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OVERWINTERING POPULATION CHANGES OF *PTERYGODERMATITES COLORADENSIS* (NEMATODA: RICTULARIIDAE) IN KENTUCKY AND OHIO[1]

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Abstract. Field studies demonstrated a 21.3% prevalence of *Pterygodermatites coloradensis* in *Peromyscus leucopus* and a decreasing mean worm burden during the October to April, 1977–79, study period. Decreasing worm burden may be due to fewer available infested intermediate hosts, the short life span of adult worms, or both. Forest and cave salamanders are probably not effective transfer hosts of *P. coloradensis* since they contained few encysted spirurid larvae and are unlikely to be eaten by *P. leucopus*. Mean worm burden ($\bar{X}=4.4$) and prevalence (15.7%) of encysted larvae *P. coloradensis* increased more significantly with host size than month of the overwintering study period in cave *Ceuthophilus* spp.

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Because of ease of collection, studies on the parasite fauna of *Peromyscus leucopus* are common. *Pterygodermatites* (= *Rictularia*) *coloradensis* is a common parasite of *P. leucopus*, first reported from North Carolina by Harkema (1936). A study in West Virginia reported a 10% infection of *Rictularia* sp. in 130 stomachs (probably small intestines) of *P. l. noveboracensis* (Wilson 1945). Tiner (1948) redescribed and reported the occurrence of *R. coloradensis* using specimens from *P. l. leucopus*, *P. l. noveboracensis* (Maryland) and *P. m. bairdii* (Wisconsin). In central Ohio, Oswald (1956) found 30 of 82 (36.6%) *P. l. noveboracensis* infected with *R. coloradensis*. Zenchak and Hall (1971) showed a relatively high prevalence (50.8%) of *Pterygodermatites* sp. (probably *P. coloradensis*) in 120 *P. leucopus* collected in West Virginia from March to November. Hall *et al* (1955) found a 20.7% and 30.2% prevalence of *R. coloradensis* in 82 and 53 *P. leucopus* from Maryland and Kentucky, respectively.

Oswald (1958a, 1958b) completed the first rictulariid life cycle in *Rictularia coloradensis* from *P. leucopus* and noted that worm burden was largely independent of number of ingested larvae, but their number declined with time; 80 days was postulated to be the maximum life span of *R. coloradensis* in the laboratory mouse. Experimentally, eggs of *R. colorandensis* were shown to be infective to the orthopterans *Parcoblatta pennsylvancia*, *P. virginica*, *Blatella germanica*, *Supella supellectilium*, *Blatta orientalis*, *Periplaneta americana*, *Acheta assimilis*, and *Ceuthophilus* sp., and he demonstrated natural infestations in *Ceuthophilus gracilipes*, *Ceuthophilus* sp., and *Parcoblatta pennsylvanica*.

Quentin's (1969) revision of the Rictulariidae recognized two valid genera, *Rictularia* and *Pterygodermatites*. *Rictularia coloradensis* was designated the type species of the subgenus *Paucipectines* in the genus *Pterygodermatites*. The primary characteristics on which this classification was based were cephalic morphology, disposition of male cloacal papillae, and number of cuticular spines.

The present study was aimed at determining overwintering mechanisms of *Pterygodermatites coloradensis* by surveying definitive, intermediate, and potential paratenic hosts.

MATERIALS AND METHODS

White-footed mice, *Peromyscus leucopus*, were collected from two forest locations in Indian

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Hill, a suburb of Cincinnati, Ohio. We set snaptraps each month from October 1977 to April 1979 at one or both study sites, and captured specimens were autopsied within 12 hr of collection. Eggs of *P. coloradensis*, from *P. leucopus*, were refrigerated (8 °C) for up to 77 days in Ringer's saline and fed to uninfected domestic crickets (*Acheta domesticus*) to test effects of cold temperature on viability; infectivity remained constant for this period, since all exposed crickets were positive for encysted larvae of *P. coloradensis*.

