ABSTRACT

Morphological similarity between malaria vectors and non-vectors occurring in sympatry has serious consequences if the killer diseases have to be controlled. Malaria in Malawi is transmitted by *Anopheles gambiae, An. arabiensis* and *An. funestus*. This vector diversity is further complicated by the recently discovered *An. funestus*-like species which is morphologically similar to *An. funestus,* and found in association with humans. Currently there is no single assay available that differentiates *An. funestus*-like from the other African members in the *An. funestus* group.

The objective of this study was to investigate the biology and behavior of the newly discovered *An. funestus*-like species and its possible role in malaria transmission. This information will assist in the implementation of vector control programs. In addition to this, the study investigated the development of a DNA based assay to differentiate between the members of the *An. funestus* group and to morphologically described *An. funestus*-like species.

Anopheles mosquitoes were collected resting indoors and outdoors from Karonga in Malawi. Specimens were identified morphologically and molecularly using chain reaction PCR. Identified samples were analyzed by ELISA for blood meal source and *Plasmodium* sporozoite infection. *Anopheles funestus*-like was morphologically compared with *An. funestus*. Real time based PCR was developed and compared to the current multiplex or allele-specific PCR (AS-PCR) assay for sensitivity and performance. The IGS region of the rDNA gene was investigated for development of AS-PCR. Phylogenetic relationship of mosquitoes was constructed from ITS2 and D3 sequences.

Adult *An. funestus* mosquitoes (n = 391) were collected during April and September, 2010. Karonga contributed 63.9% and Likoma Island 36.1%. Of the identified specimens (n = 347) *An. funestus*-like comprised 10.4%, *An. rivulorum* 31.7%, *An. funestus* 57.3% and *An. parensis* 0.6%. Most of the *An. funestus*-like species were collected resting indoors 91.7% (33/36) compared to outdoors 8.3% (3/36). The species was predominant during the dry season 63.9% (23/36) compared to the wet season. A total of 19 *An. funestus*-like females were analyzed for blood meal source. Mixed blood meal from goat and bovine was found in 7 specimens and a single blood meal from goat in 3 specimens.. The rest of the *An. funestus*-like was negative for blood meal. An overall dry season infection rate of *An. funestus*-like species by *Plasmodium vivax* was 5% (1/20) in this study and 3.1% (2/64) from samples collected in 2009 was found. However, the possibility of false positivity could not be excluded and further study is urgently needed to investigate this. Real-time PCR for the identification of members of the *An. funestus* group was found to be more sensitive (0.02ng/µl) than AS-PCR (0.04ng/µl) and had performance comparable to AS-PCR. AS-PCR developed from the intergenic spacer region of rDNA discriminates *An. funestus*, *An. rivulorum*, *An. vaneedeni* and *An. parensis*.

Of all assays developed in this study, the hydrolysis probe assay is the most reliable assay for identifying members in the *An. funestus* group. This study confirmed the existence of *An. funestus*-like species in sympatry with *An. funestus* group members. *An. funestus*-like was predominantly found resting indoors (endophilic) but preferring animal over human blood (zoophilic). No consistent morphological characters were found to discriminate between *An. funestus* and *An. funestus*-like based on morphological data, *An. funestus*-like is very similar and closely related to *An. funestus* which is supported by phylogenetic analysis. However, Restriction Fragment Length Polymorphism separates the two species.