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Prevalence of *Mesocestoides* (Eucestoda: Mesocestoididea) Tetrathyridia in Southern California Reptiles with Notes on the Pathology in the Crotalidae

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Introduction

Adult cestodes of the genus *Mesocestoides* are intestinal parasites of carnivorous birds and mammals. The taenioid larvae of this tapeworm, called tetrathyridea, occur free or encysted in the body cavity or viscera of lizards (Specht and Voge, 1965), snakes (Joyeux *et al.*, 1933), birds (Markowski, 1933) and mammals (Grunnmann, 1956; Joyeux, 1927). It has been established repeatedly that tetrathyridia, when fed to carnivores, will either develop into the intestinal strobilate phase of *Mesocestoides* or reach the coelom and remain there as tetrathyridia larvae. The report that tetrathyridia from the liver and body cavity of the lizard, *Sceloporus occidentalis biseriatus*, when injected intraperitoneally or fed by stomach tube to white mice, multiplid asexually in the body cavity and liver (Specht and Voge, 1965), prompted the studies reported here. The prevalence of tetrathyridia larva of *Mesocestoides* was investigated in the various species of lizards and rattlesnakes of Southern California and on the histopathology caused by the parasite in natural infections in lizards and both natural and experimental infections in snakes was studied.

Materials and Methods

Lizards, belonging to 15 different species and 83 rattlesnakes belonging to 6 different species were collected from Riverside, San Bernardino, San Diego, and Imperial counties of Southern California. They were killed by hyperanesthesia using sodium pentobarbital. The body cavity and viscera were examined under a dissecting scope for tapeworm cysts. All infected organs along with cysts free or attached to the mesentery were removed, washed in Mammalian Ringers solution and were fixed in 10 percent formalin or Bouin's fluid. Materials to be sectioned for detailed histopathological studies were routinely stained with hematoxylin and eosin, special stains such as Masson's trichrome and Mallory's triple stain were also used.

Westen rattlesnakes, *Crotalus ruber*, were experimentally infected with the strain of tetrathyridia isolated by Specht and Voge (1965). These were larvae originally collected from fence lizards and transferred to Swiss Albino mice by intraperitoneal injections and maintained in the laboratory. The larvae recovered from the mice were rinsed in saline and were fed to the snakes using a stomach tube. The two infected snakes were examined 19 and 48 months respectively after infection. At autopsy, the intestinal tract, body cavity, mesenteries and liver were examined. Tetrathyridia were recovered by repeated rinsing of the body cavity with saline. The infected materials were fixed in Bouins and were prepared for histological studies as described earlier.

Results

Prevalence

Eleven of the fifteen species of lizards examined were found to be infected with the tetrathyridia larva (Table 1). No larvae were found in the leopard lizard, *Crotaphytus wislizenii* or three species of *Sceloporus*. The highest incidence of tetrathyridia occurred in the two species of *Phrynosoma*. 14.3 percent and 50 percent of the male and female of *P. platyrhinos*, respectively, and 40 percent and 20 percent of male and female of *P. nicalli* respectively were infected. The percent of infected male and female lizards ranged from 2.6–40 and 5.2 to 50 respectively.

Of the six species of rattlesnakes examined, *Crotalus cerastes*, *C. mitchelli*, *C. ruber*, *C. viridis*, *C. helleri* and *C. acutulatus*, only one male *C. ruber* and one male *C. viridis* were found to be infected by the tetrathyridia larva.

Gross Pathology

Pseudocysts containing tetrathyridia which appeared as white masses were found embedded in both the mesentery and liver of the infected lizards. In the snakes the primary focus of infection was the mesentery, with very few cysts occurring in the liver. The experimentally infected snakes contained approximately 200 pseudocysts, largest number of them occurring in the mesenteries of the small intestine. Each pseudocyst contained two to five tetrathyridia. No gross pathology was evident in the organs surrounding the cystic masses.

Histopathology

Pseudocysts, when they occurred in the liver, caused far more extensive histopathology compared to the cysts attached to the mesentery. The changes produced by the infection can be described as a process of acute inflammation, stimulated by the

Table 1 Prevalence of *Mesocestoides* tetrathyridia in lizards collected from San Bernardino and Riverside Counties of Southern California

Species	Males		Females		Percent	
	No. Examined	No. Infected	No. Examined	No. Infected	Infected Males	Infected Females
Gekkonidae						
Coleonyx variegatus	7	1	6	0	14.3	0
Iguanidae						
Callisaurus draconoides	25	1	0	0	4	0
Crotaphytus wislizenii	7	0	3	0	0	0
Dipsosaurus dorsalis	39	1	24	0	2.6	0
Phrynosoma m'calli	5	2	10	2	40	20
Phrynosoma platyrhinos	7	1	4	2	14.3	50
Sceloporus graciosus	18	0	11	0	0	0
Sceloporus magister	22	0	4	0	0	0
Sceloporus occidentalis	32	0	13	0	0	0
Uma inornata	6	1	4	1	16.7	25
Uma notata	3	0	9	1	0	11.1
Uma scoparia	20	1	19	1	5	5.2
Uta stansburiana	7	0	12	1	0	8.3
Urosaurus graciosus	19	0	14	1	0	7.1
Teidae						
Cnemidophorus tigris	14	0	10	2	0	20

