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LARVAL DEVELOPMENT AND TRANSMISSION OF *FOLEYELLA FLEXICAUDA* SCHACHER AND CRANS, 1973 (NEMATODA: FILARIOIDEA) IN *CULEX TERRITANS**

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ABSTRACT: Larvae of *Foleyella flexicauda* which developed to the infective stage in 13 days in the fat body of experimentally infected *Culex territans* were transmitted by bite to laboratory-reared bullfrogs (*Rana catesbeiana*). The prepatent period was approximately 8 months. Attempts to similarly infect green frogs (*Rana clamitans*) and leopard frogs (*Rana pipiens*) were unsuccessful. Larval development within *Culex territans* is described.

Crans (1969) reported a filarial worm, now known as *Foleyella flexicauda* Schacher and Crans, 1973, in bullfrogs (*Rana catesbeiana*) in New Jersey. He also noted that other species of frogs, notably green frogs (*Rana clamitans*), did not harbor the parasite even when found in ponds where the infection was very common among bullfrogs. More recently, Crans (1970) showed that *Culex territans* feeds predominantly on frogs. These findings suggested that *Culex territans* may be a natural vector of *F. flexicauda*.

The present study describes the development of *F. flexicauda* in *C. territans* as well as the results of transmission experiments.

MATERIALS AND METHODS

Culex territans, colonized and maintained as described by Benach (1970), were fed on an infected bullfrog 7 days after emergence. Engorged females were removed by aspirator, and maintained on sucrose in screened 1-pint cartons. The insectary was maintained at 24 ± 1 C and 80% relative humidity.

Infected mosquitoes were immobilized by chilling and dissected in physiological saline; head, thorax, and abdomen were examined separately. Larval development was followed by dissecting 25 mosquitoes daily from engorgement through the 21st day.

Larvae were heat-stunned and measured; methyl green pyronin, dilute Giemsa, and Azur II were used to differentiate internal structures as described by Causey (1939). Cephalic structures of the microfilaria were studied using the staining techniques of Laurence and Simpson (1969).

Wild-caught bullfrogs from various localities in New Jersey were maintained as described by Nace (1968). Microfilarial counts were made by the method of Crans (1969).

Transmission was accomplished by exposing uninfected laboratory-reared frogs to mosquitoes which had fed on infected frogs 14 days earlier. After 2 hr exposure, the frogs were removed and both engorged and unengorged mosquitoes were dissected. Blood from the frogs was examined every month to determine the prepatent period. The laboratory-reared frogs were kept separate from the wild bullfrogs at a temperature of 17 to 21 C.

RESULTS

Larval development in *Culex territans*

As *C. territans* feeds slowly, engorged mosquitoes dissected immediately had already spent about 12 min on the host. Exsheathing microfilariae seen in the midgut immediately after engorgement; many had already penetrated into the hemocoel. Penetration of midgut epithelium accomplished by rapid slashing movements of the cephalic hook. Exsheathed microfilariae migrated directly into the fat body near intersegmental muscles and body wall in distal portion of abdomen, where they remained until 13th day postinfection.

Little development during the first 24 hr; by the 2nd day, the larvae began to undergo shortening and thickening (Table I). Shortening more obvious by 3rd day, when larvae were distinctly wider at the posterior region and had a blunt head. Nerve ring prominent, finely striated with small nuclei. Excretory pore located ventral and posterior to nerve ring. Rectum enlarged, protruding outside contour of the worm; tail prominent and hooked slightly. Alimentary canal still undifferentiated.

First-stage larva almost doubled its length on 4th and 5th days. By 5th day, the striated excretory cell occupied half the width of the worm. Esoph-

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TABLE I. *Measurements in microns of 25 larvae of* F. flexicauda *from* Culex territans *at various time intervals postinfection.*

