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SEASONAL ABUNDANCE, VECTOR BEHAVIOR, AND MALARIA PARASITE TRANSMISSION IN ERITREA

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ABSTRACT. Entomological studies were conducted over a 24-month period in 8 villages to establish the behavior patterns, seasonal densities, and variation in entomological inoculation rates (EIRs) of *Anopheles arabiensis,* the main vector of malaria in Eritrea. A total of 5,683 anopheline mosquitoes were collected through indoor sampling (1,613), human-landing catches (2,711), and outdoor pit shelters (1,359). Overall, *An. arabiensis* was the predominant species at all of the study sites, with its population density increasing during the rainy season. Peak indoor-resting densities was observed during September and October. Human landing indices for *An. arabiensis* averaged 1.9 and 3.8 per person per night in October and September, respectively. Peak biting and landing rates occurred between 2000–2200 h and 0100–0300 h. Of the total number of bites, 44.7% occurred between 1800 and 2300 h, and at least 56.5% of the total bites occurred outdoors, indicating that the species was partially exophagic. The fed to gravid ratio for *An. arabiensis* in indoor-resting collections was 2:1, indicating some degree of exophily. The sporozoite rates (SRs) for *An. arabiensis* ranged from 0.54% in the Anseba zone to 1.3% in the Gash–Barka zone. One mosquito each of *An. d'thali* (SR = 0.45%) and *An. cinereus* (SR = 2.13%) was found to be positive. Of the total positive *An. arabiensis* (n = 64), 18.2% came from human-landing collections outdoors. Blood-meal analysis by enzyme-linked immunosorbent assay for *An. arabiensis* indicated that this species was partially zoophilic with a human to bovine ratio of 2:1 being recorded. The EIR profiles indicated that malaria transmission is highly seasonal, increasing during the wet season and declining drastically during the dry season. On average, the greatest risk of infection occurs in Hiletsidi, in the Gash–Barka zone (6.5 infective bites per month). The exophilic behavior and early evening biting of *An. arabiensis* present obstacles for control with treated bed-nets and indoor residual spraying within the context of integrated malaria control, and call for greater focus on strategies such as larval control.

KEY WORDS *Anopheles arabiensis,* seasonal density, vector behavior, entomological inoculation rate, vector control

INTRODUCTION

Malaria is the most important vector-borne disease in Eritrea, viewed both in terms of the number of infections and the number of deaths. The disease accounts for 30% of outpatient morbidity and 28% of all hospital admissions and has a 7.2% mortality rate and 1.2% case fatality rate in all age groups (Ministry of Health, Asmara, Eritrea). The Malaria Control Program (MCP) in Eritrea is expending considerable effort in fighting this public health problem. However, as in other regions in sub-Saharan Africa, the task is complicated by a wide range of ecological settings and concomitant variations in endemicity of the disease. Studies conducted in Eritrea indicate that *Anopheles arabiensis* Patton is the main vector of malaria and the only member of the *Anopheles gambiae* complex in the country. Polymerase chain reaction (PCR) assays of 1,446 adult and 237 larval specimens of *Anopheles gambiae* Giles collected countrywide revealed the presence of *An. arabiensis* only when using primers specific for *An. arabiensis, An. gambiae,* and *An. quadriannulatus* (Theobald) (Shililu et al. 2003a, 2003b). This species also is responsible for malaria parasite transmission in Ethiopia and Sudan (Krafsur 1978, White et al. 1980, Hadis et al. 1997).

Malaria transmission is influenced, among other factors, by host preference, resting behavior, biting periodicity, longevity, and susceptibility to infection of the vector species. Studies conducted elsewhere indicate that *An. arabiensis* is moderately zoophilic and derives blood meals from livestock, if present in appreciable numbers (Garrett-Jones et al. 1980, Habtewold et al. 2001). The resting tendency of this species may be quite variable, depending on the climatic setting. Studies conducted in the Awash Valley in Ethiopia indicated that this species is partially exophilic (Ameneshewa and Service 1996), whereas in some areas of Africa such as Sudan, the species has become increasingly exophilic as a result of the irritant effect caused by DDT (Zahar 1985). Conversely, studies conducted

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in western Kenya indicated that this species is largely endophilic (Githeko et al. 1994). Because the behavior patterns of *An. arabiensis* are variable over diverse ecological situations, it is not easy to extrapolate findings across different ecozones. For this reason, it is important for malaria control programs in Africa to generate information on the bionomics of vector species based on their specific settings. Vector behavior studies have not been conducted in Eritrea despite the public health impact of malaria. The present study examines the seasonal distribution and behavior patterns of *An. arabiensis*, and dynamics of transmission of malaria. This information should provide a better understanding of the facets that drive the disease, which would be useful for planning purposes.

