Identification of Anopheles Species of the Funestus Group and their Role in Malaria Transmission in Sudan

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Abstract—Anopheles mosquitoes of the Funestus group are important vectors of malaria in Africa. Although species of the group were known to occur in Sudan, there are no published reports on their involvement in malaria transmission. This study was carried out to elucidate the role of species of the Funestus group in malaria transmission in eastern Sudan. A total of 540 mosquitoes morphologically identified as members of the Funestus group were collected by Pyrthrum Spray Catch method from the Tabaldia, Batut Tabut Tabut and El Rugab villages in Gadarof State, Eastern Sudan during November 1999 – November 2000 and in December 2004 and subjected to species-specific PCR typing. This analysis demonstrated the existence of three An. funestus species in Sudan; namely An. rivulorum Leeson (60% of specimens), An. funestus Giles (29%) and An. leesoni (11%). To investigate the role An. funestus in transmission of malaria parasites a group of 92 females of this species were subjected to Plasmodium species-specific ELISA analysis. The only Plasmodium species found in the salivary glands of An. funestus was P. falciparum, which showed a total infection rate of 7.6% (7/92). The presence of these three species of the Funestus group in Sudan emphasizes the need to define their spatial and temporal distribution, their behaviour and ecology and their overall role in malaria transmission within Sudan. Results are discussed in relation to the epidemiology of malaria.

Index Terms — Anopheles funestus, molecular identification, sporozoite rate

I. INTRODUCTION

The Funestus group comprises ten species of very similar morphology, eight of which are formally named, and several of which play a significant role in malaria transmission in Africa [1, 2]. Member species of the group include An. funestus Giles, An. rivulorum Leeson, An. leesoni Evans, An. vaneedeni Gillies & Coetsee, An. confusus Evans & Lessoon, An. paresis Gillies, An. aruni Sobiti, An. fuscivenosus Leeson, and An. brucei Service. Recently an An. rivulorum-like mosquito has been identified [3, 4] collected from the extremes of its range; Eastern Africa (Kenya), Southern Africa (South Africa) and Western Africa (Burkina Faso). Additionally a new species of the group from Malawi has been identified, based on ribosomal D3 region, and provisionally named An. funestus-like [2]. Of these species only An. funestus and An. rivulorum have previously been found to play a major role in malaria transmission and their vector competence is similar to that of An. gambiae [3, 5]. Of the ten species, An. funestus is the only member of the group that plays a significant role in the transmission of human malaria throughout the African continent, although other species of the group have been found naturally infected with Plasmodium falciparum [6]. An. funestuss.s. has a widespread distribution over most of the malarious areas of Africa, extending from northern Sudan to South Africa and across West Africa to northern Mali and Senegal [7]. An. rivulorum and An. leesoni are widespread, occurring from Ethiopia through to the northern parts of South Africa and across West Africa. The other members of the group are localized: An. vaneedeni occurs only in Mpumalanga and the Northern Province in South Africa, An. aruni from Zanzibar, An. fuscivenosus from Zimbabwe, and An. brucei from Nigeria [8]. Studies in South Africa have shown that four members of the An. funestus group can be collected resting indoors: An. funestus, An. rivulorum, An. paresis, and An. leesoni [9]. This underlines the importance of identifying the member species of the Funestus group. In the past species identification has mainly been performed using either morphological or cytotegenic methods. However, the development of PCR-based methods has greatly facilitated the identification of species of the group [10, 3].

It is commonly asserted that malaria transmission in Africa is maintained by members of the An. gambiae complex [11]. However, in several parts of the continent other mosquito species contribute to the transmission of the parasite, including An. funestus and An. nili. All previous studies in Sudan stressed that An. arabiensis is the main, if not the only vector responsible for transmission of malaria parasites [12, 13]. Although An. funestus, An. pharoensis and An. nili have been encountered in several malaria foci, their role in the transmission of malaria in Sudan has not been further studied. In this paper we provide evidence incriminating An. funestus in the transmission of malaria in Sudan. In addition, we demonstrate the presence of three species of the Funestus group in eastern Sudan where a malaria transmission is a serious public health problem.