We collected forest camel crickets, *Ceuthophilus* spp., from May to October 1978 and May 1979 in Indian Hill from pitfall traps modified from a design by Uetz and Unzicker (1976). The killing solution was a 1 : 1 solution of ethylene glycol and water. Specimens were rinsed repeatedly in 70% ethanol before autopsy. A small subsample was collected alive and autopsied to compare with results of pitfall trapping. Cave collected *Ceuthophilus* spp. came from two cites in Lawrenceburg, Kentucky. We collected crickets monthly by hand from walls and ceilings of the cave sites from October 1978 to April 1979 and autopsied them within 48 hr of collection. Caudal femur and ovipositor lengths were recorded as indicators of cricket size. Encysted nematode larvae were collected in Ringer's solution, fixed in hot Schaudinn's solution, stored in 70% ethanol, cleared in lactophenol, and mounted in glycerine jelly. To verify identification of *P. coloradensis* cysts from cave *Ceuthophilus* spp., we examined laboratory mice exposed to such larvae 10 days to 54 days post-exposure.

Salamanders were collected in pitfall traps from forest and by hand from Ohio stream locations in September 1978 and April 1979 and from the two Kentucky cave sites in November 1978 and April 1979. Pitfall trapped forest salamanders were rinsed repeatedly in 70% ethanol, then examined for encysted larval spirurid nematodes within 48 hr of collection. Regression and Student T-test analyses were made according to Sokal and Rohlf (1969).

RESULTS

Pterygodermatites coloradensis occurred in 21.3% of 94 *Peromyscus leucopus*. We noted a significant (r = 0.75) decrease of mean parasite burden during the October to April period (fig. 1). Worms were found in every month of this overwintering study, except for March when we collected 7 uninfected mice (fig. 2). From November to January, a relatively large number of mice (n = 13–35) was collected and we found prevalence of infection to be 9% to 15%. Embryonated eggs were commonly seen in adult females collected from October to February, but the single female worm collected in April was not gravid.

Among the salamanders examined in this study, two distinct types of larval

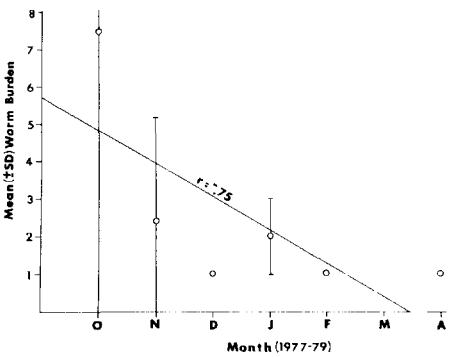

FIGURE 1. Regression model of adult *Pterygodermatites coloradensis* mean worm burden in infected *Peromyscus leucopus* collected during the overwintering period 1977–1979. r = correlation coefficient; bars = ±S.D.

spirurid cysts occurred in 30% of 20 *Eurycea lucifuga* from Kentucky caves. The two cyst types differed in worm body diameter (8 μm *vs* 40 μm) and cyst wall structure, but insufficient material prevented detailed description and exact identification. Eighteen *E. bislineata* and 9 *Desmognathus fuscus* from a stream location, and 12 *Plethodon cinereus* from forest locations were negative for spirurid cysts.

None of a large sample (390) of forest

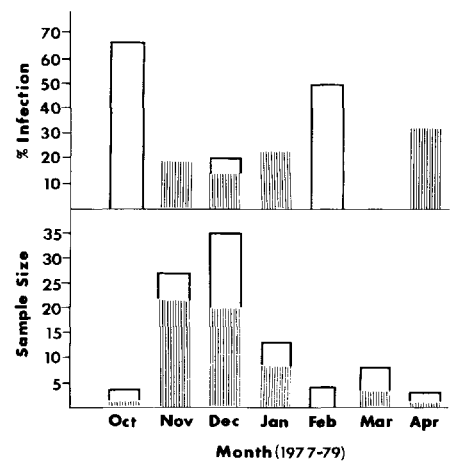

FIGURE 2. Prevalence of *Pterygodermatites coloradensis* and number of mice collected during the 1977–1979 overwintering periods. Vertical hatched bars represent the 1977–78 overwintering period; unhatched bars, 1978–79 overwintering period.

crickets, *Ceuthophilus* spp., was infested with *P. coloradensis* larvae. Forest species collected were *C. latens*, *C. tenebrarum*, and *C. divergens*. Live captured crickets (60) provided supportive evidence from pitfall trapped specimens that prevalence of *P. coloradensis* was very low in resident crickets. The small number (3) of *Parcoblatta pennsylvanica* examined prevented assessment of *P. coloradensis* prevalence in them.

