

## FACTORS AFFECTING THE HUMAN-FEEDING BEHAVIOR OF ANOPHELINE MOSQUITOES IN EGYPTIAN OASES

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**ABSTRACT.** Blood meals were tested by a direct enzyme-linked immunosorbent assay (ELISA) for 424 *Anopheles sergentii* and for 63 *An. multicolor* collected in Siwa, Farafra and Bahariya oases in the Western Desert of Egypt. Both species were highly zoophilic. Human blood-feeding by *An. sergentii* was less common in Bahariya (2.3%) and Farafra (1.3%) than in Siwa (15.3%). A likely explanation is that large domestic animals are held at night inside houses in Bahariya and in Farafra whereas in Siwa, animals are usually housed outdoors in sheds. These patterns of *An. sergentii* human-feeding behavior may contribute to the persistence of low-level *Plasmodium vivax* transmission in Siwa in contrast to negligible or no transmission in Bahariya and Farafra.

### INTRODUCTION

Zoophilic feeding behavior by anopheline malaria vectors represents an important regulatory mechanism in malaria transmission. In Egypt, the malaria vectors *Anopheles sergentii* (Theobald) and *An. pharoensis* Theobald, and a suspected vector, *An. multicolor* Cambouliu, feed to a large extent on domestic mammals. This has been observed in Aswan Governorate (Kenawy et al. 1987), in Faiyum Governorate (Beier et al. 1987), in the Nile Delta (Hurlbut and Weitz 1956), and in the Western Desert oases (Barber and Rice 1937, Kenawy et al. 1986b). However, factors affecting the degree of zoophilic feeding have not been investigated. The geographic variability in anopheline feeding patterns and sociocultural differences throughout Egypt suggest that animal holding practices may be a key factor affecting malaria transmission.

Malaria in the oases of the Western Desert of Egypt (Siwa, Bahariya, Farafra, Dakhla and Kharga) is currently being evaluated to determine appropriate measures for control. Historically, these oases were endemic for *Plasmodium vivax*, *P. falciparum* and *P. malariae* (Halawani and Shawarby 1957). Today, *P. vivax* alone persists at rates usually <5% in the villages of Siwa Oasis, and in El Gara, a small oasis near Siwa (Kenawy et al. 1986a). In contrast, Bahariya and Farafra appear to be malaria-free. Hassan et al. (1983) showed that 2,285 persons from Bahariya were slide negative, and 9,066 blood smear samples from Bahariya and 734 from Farafra were negative during surveys from 1979 to 1989 (Kenawy, unpublished data). The per-

sistence of *P. vivax* in Siwa but not in Bahariya and Farafra is interesting because residents in these ecologically similar oases employ different methods for holding domestic animals such as cows, donkeys, goats and sheep. In Bahariya and Farafra, large domestic animals are usually kept inside houses at night whereas in Siwa, animals are kept away from houses in sheds.

This study examines the possibility that traditional animal holding practices may affect the human-feeding behavior of anophelines in the oases of Siwa, Bahariya and Farafra. From these oases, blood meals were identified for *An. sergentii* and *An. multicolor* collected from inside houses, from animal sheds and from mixed sites (containing animal rooms inside houses).

### MATERIALS AND METHODS

**Study areas:** The study was conducted in 3 oases in the Western Desert of Egypt (Fig. 1). Siwa Oasis (29°07'–29°21'N, 25°16'–26°07'E) is 82 km long from east to west and lies an average of 18 m below sea level. Siwa has over 11,000 residents distributed in 5 districts and about 546 ha are under cultivation. Bahariya (27°45'–28°21'N, 28°30'–29°15'E) lies 360 km southeast of Cairo, includes 9 oases, and has >30,000 residents. Farafra oasis (26°15'–27°30'N, 26°45'–29°00'E) lies 170 km southwest of Bahariya with 3,000 residents in 5 small villages (our census, March 1988) and 250 ha are under cultivation. The water supply in the 3 oases is drawn mainly from springs and wells. Due to water infiltration from wells and improper drainage, larval development areas for mosquitoes are common in seepage areas and in irrigation canals. Numerous salt lakes exist in Siwa oasis. The main crops are dates, olives, barley, wheat and various fruits and vegetables grown in gardens around springs. Clover and alfalfa are grown in Farafra and rice is cultivated in some parts of Bahariya. Houses are constructed of mud with thick walls and ceilings supported by date-palm logs. This type of construction

