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ASEXUAL MULTIPLICATION OF TETRATHYRIDIA OF *MESOCESTOIDES CORTI* IN *CROTALUS VIRIDIS VIRIDIS*[1]

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ABSTRACT: Prairie rattlesnakes, *Crotalus viridis viridis* Rafinesque, 1818, were infected with tetrathyridia of *Mesocestoides corti* Hoeppli, 1925, by means of oral intubation. Snakes were maintained at constant temperatures of either 25, 30, or 35 C for 4–24 wk. Postmortem examinations revealed the first experimental evidence of asexual multiplication of tetrathyridia in a laboratory-infected ectothermic host. Time and temperature were statistically significant with P values of less than 0.05.

INTRODUCTION

Adult *Mesocestoides corti* Hoeppli, 1925 parasitize man (Chandler, 1942), birds, and carnivorous mammals (Webster, 1949), while the tetrathyridia have been found in snakes, lizards, frogs, toads, and mice (Voge, 1953; Gleason et al., 1973; Mankau and Widmer, 1977).

Carnivorous hosts obtain their infection from the metacestode stage (James, 1968), but attempts to determine the existence of a first intermediate host have not been successful (Webster, 1949; James, 1968). In vitro development from oncosphere to immature tetrathyridium was reported by Voge (1967).

Observational evidence of asexual multiplication of *M. corti*, in an ectothermic host, was first noted by Specht and Voge (1965). Successful transfers were made of tetrathyridia from the lizard, *Sceloporus occidentalis* Baird and Girard, to other lizards of the same species to observe penetration of the liver, but no observations on proliferation of these worms were reported. Observations were not reported beyond 44 hr post-inoculation.

The general purpose of this research was to establish laboratory infection of tetrathyridia of *M. corti* in the prairie rattlesnake, *Crotalus viridis viridis* Rafinesque. The experimental design aimed to determine the following: (1) evidence for asexual multiplication of the tetrathyridia; and (2) if multiplication of the worms occurred, the significance of temperature (25, 30, 35 C) on the rate of proliferation; and (3) effect of temperature and temperature plus parasitism on the survival time of the host.

MATERIALS AND METHODS

Preliminary procedures

Prairie rattlesnakes collected in the autumn of 1974 from Weld County, Colorado, and Pennington County, South Dakota, were placed into separate cages.

Tetrathyridia of *M. corti* were obtained from two infected mice procured from Dr. Marietta Voge at the University of California, Los Angeles. Tetrathyridia from one mouse were used to establish infections in Swiss Webster mice. The initial and subsequent maintenance in laboratory mice was by oral transfer of 25 worms per mouse (Specht and Voge, 1965).

A second mouse from Dr. Voge's laboratory was killed, the worms removed and placed in physiological saline. Isolates of 250 worms each were placed in individual 5-cm Stender dishes containing 2 ml of physiological saline solution. These were then set aside at room temperature pending preparation of the snakes.

Infection of the host

The rattlesnakes were immobilized manually and the tetrathyridia introduced into their stomachs by oral intubation. Each inoculum consisted of 250 worms. Oral gavages of 5 ml

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saline solution were given before and after the worm transfer. Control snakes were given only saline gavages.

Laboratory maintenance of host

Three environmental chambers, with constant individual temperatures set respectively at 25, 30, and 35 C, were used for this experiment. Eighteen experimental snakes and four control snakes were maintained in individual cages at each temperature.

The snakes were fed dead laboratory mice biweekly, beginning 7 days post-exposure. Mice not consumed within 24 hr were removed. This feeding practice was followed to minimize the risk of handling the snakes.

The snakes which did not eat were then fed homogenized (Alpo—beef) dog food by oral insertion of a sterile rubber catheter (Davol, 24 Fr.) attached to a metal caulking gun (Kenmark Mfg. Co., Philadelphia, Pennsylvania 19125, USA). The amount of homogenized food delivered was calculated from the weight of the unconsumed food.

Necropsy procedures

To obtain incremental data, three experimental snakes were removed from each of the three chambers every 4 wk, until termination of the experiment at 24 wk. Snakes removed from the chambers were killed and examined following the techniques described by Widmer (1970). Coelomic viscera were separated individually and examined for tetrathyridia and the total numbers recorded. All of the control snakes were examined at the end of the 24 wk period.

Statistical analysis

Analysis of covariance was done on summation data collected through 16 wk post-infection (Table 1). Tetrathyridia were declared a dependent variable with time a covariant and temperature a design variable. Three statistical tests were done to compare the validity of the three temperature groups. The three tests consisted of the following temperature combinations: 25 and 30 C, 25 and 35 C, and 30 and 35 C. Values were judged significant at $P < 0.05$.

RESULTS

All snakes force-fed with tetrathyridia were infected. All control snakes were negative.

Most of the worms were found in the small intestine (Tables 1 and 2). Fewer numbers were found in the liver, stomach, large intestine, and coelom. Worms were present in the kidney of one snake maintained for 16 wk at 30 C. None was found in the lungs or musculature of any of the snakes.

The relationship between the number of worms recovered and the length of post-infection time seemed apparent. As the number of weeks increased so did the number of worms recovered (Tables 1 and 2). Both time and temperature were statistically significant ($P < 0.0001$ and $P = 0.037$, respectively).

The specific effect of temperature on the rate of proliferation was more difficult to assess. Comparison of the 30 and 35 C temperature was the only pair combination with a significant P value ($P = 0.0144$). Since multiple tests were performed this value was adjusted by multiplying by three giving a P value of 0.0432.

