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IDENTIFICATION OF ENDEMIC FOCI OF FILARIASIS BY EXAMINATION OF MOSQUITOES FOR MICROFILARIAE

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ABSTRACT. Studies were conducted in the Nile Delta of Egypt to determine the feasibility of detecting *Wuchereria bancrofti* microfilariae (Mf) in mosquitoes as a primary surveillance method for the identification of filariasis-endemic villages. Initial experimental studies evaluated the ingestion, survival, and migration rates of *W. bancrofti* Mf in *Culex pipiens* and *Culex antennatus* after mosquitoes were fed on infected volunteers. In 2 villages, 1,684 bloodfed mosquitoes were dissected during the night immediately after collections inside houses. In the village of Kafr Tahoria, Mf were found in 27 of 519 *Cx. pipiens* and in one of 8 *Anopheles pharoensis*. In Tahoria, Mf were detected in 7 of 799 *Cx. pipiens* and in one of 302 *Cx. antennatus*. Identifying filariasis-endemic villages based on the detection of Mf in mosquitoes may be a useful strategy for epidemiologic studies or for filariasis control programs.

INTRODUCTION

Surveillance for *Wuchereria bancrofti* filariasis involves the collection of blood samples for parasitological examination or antigen detection and clinical examination (Khalil et al. 1932, Weil et al. 1987, Ramzy et al. 1991). Surveys to obtain blood samples are usually labor intensive and require an unusual degree of cooperation from residents because blood must be obtained during the evening hours when microfilaria (Mf) are present in the blood. Epidemiologic investigations for this disease are complex logistically, and sometimes extensive studies are simply impractical or ethically unacceptable.

We examined the possibility that simple strategies for detecting filarial parasites in field-collected mosquitoes might provide a useful primary surveillance method for identifying endemic villages in the Nile Delta of Egypt. Initial experimental infection studies examined the early migratory patterns of *W. bancrofti* Mf (Wharton 1960, Burton 1964, Obiamiwe 1977, McGreevy et al. 1982) in *Culex pipiens* Linn. and *Culex antennatus* (Becker), the main vectors of filariasis in Egypt (Khalil et al. 1932, Gad et al. 1989). After determining the timing of Mf escape from the midgut, we determined the prevalence of Mf in the blood meals of indoor-resting mosquitoes collected at night in 2 endemic villages.

MATERIALS AND METHODS

To study the ingestion, survival, and migration rates of *W. bancrofti* Mf in *Cx. pipiens* and *Cx. antennatus*, individual mosquitoes were killed at intervals from 0 to 24 h after imbibing an infective blood meal, and their body parts were examined microscopically for the presence of microfilaria. The *Cx. pipiens* tested originated from El Kashish (Qalubiya Governorate), a village located 20 km northeast of Cairo (Feinsod et al. 1987). *Culex antennatus* were collected from El Gabal El Asfar, a suburb 15 km northeast of Cairo. Mosquitoes were collected as larvae and maintained in an insectary at 27 ± 2°C, 70–80% RH. Adults were supplied with 10% sugar solution until used for experimental purposes. Potential microfilaremic volunteers in El Kashish were identified by taking finger-prick blood samples between 2200 and 2400 h and preparing thick blood smears (World Health Organization 1978). Only carriers with moderate infections (20–35 Mf/50 µl blood) were selected. Four- to 5-day-old mosquitoes were fed for up to 20 min on selected volunteers between 2200 and 2400 h. Several fully engorged females were killed directly after feeding and dissected to determine the number of Mf ingested. Remaining mosquitoes were dissected at 1, 3, 6, 12, and 24 h postfeeding. Midguts of mosquitoes were removed intact in distilled water without blood leakage, then body parts were separately teased apart in drops of distilled water. Microfilaria were counted in smeared midgut contents, and in thoracic and abdominal tissues of individual mosquitoes.

Field evaluations to determine rates of Mf ingestion in mosquitoes were conducted in Kafr Tahoria, an endemic focus of bancroftian filariasis 30 km north of Cairo. Data from blood surveys were used to select households containing filaria cases for mosquito collections. In Tahoria, an adjacent village 2 km north of Kafr Tahoria, households chosen for mosquito sampling were selected at random as this village had not been surveyed previously for filariasis. In both vil-

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Table 1. Early migration patterns of *Wuchereria bancrofti* microfilariae in *Culex pipiens* and *Culex antennatus* mosquitoes.

Hours post-blood meal	Mosquitoes						Anatomical location of microfilariae (% of total observed)					
	Cx. pipiens			*Cx. antennatus*			*Cx. pipiens*			*Cx. antennatus*		
	No.	% with Mf	Mean Mf ± SD	No.	% with Mf	Mean Mf ± SD	Mid-gut	Abdo-men	Tho-rax	Mid-gut	Abdo-men	Tho-rax
0	54	78.0	9.6 ± 1.1	49	75.5	4.2 ± 1.4	100	0	0	100	0	0
1	122	70.3	4.9 ± 1.1	44	81.8	3.7 ± 1.9	87	9	4	63	32	5
3	64	73.4	3.1 ± 0.8	38	72.7	3.9 ± 1.4	26	29	45	43	33	24
6	59	79.1	4.1 ± 0.9	38	57.1	3.6 ± 0.9	7	40	53	33	17	50
12	79	63.0	4.3 ± 1.4	46	53.9	4.1 ± 1.6	8	18	74	7	12	81
24	82	86.6	3.6 ± 1.3	43	55.0	3.6 ± 1.4	4	9	87	7	0	93

lages, mosquito collections were conducted during July 1990 for 3 consecutive nights. Bloodfed females resting in bedrooms of selected households were aspirated from 2200 to 0100 h. In the field, fully engorged females were killed immediately. Their midguts were dissected and smeared for the microscopic examination of microfilaria.

