ANOPHELES POPULATION DYNAMICS IN TWO MALARIA ENDEMIC VILLAGES IN FAIYUM GOVERNORATE, EGYPT[1]

SHERIF EL SAID[2], JOHN C. BEIER[3], MOHAMED A. KENAWY[2], ZAKARIA S. MORSY[2] AND ADEL I. MERDAN[2]

ABSTRACT. *Anopheles* populations were monitored for one year in 2 neighboring villages in Faiyum Governorate, Egypt, to study factors causing differences in malaria prevalence. Both villages contained the following species: *Anopheles pharoensis, An. sergentii, An. multicolor* and *An. tenebrosus.* Abundant larval breeding sites in Abheet, the village with the higher malaria rate, accounted for the higher adult densities observed from human and animal biting collections and from indoor resting collections. *Anopheles pharoensis* and *An. sergentii* were the dominant species in Abheet with seasonal biting activity extending from May to December, reaching a peak in November. In El Zawya, the village with the lower malaria rate, *An. pharoensis* dominated, reaching seasonal peaks in June and August. Inside houses, *An. sergentii* was common from May to January in Abheet, but rare in El Zawya. *Anopheles pharoensis* and *An. sergentii* were both incriminated as malaria vectors based upon their seasonal abundance and the finding of sporozoite positive specimens during the peak malaria season.

INTRODUCTION

Malaria persists in Egypt as a localized hypoendemic disease. The history and epidemiology of malaria in Egypt is detailed by Halawani and Shawarby (1957). Malaria prevalence in Egypt has shown a steady decrease in the last 60 years and in 1983, only 198 cases were recorded throughout the country by the Ministry of Health, with transmission localized primarily in Faiyum Governorate. Historically, *Plasmodium vivax, P. falciparum* and *P. malariae* were common (Halawani and Shawarby 1957), but today *P. vivax* is the dominant species, with *P. falciparum* endemic only in restricted foci of Faiyum Governorate. Only *Anopheles pharoensis* Theobald and *An. sergentii* (Theobald) are known to be malaria vectors (Barber and Rice 1937, Farid 1940).

Parasitological and serological surveys in Faiyum Governorate from 1977 to 1979 identified several malaria-endemic villages (Hassan et al. 1983). Monthly sampling in 2 neighboring villages during 1979 showed parasite rates (both *P. vivax* and *P. falciparum*) of 3.3% in Abheet and 0.8% in El Zawya, with 42% and 21% of the residents in the respective villages showing malaria antibodies (Abdel Wahab, 1983[4]). It was apparent that malaria rates in the 2 villages differed and that transmission occurred seasonally despite larval control consisting of weekly application of oil with Titron X®, as a dispensing agent to most breeding sites. These studies raised questions on how malaria rates could differ in 2 villages that are only 1 km apart.

To further examine this malaria situation, 20% of the 3000 residents in both Abheet and El Zawya were randomly selected and sampled monthly for malaria infections during 1983 (Hassan et al. unpublished data). Larval control activities were maintained during the study by the Ministry of Health. In the cohorts studied, 72 cases (22 *P. vivax* and 50 *P. falciparum*) were detected in Abheet and only 2 cases (1 *P. vivax* and 1 *P. falciparum*) were found in El Zawya. This report describes entomological studies coinciding with the longitudinal human study. The objective was to compare the anopheline population dynamics in both villages to identify factors causing differences in malaria prevalence.

MATERIALS AND METHODS

STUDY SITES. Studies were conducted in 2 villages, Abheet and El Zawya, in Sinnuris District, Faiyum Governorate. Faiyum is a large, agricultural oasis, 90 km southwest of Cairo, containing 111 villages with a rural population of 950,000. It contains an open irrigation system with 39,000 km of waterways (Mobarak 1982). The 2 study villages are 1 km apart, and are socioeconomically similar. According to a

[1] This study was supported by research contract N01 AI 22677/NIH-NIAID, entitled: "Epidemiology and Control of Arthropod-Borne Diseases in Egypt" between Ain Shams University (Research and Training Center on Vectors of Diseases), Ain Shams University, Abbassia, Cairo, Egypt, and the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA.

