

MORPHOGENESIS OF LARVAL *ONCHOCERCA VOLVULUS* IN THE PANAMANIAN BLACK FLY, *SIMULIUM QUADRIVITTATUM*

EVERETT L. SCHILLER,* JOHN L. PETERSEN,† DJAMSHID SHIRAZIAN,*
 AND HORACIO FIGUEROA MARROQUÍN‡

*Department of Immunology and Infectious Diseases, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205, †Gorgas Memorial Laboratory, Panama, Republic of Panama, and ‡Sección de Oncoercosis, Dirección General de Servicios de Salud, Guatemala City, Guatemala

Abstract. *Simulium quadrivittatum* Loew (Diptera: Simuliidae), a man-biting black fly, was shown, for the first time, to be capable of supporting development of *Onchocerca volvulus* Leuckart (Nematoda: Filarioidea) from microfilariae to third-stage (infective) larvae. The black flies were collected in Chiriqui Province, Panama and transported alive to Guatemala, where they were allowed to feed on a human subject infected with *O. volvulus*. Samples of these flies were dissected over an 11-day period to assess morphogenesis of the parasite. Vigorously motile microfilariae were recovered from the mid-gut during the first 24 hours postfeeding; second-stage larvae were found in the thoracic musculature on day 4; and fully developed third-stage larvae were obtained from the cephalic capsule by day 10. This rate of larval development is similar to that observed in Guatemalan *S. ochraceum*. Onchocerciasis is not known to occur in Panama. The results of the present study direct attention to a potential public health hazard there and possibly elsewhere in Central America.

The geographic distribution of onchocerciasis in the Western Hemisphere is sporadic. It occurs in localized areas of Mexico, Guatemala, Colombia, Venezuela, Brazil and Ecuador. Man-biting black flies of the genus *Simulium* are seasonally abundant in several other Central and South American countries in which the disease has not as yet been found. Two species responsible for the transmission of onchocerciasis in Guatemala, namely *S. ochraceum* and *S. metallicum*, were described from Panama as early as 1940,¹ but the potential role of these and other Panamanian simuliids as vectors of this disease has not been investigated, heretofore. Experimental evidence now has been obtained establishing that at least one of them, *S. quadrivittatum*, can support morphogenesis of larval *O. volvulus* to the third stage.

MATERIALS AND METHODS

During the 3rd week of August 1981, 320 live black flies, predominately *S. quadrivittatum*, were collected by two of the authors (ES and JP) near the residential compound of the Fortuna Hydro-

electric Project, Chiriqui Province, in the Republic of Panama. Pertinent environmental features of the area from which collections were made have been described previously.² The collectors served as the attractants, and the flies were captured individually in plastic tubes of the kind described by Figueroa et al.³ when they alighted and began to take a blood meal. Thereafter, they were fed ad lib on 5% sucrose solution replenished daily. These flies were transported via commercial airline from David, Panama, to Panama City and then to Guatemala City, Guatemala the following day. Eighty flies were dead on arrival in Panama City and were discarded without dissection, because facilities for microscopic examination were not available en route. Fifty-seven more flies were found dead after arrival in Guatemala City. These were dissected and examined, as controls, for the presence of filarial parasites. On the 4th day after collection, the remaining 183 live flies were transported from Guatemala City, via automobile, for a distance of about 36 km to Palín, where they were fed on an onchocerciasis-infected subject having a mean number of emergent microfilariae, after 10 min, of 60/1.5 mm² skin-snip. Feeding was accomplished by carefully removing the cap from a tube containing a fly and then quickly applying the

TABLE I

Morphogenesis of larval *O. volvulus* in Panamanian *S. quadrivittatum* experimentally infected in Guatemala

Day post-infection	No. flies dissected		No. flies with motile larvae		x no. of larvae per fly	Stage of larval development
	Without infective blood meal*	With infective blood meal	No blood meal*	Blood meal		
0	57	—	0	—	0.0	—
1	3	2	0	2	190.0	Mff†
2	7	3	0	3	30.0	Late L ₁
3	11	4	0	2	22.0	Late L ₁
4	10	11	0	4	3.3	L ₂
10	30	27	0	7	2.4	L ₃
11	39	36	0	5	1.6	L ₃

* Controls.

