

## Transmission of malaria in the Tesseney area of Eritrea: parasite prevalence in children, and vector density, host preferences, and sporozoite rate

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**ABSTRACT:** Malaria transmission was studied from July to September, 2002 in three villages of the Tesseney sub-zone, in the western lowlands of Eritrea. The three methods used for mosquito collection were light traps, pyrethrum spray catches, and pit shelter collections. All anopheline mosquitoes that were collected belonged to the *Anopheles gambiae* complex and they were identified by PCR as the sibling species *Anopheles arabiensis* (Patton). Apart from *An. arabiensis*, the only other mosquitoes caught were culicines. The vector population increased greatly for about a month after the start of the rains. The anthropophilic indices obtained from the blood-fed *An. arabiensis* resting indoors and outdoors were only 20% and 25%, respectively, with most of the other meals on goats. ELISA for *P. falciparum* circumsporozoite protein revealed only one positive out of 1,026 tested. The malaria prevalence among children <10 years was only 3.3% (all *P. falciparum*) from 300 slides examined. These low rates seem to reflect recent success in malaria control in Eritrea. *Journal of Vector Ecology* **30** (1): 27-32. 2005.

**Keyword Index:** *Anopheles arabiensis*, malaria prevalence, sporozoite rate, blood meals, indoor resting catches.

### INTRODUCTION

In many semi-arid sub-Saharan regions, including Eritrea, malaria transmission is unstable (Hamad et al. 2002, Shililu et al. 2003a, Sissoko et al. 2004). Recent studies in Eritrea (Shililu 2003a, 2003b, 2003c, 2004) recorded thirteen species of anopheline mosquitoes in different ecological settings, but of these, only *Anopheles arabiensis*, *Anopheles cinereus*, and *Anopheles d'thali* were incriminated as vectors of malaria, with *An. arabiensis* being predominant. As recorded in the Tesseney declaration, Eritrea has recently achieved a very significant reduction in its malaria burden. This has been accomplished by providing insecticide-treated bed nets to children and pregnant women free of charge so that there are now an average of two in each household in the malarious areas, organizing a regular net re-treatment service free of charge (T. Ghebremeskel, personal communication), continuation of residual house spraying with DDT in some areas, and by environmental management and larviciding (Shililu et al. 2003c).

Craig et al. (1999), and Hay et al. (2000) have demonstrated how the transmission of malaria and levels of endemicity vary across Africa as a whole, and the important small scale local variation. These variations are associated with heterogeneities in vector density, survival of the mosquitoes, vector host contact, and innate feeding preference of vectors, all of which are incorporated in the Macdonald vectorial capacity equation (Smith et al. 1995, Bøgh et al. 2001, Killeen et al. 2001). These factors are under a complex interplay of environmental influences. In endemic areas of Eritrea, estimates of exposure to infectious bites have been

made for different regions and for extended periods (Shililu et al. 2003b, 2004). However, examining just a few study villages per region inevitably misses important local differences (Greenwood 1989, Smith et al. 1995).

In this study, we investigated malaria vectors and prevalence of human infection in the Tesseney area located in the wet lowland zone of Eritrea. This area is the most malarious area in the country as described by Shililu et al. (2003a, 2003b). The objectives of the present study were to assess *Anopheles* populations in a small town and three nearby villages, determine the human blood index in mosquitoes resting indoors and outdoors, and their sporozoite rate. The entomological indices have been correlated with the parasitological findings and rainfall as the short wet season advanced.

### MATERIALS AND METHODS

#### Study sites

During the dry season of February-June, 2002, the minimum temperature in Tesseney (15° 06'N, 36° 39'E, altitude 560 m) was 20 °C, the maximum was 38 °C, and there was no rainfall. During the rainy season (July-early September, 2002), there was rainfall of 54 mm in July and 74 mm in August. The maximum temperature at this season was 42 °C and the minimum was 22 °C (meteorological data from Malaria Control Centre of Eritrea).