All snakes kept at 25 and all controls kept at 30 and 35 C survived the 24 wk of the experiment, whereas among the remaining experimentally infected snakes 83.3% survived 20 wk at 30 and only 66.7% survived 16 wk at 35 C (Table 1).

DISCUSSION

Natural infections of tetrathyridia of species of *Mesocestoides* in *C. viridis* have been reported by Voge (1953) and Mankau and Widmer (1977). The ease in establishing laboratory infection in this reptile introduces the organisms' potential availability as an intermediate or paratenic host for certain species in the genus *Mesocestoides*.

Asexual multiplication of tetrathyridia of *M. corti* in Swiss Webster mice was reported by Specht and Voge (1965). In this article they also reported observational evidence on the proliferation of tetrathyridia in the lizard, *Sceloporus occidentalis*. However, as far as the authors are aware, the present report is the first experimental evidence of asexual multiplication of tetrathyridia of a species of *Mesocestoides* in an ectothermic host.

TABLE 1. Mean numbers of tetrathyridia recovered per site from 18, 15, and 12 snakes, each fed 250 tetrathyridia (750 for 3 snakes), and each group then kept at 25, 30, or 35 C respectively. Three snakes from each temperature regimen were necropsied at 4 wk intervals.

Temperature	Location of tetrathyridia	Weeks post-infection					
		4	8	12	16	20	24
25 C	Liver	—	9 (0–14)	20 (17–24)	15 (4–21)	39 (11–66)	43 (18–67)
	Stomach	28 (23–34)[a]	41 (31–56)	96 (53–148)	63 (27–114)	73 (27–114)	202 (182–227)
	Small intestine	127 (117–142)	211 (189–249)	219 (99–289)	378 (361–396)	398 (385–421)	495 (481–507)
	Large intestine	46 (27–56)	153 (112–181)	93 (81–101)	155 (98–189)	294 (239–342)	458 (427–481)
	Coelom	16 (0–29)	—	18 (0–28)	—	—	20 (0–31)
	Total/percent recovered[b]	653/87	1,243/166	1,338/178	1,834/245	2,413/322	3,656/488
30 C	Liver	38 (22–53)	45 (14–92)	91 (42–132)	60 (20–87)	115 (105–125)	—
	Stomach	59 (42–73)	39 (12–63)	90 (40–134)	48 (23–63)	123 (119–129)	—
	Small intestine	153 (87–187)	228 (131–281)	185 (113–279)	332 (238–429)	427 (410–442)	—
	Large intestine	83 (77–88)	168 (124–194)	176 (143–229)	210 (112–290)	263 (260–265)	—
	Coelom	3 (0–8)	—	—	—	—	—
	Total/percent recovered[b]	1,007/134	1,440/192	1,625/217	1,952/260[c]	2,784/371	—
35 C	Liver	—	5 (1–8)	26 (20–38)	16 (14–18)	—	—
	Stomach	3 (0–10)	65 (33–82)	72 (29–142)	97 (91–103)	—	—
	Small intestine	96 (61–159)	148 (41–221)	336 (287–387)	225 (220–235)	—	—
	Large intestine	40 (10–62)	125 (20–187)	93 (50–171)	226 (205–239)	—	—
	Coelom	24 (0–40)	—	—	—	—	—
	Total/percent recovered[b]	490/65	1,027/137	1,581/211	1,694/226	—	—

[a] Numbers in parentheses represent range for three snakes.
[b] Actual total number of worms recovered for three snakes and percent of initial inoculum.
[c] Total does not include the 37 worms found in the kidneys of one snake.

TABLE 2. Numbers of tetrathyridia recovered per site from three and six snakes, each fed 250 tetrathyridia, and kept at 30 and 35 C respectively. These snakes died during the course of the experiment.

Temperature	Location of tetrathyridia	Weeks post-infection						
		6	7	9	11	13	15	18
30 C	Liver		41			48		31
	Stomach		28			101		29
	Small intestine		192			217		343
	Large intestine		127			149		171
	Total/percent recovered[b]		388/155			515/206		574/230
35 C	Liver	5		4	31 (28–34)[a]		16 (15–17)	
	Stomach	38		43	88 (74–101)		99 (97–101)	
	Small intestine	111		171	306 (300–311)		230 (229–231)	
	Large intestine	18		23	106 (87–124)		211 (207–214)	
	Total/percent recovered[b]	172/69		241/96	1,059/212		1,111/222	

[a] In the two snakes that died during the 11th week and the two during the 15th week numbers listed represent the mean and the (range).

[b] Actual total numbers of worms recovered and percent of initial inoculum.

The liver is the principal organ for natural infections in lizards (Specht and Voge, 1965) and experimental infections in mice (Specht and Widmer, 1972). In this study necropsies, as early as 4 wk post-infection, showed that the worms preferred the stomach, small intestine, and large intestine (Table 1). Data on the distribution of the worms in the gastrointestinal tract are consistent with data on location studies using *C. v. helleri* as a host (Widmer and Hanson, 1983).

The optimum behavioral temperature for the Crotalidae is between 26.5 and 32 C (Klauber, 1972). This knowledge aided in forming the hypothesis that a constant temperature of 30 C would prove optimal for multiplication. Statistical analysis confirmed the significant role of temperature as a variable but did not permit a strong statement for the 30 C. Perhaps an experimental design using other temperatures or a larger number of snakes would be more supportive of efforts to determine an optimum temperature.

The absence of fatalities in control animals and from experimental snakes kept at 25 C suggested that, at the higher temperatures, parasitism by tetrathyridia may have been an important stress factor.

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