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SHORT COMMUNICATION

Resting behaviour of *Anopheles gambiae s.l.* and its implication on malaria transmission in Uyui District, western Tanzania

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Abstract: An entomological survey to determine resting behaviour and species composition of malaria vectors was carried out in Uyui District in western Tanzania in May 2009. Mosquitoes were collected using indoor resting catch, window exit trap and outdoor "*bed-net*" techniques. They mosquitoes were identified using morphological key and polymerase chain reaction (PCR). A total of 672 *Anopheles gambiae* sensu lato were collected. Of these, 661 (98.4%) were collected outdoor whereas few (1.6%) were collected indoor. The exit trap catch: mechanical aspirator catch ratio was 1:1.75. The overall indoor resting density of *An. gambiae s.l.* as determined by mechanical aspirator and exit trap was 0.7 and 0.5 mosquitoes per room, respectively. The overall density of the host-seeking as determined by bed net trap outdoor was 44.1 mosquitoes per person. A sample of 44 specimens taken randomly from morphologically identified *An.gambiae s.l.* population was further analyzed to species level using PCR techniques. Of these 44 specimens 26 (59%) and 18 (41%) were *Anopheles arabiensis* and *Anopheles gambiae sensu stricto* respectively. This study contributes to the understanding of the distribution of malaria vectors with respect to species composition and their resting behaviour that could contribute to vector control operations in western Tanzania. A longitudinal study considering dry and wet seasons is recommended to provide more information on the seasonal distribution, abundance and biting behaviour of malaria vectors in the study area.

Key words: *Anopheles gambiae*, resting behavior, malaria transmission, Tanzania

Factors that influence the role of mosquitoes in malaria transmission include host preference, resting and feeding behaviour, adult longevity and density as they determine the degree of anthropophily, the human biting rate and host location strategy (Black & Kondratieff, 2005). *Anopheles gambiae* s.l, the principal vector of malaria in sub-Saharan Africa (Coetzee, 2004) locates its hosts largely based on olfactory cues and that human body odours play a significant role in host seeking behaviour (Mboera *et al.*, 1997).

Studies in Tanzania have shown that the vector species vary greatly between different ecological zones (Brooke *et al.*, 2001). Despite a number of studies on the distribution of malaria vectors in Tanzania (Mnzava & Kilama, 1986), those that looked at species composition and their resting behavior in Uyui District of western Tanzania are scarce. Most of the researches on malaria and vectors have concentrated in the northeast, northern, and southeast of the country. Information of mosquito species composition, abundance and resting behaviour are important

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in designing appropriate malaria control interventions. The objective of this study was to determine the malaria vector species composition and resting behaviour in a rural district of Uyui in Tabora, western Tanzania.

This study was carried out in Kigwa village (33.13255° E; 5.11897° S) in Uyui District about 40km from Tabora town in western Tanzania. Kigwa is one of the sentinel surveillance sites for monitoring of insecticide susceptibility in malaria vectors in Tanzania. The climate of the district is generally hot (20 to 32° C), with relative humidity ranging from 25 to 65%, and the rainfall ranges from 650 to 850 mm per year. Most of inhabitants in the study practice subsistence farming of maize, rice and tobacco. Livestock keeping and beekeeping are also important types of livelihoods. The village has more or less homogenous characteristics in vegetations and house style.

Three methods were used in the collection of mosquito samples. These are indoor resting catch, window exit trap and outdoor *"bed-net"* collections. Indoor resting adult mosquitoes were collected from a sentinel of five houses selected randomly. A second sentinel of five houses was also randomly selected for window exit trap that was fixed in one window in each of the houses. A third sentinel of five houses was randomly selected for outdoor collections. Indoor mosquito collections were made from 0600hrs to 0830hrs for five consecutive nights using mechanical aspirator and window exit trap (WHO, 1975) whereas outdoor collections were made using double mosquito net trap (Service, 1977) with some modifications (Figure 1). Briefly, one set of the double mosquito net trap comprised of a pyramid shape and a rectangular non-insecticide treated mosquito net each measuring 6X4X5ft in size (C. Sindato *et al*. *unpublished*).

The rectangular mosquito net was perforated at the height of about 30cm from the ground base to allow mosquito entry and was fixed outside the pyramid shape mosquito net. Each of five houses selected for outdoor collections was supplied with three sets of untreated double mosquito nets that were set outside their houses at locations where household members reported to rest in the evenings before going to bed. The adult members of the family (with exception of breast feeding mothers and those with history of ill health especially of respiratory nature) were requested to sleep under the double mosquito net trap (one person per one set of the double mosquito net trap). Alternation of the members of the family sleeping in the set of double mosquito net traps was made every other day. Immediately the collected mosquitoes were given fresh 5% glucose solution for them to survive. Mosquito collections from respective sampling techniques were kept separately. The number collected in a day from respective houses/homesteads was recorded. As much as possible, the houses selected were of similar construction to avoid the effect of variability caused by differences in construction.

Informed consent from the participants was sought and the participation was absolutely voluntary. The study received ethical approval from the Medical Research Coordination Committee of the National Institute for Medical Research, Tanzania .

Mosquitoes collected were sorted and identified using morphological key (Gillies & de Meillon, 1968; Gillies & Coetzee, 1987). The female *Anopheles gambiae* sensu lato were preserved dry over silica gel in well-labelled eppendorf tubes. DNA were extracted from 44 adult female mosquitoes using a method described by Collins *et al*. (1987). These 44 specimens were

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randomly selected from the preserved mosquito samples. Molecular species identification of the *Anopheles gambiae* sibling species was carried out according to the polymerase chain reaction (PCR) method (Scott *et al*., 1993). Five oligonucleotide primers, GA, ME, AR, QD and UN designed from the DNA sequences of the intergenic spacer region of complex ribosomal DNA (rDNA) was used to amplify species-specific DNA sequences.