[2] Ain Shams Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

[3] National Institutes of Health (NIAID) resident consultant to the Ain Shams Center. Present address: Department of Immunology, WRAIR, Walter Reed Army Medical Center, Washington, DC 20307-5100.

[4] Abdel Wahab, M.S. 1983. Parasitological and serological studies on malaria in relation to anopheline distribution in Faiyum Governorate. M.Sc. thesis, Department of Entomology, Ain Shams University. 136 pp.

1981 census, each village contains 650 houses and around 3,000 residents. Houses are constructed of either mud or fired brick. An animal census determined that 50% of all houses contain at least one room inside the house where domestic mammals, such as buffalo, goats, sheep and donkeys are kept at night. Land surrounding the villages is irrigated throughout the year and crops such as alfalfa, corn, rice, sugar cane, cotton and vegetables are rotated in fields. Some areas also contained perennial date palm and lemon orchards.

ENTOMOLOGICAL SAMPLING TECHNIQUES. Anopheline populations in both villages were sampled over a 1 year period beginning in February 1983. Each village was usually sampled twice per month. Larval surveys, and spray captures of resting adults inside houses and animal sheds were done throughout the year. All-night collections of biting mosquitoes were made from human and animal baits, and outdoor collections of resting adults were conducted from April to December 1983, the normal period of adult mosquito activity in this area.

Larval surveys determined the monthly presence of anopheline species in both villages. Larvae were collected by dipping from 8 regularly productive sites in each village. Samples were placed in 300 ml tissue culture flasks and transported to the insectary for identification.

Indoor and outdoor captures of mosquitoes attracted to human baits were made by aspiration to determine the degree of man-biting contact. Three collection stations were used in representative sectors of the villages, usually employing the same houses on each trip. At each station a collector worked inside the house while another worked outside, usually more than 10 m from indoor collector. Observations began just before sunset and ended at sunrise, with 30 min collections made each hour throughout the night.

Four donkey-baited traps were used to sample anophelines in 4 representative sectors of the villages. The trap consisted of a fine mesh tent (3 × 3 × 2 m) supported at the corners by four 3 cm diam steel rods, with the tent suspended 30 cm above the ground. Donkeys were secured inside traps at sunset and anopheline collections were made every 2 hr throughout the night.

Anopheles resting inside houses and animal sheds were collected by spray capture using the index sheet method. A 1 m^2 white sheet supported by 2 sticks was spread and moved around the room and under furniture by one collector while a second sprayed a solution of 0.2% pyrethrum in kerosene. This technique is commonly used in Egypt and differs from the pyrethrum spray capture technique using floor sheets (World Health Organization 1975) in that the index sheet method collects only a proportion of mosquitoes resting in a room. Normally, 12 houses and 2–5 animal sheds were sampled each trip from 6 fixed sampling areas in each village. The 6 areas were initially selected at random.

Resting mosquitoes were collected from outdoor areas using a battery-powered aspirator (Nasci 1981). A series of 5 min collections was made in the evening 1–2 hr before sunset and again in the morning. Collections were made within 0.25 km of houses in vegetation surrounding houses, along irrigation canals, and in irrigated fields.

Anopheles from human and animal collections were identified and dissected throughout the night by a 2-man team. Each mosquito was examined for insemination status, parity based on the coiling of ovarian tracheoles, and the Christophers' stage of oocyte development. Parous mosquitoes were further examined for the number of ovarian dilations and salivary glands were inspected for sporozoites.

Daily survivorship (p) was calculated as $p=\sqrt[g]{P}$, where P = proportion parous, and g = the duration of the gonotrophic cycle (Macdonald 1957). Gonotrophic cycle estimates were based on laboratory observations of colonized species under different temperatures, and this parameter was calculated as the time from blood feeding to oviposition.