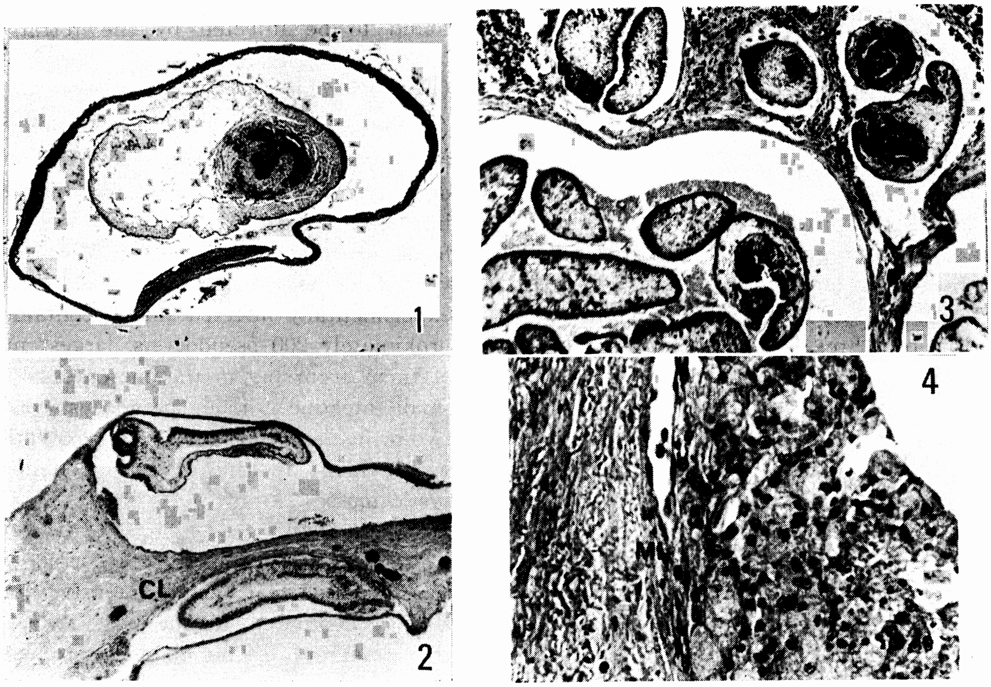

Figure 1. *Mesocestoides* tetrathyridium in pseudocyst recovered from mesenteries of *C. ruber*. Note signet ring appearance of pseudocyst wall. (× 136).
Figure 2. Multiple pseudocysts from the mesenteries of experimentally infected *C. viridis helleri*. A connective tissue layer (CL) separates the pseudocyst. (× 340).
Figure 3. Pseudocyst from the liver of experimentally infected *C. ruber*; a number of multiple cysts and connective tissue encapsulation.
Figure 4. Pseudocyst wall from experimental infection in *C. viridis helleri* showing middle layer (ML). (× 1,000).

entrance of the foreign body into the host. Large numbers of polymorphonuclear cells surround the infection site and many of the hepatic cells undergo necrotic changes (Fig. 1). The nuclei of some of the host cells shrink in size and appear hyperchromatic (Fig. 1). The sinusoids become distended, often filled with extravasated erythrocytes and mononuclear cells. The larva appears to liberate toxic substances into the encapsulating host tissue causing general eosinophilia. In both hepatic and mesenteric infections, there is increased deposition of collagen which become more evident when treated with Mallory's trichrome. The collagenized stroma occurs as concentric bands, lined with epitheloid cells encapsulating the tetrathyridia (Figs. 2, 3).

Mesenteric pseudocysts, measuring on the average 388 μm in diameter from the rattlesnakes *C. ruber* (Fig. 2) and *C. viridis helleri* (Figs. 1, 3, 4) showed the typical chronic inflammatory response with a few lymphocytes scattered around the connective tissue capsule. Multiple cysts were found both in hepatic (Fig. 1) and mesenteric infections (Fig. 3).

Discussion

It is well known that the adult stage of the tapeworm *Mesocestoides* is found in the intstine of numerous species of birds and mammals and the second stage larva, and tetrathyridia is commonly found in the coelom, peritoneum or liver of the second intermediate host, namely: mice, snakes, lizards, etc. The nature of the first intermediate host or the way in which the first stage larva reaches the second intermediate host is still unknown. Information on the range of the second intermediate host, their distribution, food habits, etc., should provide valuable insight into the mysterious life cycle of this tapeworm. Our data indicates that natural infectons of *Mesocestoides* tetrathyridia occur in a wide variety of reptiles of Southern Cailfornia. The prevalence of these parasites in the lizards all of which are insectivorous except for *D. dorsalis* supports the hypothesis that an insect might serve as the first intermediate host. Webster (1949) attempted to infect 36 different species of invertebrates including many groups of insects such as grasshoppers, cockroaches, several species of beetles, etc., but the results were negative. The authors feel that number of insects used were too few to definitely rule out the role of insects in the life cycle.

Crotalus sp. used in the study are carnivorous and feed on relatively large prey, and quite probably obtain their tetrathyridia infection upon feeding on small vertebrates infected with the second stage larva.

The ability to multiply asexually during larval stages is common among many tapeworms. Large numbers of infective second stage larvae are thus produced within the same host by a single infection which greatly enhances the probability of at least a few reaching the final host to complete the life cycle. Asexual multiplication of a species of *Mesocestoides* has been demonstrated in the past only in experimental infections (Specht and Voge, 1965; Voge and Coulombe, 1966). Multiplication occurs either by anteroposterior fission resulting in two individuals, or by lateral outgrowth or budding, the buds later separate and become a new individual. The infected rattlesnakes collected in the field for the present study contained multiple tetrathyridia demonstrating that asexual multiplication also occurs in natural infections.

It is hoped that further investigation would provide information on the nature of the first host, hatching and development of the tapeworm ova and mode of infection of reptiles and other vertebrates in nature.

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