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II. MATERIALS AND METHODS

Study area
The study was carried out in three villages in Rahad province, Gedaref State, eastern Sudan: Batta (12.53N-35.7E), El Rugab (12.47N – 35.16E) and Tabaldia (12.53N-35.9E). The villages lie along eastern bank of El Rahad River, which is a seasonal watercourse that flows during and after the rainy season, June - December and then fragment into small water pools in the dry hot season. The pools that are formed by the river become adequate breeding habitats for mosquitoes during this period. The study was performed during November 1999 – November 2000 and December 2004. The study area has a tropical continental climate with two seasons: a rainy season lasting from June - October and a dry season between November to May. The average annual rainfall in the study area is 815 mm. The dry season is divided into two phases, warm winter, with an average min-max temperature of 17.9-26.2°C and a hot summer, with an average min-max temperature of 34-40°C.

Mosquito collection and identification
Mosquitoes were collected by a standard Pyrethrum Spray Catch (PSC) knock down technique [14] from indoor sites in the three villages. Collected mosquitoes were identified morphologically using the key described by Gillies & DeMeillon [7] and Gillies & Coetzee [8].

Molecular identification of the An. funestus group members
Following morphological identification, representative specimens of Anopheles funestus were subjected to molecular typing, using the species-specific protocols described by [10] and [3]. Briefly, genomic DNA was extracted and purified using Phenol-chloroform extraction method of Ballinger-Crabtree et al. [15] with minor modifications. A final 25 μL reaction volume of PCR contained 2.5 μL of 10× buffer including 15 mM MgCl₂, 5 pmol of each primer, 200 μM of each dNTP, and 0.5 units of Taq polymerase unit. Amplification started with an initial denaturation step at 94°C for two minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 30 seconds, and elongation at 72°C for 40 seconds, with a final extension step at 72°C for five minutes. The PCR products were loaded and visualized on regular 1.5% agarose gels following staining with ethidium bromide.

Estimation of sporozoite rate within collected Anopheles funestus mosquitoes
To determine role of An. funestus in transmission of malaria parasites in the area, a total of 92 females were subjected to sporozoite detection using ELISA technique [16].

III. RESULTS AND DISCUSSION

A total of 540 Funestus group mosquitoes were collected from three villages in Gadaref State on two occasions. The first covered the period between November 1999 and November 2000 and the second collection was carried out in December 2004 (Table 1).

Table 1: Density of Anopheles funestus group mosquitoes collected from Tabaldia, Batta and El Rugab villages (Rahad Province, Gadaref State, Eastern Sudan) during November 1999- November 2000 and December 2004.

<table>
<thead>
<tr>
<th>Time of survey</th>
<th>No. of An. funestus mosquitoes collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov.99-Feb.2000</td>
<td>225</td>
</tr>
<tr>
<td>Oct.-Nov.2000</td>
<td>183</td>
</tr>
<tr>
<td>Dec., 2004*</td>
<td>132</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>540</strong></td>
</tr>
</tbody>
</table>

*These specimens have been submitted for molecular identification.

Molecular identification of An. funestus group species
The 540 individuals found resting indoors during the first study period were identified as members of the Funestus group, but were not assigned to species. All 132 specimens collected during December 2004 were identified to species using a species-specific method PCR based on the internal transcribed spacer ITS2 [10]. This revealed the presence of three species of the Funestus group: Anopheles funestus Giles, An. rivulorum Leeson, and An. leesoni Evans (Table 1). These species showed the expected bands of 505bp, 411bp and 146bp respectively (Figure 1).
Figure 1: PCR identification of *Anopheles funestus* specimens collected from Tabaldia, Batta and El Rugab villages in eastern Sudan. L = ladder (1000bp), N = negative control, lane 1 & 2 *An. funestus* s.s. (505bp), lane 3 & 4 *An. rivulorum* (411bp), lane 5 & 6 *An. lessoni* (146bp).