RESULTS

ADULT SEASONAL PREVALENCE. The seasonal abundance and degree of human contact was determined for adult *Anopheles* by night observations with human baits, donkey-baited traps, and by daytime spray capture inside houses (Fig. 1). Both *An. pharoensis* and *An. sergentii* were common in Abheet. In El Zawya, *An. pharoensis* was the only common species. The 3 techniques provided a basis for comparing seasonal patterns of *Anopheles* populations within each village.

Limited numbers of *Anopheles* were collected using the human bait technique. In Abheet, 84 man nights (with collectors working 30 min every hour) yielded 68 *An. pharoensis,* 28 *An. sergentii,* 7 *An. multicolor* Cambouliu, and 1 *An. tenebrosus* Doenitz. In El Zawya during 80 man nights, collections included 38 *An. pharoensis,* 1 *An. multicolor* and 3 *An. tenebrosus*; no *An. sergentii* were collected. Indoor and outdoor biting rates were similar except for *An. sergentii,* where 68% (19/28) were collected indoors. Man-biting rates in Fig. 1 represent collections from both indoor and outdoor stations. Seasonal trends

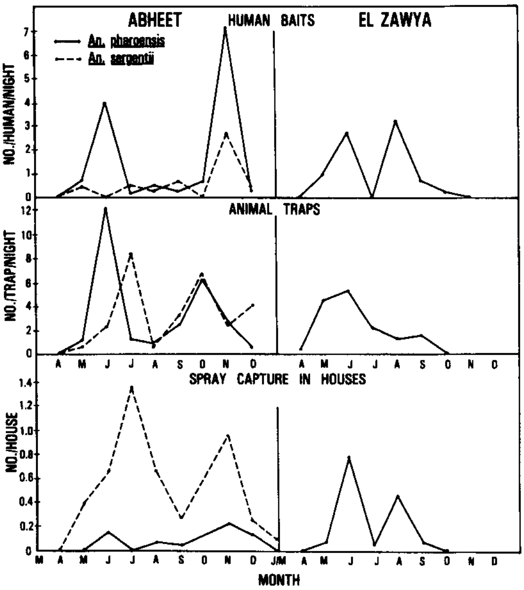

Fig. 1. Seasonal distribution of predominant *Anopheles* species determined by human bait, animal trap and indoor spray collections in Abheet and El Zawya.

for *An. pharoensis* showed a bimodal distribution in Abheet with peak biting rates of 4.0 and 7.2 bites/man/night in June and November, respectively. The seasonal patterns for *An. pharoensis* differed in El Zawya, with peak biting rates of 2.7 and 3.2 in June and August; no activity was detected after October. *Anopheles sergentii* was collected in Abheet from May to December with highest densities in November (2.7 bites/man/night). *Anopheles multicolor* were collected only from April to June. Donkey-baited traps in Abheet during 54 trap nights yielded 146 *An. pharoensis,* 185 *An. sergentii,* 34 *An. multicolor* and 4 *An. tenebrosus.* In El Zawya, collections from 56 trap nights included 104 *An. pharoensis,* 1 *An. sergentii,* 17 *An. multicolor* and 5 *An. tenebrosus.* Comparison of biting rates per night for human and animal baits showed the following ratios (animal:human): 1.8:1 *An. pharoensis,* 5.1:1 *An. sergentii,* 4.7:1 *An. multicolor,* 2.4:1 *An. tenebrosus.*

Collections inside houses and animal sheds by space spraying included 3 species, with higher densities in Abheet (Table 1). *Anopheles sergentii* was the dominant species in Abheet accounting for over 80% of the anophelines in houses and 90% of the specimens in sheds; only 7 *An. sergentii* were collected in El Zawya. The seasonal trends (Fig. 1) show that *An. sergentii* in Abheet was found in houses from May to January with peak densities in July and November. *Anopheles sergentii* sometimes reached high densities in animal sheds as 185 of the 257 specimens were from a single collection in one shed. *Anopheles pharoensis* occurred at low density in Abheet, but was the predominant indoor species in El Zawya. *Anopheles multicolor* was collected in low numbers in both villages from April to August, with one specimen in October.