RESULTS

Experimental infection of *Cx. pipiens* with *W. bancrofti* from microfilaremic volunteers revealed that more than 80% ingested Mf during bloodfeeding (Table 1). Infected females ingested from 1 to 23 Mf (mean 9.6 ± 1.1). The kinetics of microfilarial migration from the midgut to the abdomen and thorax (Table 1) indicated that at 1 h postinfection, about 13% of the surviving Mf had migrated from the midgut to either the abdominal hemocele or the thoracic muscles. By 3 h postfeeding, only 26% of the recovered Mf were still in the midgut. Migration of Mf through the midgut wall seemed to stop after 6 h postfeeding, because the proportion of Mf recovered in the midgut was the same at 6 and 12 h postfeeding. By 12 h, 74% of the surviving Mf had reached the thoracic muscles. At 24 h postfeeding, more than 95% of the Mf had escaped the midgut and most recovered Mf were found in the thoracic muscles (87%).

The proportion of *Cx. antennatus* that had ingested Mf with the infected blood meal was 75.5% (Table 1). At 1 h postfeeding, the rate of Mf escape from *Cx. antennatus* midgut was more rapid than for *Cx. pipiens* with 37% of the surviving Mf (3.7 ± 1.9) recovered either from the abdominal hemocele or the thoracic tissues. Afterwards, migration slowed, compared to rates observed for *Cx. pipiens* (Table 1).

Field studies were conducted to detect Mf in blood meals of freshly fed mosquitoes. A total of 1,684 fully engorged mosquitoes representing 3 species were collected from bedrooms and dissected (Table 2). In the more highly endemic village of Kafr Tahoria, Mf were recovered from 27 of 519 *Cx. pipiens* and one of 8 *Anopheles pharoensis* Theobald. No Mf were detected in 12 *Cx. antennatus.*

In Tahoria, 1,145 engorged females were collected in 64 households and dissected (Table 2).

Table 2. Rates of *Wuchereria bancrofti* microfilariae (Mf) in blood meals of mosquitoes collected from bedrooms in Kafr Tahoria and Tahoria villages.

Site	Households sampled[1]		Blood-engorged mosquitoes					
			Cx. pipiens		*Cx. antennatus*		*An. pharoensis*	
	No.	% with Mf in mosquitoes	No.	% with Mf	No.	% with Mf	No.	% with Mf
Kafr Tahoria	44	15.9	519	5.2	12	0.0	8	12.5
Tahoria	64	12.5	799	0.9	302	0.3	44	0.0

[1] Households studied in Kafr Tahoria were known to harbor at least one Mf carrier but households in Tahoria were selected without information on Mf carrier status of inhabitants.

Microfilariae were detected in midguts of 7 of 799 *Cx. pipiens* and one of 302 *Cx. antennatus,* but no Mf were detected in 44 *An. pharoensis.*

DISCUSSION

Patterns of microfilarial elimination and migration in *Cx. pipiens* and *Cx. antennatus* indicate that Mf can be recovered successfully in the midgut of both mosquito species up to 1 h after bloodfeeding. After that time, the chances of detecting Mf in the midgut decrease dramatically and more than 70% of the Mf can be recovered from the thoracic muscles of both species 12 h postinfection. The rate of Mf migration to the thorax continues to increase from 12 to 24 h. Thus, thoracic dissections for Mf may be fruitful anytime during the day after mosquito bloodfeeding.

In 2 endemic villages in the Nile Delta, microfilariae were detected readily in the blood meals of several mosquito species. Mosquito sampling was time-limited and simple methods were used for both collection and processing of mosquitoes. In examining freshly fed mosquitoes from houses, most of which had fed on humans (Zimmerman et al. 1985), we were able to demonstrate the presence of Mf directly in the blood meals. Overall, 5.2% of 539 mosquitoes dissected in Kafr Tahoria and 0.7% of 1,145 mosquitoes in Tahoria contained Mf in their midguts. Based on parallel experimental infection studies indicating that Mf can be detected in the abdomen and thorax for at least 24 h, it is possible that even higher infection rates could have been detected had it been logistically possible to also sample and dissect mosquitoes during the early morning hours.

The use of vector-based methods for detecting filariasis-endemic villages may be especially useful in the Nile Delta of Egypt, where the disease is highly focal and there is a high rate of inapparent clinical infections (Khalil et al. 1932, Ramzy et al. 1991). Many factors will affect the successful use of vector-based techniques for detecting endemic areas of filariasis. Critically important are seasonal patterns of vector density and the timing of biting activity. In Egypt, *Cx. pipiens* is most abundant from June through October. Biting begins at sunset and peaks around midnight, coinciding with peak microfilaremia densities in humans. *Culex antennatus* is most abundant from June through November with a peak in July (Zimmerman et al. 1985). Another important factor in applying this technique is selection of houses for mosquito sampling. Filariasis prevalence rates are higher in houses that lie near vector breeding sites, usually on the periphery of villages.

In conclusion, we demonstrate the potential value of using simple methods for detecting *W. bancrofti* Mf in field-collected mosquitoes as an approach for identifying filariasis-endemic villages in the Nile Delta of Egypt. Such techniques have broad applicability for other endemic countries and can be adapted to suit the nature of filaria transmission and the specific behavior patterns of vector species. An important use of vector-based strategies for filariasis surveillance would be to define focal areas of endemicity prior to more detailed epidemiologic surveys, or in conjunction with drug treatment or vector control programs.

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