Days after feeding	Length	Width*	Head to nerve ring	Esophagus length	Anus to tail
First stage					
2	100 (99–107)	5 (5–6)	15 (15–17)		
3	91 (82–99)	10 (9–12)	13 (13–14)		15 (13–16)
4	138 (122–153)	16 (13–18)	24 (22–27)		18 (16–19)
5	279 (180–323)	17 (13–21)	30 (29–31)	149 (143–157)	20 (16–24)
6	462 (411–500)	19 (18–21)	34 (26–46)	224 (214–236)	23 (19–24)
Second stage					
7	688 (680–712)	12 (18–22)	68 (65–70)	358 (351–362)	32 (27–34)
8	705 (700–711)	21 (18–22)	68 (67–69)	358 (351–362)	33 (27–34)
9	710 (704–720)	20 (18–22)	73 (70–75)	359 (352–361)	33 (27–34)
11	720 (716–734)	20 (18–22)	75 (68–79)	373 (370–376)	33 (27–34)
13†	734 (729–740)	21 (17–22)	40 (36–43)	378 (267–389)	39 (33–42)
Third stage					
13	921 (848–1,026)	21 (19–22)	93 (88–94)	563 (525–589)	41 (39–43)
15	912 (843–1,018)	21 (19–22)	92 (88–94)	567 (532–582)	41 (39–43)

* Width at nerve ring level.
† Molting forms.

agus, longer than the intestine, comprised finely granular cells; intestinal cells larger with coarser granules. Rectum composed of 3 large cells, most anterior of which protruded beyond the cuticle. Tail pointed and usually bent laterally.

Second-stage larvae found from 7th through 13th days. Length 680 to 740 μ; body shape uniformly cylindrical 18 to 22 μ wide. Head bluntly rounded; nerve ring prominent but no excretory cell could be observed. Finely granular esophagus more than half the length of the larva. Well-differentiated intestine coarsely granular. Rectum pyriform, protruding beyond body outline. Tail ending in blunt point. Three glandlike structures lateral to esophagus and posterior to nerve ring, their function unknown.

On 13th day, many 2nd-stage larvae molting, many 3rd-stage larvae also present. Third-stage larvae 848 to 1,026 μ long; cylindrical, 18 to 22 μ wide. Four submedian cephalic circumoral papillae; amphids could not be detected from lateral aspect. Esophagus, as in previous stages, longer than intestine. Posterior region of esophagus wider than anterior, but without distinct division between glandular and muscular regions. Rectum simple. Tail blunt with 2 lateroventral subterminal papillae. Third-stage larvae migrated from the abdomen toward the head and labium of the mosquito shortly after the final molt.

Transmission of infective larvae by *C. territans* in the laboratory

Mosquitoes infected 14 days previously and fed on uninfected, laboratory-reared frogs had few or no 3rd-stage larvae remaining at dissection (Table II); the few that remained were found in the abdomen. Conversely, those mosquitoes which did not feed had numerous 3rd-stage larvae in the head and labia.

The time between exposure of the bullfrogs to infected mosquitoes and the appearance of microfilariae in the blood was between 228 and 232 days, or roughly 8 months (Table II). Green frogs (*Rana clamitans*) and leopard frogs (*Rana pipiens*) did not become patent even 1 year after exposure to infected mosquitoes. Necropsy of the latter yielded neither immature nor adult filariae. Bullfrogs Nos. 4 and 6 (Table II) which died during the prepatent period contained only immature worms embedded in the mesenteries at necropsy.

DISCUSSION

Microfilariae of *F. flexicauda* probably penetrate the midgut of *C. territans* when the mosquito is still in the act of feeding. The rate of penetration of the microfilariae of other *Foleyella* species have not been studied, but there are many reports which attest to rapid penetration in other filarial genera.

Kotcher (1941) working with the microfilariae of *F. brachyoptera*, noticed "lips that were inverted and then everted regularly," and thought that a stylet might be present at the cephalic end. The presence of such hooks is now confirmed in the microfilariae of *F. flexicauda*.

There are few marked differences in the morphogenesis of the larval stages of *F. flexi-*

TABLE II. *Results of exposure of three species of uninfected, laboratory-reared frogs to* C. territans *infected with* F. flexicauda *14 days earlier.*

Frog no.	Mosq. refusing 2nd blood meal		Mosq. accepting 2nd blood meal		Prepatent period (days)
	No.	Mean No. of 3rd-stage larvae per mosquito	No.	Mean no. of 3rd-stage larvae per mosquito	
BF 1	26	10.8	16	0	228
2	19	11.1	19	0	228
3	15	9.7	21	2.8	230
4	25	9.6	15	0	Died
5	20	9.9	18	0	232
6	23	10.0	12	0	Died
LF 1	21	10.2	12	0	—
2	25	8.4	10	1.8	—
3	21	9.3	17	1.1	—
4	25	8.6	13	0	—
5	28	10.0	9	0	—
6	22	11.2	12	0	—
GF 1	20	9.4	14	0	—
2	3	9.2	18	0	—
3	32	10.2	8	0	—
4	33	10.4	13	0	—