Tesseney is a small town in a largely arable area. At the time of the study some larval control was being carried out in Tesseney but not in the nearby villages. Hiletsidi (15° 07'N, 36° 39'E) is a peri-urban village with a well that supplies

potable water to most of the people of Tesseneay and Hiletsidi itself. It has small farms at the periphery of the village and is a monitoring site for the national malaria control program of Eritrea. Adihassan (15° 08'N, 36° 39'E) and Adiomer (15° 08'N, 36° 42'E) are also rural villages with small farms at the periphery. It is estimated that at least two insecticide-treated bed nets are owned by almost every household in the study sites, but there are not enough nets to cover all beds in each house. All the inhabitants in the study sites use the same hospital situated in Tesseneay. The community sizes of Tesseneay, Hiletsidi, Adihassan, and Adiomer are 13,664, 1,340, 579 and 1,124, respectively (data from the Malaria Control Centre of Eritrea). In this paper, Tesseneay town will be distinguished from the Tesseneay administrative sub-zone which includes the town itself as well as the study villages and some other villages. Populations of mosquitoes vary greatly with season in this area (Omer and Cloudsley-Thompson 1970, Shililu et al. 2003b, 2004), but they can breed to some extent throughout the year due to the spillage of well water into a ditch around the well.

### Mosquito sampling

Intensive mosquito collections were made using CDC miniature light traps (Model 1012 by John W. Hock), pyrethrum spray catches, and pit shelter collections in the wet season (from mid-July to mid-September, 2002) in Tesseneay town, Hiletsidi, and Adihassan. Unlike the studies of Shililu et al. (2003a, 2003b, and 2004), human landing catches were not used. A total of fourteen trap-night collections of mosquitoes were carried out over five nights in Adihassan, Hiletsidi, and Tesseneay town using three light traps each with a photo-switch that turns the trap on at dusk and off at dawn. Traps were operated from batteries that were rechargeable each day with a solar charger. Light traps were set at the foot of occupied beds and all the beds in the house were covered with untreated bed nets including the one with the light trap hung beside it. Lines et al. (1991) showed that under these conditions catches of *An. gambiae s.s.* and *Culex quinquefasciatus* are proportional to what would have bitten humans without nets, three traps catching about as many as two human catchers. Magbity et al. (2002) confirmed that the presence of treated bed nets in nearby houses had little impact on the efficiency of light traps at moderate population densities, as in the present study.

Mosquitoes were collected on 16 mornings from 06:00 hrs to 09:30 hrs from a total of 175 houses using pyrethrum spray catches. All the openings in the houses were covered carefully and white collection sheets were placed on the floor. The houses were then sprayed with 0.3% pyrethrum in kerosene and the fallen mosquitoes were collected from the sheets. Two pit traps, one of which had been dug at Hiletsidi for the studies of Shililu et al. (2003b, 2004) and the other dug for this study, were monitored during the present study. The pits were 1.5 m deep and 1 m wide and collections were made from the horizontal 'pockets' dug in the four walls of each (Service 1993).

### Entomological indices

The collected mosquitoes were sorted both by sex and whether they were anophelines or culicines. The anopheline mosquitoes were identified morphologically following Gilles and De Meillon (1968). Each anopheline was kept in a tube with silica gel for later sporozoite testing or sibling species identification using polymerase chain reaction (PCR) following the procedures of Scott et al. (1993). Ninety-two females of the *An. gambiae* complex, which had fed on blood and collected from Tesseneay town and its nearby village, Hiletsidi, were squashed onto filter paper. The origins of the blood meals were identified later by precipitin tests. The blood meals were tested either with anti-serum to human or goat as goats and sheep are frequently kept in compounds outside the houses. The laboratory testing (ELISA, precipitin, and PCR) were performed at the London School of Hygiene and Tropical Medicine.

Anophelines from all the study sites and collected by all three methods were tested for *P. falciparum* by ELISA for circumsporozoite protein (CSP), using a double monoclonal antibody (sandwich) ELISA as described by Burkot et al. (1984). The wells of microtiter plates were coated with monoclonal antibody to *P. falciparum* CSP. Blocking buffer (consisting of phosphate buffered saline, casein, egg albumin, thimerosal, and phenol red and containing Nonidet P-40) was then applied. The heads and thoraces of individual mosquitoes were ground in buffer and then transferred to the wells of the antibody-coated plates. Positive and negative controls were added to specific plate wells and the plates were incubated for 2 h at room temperature. The same monoclonal antibody was then added, but this time it was conjugated with peroxidase enzyme. The plates were incubated for another hour at room temperature and results were read visually after the addition of peroxidase substrate. The presence of a yellow color was a positive indication for CSP. In all cases, the positive and negative controls of each plate gave the expected results.