Figure 1: Double mosquito net trap

The relative abundance of mosquito species was expressed as the percentage of the proportion of the total number of *Anopheles* collected. Mosquito density per room was calculated for specific indoor collection techniques as total number of mosquitoes collected divided by total number of rooms in the sampled houses. The mean house density of mosquitoes was calculated as the total number of mosquitoes collected indoor divided by total number of houses involved in the indoor collection exercise. The overall mean density of host seeking mosquitoes (outdoor) was calculated as the total number of mosquitoes collected outdoor divided by total number of individuals who slept under bed net traps.

Table 1: Number and density of *An.gambiae* per room collected using mechanical aspirator and exit trap

Day	House 1	House 2	House 3	House 4	House 5	Total	Density/room
Mosquito collection using mechanical aspirator							
1	1	1	0	0	0	2	0.2
2	0	1	2	1	0	4	0.4
3	0	0	0	0	1	1	0.1
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
Total	**1**	**2**	**2**	**1**	**1**	**7**	**0.7**
Mosquito collection using window exit trap							
1	0	1	0	0	0	1	**0.1**
2	1	1	0	0	0	2	**0.3**
3	0	0	0	1	0	1	**0.1**
4	0	0	0	0	0	0	**0**
5	0	0	0	0	0	0	**0**

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Total	**1**	**2**	**0**	**1**	**0**	**4**	**0.5**

A total of 672 *An. gambiae* s.l. were collected, of these, 98.4% were collected outdoor whereas 1.6% were collected indoor. The exit trap catch: mechanical aspirator catch ratio was 1:1.75. The overall indoor resting density of *An. gambiae s.l.* as determined by mechanical aspirator and exit trap was 0.7 and 0.5 (Table 1) mosquitoes per room, respectively, whereas the overall density of the host-seeking as determined by bed net trap outdoor was 44.1 mosquitoes per person (Table 2). A sample of 44 specimens taken randomly from morphologically identified *An.gambiae* s.l. population was further analyzed to species level using molecular techniques. Of these 44 specimens 26 (59%) and 18 (41%) were *Anopheles arabiensis* and *Anopheles gambiae* sensu stricto respectively. Bed net trap has proved exceptionally useful for catching exophagic malaria vectors in the study area and can be used to target host seeking mosquitoes.

An. arabiensis was the predominant malaria vector in the study area. *An. gambie s.s.* is known to be most prevalent along the coastal belt of eastern Tanzania. This is the first report on *An. gambiae* s.s. in western Tanzania in an area located about 900km from the coastal belt. A study by Mboera (2000) also reported occurrence of *An. gambiae s.s.* in the Kilombero Valley located about 500km from the coast. *An. gambiae* s.s and *An. arabiensis* have been identified to be the major vectors of malaria in Tanzania (Mboera, 2000). In this study quite higher number *An. gambiae s.l.* was collected outdoor contrary to indoor. On the speciation relatively large number comprised of *An. arabiensis* that has been reported elsewhere by Mnzava & Kilama (1986) to be endophagic but exophilic. This implies few of them are likely to be caught indoor as most of the mosquitoes would rest outdoors after a blood meal although very few mosquitoes were collected using exit traps. Furthermore it may indicate that majority of the mosquitoes collected were host-seeking than resting and that it was relatively easier to locate hosts oudoor.

Table 2: Number and average density of *An.gambiae* s.l. per person collected outdoor in Uyui using bed net trap

Day	H*1	H* 2	H*3	H* 4	H*5	**Total**	Density/person**
1	10	14	17	13	15	**69**	**4.6**
2	8	17	23	19	12	**79**	**5.3**
3	25	21	37	24	32	**139**	**9.3**
4	28	25	42	34	38	**167**	**11.1**
5	35	39	50	45	38	**207**	**13.8**
Total	**106**	**116**	**169**	**135**	**135**	**661**	**44.1**

* Homestead; **Average density

It is recognized that the anthropophilic behaviour of *An.gambiae s.s.* and the opportunistic behaviours of *An. arabiensis* are genetically fixed (Coluzzi *et al*. 2002). However, in the study area *An. gambiae* s.s might be exihibiting exophagic behaviour that needs further investigation. This behavior is of practical importance because it makes them less vulnerable to indoor residual spraying or treated mosquito nets (ITNs/LLINs). Besides the fact that *An. gambiae s.s.* is extremely well-adapted for entering houses feeding on people and resting indoor (Gillies & De

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Meillon,1968) exophily has been reported elsewhere in northern Tanzania (Mahande *et al.*, 2007). This could be contributed by the presence of barrier such as ITNs that might influence the host seeking and resting preferences of this species. Repellent or irritancy effect of ITNs has been reported to divert mosquitoes biting outdoors instead of indoors in the early evening when people have not yet gone to bed (Zoulani *et al.,* 1994). Contribution of such physical barriers/irritancy effect in the current study area needs further investigation.

Although in our study the evidence regarding diversion to outdoor resting and biting is inconclusive, the higher number of host seeking *An. gambiae s.l.* collected outdoor suggests increased chances of outdoor malaria transmission in the study area. This study only covered part of the year i.e. rain season with limited time of data collection. Conclusively, the study contributes to the understanding of the distribution of malaria vectors with respect to species composition and their resting behaviour that could contribute to vector control operations in western Tanzania. A longitudinal study considering dry and wet seasons is likely to provide more information on the distribution, abundance and biting pattern & resting behavior of malaria vectors in the study area.

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