MATERIALS AND METHODS

Study sites: Two villages were selected from each of the 4 political zones in Eritrea, the Anseba, Gash–Barka, Debub, and North Red Sea (NRS) zones. The villages were selected based on past high malaria morbidity and accessibility. Ten houses were randomly selected per village for assessing seasonal variation in anopheline mosquito densities. In each of the selected villages, 2 further houses were selected based on the relative location in the village. One house was located at the center of the village, whereas the 2nd was located at the periphery. The 2 houses served as sentinel stations for studies involving human-landing catches. Two pit shelters (1.5 × 1.0 × 2.0 m) were constructed, 1 at the center of the village and the other at the periphery, to serve as artificial outdoor mosquito shelters in each of the villages and served for determining seasonal variations in outdoor mosquito resting densities. Vector control in Eritrea is accomplished through selective application of indoor residual spraying, with DDT 75% water-dispersible powder. The 8 villages were routinely sprayed from 1995 to 1999, but with greater frequency and intensity in the Debub zone. No spraying was conducted from 2000 to 2002 in the study villages. Use of insecticide-impregnated bed-nets and environmental management at the community level also were implemented in the study sites on a routine basis.

Mosquito sampling: Entomological surveys were conducted for 24 months in the 8 villages from October 1999 to September 2001 by using 3 collection techniques. In indoor-resting collections (IRCs), adult indoor-resting anopheline mosquitoes were sampled once every month in 10 houses per site from 0630 to 0900 h by using pyrethrum spray collections (PSCs). In human-landing collections (HLCs), collections were made on adult volunteers from 1800 to 0600 h. Two teams of collectors in each village conducted the HLCs once a month for 24 months at 2 sentinel houses in each selected village. The collectors worked in pairs, with 1 pair working from 1800 h to midnight and the next from midnight until 0600 h. One indoor collector and 1 outdoor collector, positioned about 20 m away from the sentinel house, conducted the collections at each village and rotated hourly for the specified time. Relative humidity and temperature were recorded. The human biting rate (HBR) was expressed as the mean number of mosquito bites per person per night. In outdoor-resting collections (ORCs), mosquito collections were conducted monthly for 3 consecutive days by use of mouth aspirators from 2 pit shelters within each sentinel village. Based on previous investigations on adult mosquitoes in Eritrea that used molecular (PCR) identification to distinguish members of the *An. gambiae* complex (Shililu et al. 2003a), all mosquito specimens collected were preserved either on moist filter paper (PSCs) or in paper cups (HLCs and ORCs) and were identified to species based on morphological characters (Veronne 1962, Gillies and Coetzee 1987).

Enzyme-linked immunosorbent assay for sporozoites of Plasmodium falciparum *and blood-meal analysis:* The head and thorax of each mosquito were separated from the abdomen and tested for the presence of circumsporozoite protein of *Plasmodium falciparum*, as described by Beier et al. (1987). Mosquitoes were ground in 50 μl of boiled casein containing Nonidet 40 (Amphotech Ltd., Beverly, MA), with a final volume brought to 250 μl with blocking buffer. Fifty microliters of the triturate was used in the sporozoite enzyme-linked immunosorbent assays (ELISAs). Blood-meal ELISAs were carried out according to Beier et al. (1988). Positive reactions were determined visually for the 2 tests. Data analysis was performed by using the chi-square test to establish associations between HBR and location of host, and variations in sporozoite rates among sites.

RESULTS

Seasonal densities of *An. arabiensis* and other anopheline species

A total of 1,613 anopheline mosquitoes belonging to 7 species were obtained from IRCs during the 24-month study in the 8 study villages (Table 1). *Anopheles arabiensis* predominated, comprising 97.4% (n = 1,571) of the total indoor-resting anophelines. This species was most abundant in the western lowlands, with at least 77.1% (n = 1,211) of the *An. arabiensis* coming from the Gash–Barka zone (approximately 500 m above sea level [asl]). The density of this species from the high-altitude villages (>1,400 m asl) sampled in the Anseba (16.2%) and Debub (4.9%) zones was generally low. The 2 villages sampled in the NRS zone, Ghinda (800 m asl) and Gahtelay (290 m asl), yielded only 1.7% of the total *An. arabiensis* collected over the 24-month study period. The mean

Table 1. Number of anopheline mosquitoes collected in indoor-resting collections in 4 zones in Eritrea over 24 months.[1]

	Anseba		Debub		Gash–Barka		NRS[2]		
Month	*An. arabiensis*	Other species	*An. arabiensis*	Other species	*An. arabiensis*	Other species	*An. arabiensis*	Other species	Total
Jan.	1	2	2	2	9	0	4	0	20
Feb.	0	3	0	0	28	0	3	0	34
March	0	3	0	5	224	0	2	0	234
April	2	1	0	0	153	0	4	0	160
May	0	0	1	0	6	0	0	0	7
June	0	1	0	0	4	0	0	0	5
July	18	2	5	0	148	0	1	0	174
Aug.	19	0	14	0	139	0	0	0	172
Sept.	134	0	23	2	257	1	7	0	424
Oct.	64	0	31	6	109	0	0	0	210
Nov.	17	4	2	5	122	1	5	0	156
Dec.	0	2	0	2	12	0	1	0	17
Total	255	18	78	22	1,211	2	27	0	1,613

[1] *An.*, *Anopheles*. Other species include *An. cinereus, An. d'thali, An. pretoriensis, An. demeilloni, An. rhodesiensis,* and *An. rupicola.*
[2] NRS, North Red Sea.

annual indoor-resting densities for *An. arabiensis* were 2.8 mosquitoes per house for the Gash–Barka zone, and 0.5, 0.2, and 0.1 mosquitoes per house for the Anseba, Debub, and NRS zones, respectively.