† Mf, microfilariae. Approximately 50% were motile.

open end of the tube to the skin on the back, neck or upper arm of the infected subject. The tube was held perpendicular to the surface of the skin and, upon release after engorgement, the fly remained trapped inside the tube. During removal of the tube from the skin, its open end was covered by a thumb or finger to prevent the fly from escaping while the cap was being replaced. Forty-six flies were fed successfully on the first day and 37 additional flies were fed on the same subject the following day. The remaining 100 flies which did not take a blood meal served as additional controls to make certain that these simuliids had not acquired any filarial parasites in their native environment. Samples of the 83 flies receiving an infective blood meal were dissected and examined by both phase contrast and bright field microscopy over an 11-day period post-feeding to assess the occurrence, viability and stage of development of onchocercal larvae.

RESULTS

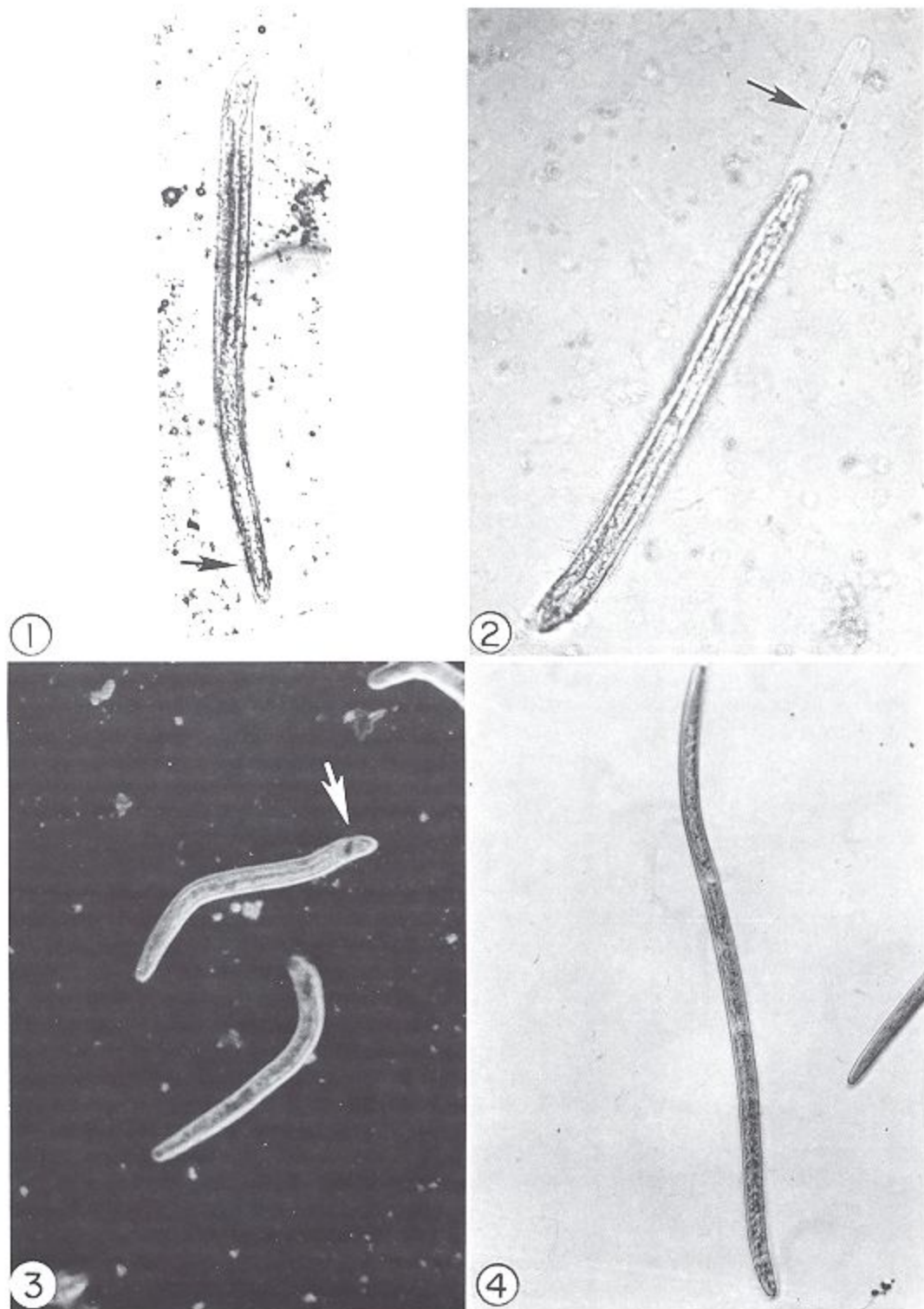
The data resulting from the dissections of the Panamanian *S. quadrivittatum* experimentally infected in Guatemala are shown in Table I. None of the 157 black flies which served as controls in this study harbored any larval nematodes. Eighty-three (45%) of the 183 captive flies succeeded in obtaining a bloodmeal from the infected subject. Microfilariae, about one-half of which exhibited vigorous motility, were found