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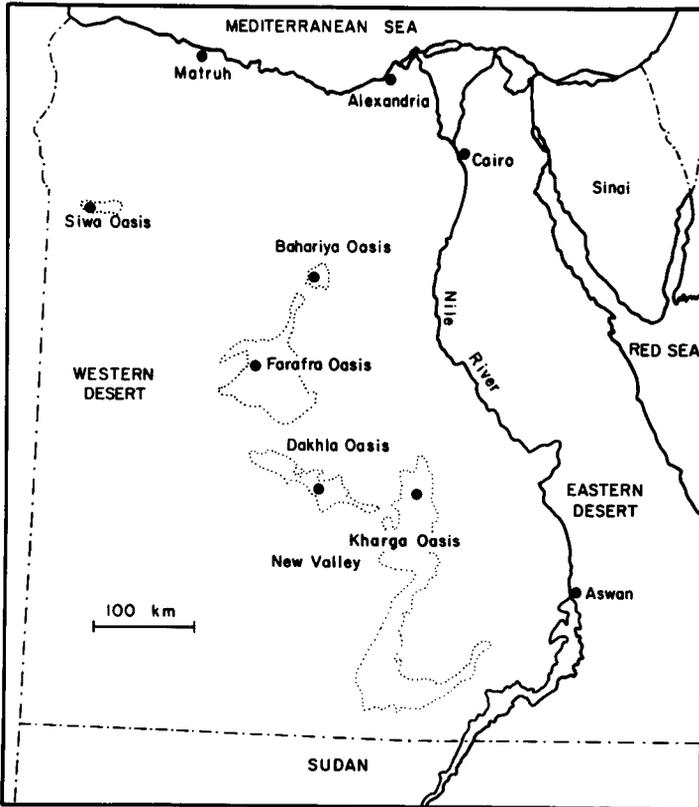


Fig. 1. Map showing the location of Siwa, Farafra and Bahariya oases in the Western Desert of Egypt.

struction provides insulation from the desert heat. The main difference in housing between the 3 oases is that animal sheds in Siwa are usually separated from houses, whereas in Farafra and Bahariya, most of the houses include one or more animal rooms where animals are held during the night.

**Mosquito collection and handling:** Mosquitoes were collected in 4 Siwa villages (Meshindit, Bahi El Din, Aghurmi and Abu Shuruf) during April and May 1986 and in July 1987, in 2 Farafra villages (Qasr Farafra and El Sheikh Marzouk) during November 1987 and March 1988, and in 4 Bahariya villages (Mandisha, El Qubala, Bawiti and El Qasr) during November and December 1987.

Indoor resting sites were surveyed by space-spraying of pyrethroid insecticide (0.2% neopybuthrin in kerosene) with a hand sprayer. A 1-m<sup>2</sup> white sheet supported by 2 sticks was spread and moved around the walls and under furniture by one collector while another sprayed the insecticide. Collection sites included 30 houses (22 in Siwa and 8 in Farafra), 46 animal sheds (31 in Siwa and 15 in Farafra) and 59 mixed sites (3 in Siwa, 26 in Farafra and 30 in Bahariya) in

which animal rooms were inside the houses. Mosquitoes were placed in small paper cartons and transported to the laboratory in Cairo for processing. Mosquitoes were identified, placed in 1.5-ml capped plastic vials (up to 25/vial) by species and site of collection, and kept desiccated at room temperature until tested. For the Siwa collection in 1986, samples were frozen at -70°C and then desiccated 1 week before testing.

**Preparation of mosquitoes for ELISA testing:** Each blood-fed mosquito was cut transversely with a scalpel blade at the juncture of the thorax and abdomen. Abdomens were prepared individually for testing by trituration in 0.2-ml glass microtissue grinders to which 50- $\mu$ l 0.01 M phosphate buffered saline (PBS), pH 7.4 was added. Of this, a 25- $\mu$ l aliquot was then diluted with PBS (1:50) and frozen at -20°C.