A bimodal pattern in the seasonal distribution was observed in the Gash–Barka zone for *An. arabiensis.* Overall, indoor-resting densities of the vector species increased in March and April and from July and November in the Gash–Barka zone. In January and February, the densities were generally low but rose in March and April to a mean of 5.6 anophelines per house. A 2nd peak was recorded in September with a mean density of 6.4 anophelines per house. A unimodal distribution pattern was observed in the Anseba and Debub zones, with densities of *An. arabiensis* being greatest between August and November. The indoor-resting densities of *An. arabiensis* in the Anseba and Debub zones increased after July, after the onset of the rains. Peak indoor densities in these 2 zones were attained in September and October, with 3.6 and 0.8 anophelines per house, respectively. No trend was apparent for the sites in the NRS zone because of the low number of vector mosquitoes collected.

Six other anopheline species collected from IRCs formed only a very small proportion (2.7%, n = 44) of the total. These included *An. cinereus* Theobald (1.3%), *An. rhodesiensis* Theobald (0.6%), *An. d'thali* Patton (0.4%), *An. demeilloni* Evans (0.2%), *An. pretoriensis* (Theobald) (0.1%), and *An. rupicola* Lewis. (0.1%).

Biting habits and densities of *An. arabiensis*

Anopheles arabiensis accounted for 97.6% (n = 2,645) of the total mosquitoes collected in HLCs over the 24-month study period. Of these, 43.3% (n = 1,150) and 56.7% (n = 1,495) were collected in HLCs indoors and outdoors, respectively. The endophagy to exophagy ratio was 1:1.3 and this trend was apparent across all study sites (Table 2). The peak HBRs, expressed as number of bites per person per night (b/p/n) for the predominant species, *An. arabiensis,* varied by season and site. In all zones, biting activity was concentrated between July and December with peak biting density vary-

Table 2. The number of *Anopheles arabiensis* and other anopheline species caught in indoor resting collections (IRCs) and in outdoor resting collections (ORCs) in outdoor shelters in Anseba, Debub, Gash–Barka, and North Red Sea (NRS) zones over the 24-month study period. Values in parentheses represent the total number of mosquitoes collected.

		Zone			
Species	Collection method	Anseba	Debub	Gash–Barka	NRS
An. arabiensis	IRC	48% (255)	17% (78)	71% (1,211)	34% (27)
	ORC	52% (267)	83% (380)	29% (487)	66% (52)
Other *Anopheles* species[1]	IRC	18% (18)	22% (22)	67% (2)	0% (0)
	ORC	82% (80)	78% (82)	33% (1)	100% (10)

[1] Other *Anopheles* species include *An. cinereus, An. pretoriensis, An. d'thali, An. squamosus, An. demeilloni, An. garnhami,* and *An. rupicola.*

ing among sites. In the Gash–Barka zone, the peak biting activity was recorded in September (HBRs of 20.1 b/p/n indoors and 28.8 b/p/n outdoors, respectively). The peak indoor and outdoor biting densities for *An. arabiensis* in the Anseba (20.1 b/p/n indoors and 26.9 b/p/n outdoors) and Debub (16.3 b/p/n indoors and 23.6 b/p/n outdoors) zones also were recorded in September. Overall, the numbers of *An. arabiensis* caught on human baits did not vary significantly with regard to host location ($\chi^2 = 6.61$, df = 3, $P = 0.085$). *Anopheles pharoensis* Theobald (0.96%, $n = 26$) was the 2nd most abundant species caught, at proportions of almost 50% indoors ($n = 14$) and outdoors ($n = 12$). *Anopheles cinereus* comprised only 0.41% of the total anophelines, with 63.6% of these species collected in HLCs outdoors.

Biting periodicity of *An. arabiensis*

The hourly peak biting activity for *An. arabiensis* was variable among sites. In the Gash–Barka zone, mosquito biting commenced at 1800 h but steadily increased, with peak biting activity occurring between 2100 and 2300 h. Although a general decrease in landing collections was observed from 2300 h, appreciable levels of biting activity were maintained throughout the night. The proportion of landing catches recorded between 1800 and 2300 h was 39.8% of the total bites throughout the night indoors and 49.6% outdoors. No clear biting pattern was evident for *An. pharoensis* and *An. cinereus* because few were caught on human baits in this zone.