Smears from each blood-fed mosquito were eluted in 400 µl of PBS and left overnight at 4°C. The mixture was centrifuged at 5,000 rpm for 5 min. Two hundred µl of anti-goat whole serum (Sigma-Aldrich) or human antiserum was transferred into a tube, followed by 200 µl of the blood meal eluate. If the blood meal was derived from a particular host for which the antiserum was prepared, a white band of precipitate formed at the interface between the blood meal extract and the antiserum (Weitz and Jackson 1955, Weitz 1956).

Thirty-three mosquitoes, including some males, of the *An. gambiae* complex, were selected randomly from the different collection sites and collection methods and analysed using PCR to identify the sibling species. The DNA was extracted from individual mosquitoes and was amplified after adding species specific primers (to *An. gambiae s.s.*, *An. quadriannulatus* and *An. arabiensis*) and DNA polymerase in a PCR machine. After the PCR was completed, 10 µl of the amplified DNA was mixed with 2 µl agarose gel loading dye and electrophoresed through a 1.5% agarose-tris-borate-EDTA gel containing Ethidium Bromide. The amplified

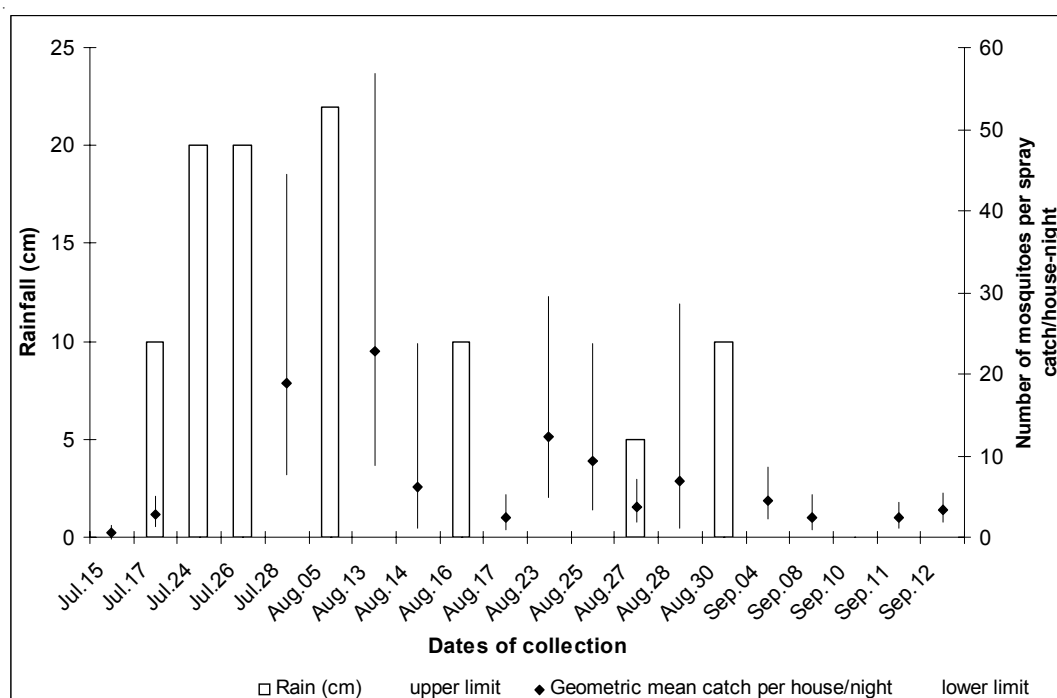


Figure 1. Resting catches of mosquitoes versus rainfall in the village Hiletsidi by spray catch, (July to September, 2002).

fragments were visualised by illumination with ultraviolet light (Scott et al. 1993).

#### Parasitological survey

A cross-sectional parasitological prevalence survey in children was carried out at the end of the wet season on August 26-28, 2002 in Tesseney town, in one of the villages on the outskirts of the town, Hiletsidi, and in another village situated about 10 km north of Tesseney town, Adiomer. The study was conducted after permission was obtained from the administrator of the region and with the consent of the children's parents. The blood samples were collected and diagnosed by a well-trained clinical technician who works at the Tesseney hospital and it was carried out in the morning (8:00-12:00), except in Adiomer which was carried out from 12:00-16:00. This survey covered 300 children <10 years of age (100 children from each site). Detection of parasites in the peripheral blood was by examination of Giemsa stained thick and thin blood films. Microscopic examination of the

slides was done on site. Each slide was examined for at least 20 min. No special criteria were used to select children; the first 100 in the queue were examined.