**Blood meal identification:** Mosquito blood meals were identified by a direct enzyme linked immunosorbent assay (ELISA) (Beier et al. 1988) at the U.S. Army Medical Research Unit, Kenya Medical Research Institute, Nairobi, Kenya. Each mosquito sample was tested for blood meals of 9 hosts: human, cow, goat, horse,

dog, cat, rabbit, rat and chicken. Negative samples were retested for all hosts. Positive and negative controls and methods for determining positivity were described previously (Beier et al. 1988).

**Host census:** Humans and domestic animals were counted in Siwa whereas in Farafra and Bahariya oases census data were based on information from local councils, veterinary stations and malaria stations. A census in May 1986 was conducted in all houses ( $n = 62$ ) and their adjacent animal sheds in Bahi El Din, one of 4 villages sampled in Siwa Oasis. Potential hosts included: humans (450), cows (81), sheep and goats (719), donkeys (80), rabbits (142), geese (50), ducks (43) and chickens (377). In Farafra, the estimated numbers of hosts were 3,000 humans, 665 cows and buffaloes, 1,740 sheep and goats, 311 donkeys and horses and 370 camels. In Bahariya, the estimated numbers of hosts in 3 areas of mosquito collections were 13,500 humans, 2,650 cows and buffaloes, 5,550 goats and sheep, 2,500 donkeys and 600 camels. Other common animals included cats, dogs, rabbits, ducks, geese, chickens, wild birds, rodents, bats and reptiles but these were not counted.

**Forage ratios:** Forage ratios were calculated for *An. sergentii* and *An. multicolor* in Siwa and Farafra oases to provide a standard index of host selection. This ratio was calculated for humans and domestic animals as the percentage of positive blood meals divided by the percentage of these hosts. Ratios  $> 1$  indicate preferences, ratios  $< 1$  indicate avoidance, and ratios approaching 1 indicate no preference or avoidance (Hess et al. 1968).

## RESULTS

A total of 544 *An. sergentii* and 71 *An. multicolor* females were collected in Siwa, Farafra and Bahariya oases. *Anopheles sergentii* represented 98.2% of the total anophelines collected in Siwa ( $n = 164$ ), 70.7% in Farafra ( $n = 232$ ) and 100% in Bahariya ( $n = 219$ ).

Blood meals from 424 *An. sergentii* were tested by ELISA (Table 1). Positive reactions were obtained for 80.4%, 66.7% and 76.3% of the samples tested in Siwa, Farafra and Bahariya, respectively. *Anopheles sergentii* collected inside houses in Siwa fed mainly on humans (42.9%), and in animal sheds, most of the feedings were on donkeys (35.1%) and cows (31.1%). In Farafra, only 1.3% (1/78) of the *An. sergentii* contained human blood. This species fed predominantly on donkeys (70%) inside houses, and on cows in animal sheds (70.8%) and in mixed sites (59.1%). In Bahariya, 55.0% and 35.7% of the 129 blood meals were identified as donkey and cow, respectively; only 2.3% contained human blood. Overall, the proportion of *An. sergentii* feeding on humans in Siwa (15.3%,  $n = 111$ ) was significantly higher than in Farafra ( $\chi^2 = 9.3$ ,  $df = 1$ ,  $P < 0.005$ ) or in Bahariya ( $\chi^2 = 14.1$ ,  $df = 1$ ,  $P < 0.005$ ).

Blood meals from 63 *An. multicolor* were tested by ELISA (Table 2). In Siwa, 3 fed mosquitoes contained human, goat and horse blood. In Farafra, a low percentage of human feedings (2.9%) was observed and most of the feedings were on cows.

Mixed blood meals were detected for 6% (19/318) of *An. sergentii* and for 10.8% (4/37) of *An.*

Table 1. Host blood meal sources for *Anopheles sergentii* from inside houses, animal sheds or mixed sites containing animal rooms inside houses in Siwa, Farafra and Bahariya oases, Egypt.