Biting activity of *An. arabiensis* in the Anseba zone commenced at 1800 h, with peak activity being observed outdoors between 1900 and 2300 h. An extended period of biting activity indoors continued from 1900 h and peaked at 0100 h. The hourly biting cycle of *An. arabiensis,* expressed as number of bites per person per hour, is shown in Fig. 1. The biting pattern observed in the Anseba and Gash–Barka zones evidently was similar. In the Anseba zone, 49.4% (indoors) and 59.1% (outdoors) of the total HLCs were concentrated between 1800 and 2300 h. *Anopheles pharoensis,* the 2nd most abundant species at this site (3.5%, $n = 24$), was collected in HLCs between 1800 and 2100 h in equal proportions indoors and outdoors. In the Debub zone, sustained high levels of biting activity by *An. arabiensis* indoors and outdoors were concentrated between 2000 and 0300 h. Overall, examination of the data shows a significant biting activity indoors and outdoors throughout the night.

Resting patterns of malaria vectors

Outdoor resting collections in pit shelters yielded 1,359 anophelines over the 24-month study period, of which *An. arabiensis* comprised 87.3%. Other species included *An. cinereus* (10.4%), *An. pretoriensis* (0.3%), *An. d'thali* (0.9%), *An. squamosus* Theobald (0.2%), *An. demeilloni* (0.8%), *An. garnhami* Edwards (0.1%), and *An. rupicola* (0.1%). Outdoor resting densities of *An. arabiensis* rose in July and peaked in September in all the study sites, with mean densities of 12.75, 9.82, 16.63, and 0.4 mosquitoes per pit shelter being recorded in the Anseba, Debub, Gash–Barka, and NRS zones, respectively. The proportion of each gonotrophic stage of *An. arabiensis* in the ORCs was unfed 22.6%, bloodfed 42%, half-gravid 12.8%, and fully gravid 22.6%. Comparison of ORCs and IRCs for the same study sites indicated some variability in resting tendencies. In the Debub zone (>1,500 m asl), a high proportion of *An. arabiensis* was collected in outdoor shelters. In the Anseba zone (1,400 m asl), the number of *An. arabiensis* collected resting indoors and outdoors generally was similar; whereas in the Gash–Barka zone, higher proportions of the species were collected indoors, an indication that the species was predominantly endophilic in this zone (Table 2). The proportion of species other than *An. arabiensis* was greater in ORCs than in IRCs, an indication that these rare species exhibited greater outdoor-resting tendencies.

Anopheles arabiensis comprised 18.1% unfed, 47.9% fed, 12.2% half-gravid, and 21.9% fully gravid from IRCs. The ratio of fed to gravid was approximately 2:1, indicating that a high proportion (50%) of *An. arabiensis* left the houses before they became gravid (Table 3). The species exhibited the tendency to leave houses the same night after feeding in all zones, but to a lesser extent in the Anseba zone. However, the high fed to gravid ratio (30:1) in the Debub zone indicates the presence of inherent variations in the partial exophily observed for this species among zones and this behavioral tendency may not be conserved across ecological strata. The results show that although *An. arabiensis* was only partially exophilic in the Anseba and Gash–Barka zones, the same species exhibited great exophilic tendencies in the Debub zone. The rest of the species in IRCs were too scarce to allow for meaningful interpretation.

Sporozoite rates for *P. falciparum*

Overall, a 0.99% ($n = 66$) infection rate was recorded from the total number of anopheline mosquitoes tested ($n = 6,634$). Sporozoite rates (SRs) for *An. arabiensis* were significantly different among zones ($\chi^2 = 8.00$, df = 3, $P = 0.046$). Partitioning the infection rates by zone showed a 1.3% infection rate in the Gash–Barka zone (500–1,000 m asl), which is the most highly malarious zone in the country. The sporozoite rates recorded in the Anseba and Debub zones were 0.5 and 1.01%, respectively. Of 162 anophelines tested from the NRS zone, none was positive for the circumsporozoite antigen for *P. falciparum.* Analysis of the data at the village level showed that 65.1% ($n = 44$) of the