in abundance in the mid-gut up to 24 hours post-feeding. Comparative measurements of these microfilariae and those of *O. volvulus* obtained in previous investigations in Guatemala⁴ revealed no statistically significant differences. Late first-stage larvae (L₁), just beginning to molt, were found in the thoracic musculature on day 2 post-infection (Fig. 1). Late L₁ larvae in an advanced stage of the molting process were found on day 3 (Fig. 2). Molting was completed by day 4, when typical L₂ larvae were dissected from the thoracic musculature of the flies (Fig. 3). Fully developed L₃ larvae (the stage infective for humans when transmitted by the bite of the black fly) were recovered from the cephalic capsule on days 10 and 11 (Fig. 4). The infectivity of these third-stage larvae was not determined.

DISCUSSION

The rate of morphogenesis of larval *O. volvulus* in *S. quadrivittatum* is similar to that observed in experimentally infected Guatemalan *S. ochraceum*.⁵ Although the sample size in the case of the former species is relatively small, it appears that both the percentage of flies infected and the mean number of L₃ larvae per infected fly also are very similar.

In preparing the protocol for this study, some skepticism was expressed about whether the captive flies would take a blood meal. Gibson found that the three species of *Simulium*, i.e., *S. ochraceum*, *S. metallicum* and *S. callidum*, which commonly bite man in Guatemala, would not feed in captivity.⁶ Several thousand flies that had emerged from pupae in the laboratory were given the opportunity to feed on human volunteers under a "great variety of conditions," but only three of these took a blood meal.⁶ By contrast, 45% of the 183 *S. quadrivittatum* given the chance to bite the infected subject at Palín in this investigation, did so and fed to repletion. The reason(s) for the contrasting results of these two studies is not known but it may be attributable to one or more of the following: 1) a difference in the feeding behavior of the two species of *Simulium*; 2) differences in feeding requirements relative to the conditions of emergence (i.e., laboratory vs. natural environment); and/or 3) method by which the captive flies were given the opportunity to feed. According to observations made in this study, successful feeding of *S. quad-*



FIGURES 1-4. Morphogenesis of larval *O. volvulus* in *S. quadrivittatum*. 1. Late L_1 stage at beginning of molt. Note separation and wrinkling of the tegument (arrow). $\times 380$. 2. L_1 form in an advanced stage of molting. Note shed portion of old tegument (arrow). $\times 380$. 3. Early L_2 forms after completion of the molt. Note well developed anal pore (arrow). Phase contrast. $\times 128$. 4. Fully developed third-stage larvae (L_3). $\times 154$.

rivittatum was achieved only when the collection tubes used in the process each contained a single fly. Gibson did not indicate whether he exposed his flies in large numbers or individually in his attempts to infect them.⁶

A new focus of onchocerciasis recently has been reported from Ecuador.^{7,8} In one study three species of man-biting simuliids were identified, among which *S. quadrivittatum* was predominant.⁷ This species constitutes a serious pest problem in Panama. More than 90% of the adult black flies collected on human bait in the vicinity of the Fortuna Hydroelectric Project belonged to this species.² As shown for the first time by this study, *S. quadrivittatum* is a potential vector for human onchocerciasis. Surveys employing skin biopsies have been conducted in several localities where black flies are abundant in Panama, but as yet this disease has not been found in the indigenous population there. Upon completion, the Panamerican Highway will interconnect areas of high endemicity, both north and south of Panama. Interamerican travel by infected persons now residing in these endemic areas could bring onchocerciasis into this and other non-endemic countries of Central America. Extensive migration of refugees from politically unstable countries in that region would enhance such introduction and increase the likelihood that new foci of this disease eventually will become established.

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