Sites/ habitats	Total no. tested	No. (%) identified	% bloodmeal hosts*				
			Human	Cow	Goat/ sheep	Donkey/ horse	Mixed meals**
<b>Siwa</b>							
House	34	28 (82.4)	42.9	3.6	17.9	17.9	17.9
Shed	95	74 (37.9)	4.1	31.1	21.6	35.1	8.1
Mixed	9	9 (100.0)	22.2	22.2	22.2	22.2	11.1
Total	138	111 (80.4)	15.3	23.4	20.7	29.7	10.8a
<b>Farafra</b>							
House	12	10 (83.3)	0.0	30.0	0.0	70.0	0.0
Shed	23	24 (85.7)	0.0	70.8	16.7	8.3	4.2
Mixed	77	44 (57.1)	2.3	59.1	4.5	29.5	4.5
Total	117	78 (66.7)	1.3	59.0	7.7	28.2	3.8b
<b>Bahariya</b>							
Mixed	169	129 (76.3)	2.3	35.7	3.9	55.0	3.1c

\* No reactions to rat, cat, dog and chicken antisera.

\*\* Mixed meals included: (a) cow + donkey ( $n = 1$ ), goat + donkey ( $n = 3$ ), donkey + rabbit ( $n = 1$ ), human + donkey ( $n = 6$ ) and human + rabbit ( $n = 1$ ), (b) cow + donkey ( $n = 1$ ), human + cow ( $n = 1$ ), and human + rabbit ( $n = 1$ ), and (c) cow + donkey ( $n = 2$ ) and human + cow ( $n = 2$ ).

Table 2. Host blood meal sources for *Anopheles multicolor* from inside houses, animal sheds or mixed sites containing animal rooms inside houses in Siwa and in Farafra oases, Egypt.

Sites/ habitats	Total no. tested	No. (%) identified	% bloodmeal hosts*					
			Human	Cow	Goat/ sheep	Donkey/ horse	Mixed meals**	
Siwa								
House	1	1 (100.0)	0.0	0.0	0.0	100.0	0.0	
Shed	2	2 (100.0)	50.0	0.0	50.0	0.0	0.0	
Mixed	0	—	—	—	—	—	—	
Total	3	3 (100.0)	33.3	0.0	33.3	33.3	0.0	
Farafra								
House	3	1 (33.3)	0.0	0.0	0.0	100.0	0.0	
Shed	56	33 (58.9)	3.0	57.6	18.2	9.1	12.1	
Mixed	1	0 (0.0)	—	—	—	—	—	
Total	60	34 (56.7)	2.9	55.9	17.6	11.8	11.8	

\* No reactions to rat, cat, dog and chicken antisera.

\*\* Mixed meals included cow + donkey ( $n = 2$ ), cow + rabbit ( $n = 1$ ) and cow + donkey + goat ( $n = 1$ ).

*multicolor*. These were mostly feedings on more than one type of large mammal. In 3 *An. sergentii* and in one *An. multicolor*, rabbit blood was detected in conjunction with blood from large mammals, including human. A triple feeding on cow, donkey and goat was detected for one *An. multicolor* in Farafra.

Forage ratios were calculated for *An. sergentii* and *An. multicolor* from Siwa, Farafra and Bahariya (Table 3). Around 93.5% (332/355) of the feedings were on humans or on large domestic mammals (excluding mixed meals); thus, forage ratios were calculated only for humans, bovines, ovines and equines. Highest forage ratios were observed for equines and bovines. *Anopheles sergentii* forage ratios for humans were low in Siwa (0.45) but were 15 times lower in Farafra (0.03) and 11 times lower in Bahariya (0.04). Low forage ratios for humans were also observed for *An. multicolor* in Farafra (0.06).

## DISCUSSION

*Anopheles sergentii* and *An. multicolor* from 3 Western Desert oases fed primarily on large domestic hosts. Such zoophilic feeding behavior has been reported previously for these 2 species in Siwa (Barber and Rice 1937, Kenawy et al. 1986b) and in areas outside Egypt (Bruce-Chwatt and Gockel 1960). Over 95% of the *An. sergentii* in Bahariya and Farafra, and *An. multicolor* in Farafra, fed on large domestic animals. This is the first evidence that anophelines in Bahariya and in Farafra feed to a greater extent on large domestic animals than in Siwa.