Cave collections revealed a relatively high prevalence (15.7%) of larval *P. coloradensis* cysts free in the hemocoel of 460 *Ceuthophilus stygius* and *C. divergens*. Prevalence of *P. coloradensis* increased with both month of overwintering period and size of cricket. A more significant relationship was observed for prevalence with host size ($Y = 2.7X - 17.4$: $r = 0.96$) (fig. 3) than with month ($Y =$

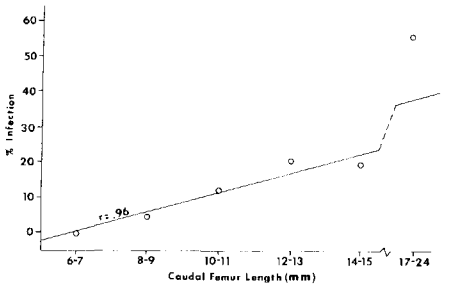

FIGURE 3. Regression model of larval *Pterygodermatites coloradensis* prevalence by size of cave *Ceuthophilus* spp. during overwintering study period, 1978–79. r = correlation coefficient; dashed line = predicted value for adult cohort; far right hand point = observed value for adult cohort (not included in regression model).

$1.86X + 5.36$; $r = 0.56$). Mean worm burden also was correlated more closely with host size ($Y = .49X - 2.7$; $r = 0.85$) (fig. 4) than with month of overwintering period ($Y = .37X + 1.79$; $r = 0.68$). Analyses of prevalence and mean worm burden were made using paired caudal femur lengths (F.L.) (*i.e.* 6–7, 8–9, *etc.*) to increase sample size of individual points. The group of 16 adults *Ceuthophilus* spp. (X F.L. = 20.3) collected in October and November were not included in the regression analyses, since they represented a cohort one year older, but prevalence of infection (56.3%) and mean

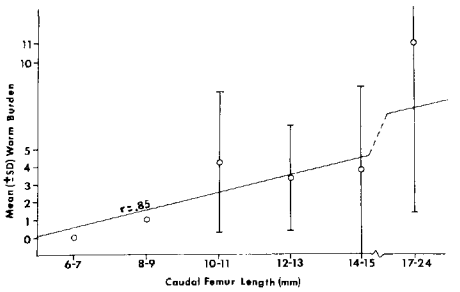

FIGURE 4. Regression model of larval *Pterygodermatites coloradensis* mean worm burden by size of cave *Ceuthophilus* spp. during the overwintering study period, 1978–79. r = correlation coefficient; dashed line = predicted value for adult cohort; far right hand point = observed value for adult cohort (not included in regression model).

worm burden (10.9) in adult hosts were higher than predicted by regression analyses of smaller crickets (X F.L. = 11.2).

Infected crickets were significantly larger than uninfected crickets (99.5% C.I.), but hosts with cysts with multiple larvae were not significantly larger (60% C.I.) than those with cysts containing single larvae (table 1). Mean worm burden of 13 Crickets with multiple cysts (12.2 ± 7.51) was significantly greater (99.95% C.I.) than for 59 specimens with single larva cysts (2.9 ± 2.41). The months in which infected crickets were significantly larger in size than uninfected were January (80% C.I.), February (80% C.I.) and April (99.5% C.I.).

DISCUSSION

The observed decrease in mean worm burden in the present study during the overwintering periods, and absence of *P. coloradensis* in *P. leucopus* in March may be due to fewer infested intermediate hosts, the short life span of adult worms, or both. Numbers of overwintering infested nymphs probably decrease due to predatory pressure of shrews, raccoons, and mice. Oswald (1958a) predicted that few adult worms would be present in laboratory mice after 80 days post-exposure. The decrease in mean worm burden observed in natural hosts in the present study from October to March may be due to limited longevity of adult worms. While seven mice collected in

TABLE 1

Student's T-test analysis of worm burden with month and caudal femur length of cave Ceuthophilus with Pterygodermatites coloradensis.