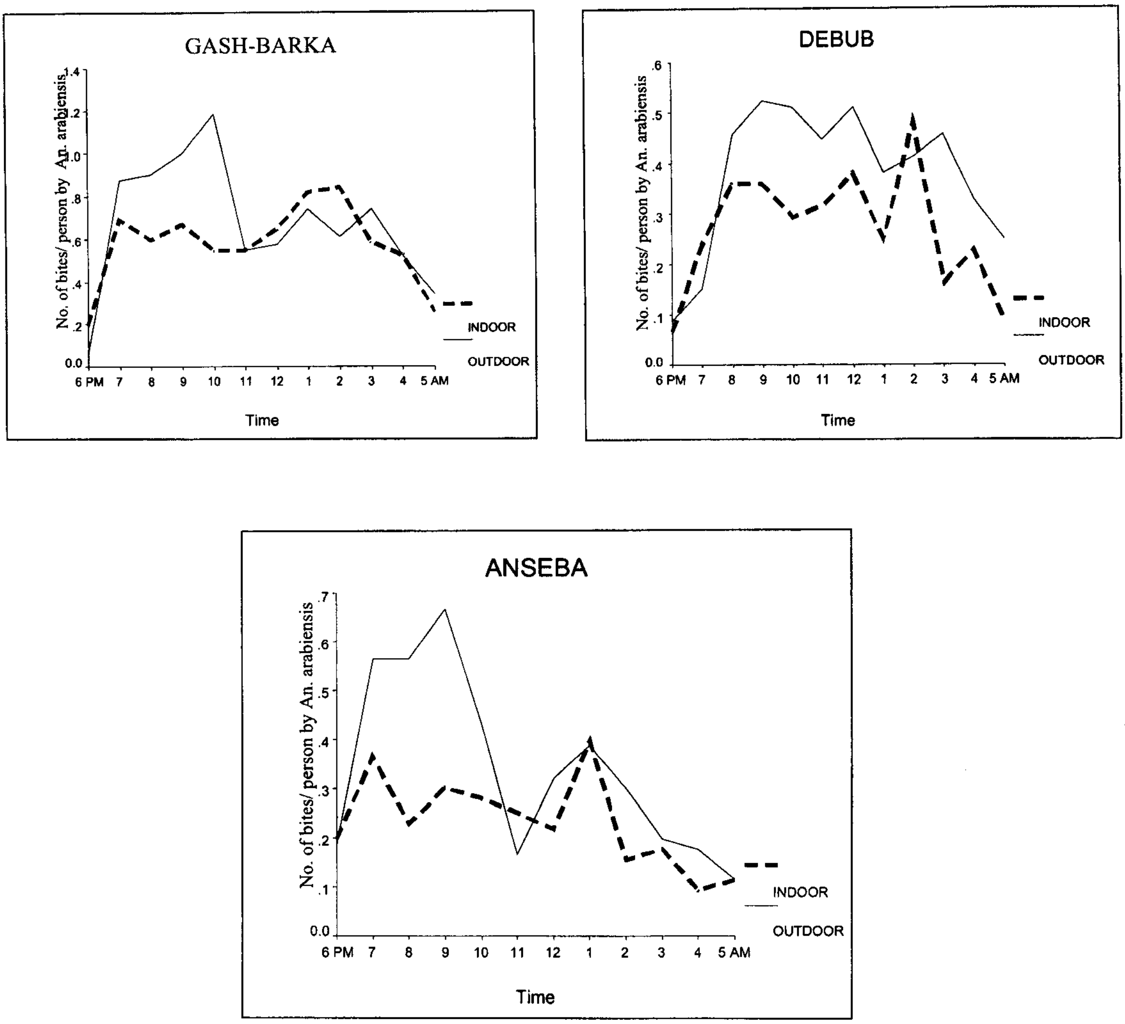


Fig. 1. Biting cycle of *Anopheles gambiae* s.l. in the Anseba, Gash–Barka, and Debub zones in Eritrea. The North Red Sea zone is not shown because low numbers of adult mosquitoes were collected there during the study.

Table 3. The number of species of *Anopheles* by gonotrophic stages and their calculated fed to gravid ratios collected resting indoors at the 4 different study sites in Eritrea.[1]

Species	Zone	UF	F	HG	G	Fed to gravid ratio
An. arabiensis	Gash–Barka	241	556	164	248	2.2:1
	Anseba	24	123	21	87	1.4:1
	Debub	14	59	3	2	30:1
	NRS	2	21	4	0	—
An. cinereus	Anseba	2	3	0	0	—
	Debub	5	8	1	2	4:2
An. d'thali	Anseba	1	2	1	2	1:1
An. rhodesiensis	Anseba	1	4	2	0	—
	Debub	3	0	0	0	—
An. pretoriensis	Gash–Barka	0	0	0	1	—
	Anseba	1	0	0	0	—
An. demeilloni	Debub	2	1	0	0	—
An. rupicola	NRS	2	0	0	0	—
Total		298	777	196	342	2.3:1

[1] UF, unfed; F, fed; HG, half-gravid; G, gravid; NRS, North Red Sea.

Table 4. Human biting rates, sporozoite rates, and entomological inoculation rates (EIRs) for *Anopheles arabiensis* at the study villages over the 24-month period in Eritrea.[1]

Zone	Village	Biting rate (b/p/n)	Sporozoite rate (%)	EIR (ib/p/n)	EIR (ib/p/month)	Annual EIR (ib/p/year)
Gash–Barka	Hiletsidi	12.4	1.838	0.218	6.54	78.5
	Dasse	1.22	1.01	0.015	0.45	5.4
Anseba	Adibosqual	6.70	0.119	0.014	0.42	5.0
	Hagaz	0.16	0.159	0.0008	0.024	0.3
Debub	Mai-Aini	6.80	0.655	0.093	2.79	33.6
	Shekaeyamo	0.60	0.000	0.000	0.000	0.0
NRS	Gahtelay	0.58	0.000	0.000	0.000	0.0
	Ghinda	0.16	0.000	0.000	0.000	0.0

[1] b/p/n, bites per person per night; ib/p/n, infective bites per person per night; NRS, North Red Sea.

positive mosquitoes were collected from 2 sites in the Gash–Barka zone, with Hiletsidi (≈500 m asl) alone comprising 59.1% (n = 39) of the total positive mosquitoes. One mosquito each of *An. d'thali* (SR = 0.45%) and *An. cinereus* (SR = 2.13%) was found to be positive.