Biting periodicity. Nocturnal biting periodicity examined for animal trap collections showed that all 4 species were collected throughout the night (Table 2). Peak periods of biting for *An. pharoensis* and *An. sergentii* occurred during the first sampling period, while lowest activity levels for these species and *An. multicolor* occurred during the last period before sunrise (i.e., values exceeded 1 standard deviation from the mean). These patterns differed from the human bait results where 60% (62/104) and 79% (22/28) of the *An. pharoensis* and *An. sergentii,* respectively, were collected during the first 3 hourly periods after sunset. Differences in feeding periodicity between indoor and outdoor stations were not detected for either species.

Resting behavior. Outdoor resting collections in over two hundred 5 min aspirations yielded 66 *An. pharoensis* females and 1 *An. multicolor* female. Specimens were collected most commonly in wet areas near breeding sites, in irrigated fields, and in vegetation along irrigation canals.

Specimens from outdoor collections and from space spraying inside houses and sheds were classified according to blood feeding stages (unfed, fed, half-gravid, or gravid) to examine relative degrees of endophily. *Anopheles sergentii* showed the highest degree of endophily as 29% (17/59) from houses were

Table 1. *Anopheles* collected by spray capture inside houses and animal sheds by the index sheet technique in Abheet and El Zawya, February 1983 to February 1984.

Village	Type of building	No. inspected	No. of female *Anopheles*			
			pharoensis	*sergentii*	*multicolor*	*tenebrosus*
Abheet	house	162	9	82	10	0
	animal shed	38	11	257	12	0
El Zawya	house	153	20	2	1	0
	animal shed	47	2	5	6	0

Table 2. Nocturnal periodicity of biting activity for four *Anopheles* species captured in donkey baited animal traps in Abheet and El Zawya (April to December 1983).

	Percentage of total female *Anopheles*/period			
Period (hr)	*pharoensis*	*sergentii*	*multicolor*	*tenebrosus*
2000–2200	37	32	19	56
2200–2400	20	22	19	0
2400–0200	21	26	24	11
0200–0400	21	15	24	11
0400–0600	1	5	14	22
Total no.	247	186	58	9

either half or fully gravid; this was the most common species collected indoors, but none were found outdoors by aspiration. Limited evidence suggests endophilic tendencies for *An. multicolor* as well, since 7/9 from indoor collections were gravid and this species was seldom found outdoors. *Anopheles pharoensis* was the least endophilic species as indoor densities were low, only 6% (2/34) were gravid from indoor collections while from outdoor collections 50% (11/22) were either half or fully gravid.

SPOROZOITE AND SURVIVAL RATES. A total of 567 *Anopheles* from Abheet and 163 from El Zawya were dissected (Table 3). All were collected from human bait collections and donkey-baited traps. Sporozoites were detected in the salivary glands of 2 *An. sergentii* and 1 *An. pharoensis*. All 3 were from a collection in Abheet on November 11, 1983. Single positive specimens of each species were from outdoor human bait collections and the other *An. sergentii* was from a donkey-baited trap. All 3 were in the saculate condition and could not be age graded. No infected specimens were detected in El Zawya. Oocyst infections were not detected. Sporozoite rates for *An. pharoensis* and *An. sergentii* in Abheet were 0.36 and 0.85%, respectively.

Parity rates were less than 50% for *An. pharoensis* and *An. sergentii* and none of the dissected specimens contained more than 2 dilatations. However a high proportion contained saculate ovarioles, indicating oviposition during the night of collection, and dilatations could not be determined. Insemination rates ranged from 53 to 96% with *An. sergentii* showing the highest rate. At least 85% of all species contained oocytes in Christophers' stages IIA and IIB, which is reasonable since all specimens were captured while seeking a blood meal.