Month (n)	Caudal Femur Length* ($\bar{X}$ in mm) (S. D.)			Mean Worm Burden** (S. D.)	
	Uninf.	Inf.	Inf. w/ mult. cysts	Inf. w/ single	Inf. w/ mult. cysts
Oct	11.8+	11.0	—	3.67	—
(13)	(.96)	—	—	(3.06)	—
Nov	11.5+	11.3	13.0	3.90	14.17
(68)	(1.93)	(1.73)	—	(3.45)	(9.81)
Dec	10.1+	10.0	—	4.00	—
(41)	(1.52)	—	—	—	—
Jan	10.5++	11.3	11.5	1.75	6.50
(60)	(2.10)	(.95)	(.71)	(1.16)	(4.95)
Feb	10.7++	11.4	13.0	3.71	6.0
(60)	(1.61)	(1.19)	—	(3.45)	—
Mar	11.4+	11.7	11.0	2.78	12.00
(54)	(1.54)	(1.35)	(1.41)	(2.44)	(5.66)
Apr	11.8++	12.9	13.5	2.48	14.00
(149)	(1.84)	(1.52)	(.71)	(1.60)	(2.83)
Total	11.2†	11.9+	12.1	2.90††	12.15
(445)	(1.64)	(1.35)	(1.13)	(2.41)	(7.51)
	(n=388)	(n=63)	(n=8)	(n=59)	(n=13)

*Includes only small cohort of crickets.
**Includes all crickets.
+Not significant at 75% Confidence Interval.
++80% C.I.
†99.5% C.I.
††99.95% C.I.

March in the present two year study is not a large sample, it represents a relatively high number of resident mice during the seasonal population low. During February and April few mice were collected, but infestation was still observed in each month. The high prevalence in April observed in this study (33.3%) and by Oswald (1956) (34.4%) is likely due to ingestion of infested overwintering orthopterans and the lack of gravid female worms in mice collected in April supports this view.

Spirurid cysts found in the cave salamander, *Eurycea lucifuga*, were not identified, but it is virtually certain that the larger encysted worms (40 μm) were *P. coloradensis*. We collected these salamanders in the twilight and entrance zones, where many infected *Ceuthophilus* spp. also were collected, and where they could be preyed upon by salamanders during the winter when other food is not available. Norton (1978) suggested that *Hadenoecus* is one of the principle food items in the diet of *E. lucifuga* in cave entrances and neighboring cliffs. We did not observe large numbers of *P. coloradensis* cysts in *E. lucifuga* in our study, suggesting that these salamanders may not accumulate such larvae as other paratenic hosts do. Further, salamanders are not readily eaten by *P. leucopus* and therefore may not be ecologically effective transfer hosts for *P. coloradensis*. The lack of encysted spirurid larvae observed in forest and stream salamanders in a location endemic for *P. coloradensis* supports this hypothesis.

Increased prevalence and numbers of larval *P. coloradensis* with body size of cave *Ceuthophilus* spp. are probably due to accumulation of larvae. Clarke (1957) showed that increased hind femur length of *Locusta* is correlated with time, and caudal femur length of *Ceuthophilus* is similarly correlated with host age. Exposure to sources of infection for a longer time would increase the probability of infestation of older hosts, and increased

burden is related to the number of eggs ingested and the number of meals with infestive material. Since both cave locations were essentially closed systems for cave crickets during the winter, they were observed further into each cave as external temperature decreased. While not entirely dormant, both growth and sexual development of crickets were found to be much slower during the winter period. Cannibalism of *Ceuthophilus* during ecdysis was observed by Hubbell (1936) in crowded experimental conditions, a phenomenon that would magnify infestation in larger hosts in natural populations. He observed that sexual maturation of *C. stygius* and *C. divergens* occurred during the summer, but adults were collected in our study only in October and November. We did not observe gravid females overwintering, but eggs probably can do this. Both prevalence and worm burden of adult crickets were above that predicted by regression analyses of data from smaller crickets, and may be due to increased food demands associated with warmer temperatures, increased range of activity, and reproduction. Feeding outside the cave from spring to fall would obviously increase the exposure of cave crickets to infestive material. Mice, probably *P. leucopus*, were observed in one cave location and may be a common cave dweller in the winter; since caves are a constant, tolerable thermal environment, these mice probably contributed infective material to caves.