The number of infected anopheline mosquitoes collected varied significantly with the method of collection, that is, IRC, ORC, or HLC indoors and outdoors (χ^2 = 15.59, df = 3, P = 0.01). Of the total positive *An. arabiensis* (n = 64), 31.8 and 18.2% came from HLCs indoors and HLCs outdoors, respectively. The results indicate appreciable outdoor biting by infective mosquitoes, and therefore the risk of infection associated with outdoor biting may not be underrated. The proportion of infected *An. arabiensis* also was variable, depending on season. Sporozoite rates for *P. falciparum* generally were low in the dry season (January–June), but increased between June and October, coinciding with the rainy season. The sporozoite rates decreased drastically from October to December in the Anseba, Debub, and Gash–Barka zones. The seasonal trends of infection rates among *An. arabiensis* were similar in all 3 zones. The highest proportion of infected *An. arabiensis* was recorded in September in all 3 zones. Table 4 shows the site-specific variation in biting and sporozoite and inoculation rates among the study villages over the 24-month study period.

Entomological inoculation rate

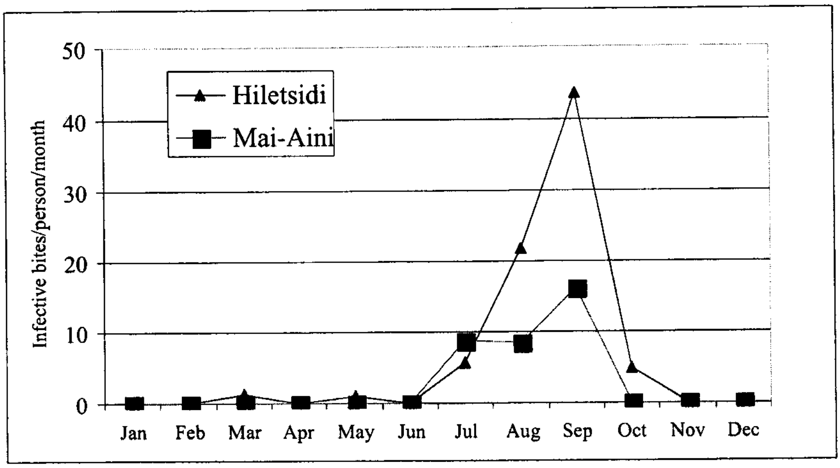
The entomological inoculation rate (EIR), derived from the product of the sporozoite rate and landing rates for *An. arabiensis,* tended to vary among the study villages. Each resident was estimated to be exposed on average to 6.5 and 2.8 infective bites per month in Hiletsidi in the Gash–Barka zone and Mai-Aini in the Debub zone, respectively, and the annual EIRs for the 2 sites are 78.5 and 33.6 infective bites per person (Table 4). The patterns of malaria transmission as depicted by the EIR seasonal profiles were generally similar and indicated that transmission of malaria is highly seasonal, increasing during the wet season (June–November) and decreasing drastically during the dry season (December–June). However, in Hiletsidi in the Gash–Barka zone, a 2nd peak was observed in March. The highest inoculation rate of 1.45 infective bites per person per night was recorded in this village in September (Fig. 2).

Feeding preferences of the anopheline mosquitoes in Eritrea

Results of the ELISA on blood-meal analysis of the anopheline species tested are shown in Table 5, and indicate that the host preferences of the different species were variable. The bovine to human blood-meal ratio for *An. cinereus* was 1:1, indicating that the species fed equally well on nonhuman hosts when present. *Anopheles d'thali* was highly anthropophilic, with a human to bovine blood-meal ratio of almost 2:1. The results further show that *An. arabiensis,* the most abundant species, was partially zoophilic. The human to bovine ratio for this species was 1.6:1. At least 44% of the *An. arabiensis* identified had received at least 1 blood meal from a nonhuman host. A proportion (15.5%) of this species had a mixed blood meal derived from both human and bovine hosts. A high proportion (77%) of anophelines positive for a human blood meal were collected indoors from PSCs and at least 23% came from pit shelters.

