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Source: Journal of Wildlife Diseases, 27(1) : 161-163

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-27.1.161>

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## Asynchronous Capsule Formation in the Gastrointestinal Tract of the Prairie Rattlesnake (*Crotalus viridis viridis*) Induced by *Mesocestoides* sp. Tetrathyridia

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**ABSTRACT:** The prairie rattlesnake (*Crotalus viridis viridis*) was experimentally infected with tetrathyridia of *Mesocestoides* sp. Individual snakes were killed at 4 wk increments, and sections of the stomach, small intestine, large intestine and attached mesenteries were examined for nonencapsulated and encapsulated tetrathyridia. Capsule formation was asynchronous with 9 to 80% encapsulated metacestodes. The distribution of tetrathyridia in the wall of all segments of the gastrointestinal tract is presented as evidence that this metacestode is principally a tissue dwelling parasite.

**Key words:** Prairie rattlesnake, *Crotalus viridis viridis*, *Mesocestoides* sp., capsule, tetrathyridium, experimental infection.

Experimental infection with tetrathyridia of *Mesocestoides* sp. has been reported from the prairie rattlesnake (*Crotalus viridis viridis*) (Mankau and Widmer, 1977). (The original report listing the subspecies as *Crotalus viridis helleri* was incorrect.) Capsules containing this proliferative metacestode were located in the liver and mesenteries of infected snakes. The results of this preliminary experiment stimulated interest in additional studies with a larger number of snakes and was designed primarily to obtain evidence for asexual multiplication of the tetrathyridia of *Mesocestoides* sp. in the same reptile (Hanson and Widmer, 1985). The data collected on the numbers of tetrathyridia recovered and their anatomical distribution were by macroscopic observation. For the present study, tissue sections from five experimental and four control snakes, used in the original experiment, were chosen for microscopic analysis. Snakes were maintained at a constant temperature of 30 C. The other general procedures of the experimental design were as stated by Hanson and Widmer (1985).

At each 4 wk interval one experimental and one control snake was removed from the environmental chamber, killed and examined. The four control snakes were killed and examined at the end of 24 wk. Sections of the stomach, small intestine, large intestine and attached mesenteries were removed, washed in physiological saline solution and fixed in Bouin's fixative. These tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin and Mallory's and Masson's trichrome stains. Stained sections were examined to determine the presence of nonencapsulated and encapsulated tetrathyridia. Tetrathyridia surrounded by a continuous layer of epithelioid cells or fibrous connective tissue or both were designated as encapsulated (Mankau and Widmer, 1977).

Nonencapsulated and encapsulated tetrathyridia were seen in each of the three gastrointestinal organs and their associated mesenteries in all of the snakes where tissue sections were available and examined (Table 1). Both nonencapsulated and encapsulated metacestodes were observed in each of the three main histologic layers (i.e., mucosa, muscularis, and serosa). The muscularis mucosa layer is discontinuous throughout the gastrointestinal tract and therefore no distinct submucosa was recognizable. Two degenerating capsules were observed; one in the serosa of the large intestine of a snake 4 wk post-infection, the other in the mucosa of the small intestine of a snake 8 wk post-infection. All control snakes were negative for infection.

The distribution of the nonencapsulated tetrathyridia, in the three layers of the gas-

TABLE 1. Numbers of nonencapsulated and encapsulated tetrathyridia observed in the gastrointestinal tracts of five snakes.

Location of tetrathyridia <sup>a</sup>	Weeks post-infection				
	4	8	12	16	20
Stomach	— <sup>b</sup>	1/1 <sup>c</sup>	3/0	— <sup>b</sup>	9/7
Small intestine	5/0	1/2	3/4	5/2	1/4
Large intestine	5/1	0/5	10/1	4/6	7/6
Totals	10/1	2/8	16/5	9/8	17/17
Encapsulated (%)	9	80	24	47	50

<sup>a</sup> Segments of gastrointestinal tract include bases of attached mesenteries.

<sup>b</sup> Tissue sections not available for study.

<sup>c</sup> Nonencapsulated/encapsulated tetrathyridia.

gastrointestinal tract, is additional evidence of their invasive ability (Specht and Widmer, 1972; White et al., 1983). The specific location of capsules in the wall of the gastrointestinal tract is an apparent function of the size of inoculum, rate and frequency of the movement of nonencapsulated tetrathyridia, rate of asexual proliferation, and frequency of host feeding schedules. Additional studies are needed to determine the significance of these and perhaps other as yet unrecognized factors.

Experimental infections with encapsulated tetrathyridia have been reported from mesenteries of *C. viridis viridis* (Mankau and Widmer, 1977), and nonencapsulated tetrathyridia from the gastrointestinal tract of the southern Pacific rattlesnake (*Crotalus viridis helleri*) (Widmer and Hanson, 1983). No asynchronous capsule development was reported in either study. One possible explanation for the asynchronous capsule development may be the exiting of tetrathyridia from capsules in early stages of development or even mature capsules and their migration to other segments of the gastrointestinal tract. No observations were made to support this hypothesis.

Tetrathyridia have been observed projecting through the intestinal wall of laboratory mice, suggesting that extraintestinal infections may develop through this route (Specht and Voge, 1965). The specific route of coelomic or other extraintestinal infections in lizards or snakes has not been ascertained. Capsules in the wall

of the gastrointestinal tract may suggest resistance of the serosa to penetration by tetrathyridia.

Chernin and McLaren (1983) suggested that in laboratory rats, the metacestode of *Mesocestoides* is principally a tissue-dwelling parasite. This conclusion was reached in spite of finding nonencapsulated forms in the peritoneal cavity. Our study with the ectothermic host, *C. viridis viridis*, reinforces this position.

The authors express appreciation for the technical assistance of J. Howard Carbo, Paul C. Engen, and Gary B. Hanson. The editorial assistance of Burton N. Brin is also gratefully acknowledged.

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*Received for publication 5 December 1989.*