Daily survival estimates could not be evaluated monthly due to the low number of collected specimens. If all collections from April to December are considered using gc = 4, based on laboratory observations for temperatures above 22°C (Beier, unpublished), survival rates were 0.806 and 0.762 for *An. pharoensis* in Abheet and El Zawya, respectively, and 0.835 for *An sergentii* from Abheet. Estimates for November collections in Abheet, the peak period of biting activity, were 0.912 for *An. pharoensis* and 0.897 for *An. sergentii*, based on gc = 6 estimated for temperatures less than 20°C.

LARVAL SURVEYS. *Anopheles pharoensis* and *An. sergentii* were the most common anopheline larvae encountered in both villages. *Anopheles pharoensis* was collected from May to January in both villages and *An. sergentii* occurred during the same period, but persisted longer into the winter months. *Anopheles multicolor* and *An. tenebrosus* larvae were found in Abheet, but not in El Zawya. These species showed a limited seasonal range with *An. multicolor* collected from April to June, and *An. tenebrosus* was found only in May.

Larval development sites were much more abundant in Abheet than in El Zawya, with most

Table 3. Dissections of host-seeking *Anopheles* captured by human bait and animal trap collections in Abheet and El Zawya (April to December 1983).

	Abheet				El Zawya			
Category	*An. pharoensis*	*An. sergentii*	*An. multicolor*	*An. tenebrosus*	*An. pharoensis*	*An. sergentii*	*An. multicolor*	*An. tenebrosus*
No. dissected	279	234	48	6	139	1	15	8
Nulliparous	161	120	36	2	92	1	14	6
1 parous	50	39	7	0	28	0	1	1
2 parous	7	6	0	0	1	0	0	0
Saculate	61	69	5	4	18	0	0	1
% parous	42.3	48.7	25.0	66.7	33.8	—	6.7	25.0
% inseminated	80.5	96.4	53.3	100.0	72.0	—	66.7	75.0
No. with sporozoites in salivary glands	1	2	0	0	0	0	0	0
Sporozoite rate	0.36	0.85	0	0	0	0	0	0

sites occurring close to houses. In Abheet, the most productive sites for all species included 5 low areas (<25 m^2) where drainage water accumulated from irrigation canals. Most contained water from 5-30 cm deep, algae, low grasses with sedges and other herbaceous plants common around the periphery; one site contained dense reeds over 2 m high. Anopheline larvae were most abundant in these sites in May and June, and October to December when the water level was high. During July to August these areas dried considerably and breeding was limited. Abheet also contained a permanent pond (>60 m^2), but larvae were seldom found. El Zawya contained no large areas of standing water except for one semi-permanent pool (>25 m^2) 0.5 km south of the villages. Sites in El Zawya consisted primarily of small pools (1–5 m^2) of seepage water from irrigation canals. Secondary sites included irrigated fields of sugar cane and other crops. Rice fields in El Zawya contained numerous *An. pharoensis* larvae from July to September; rice was not cultivated in Abheet during the study.

DISCUSSION

Anopheles pharoensis and *An. sergentii* were incriminated as malaria vectors in this area based upon their seasonal population dynamics and the findings of sporozoite-positive specimens. *Anopheles pharoensis* has been found naturally infected with sporozoites several times in Egypt (Barber and Rice 1937; e.g., Halawani and Shawarby 1957, El Said 1975[5]). However, this is only the second time that sporozoites have been found in *An. sergentii* (Farid 1940), although infections have been detected from this species in other countries (Farid 1956). In the 2 study villages, *An. multicolor* and *An. tenebrosus* occurred at such low densities that they could not have accounted for significant malaria transmission; neither are considered vectors in Egypt.

Man-biting and sporozoite rates provide evidence why more malaria cases were detected in Abheet (72 cases) than in El Zawya (2 cases) during concurrent clinical studies, even though these 2 villages are only 1 km apart. Based on human-biting rates for the 2 vector species, residents in Abheet received ca. 308 bites/man/season (April–December, the period of adult anopheline activity) compared to 128 bites/man/season for El Zawya residents. Considering the sporozoite rate of 0.585% for the 2 vector species in Abheet, the inoculation rate was estimated to be 1.80 infective bites/man/season. An inoculation rate could not be estimated for El Zawya since infected anophelines were not detected. The presence of both *An. pharoensis* and *An. sergentii* in Abheet served to maintain a higher malaria transmission rate than in El Zawya, where *An. pharoensis* dominated.