DISCUSSION

Our results on the seasonal abundance of *An. arabiensis* and other anopheline species have improved our understanding of the patterns of malaria transmission and the role of this species in malaria transmission. Our results confirmed that the density of *An. arabiensis* was influenced by rainfall and increased at the beginning of the rainy season, between June and July. However, the peak densities were observed toward the end of the rainy season, from September to October. This occurrence is likely due to an increase in the number of larval habitats and the persistence of such habitats after the rains subside. Larval studies conducted in Eritrea have confirmed that mosquito abundance is strong-



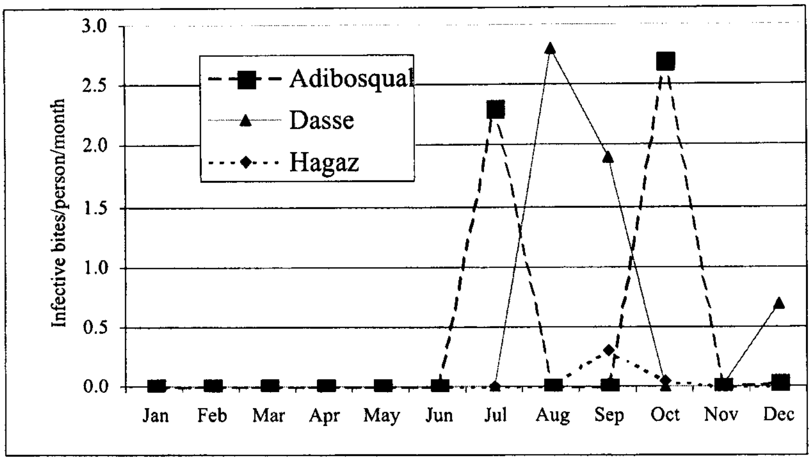


Fig. 2. Seasonal variation in inoculation rates of *Plasmodium falciparum* in 5 study villages in Eritrea (October 1999–September 2001). Ghinda, Gahtelay, and Shekaeyamo are not shown because infective mosquitoes were not present at these study sites.

Table 5. The bloodfeeding preferences of *Anopheles arabiensis* and other anopheline mosquitoes collected over 24 months during the vector behavior studies in Eritrea.

Species	Bovine	Human	Mixed blood meal	Human to bovine ratio
An. arabiensis	510	991	260	1.6:1
An. cinereus	45	39	19	0.9:1
An. d'thali	11	29	21	1.6:1
An. squamosus	2	12	0	6:1
An. rupicola	1	5	4	1.5:1
An. pretoriensis	1	4	0	4:1
An. demeilloni	2	2	0	1:1
An. garnhami	1	3	0	3:1
An. chrysti	0	2	0	—
An. pharoensis	0	2	0	—
Total	573	1,089	304	1:1.6

ly associated with rain pools, stream edge pools, and streambed pools, which often are present after the rains and persist well into the dry season (Shililu et al. 2003b).

We found significant spatial heterogeneity in abundance of *An. arabiensis* among the villages investigated. Higher densities of anophelines were sampled from sites in the western lowlands. This can be attributed to variability in environmental variables such as rainfall amounts, temperature, and presence of suitable larval habitats. Lindblade et al. (2000) found indoor-resting densities of *An. gambiae* s.l. to be significantly associated with the average minimum temperature of a village. Furthermore, variation in abundance of adult *An. arabiensis* also could be affected by habitat-specific physicochemical and biological variables that influence larval production (Robert et al. 1998, Minakawa et al. 1999, Gimnig et al. 2001).

Large seasonal variation in the HBRs of *An. arabiensis* also was found in all villages. The number of bites by *An. arabiensis* was 9 times higher in the wet season than in the dry season. The peak biting density for *An. arabiensis* was achieved in September in the Gash–Barka and Debub zones, and in October in the Anseba zone. Overall, the mean densities from the HLCs strongly correlated with indoor-resting densities ($r = 0.687$, df = 94, $P < 0.01$), confirming that biting rate is a function of vector abundance and that it is influenced by environmental variables such as rainfall, temperature, and larval habitat, as observed above (Lindblade et al. 2000).

Thirty-five percent of the biting activity of *An. arabiensis* occurred between 1800 and 2200 h, with 58.6% of the total mosquito bites during this time occurring outdoors. A concurrent questionnaire conducted on sleeping behavior indicated that 22.2% of the people went to bed only after 2100 h. This confirms that a significant proportion of the population was exposed to vector bites during the peak biting hours (2100–2300 h) both indoors and outdoors before going to bed. The situation was further complicated by the fact that almost 20% of the total infected *An. arabiensis* were collected in HLCs outdoors. This behavioral tendency would influence the success of insecticide-impregnated bed-net programs because of the substantial prebedtime and outdoor exposure of residents to infective bites. This scenario presents even stronger reason for the malaria control program in Eritrea and other affected areas of Africa to focus strongly on integrated malaria control strategies by strengthening their larval control program.