Abbreviations: BF, bullfrogs (*Rana catesbeiana*). LF, leopard frogs (*Rana pipiens*). GF, green frogs (*Rana clamitans*).

cauda from other amphibian species of the same genus. The most salient morphological feature of the larvae of this species is their long esophagus relative to the body length. Kotcher (1941), however, observed a similar feature in *F. brachyoptera* and *F. ranae*; and Witenberg and Gerichter (1944) also observed it in the second larval stage of *F. duboisi*.

This contrasts sharply with third-stage larvae of reptilian *Foleyella*, wherein the esophagus is much shorter relative to the body length (Schacher and Khalil, 1968; Bain, 1969). Length of the larval esophagus has recently been proposed as one of the morphological characters supporting the erection of two subgenera of the genus *Foleyella* (Schacher and Crans, 1973).

Transmission of third-stage larvae to bullfrogs via the mosquito was readily accomplished in the laboratory (Table II). However, the complete refractoriness of *Rana pipiens* and *Rana clamitans* to *F. flexicauda* may indicate that this filarioid is species-specific for the bullfrog. These results agree with the observations of Crans (1969), who could find no other species of frogs naturally infected with *F. flexicauda* even in locations where infections were both abundant and widespread among bullfrogs.

The prepatent period of almost 8 months appears to be long. It is possible that more carefully controlled environmental conditions in the laboratory could significantly alter the time needed for full development of *F. flexicauda* in frogs. Present techniques for maintaining frogs in the laboratory do not completely satisfy many environmental needs of aquatic poikilotherms since many periodic natural processes such as mating and hibernation are largely by-passed or obliterated. However, better techniques in rearing frogs are being rapidly developed and the presence of large numbers of microfilariae in bullfrogs, and the ease with which *C. territans* can be infected in the laboratory, make this parasite a useful tool for investigating general aspects of development and transmission of filarial worms as well as more specific questions concerning fat-body development.

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LITERATURE CITED

BAIN, O. 1969. Étude morphologique du developpement larvaire de *Foleyella furcata* chez *Anopheles stephensi*. Ann. Parasit. **44:** 165–172.

BENACH, J. L. 1970. Observations of a colony of *Culex territans* Walker. New Jersey Mosq. Extern. As. Proc. **57:** 70–76.

CAUSEY, O. R. 1939. Description of three species of frog microfilariae, with notes on staining methods. Am. J. Hyg. **39** (D): 117–121.

CRANS, W. J. 1969. Preliminary observations of frog filariasis in New Jersey. Bull. Wildl. Dis. As. **5:** 342–347.

———. 1970. The blood feeding habits of *Culex territans* Walker. Mosq. News **30:** 445–447.

KOTCHER, E. 1941. Studies on the development of frog filariae. Am. J. Hyg. **34** (D): 36–65.

LAURENCE, B. R., AND M. G. SIMPSON. 1969. Staining microfilaria for structures other than nuclei. Tr. Roy. Soc. Trop. Med. Hyg. **63:** 801–810.

NACE, G. W. 1968. The amphibian facility of the University of Michigan. BioScience **18:** 767–775.

SCHACHER, J. F., AND W. J. CRANS. 1973. *Foleyella flexicauda* sp. n. (Nematoda: Filarioidea) from *Rana catesbeiana* in New Jersey with a review of the genus and erection of two new subgenera. J. Parasit. **59:** 685–690.

———, AND G. M. KHALIL. 1968. Development of *Foleyella philistinae* Schacher and Khalil, 1967 (Nematoda: Filarioidea) in *Culex pipiens molestus*, with notes on pathology in the arthropod. J. Parasit. **54:** 869–878.

WITENBERG, G., AND C. GERICHTER. 1944. Morphology and life history of *Foleyella duboisi* with remarks on allied filariids of amphibia. J. Parasit. **30:** 245–256.