Additional entomological parameters account for low malaria transmission rates. Concurrent host-feeding studies in Abheet and El Zawya showed that *An. pharoensis* and *An. sergentii* feed primarily on large domestic mammals. Human blood indices were 0.28 for *An. pharoensis* and 0.14 for *An. sergentii* (Beier et al. 1986). Comparison of human-baited and donkey-baited collection further confirmed zoophilic preferences for both species. Since only a small proportion of *An. pharoensis* and *An. sergentii* that feed on humans with infective gametocytes could be expected to complete more than 2 gonotrophic cycles, based on daily mortality rates of 15–20%, zoophilic feeding behavior dramatically reduces the probability of malaria transmission.

Mechanisms for maintenance of hypoendemic malaria in Faiyum deserve further consideration if malaria control efforts are to be successful. This study shows that the occurrence of both An. *pharoensis* and *An. sergentii* in Abheet served to maintain a higher malaria transmission rate than in El Zawya where only one vector dominated. Under the prevailing conditions of low malaria endemicity it would be unrealistic to speculate or to pursue which is the more efficient vector species. The extensive larval development sites in Abheet are clearly responsible for higher adult mosquito densities in this village. Efforts to improve drainage of irrigation runoff water perhaps offers the best long-term solution for interrupting malaria transmission.

[5] El Said, S. 1975. Biology of some Egyptian anopheline mosquitoes and their relation to transmission of human malaria under laboratory conditions. Unpublished PhD thesis. Faculty of Science, Ain Shams University, Cairo, Egypt.

ACKNOWLEDGMENTS

We are grateful to the Egyptian Ministry of Health for facilitating this research, and to the MOH Malaria Station staff in Sinnuris, Faiyum for assisting with field work. We sincerely thank the Ain Shams Center field staff for providing expert assistance in the field, Miss Eptesam El Kordy for assistance in specimen processing, and Dr. William Reisen (University of California) and Dr. Roger Nasci (McNeese State University) for consulting on project organization and for comments on the manuscript.

References Cited

Barber, M. A. and J. B. Rice. 1937. A survey of malaria in Egypt. Am. J. Trop. Med. 17:413–436.

Beier, J. C., J. H. Zimmerman, M. A. Kenawy, S. El Said and M. M. Abbassy. 1986. Host-feeding patterns of the mosquito community (Diptera: Culicidae) in two Faiym Governorate villages, Egypt. J. Med. Entomol. 23:(in press).

Farid, M. A. 1940. Malaria infection in *Anopheles sergentii* in Egypt. Riv. Malariol. 19:159–161.

Farid, M. A. 1956. The implication of *Anopheles sergentii* for malaria eradication programmes east of the Mediterranean. Bull. W.H.O. 15:821–828.

Halawani, A. and A. A. Shawarby. 1957. Malaria in Egypt. J. Egypt. Med. Assoc. 40:753–792.

Hassan, Z., M. Gebril, M. Kenawy, F. Mohammed and S. El Said. 1983. Seroepidemiological malaria surveys in Egypt. J. Egypt. Pub. Hlth. Assoc. (Special issue No. 1 and 2) 58:168–179.

Macdonald, G. 1957. The epidemiology and control of malaria. Oxford Univ. Press. London.

Mobarak, A. B. 1982. The schistosomiasis problem in Egypt. Am. J. Trop. Med. Hyg. 31:87–91.

Nasci, R. S. 1981. A lightweight battery-powered aspirator for collecting resting mosquitoes in the field. Mosq. News. 41:808–811.

World Health Organization. 1975. Manual on practical entomology in malaria. Part II. Methods and techniques. WHO Offset Publ. No. 13. 191 p.