The fed to gravid ratios and the proportions of *An. arabiensis* caught in IRCs and ORCs shows that a proportion of the species rests in outdoor shelters, an indication of partial exophily in this species. Similar observations for this species have been noted in sites in the Awash River valley in neighboring Ethiopia (Ameneshewa and Service 1996). Furthermore, examination of the data shows that the resting tendencies were variable among the study sites. For example, a higher degree of exophily was observed for this species in the Debub zone compared to the other sites. Ameneshewa and Service (1996) observed seasonal differences in the degree of exophily for *An. arabiensis* to be associated with rainfall and possibly the availability of outdoor resting sites. The site-specific behavioral variability likely could be attributed to the amount of rainfall and subsequent relative humidity prevailing at the specific sites. Although the partial exophily observed would be associated with natural behavior patterns of the species, it would be likely to result from an irritant action of DDT, especially at sites in the Debub zone where DDT is used extensively. Results from Khashm Elgirba in eastern Sudan indicated that high exophilic tendencies of *An. arabiensis* were associated with DDT spraying in this area (Haridi 1972). Studies have further shown that *An. arabiensis* tends to avoid DDT-sprayed surfaces and would usually rest on unsprayed surfaces, thus minimizing insecticidal contact (Ameneshewa and Service 1996). Excito-repellency due to the extensive use of insecticide-treated bed-nets, as observed in the study villages, also is likely to have influenced the exophilic behavior of *An. arabiensis* observed in the present study.

The number of infected *An. arabiensis* varied significantly with season and host location. Although twice as many infected mosquitoes were found among indoor collections, the risk of infection associated with outdoor biting was appreciable. These observations underline the inherent problems associated with protection based only on insecticide-treated bed-nets. It is worth noting that insecticide-treated bed-nets have been shown to offer protection against malaria infection in endemic areas of Africa and use of insecticide-treated bed-nets would benefit greatly from integration of other vector control measures in such areas of highly seasonal malaria transmission.

Malaria transmission is highly seasonal, occurring for only 5 months and with a peak during September and October, depending on the site. This is partially influenced by variations in biting densities of the vector species, *An. arabiensis*. In Hiletsidi in the Gash–Barka zone, transmission is maintained almost throughout the year. This is a site where vector production is maintained throughout the year from man-made breeding sites (Shililu et al. 2003b). During the same period as the study, 5 of the 6 zones in the country were surveyed for malaria. A total of 12,937 individuals from 176 villages were screened for both *P. falciparum* and *P. vivax* by using the OptiMal Rapid Diagnostic Test® kit (MOH, Asmara, Eritrea). Malaria prevalence was found to be generally low, yet highly focal and variable, with the proportion positive for malaria parasites at 2.2% (range 0.4–6.5%; Table 6).

Table 6. Malaria parasites and prevalence found at the 5 zones in Eritrea.[1]

Zone	Villages sampled	Population screened	Pf positive (%)	Pv positive (%)	Total positive	Prevalence rate (%)
Anseba	52	4,892	39 (88.6)	5 (11.4)	44	0.90
Debub	32	1,686	33 (91.7)	3 (8.3)	36	2.14
Gash–Barka	32	1,691	109 (92.4)	9 (7.6)	118	6.98
Maekel	15	763	1 (100)	0 (0)	1	0.13
NRS	46	4,247	76 (88.4)	10 (11.6)	86	2.02
Total	177	13,279	258 (90.5)	27 (9.5)	285	2.15

[1] Pf, *Plasmodium falciparum*; Pv, *Plasmodium vivax*; NRS, North Red Sea.

However, the results indicate that despite the overall seasonal pattern of malaria transmission, observed variations in EIR were likely dependent on local and site-specific factors that would facilitate vector production. It is imperative that vector control measures should take into consideration both seasonal and local variations (Fontenille et al. 1997, Shililu et al. 1998). Even though some of the other *Anopheles* species sampled were ELISA-positive, their actual vector status remains unknown, as the demonstration of circumsporozoite antigen by ELISA does not establish infectivity. Moreover, the low numbers of the other species would render them unimportant in malaria transmission in the country. Although EIRs of zero were recorded in Shekaeyamo, Ghinda, and Gahtelay (Table 4), it is likely that transmission goes on below thresholds for detection by the entomological techniques employed. The high EIR values for *An. arabiensis* in relation to the other species would probably be explained by vector longevity. Studies addressing vector longevity for the different *Anopheles* species would shed more light on this important vectorial capacity parameter.

Examination of our data indicated that *An. arabiensis* was moderately zoophagic, a feature that would lower the level of malaria transmission where animals are abundant. Similar results have been obtained in studies in Ethiopia in mixed dwellings of humans and animals (Hadis et al. 1997, Habtewold et al. 2001). Initial studies have indicated that this species takes up a large proportion of blood meals from livestock where such hosts are plentiful (Garret-Jones et al. 1980). This feeding tendency on animals also would arise from changes in behavioral mechanisms as a result of sustained indoor residual spraying. Sharp and Le Sauer (1991) demonstrated that *An. arabiensis* shifts from feeding on humans to feeding on livestock because of indoor residual spraying. Based on this zoophagic tendency, the use of livestock for protection against malaria, through either diversion or by treating livestock with insecticides, is gaining prominence (Hewitt and Rowland 1999). However, the inherent excito-repellency of pyrethroid insecticides used for livestock treatment would reduce the efficiency of this approach.

Our study at 8 sites in Eritrea has provided basic data on malaria transmission and vector behavior that will be useful for planning malaria control operations. Vector control measures must address site-specific variations in transmission intensity and vector behavioral mechanisms.

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