Present experimental data show that eggs of *P. coloradensis* remain viable up to 77 days in refrigerated saline. Eggs deposited in the feces of *P. leucopus* in caves would thus probably remain viable for an extended period, perhaps the entire overwintering period, at the cool temperatures of damp cave soil.

From 1 to 29 *P. coloradensis* cysts were found free in the hemocoel of cave crickets; infected crickets were larger than uninfected ones in January, February, and April when prevalence of infection was high. Identity of these cysts was confirmed from adult worms recovered from experimentally infected laboratory mice. Crickets containing cysts with more than one larva per cyst capsule were not larger, but had a higher mean worm burden than crickets infected with only single cysts. Formation of multiple cysts is probably not a function of host size as much as number of eggs ingested, since large numbers of ingested eggs would result in many penetrating larvae in the intestine and increase the likelihood of incorporating several larvae in each cyst. Oswald (1958b) observed multiple cysts 480 μm in diameter with up to 12 worms per cyst from experimental *Ceuthophilus* sp. We also commonly observed multiple cysts with three larvae and over 500 μm in diameter in our study of cave *Ceuthophilus*.

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LITERATURE CITED

Clarke, K. U. 1957 On the increase in linear size during growth of *Locusta migratoria* L. Proc. Roy. Ent. Soc. (London). A 32: 35–39

Hall, J. E., B. Sonnenberg and J. R. Hodes 1955 Some helminth parasites of rodents from localities in Maryland and Kentucky. J. Parasitol. 41: 640–641.

Harkema, R. 1936 The parasites of some North Carolina rodents. Ecol. Mongr. 6: 153–232.

Hubbell, T. H. 1936 A monographic revision of the genus *Ceuthophilus*. Univ. Fla. Publ., Biol. Sci. Ser. 551 pp.

Norton, R. M. 1978 The biology of *Hadenoecus subterraneus* and *H. cumberlandicus*. *In:* Hubbell, T. H. and R. M. Norton (eds) The systematics and biology of the cave-crickets of the North American tribe Hadenoecini (Orthoptera Saltatoria: Ensifera: Rhaphidophoridae: Dolichopodinae). Univ. Mich. Misc. Publ., Mus. Zool. pp. 100–117.

Oswald, V. H. 1956 Studies on the life cycle of *Rictularia colorandensis* Hall, 1916 (Nematoda: Thelaziidae) a parasite of *Peromyscus leucopus noveboracensis* (Fishcher). Unpubl. Ph. D. thesis, Ohio St. Univ. Columbus.

——— 1958a Studies on *Rictularia coloradensis* Hall, 1916 (Nematoda: Thelaziidae) II. Development in the definitive host. Trans. Amer. Micr. Soc. 77: 413–422.

——— 1958b Studies on *Rictularia coloradensis* Hall, 1916 (Nematoda: Thelaziidae) I. Larval development in the intermediate host. Trans. Amer. Micr. Soc. 77: 229–240.

Quentin, J. C. 1969 Essai de classification des nematodes Rictularies. Mem. Mus. Nat. Hist. Nat. Ser. A. Zool. 54: 55–115.

Sokal, R. R. and F. J. Rohlf 1969 *Biometry*. W. H. Freeman, San Francisco. 776 pp.

Tiner, J. D. 1948 Observations on the *Rictularia* (Nematoda: Thelaziidae) of North America. Trans. Amer. Micr. Soc. 67: 192–200.
Uetz, G. W. and J. D. Unzicker 1976 Pitfall trapping in ecological studies of wandering spiders. J. Arachnol. 3: 101–111.
Wilson, L. W. 1945 Parasites collected from the wood mouse in West Virginia. J. Mammal. 26: 200.
Zenchak, J. J. and J. E. Hall 1971 Helminths from *Peromyscus leucopus* and *P. maniculatus* in West Virginia. J. Parasitol. 57: 542.