, 6, 9, 10, 12</sup> except one in which a 100% prevalence was detected by means of a concentration technique.<sup>7</sup> Since techniques used for this study were not as efficient as concentration methods,<sup>7</sup> the prevalence (41%) reported probably was below the actual value. Standard procedures utilized in this study, however, afforded data that could be compared to establish relative values.

TABLE 1. Seasonal data on naturally occurring *Hepatozoon griseisciuri* infection in 270 gray squirrels from the Southeast.

Season	Number Infected/Number Examined (% Infected)			
	Circulating Gametocytes	Lung Gametocytes	Schizonts	Total*
Spring	20/90 (22)	10/90 (11)	11/90 (12)	27/90 (30)
Summer	31/90 (34)	18/90 (20)	17/90 (19)	37/90 (41)
Winter	39/90 (43)	20/90 (22)	14/90 (16)	46/90 (51)
Total	90/270 (33)	48/270 (18)	42/270 (16)	110/270 (41)

\* Total infected per season; many infections were of all stages.

TABLE 2. Prevalence of naturally occurring *Hepatozoon griseisciuri* infection in 270 gray squirrels from the Southeast according to sex, age, and vegetative type.

Parameter	Number Infected	Number Examined	Percent Infected
<b>Sex</b>			
Male	54	122	44
Female	56	148	38
<b>Age</b>			
Juvenile	1	19	5
Subadult	32	94	34
Adult	77	157	49
<b>Vegetative Type</b>			
Southern floodplain (Clarke Co., Ala.; Jeff Davis Co., Ga.; Hampton Co., S.C.)	17	45	38
Southern mixed (Decatur Co., Ga.; Jeff Davis Co., Ga.; Forest Co., Miss.)	14	45	31
Oak-hickory-pine (Oglethorpe Co., Ga.; Dorchester Co., Md.; Montgomery Co., N.C.)	21	45	48
Oak-hickory (Lawrence Co., Ala.; Stone Co., Ark.; Trigg Co., Ky.)	17	45	38
Appalachian oak (Buncombe Co., N.C.; Monroe Co., Tenn.; Montgomery Co., Va.)	27	45	60
Mixed mesophytic (Rowan Co., Ky.; Cumberland Co., Tenn.; Mason Co., W. Va.)	14	45	31

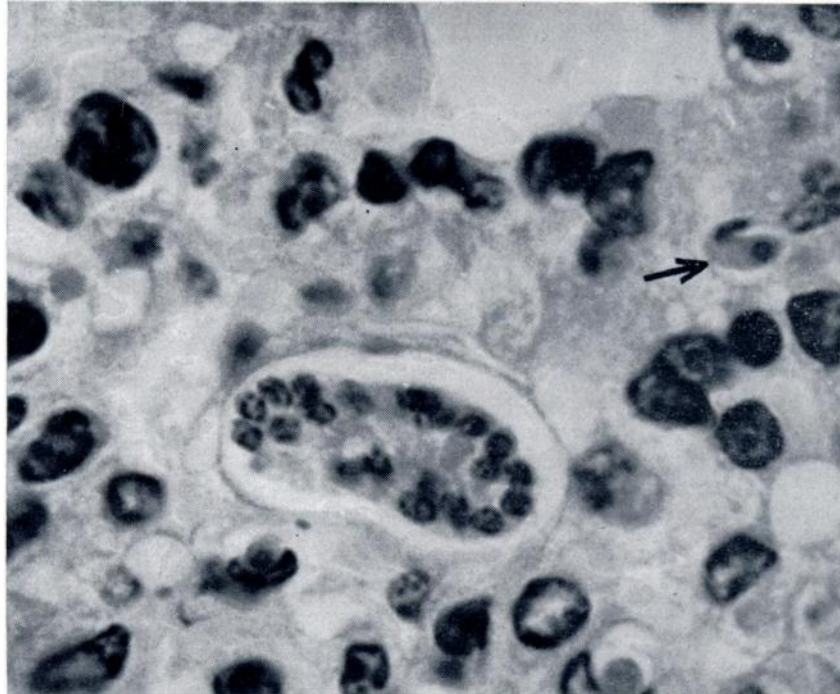


FIGURE 1. Photomicrograph of gray squirrel lung containing schizogonic stages of *Hepatozoon griseisciuri*. Note schizont in center of field with nuclei around perimeter and merozoite (arrow). X1634.

*H. griseisciuri* is widely distributed in the Southeast, and apparently no differences in prevalence occur between different vegetative types. *H. griseisciuri* probably is ubiquitous throughout the range of the gray squirrel. The increasing prevalence of infection in successive age classes is considered to be a function of the length of exposure to infected intermediate hosts.

The prevalence of *H. griseisciuri* infection increased from the spring to summer and was highest in winter. Gametocytemias had a similar pattern. Schizogonic stages increased from spring to summer but declined slightly in winter. Therefore, transmission apparently occurs throughout the year as was shown by the presence of schizonts. Transmission also seems to increase in intensity

chronologically from spring as indicated by the increased prevalence of schizonts and the increased prevalence of infection. The effects of immune responses on the intensity or duration of infection are unknown.

Schizogonic stages found in the lungs were morphologically similar to those described previously in young squirrels<sup>1,4,10</sup> and squirrel kidney cell cultures.<sup>5</sup> Only one other investigator<sup>5</sup> found schizonts in the lungs. However, the confinement of schizogonic stages within the lungs during this study indicates this organ is a primary site of schizogony.

Previous authors have not attributed significant pathologic responses to *H. griseisciuri* infection in gray squirrels.<sup>1,3,4,6,7,10,12</sup> Although histopathologic examinations of lung in this study was often

complicated by traumatic damage caused by shooting, changes associated with the infection were found. There was a thickening of the alveolar walls, pulmonary congestion, and multifocal areas of atelectasis. In lung sections with intense schizogony, where more than 60% of the endothelial cells were infected, the amount of tissue parasitized was considered to constitute a significant loss of respiratory function.

Data from this study indicate that infection by *H. griseisciuri* is capable of affecting extensive portions of the lungs and is more pathogenic than previously realized. The concomitant occurrence of a high prevalence of infection with high seasonal mortality during the late winter suggests a possible relationship between *H. griseisciuri* and squirrel mortality. *H. griseisciuri* infection may also increase susceptibility to other respiratory pathogens.

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