*Anopheles rivulorum* was the most abundant species in the collections, followed by *An. funestus* and *An. lessoni*.

**Sporozoite rate in An. funestus collected from Batta village during study period**

Relatively high rates of mature infections of *Plasmodium malariae* were detected in *An. funestus* collected in the study area. In the two collection periods, four out of 15 and 3 out of 77 of *An. funestus* collected from Batta village were found to be infected with sporozoites of the parasite. Interestingly, no infected mosquitoes were found in the Tabaldia village, which was under an insecticide impregnated bednet programme.

**Table 2: Three species of An. funestus group were identified based on ITS2 (Koekemoer et al., 2002).**

<table>
<thead>
<tr>
<th>Mosquito spp</th>
<th>Band size (bp)</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. funestus</em></td>
<td>505</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td><em>An. rivulorum</em></td>
<td>411</td>
<td>21</td>
<td>60</td>
</tr>
<tr>
<td><em>An. leesoni</em></td>
<td>146</td>
<td>04</td>
<td>11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>35</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

This is the first report on molecular typing and plasmodium infection in *Anopheles funestus* in Sudan. Although this vector has been reported from different sites in East Africa and has been shown to play a major role in malaria transmission in Africa, no information is available on its infection with malaria parasites or related species in Sudan.

Our results show presence of three members of the Funestus group in eastern Sudan, *An. funestus* s.s., *An. rivulorum*, and *An. leesoni*. The abundance of *An. funestus* and *An. rivulorum* is higher than *An. leesoni*.

It is interesting that the three species were found sympatrically in the study villages. Similar results have also been reported in western and coastal Kenya [17], suggesting existence of a strong reproductive isolation mechanisms between them [9, 18]. Molecular characterization of the intergenic transcribed spacer (ITS) region of the ribosomal DNA (rDNA) is required for further studies in these species.

In this study we found moderately high sporozoite rate of *Plasmodium malariae* in *An. funestus* collected from the study villages. No other *Plasmodium* species was detected in these mosquitoes.

The importance of different members of *An. funestus* group in malaria transmission has been reviewed by Temu et al. [5]. Of the three members identified in current study, only *An. funestus* s.s. and *An. rivulorum* are suspected to play role in malaria transmission. Although sporozoites of malaria parasites were detected in *An. funestus* females collected in the study, no species typing was done for these specimens. Similarly, the two species *An. funestus* s.s., *An. rivulorum* were reported as an important malaria vectors in the Tanzania [19, 20].

We did not attempt to identify the breeding habitat of *An. funestus* in the study area. Based on previous literature reports, it is likely that this mosquito breeds in the water pools in the seasonal Atbar and Rahad rivers in Gadaref State. This area is characterized by dense vegetation that provides suitable conditions of breeding and resting sites *An. funestus* group that are typical throughout Savannah environment in most parts of Africa [21].

The collection method used in our study was restricted to the PSC method which identifies indoor resting mosquitoes. The study not investigated the nocturnal biting activities of these mosquitoes. In other studies, clear variation was found in the behaviour of different members of the *An. funestus* group. For example, Kamau et al., [22] and Awolola et al., [18] reported that in Kenya and Nigeria, *An. funestus* s.s. *An. Rivulorum* were
found almost exclusively either inside or outside human dwellings respectively.

In conclusion, since mosquito abundance displays temporal and spatial fluctuation and since more than one species within the *An. Funestus* group was found infected with *P. falciparum* as reported in Tanzania [5], it will be important to define the distribution of the *An. funestuss.s.*, *An. rivulorum*, and *An. leesoni* in areas of differing levels of malaria endemicity. Among members of the Funestus group, the species composition and species diversity is likely to differ locally and hence result in a significant impact on the effective management of malaria vector control.

**Authors’ contributions**

Listed authors contributed to the design of the study and critical review of the draft manuscript. All authors read and approved the final manuscript.

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**REFERENCES**