## RESULTS

#### Anopheline species and sporozoite detection

Anopheline species caught in this area were found to consist entirely of the *An. gambiae* complex and, as indicated in Table 1, the catches also included a minority of culicines that were not identified to species. All of the 33 *An. gambiae* s.l. tested by PCR were identified as *An. arabiensis* Patton.

Only one anopheline out of 1,026 tested by ELISA was positive for *P. falciparum* circumsporozoite protein. The positive and negative controls for each plate during the analysis gave the expected results.

#### Resting catches of *An. arabiensis* during the rainy season

The geometric mean captures of mosquitoes per house

Table 1. Species composition of female mosquitoes caught in Tesseney, July-Sep., 2002.

| Collection area | Light trap            |           | Spray catch           |           | Pit shelter collection |           |
|-----------------|-----------------------|-----------|-----------------------|-----------|------------------------|-----------|
|                 | <i>An. arabiensis</i> | Culicines | <i>An. arabiensis</i> | Culicines | <i>An. arabiensis</i>  | Culicines |
| Adihassan       | 4 (4)                 | 3         | 93 (15)               | 3         | -                      | -         |
| Hiletsidi       | 125 (5)               | 8         | 665 (65)              | 12        | 99 (22)                | 18        |
| Tesseney town   | 47 (5)                | 17        | 16 (20)               | 3         | -                      | -         |
| Total catches   | 176 (14)              | 32        | 774 (100)             | 18        | -                      | -         |

Number of light-trap nights, number of collections from pit shelters, and number of house spray catches are in parenthesis.

Table 2. Blood meal identification.

| Methods of collection and number of positives |             |                    |               |
|---|-------------|--------------------|---------------|
| Anti-serum                                    | Pit shelter | Indoor spray catch | Overall total |
| Human   | 8 (25%)     | 12 (20%)           | 20 (22%)      |
| Goat  | 18 (56.3%)  | 20 (33.3%)         | 38 (41%)      |
| Human and goat<br>(Mixed blood meals)         | 3 (9.4%)    | 6 (10%)            | 9 (10%)       |
| Negative for human<br>and goat                | 3 (9.4%)    | 22 (36.7%)         | 25 (26%)      |
| <b>Total tested</b>                           | <b>32</b>   | <b>60</b>          | <b>92</b>     |

ranged from 0 to 22.8 mosquitoes (Figure 1). From a very low dry season level, the density of the mosquito population increased to a maximum for about a month after the rains began. Although rains fell intermittently for about another month, mosquito catches slowly declined.

#### Human blood index

Sixteen percent (60 out of 370) of the outdoor and 14 % (41 out 299) of the indoor collected mosquitoes had blood fed. Humans were not the predominant blood source for the mosquitoes caught either indoors or outdoors (Table 2). Goats were the most common large domestic animals and tests with anti-goat antiserum showed a majority of positives. Meals which scored negative to human and goat antiserum were presumably from donkeys, chickens or the cows in one of the houses in the area, but these species were not tested. Omitting a few mixed human and goat feeds, the proportions of feeds on humans compared to other animals was 8:21 from the outdoor pit shelter collection and 12:42 in the indoor spray catch collection. A  $\chi^2$  test showed no significant difference between these proportions.

#### Parasite prevalence

The overall malaria prevalence among the symptomatic or asymptomatic children (<10 years old) in the study region was 3.33% (10 positives out of 300) at the end of the wet season. However, the 10 positives out of 100 children were from one village (Adiomer), while no positives out of 200 slides were found in the other two sites, Tesseney town and its neighboring village, Hiletsidi. The difference between the prevalence in Adiomer and the other sites was statistically significant ( $P > 0.001$  by Fisher's exact test). All the malaria-infected children were infected with *P. falciparum*. All the children who were infected with *P. falciparum* had fever, while all the uninfected ones were asymptomatic.