Patterns of human-feeding by *An. sergentii* may be affected strongly by animal holding practices. In Siwa, where animals are usually kept away from houses, 15.3% of the feedings were on humans. This was 11.8 and 6.7 times higher than the proportion of human feedings in Farafra and Bahariya, respectively, 2 areas where animals are usually kept inside houses at night.

*Anopheles sergentii* forage ratios for humans were 11 times lower than the highest nonhuman forage ratio (equines) in Siwa, and 174 and 134 times lower than forage ratios for equines in Farafra and Bahariya, respectively. Thus, the inherent zoophilic feeding behavior of *An. sergentii* appears to be amplified when large domestic animals are housed inside houses at night.

Both *An. sergentii* and *An. multicolor* may feed on more than one type of host per gonotrophic cycle as evident by mixed blood meals in 6.0% (19/318) of the *An. sergentii* and in 10.8% (4/37) of *An. multicolor* blood meals. Host-seeking behavior after blood meal interruption may be related to animal holding practices. Mixed blood meals for *An. sergentii* collected in houses, sheds, or in mixed structures in Siwa (10.8%) were more common than in Farafra (3.8%) or in Bahariya (3.1%). The low percentage of mixed meals in Farafra and Bahariya, where animals are kept inside houses, may simply indicate that *An. sergentii* has a high degree of feeding success on large mammals.

The percentage of negative ELISA reactions was relatively high (19.6 to 33.3% for *An. sergentii* and 43.3% for *multicolor*). Using the same assays, Beier et al. (1988) identified 94.6% of blood meals from mosquitoes collected in western Kenya. The non-reacting samples could indicate feedings on hosts which were not tested but this is unlikely; precipitin tests identified 100% of 497 blood meals from Siwa using antisera to human and domestic hosts (Kenawy et al. 1986b). A more likely explanation is that handling procedures (e.g., drying and storage for up to 2 years) degraded host IgG in blood meals.

The differences in host-feeding patterns for *An. sergentii* in the 3 Western Desert oases may

Table 3. Forage ratios (FR)\* for *Anopheles sergentii* and *An. multicolor* collected in Siwa, Farafra and Bahariya oases to examine the proportions of feedings on humans, bovines, ovines and equines in relation to relative host abundance.

Host	Siwa				Farafra				Bahariya			
	<i>An. sergentii</i>		<i>An. sergentii</i>		<i>An. sergentii</i>		<i>An. multicolor</i>		<i>An. sergentii</i>		<i>An. sergentii</i>	
	No. (%) host	% blood meal	FR	No. (%) host	% blood meal	FR	No. (%) host	% blood meal	FR	No. (%) host	% blood meal	FR
Human	450 (33.8)	15.3	0.45	3,000 (52.5)	1.3	0.03	13,500 (55.8)	2.9	0.06	2,650 (11.0)	2.3	0.04
Bovine	81 (6.1)	23.4	3.84	665 (11.6)	59.0	5.09	55.9	55.9	4.82	2,650 (11.0)	35.7	3.25
Ovine	719 (54.1)	20.7	0.38	1,740 (30.4)	7.7	0.25	17.6	17.6	0.58	5,550 (22.9)	3.9	0.17
Equine	80 (6.0)	29.7	4.95	311 (5.4)	28.2	5.22	11.8	11.8	2.19	2,500 (10.3)	55.0	5.34

\* Forage ratio calculations explained in the methods.

have important implications for the low-level maintenance of *P. vivax* transmission. The extremely low levels of human-feeding (<3.0%) by the main vector species in Farafra and in Bahariya are consistent with recent parasite surveys which indicate a complete interruption of transmission in these oases (Hassan et al. 1983; Kenawy et al., unpublished data). The higher proportions of human-feedings in Siwa (up to 42.9% inside houses) appears sufficient to maintain low rates of *P. vivax* transmission (Kenawy et al. 1986a). Ultimately, vector infectivity in the oasis environment depends upon susceptible vectors feeding on infective human hosts during the first or the second gonotrophic cycle. Based upon limited sampling, the probability of human-feeding by *An. sergentii* appears to be at least 5 times more likely in Siwa than in Farafra or in Bahariya. In the absence of other obvious social or ecological differences among the oases, animal holding practices appear to be a major factor affecting vector-feeding patterns.

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