#### DISCUSSION

Our data confirm that *An. arabiensis* is the only major vector of malaria in Tesseney, as recently reported for this district and elsewhere in Eritrea by Shililu et al. (2003a), Carrara et al. (1994) and Nyanjom et al. (2003). *An. arabiensis* is associated with more arid habitats (Coetzee et al. 2000, Davidson 1967, Omer and Cloudsley-Thompson 1970, El Rayah and Abu Groun 1983, Hamad et al. 2002) and the climate of the study site is indeed arid, with only a short rainy season (Figure 1). The density of *An. arabiensis* resting indoors increased rapidly following the beginning of the rains and is consistent with the usual pattern of annual outbreaks of malaria being dependent on rainfall. The direct influence of rainfall on the density of *An. arabiensis* is in agreement with a previous study which was done in Tanzania by Charlwood et al. (1995).

During the rainy season of 2002, the sporozoite rate of *An. arabiensis* (1/1,026) in this area was very low. However, in the study of Shililu et al. (2003b) in Hiletsidi from October, 1999 to January, 2002, the sporozoite rate of *An. arabiensis* was significantly higher (39/2,437;  $\chi^2 = 13.0$ ,  $P < 0.001$ ). This very recent reduction in sporozoite rate may be due to the widespread adoption of treated bed nets that are known to be able to reduce the mean survival of vector populations to such an extent that few survive long enough for sporozoites to reach maturity (Magesa et al. 1991). The present low sporozoite rate may be said to be both the cause and the effect of the low malaria prevalence in children observed in the study area and especially in Hiletsidi and Tesseney town, from which most of the mosquitoes for the sporozoite tests were collected.

The HBI of *An. arabiensis* was found to be about 22%. The fed mosquitoes collected from indoor and outdoor resting sites had mainly fed on goats and other unidentified animals. Thus, in Tesseney, *An. arabiensis* is more zoophilic than anthropophilic. The host-choice behavior of *An. arabiensis* is less rigidly directed to man than *An. gambiae s.s.* and shows different degrees of zoophagic behavior, with higher rates of

zoophagy when many large mammals are available, apparently more so in East than West Africa, (Coluzzi 1984, White et al. 1972, Takken et al. 1996, Githeko et al. 1994). The data from Hiletsidi support this general pattern. The *An. arabiensis* found in the study area rest both indoors and outdoors after feeding. This finding is in agreement with the data of Mnzava et al. (1994) from Kenya. Few people sleep outdoors in Hiletsidi and domestic animals, except chickens, are rarely kept indoors. Thus, many of the indoor-resting *An. arabiensis* probably fed outside before entering to rest. The human blood in some of the mosquitoes in the outdoor pit shelters may have derived from outdoor feeds or from mosquitoes which exited after feeding. There was no significant difference in the HBI in mosquitoes caught indoors or outdoors (Table 2). The predominance of blood meals from goats reflects their ready availability. Other studies on blood meal source analysis for *An. arabiensis* in the region showed relatively low human blood indices with a wide range of other alternative hosts, including bovid, goats, sheep, donkeys, and probably camels (Carrara et al. 1994, Ameneshewa and Service 1997). The number of goats and other domestic animals (sheep, cattle, donkeys, and chickens) in the village where the present study was conducted varies greatly among different households. However, except in one house, no cattle were kept in the village during the present study, as cattle are moved from place to place in search of grass in different seasons. Shililu et al. (2004) recorded numerous bovine feeds not far from the present study area, but they did not test for goat blood.

The prevalence of *P. falciparum* infection found in children aged <10 years in Tesseney is relatively low compared to many other parts of Africa. Apart from the relatively zoophagic vectors, this presumably reflects the recent success in Eritrea in ensuring high population coverage with effectively treated nets. However, the data presented here for *P. falciparum* infection show that there is a marked difference in the three nearby sites in Tesseney district although they are only a short distance apart. There were no vector data for the one village in which human infections were found. Clarke et al. (2002) discussed the likely causes for local variation in malaria prevalence. In situations like Tesseney, with low overall malaria endemicity, elimination of identified pockets of relatively high malaria risk may be feasible and affordable using the available tools, and this might eliminate the malaria problem from the whole region.

Shililu et al. (2004) reported that a considerable proportion of *An. arabiensis* biting occurred before people go to bed. Thus, use of plant-based repellents which Eritreans already use to a considerable extent (Waka et al. 2004) can be expected to have a beneficial impact on the disease as they are likely to divert such zoophilic mosquitoes from biting humans to biting animals rather than other humans. We consider that the presence of a single vector species with a low rate of anthropophagy in the study area favors the prospects for interruption of malaria transmission by integrated control (including larval control) when compared to many other African countries with multiple vectors and more anthropophagic vector species.

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