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ORIGINAL PAPER

Spatial distribution, blood feeding pattern, and role of *Anopheles funestus* complex in malaria transmission in central Kenya

Ephantus J. Muturi · Luna Kamau · Benjamin G. Jacob · Simon Muriu · Charles M. Mbogo · Josephat Shililu · John Githure · Robert J. Novak

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Abstract Studies were conducted to determine the role of sibling species of *Anopheles funestus* complex in malaria transmission in three agro-ecosystems in central Kenya. Mosquitoes were sampled indoors and outdoors, and rDNA PCR was successfully used to identify 340 specimens. *Anopheles parensis* (91.8%), *A. funestus* (6.8%), and *Anopheles leesoni* (1.5%) were the three sibling species identified. *A. parensis* was the dominant species at all study sites, while 22 of 23 *A. funestus* were collected in the non-irrigated study site. None of the 362 specimens tested was positive for *Plasmodium falciparum* circumsporozoite proteins by enzyme-linked immunosorbent assay. The most common blood-meal sources (mixed blood meals included) for *A. parensis* were goat (54.0%), human (47.6%), and bovine (39.7%), while the few *A. funestus* s.s. samples had fed mostly on humans. The human blood index (HBI) for *A. parensis* (mixed blood meals included) in the non-irrigated agro-ecosystem was 0.93 and significantly higher than 0.33 in planned rice agro-ecosystem. The few samples of *A. funestus* s.s. and *A. funestus* s.l. also showed a trend of higher HBI in the non-irrigated agro-ecosystem. We conclude that agricultural practices have significant influence on distribution and blood feeding behavior of *A. funestus* complex. Although none of the species was implicated with malaria transmission, these results may partly explain why non-irrigated agro-ecosystems are associated with higher risk of malaria transmission by this species compared to irrigated agro-ecosystems.

E. J. Muturi (✉) · B. G. Jacob · R. J. Novak
Department of Medicine, William C. Gorgas Center for Geographic Medicine, University of Alabama at Birmingham, 206-C BBRB, 845 19th Street South Birmingham, Birmingham, 35294 AL, USA
e-mail: emuturi@uab.edu

L. Kamau
Center for Biotechnology Research and Development, Kenya Medical Research Institute, Nairobi, Kenya

S. Muriu · J. Shililu · J. Githure
Human Health Division, International Centre of Insect Physiology and Ecology, Nairobi, Kenya

C. M. Mbogo
Kenya Medical Research Institute, Centre for Geographic Medicine Research—Coast, Kilifi, Kenya

Introduction

Anopheles funestus Giles is one of the major vectors of malaria in sub-Saharan Africa. This species is an important bridge vector for malaria transmission during the dry season because their larvae develop in permanent swampy habitats that continue to be productive when *Anopheles gambiae* Giles habitats shrink (Gillies and De Meillon 1968). At taxonomic level, *A. funestus* comprises at least nine sibling species that are morphologically indistinguishable as adults and barely as eggs and larvae, yet they differ in their behavioral and vectorial attributes (Gillies and Coetzee 1987). *A. funestus* sensu stricto (henceforth referred to as *A. funestus*) is highly anthropophilic and a major vector of malaria, while the other species, *Anopheles vaneedeni* Gillies and Coetzee, *Anopheles parensis* Gillies, *Anopheles aruni* Sobti, *Anopheles confusus* Evans and Leesoni, *Anopheles rivulorum* Leesoni, *Anopheles fuscivenosus* Leesoni, *Anopheles leesoni* Evans, and *Anopheles brucei* Service are mainly zoophilic and non-vectors.

However, *A. rivulorum* is known to be a minor vector in Tanzania (Wilkes et al. 1996) and *A. vaneedeni* has shown experimental susceptibility to *Plasmodium falciparum* in the laboratory (De Meillon et al. 1977) but no evidence of malaria transmission in nature. Analysis of rDNA sequences has revealed the occurrence of a new taxon provisionally referred to as *A. rivulorum*-like because of its relatedness to *A. rivulorum* (Cohuet et al. 2003).

Precise identification of members of the *A. funestus* complex is essential for vector control programs because misidentification of non-vector species as *A. funestus* can lead to massive wastage of time and resources through misdirected vector control efforts. The choice of vector control tactic to be used is also dependent on behavior of the target vector species. For instance, indoor residual spraying relies upon mosquitoes resting on the sprayed surface and picking up lethal doses of insecticide. This method is therefore ineffective against exophilic and exophagic members of the *funestus* complex.

Despite the presence of larval habitats that appear conducive for *A. funestus* in irrigated rice agro-ecosystems in Africa, this species is less frequently encountered compared to *A. gambiae* s.l. Ahero rice irrigation scheme; Kenya and the highlands of Madagascar are the only two places where this species occurs abundantly in relation to rice cultivation (Marrama et al. 1995; Githeko et al. 1996). It is unclear why *A. funestus* does not do well in African rice agro-ecosystems but it could be due to some unknown aspects of its biology as well as vector control activities. Interestingly, this species plays a significant role in malaria transmission in these areas (Ijumba et al. 1990; Ijumba et al. 2002; Dolo et al. 2004; Muturi et al. 2008), yet its species composition, and the role of different members of the complex in malaria transmission, has been least studied (Kamau et al. 2003a).

In a recent study conducted in the Mwea Rice Irrigation Scheme Central Kenya, the numbers of *A. funestus* were 11-fold lower than those of *Anopheles arabiensis* but the human blood index (HBI) for *A. funestus* was 3-fold higher than for *A. arabiensis* (Muriu et al. 2008). Further studies revealed that adult mosquito densities, HBI, and the risk of malaria transmission by *A. funestus* complex were significantly lower within the scheme than in neighboring non-irrigated villages (Muriu et al. 2008; Muturi et al. 2008). Earlier studies based on indoor collected samples within the rice scheme had shown that *A. parensis* (99.8%) and *A. leesoni* (one specimen) were the two species of *A. funestus* complex occurring in the area, and none was positive for malaria parasites (Kamau et al. 2003a). Apart from these studies, little is known about the species composition of *A. funestus* complex and their role in malaria transmission in Mwea Rice Irrigation Scheme and adjacent areas. This information is critical for development of effective evidence-based vector control programs specifically tailored to malaria vectors within this species complex.

The objective of this study was to determine how different agricultural practices influence the distribution and blood feeding pattern of sibling species of *A. funestus* complex and the role of different species within the complex in malaria transmission. Because the HBI for *A. funestus* s.l. increases with increasing distance from the rice scheme (Muturi et al. 2008), it was our hypothesis that species composition shifts from *A. parensis* or other zoophilic members of the complex within the scheme to anthropophilic *A. funestus* outside the scheme.

Materials and methods

Study sites

The study was conducted in five villages, Mbuinjeru, Karima, Rurumi, Kiamachiri, and Murinduko located in Mwea division, Kirinyaga District, 100 km North East of Nairobi. The study sites have been described in details previously (Muturi et al. 2006; Muturi et al. 2007). The study area has two annual rainfall seasons, the long rains in April/May and the short rains in October/November. The annual average rainfall is 950 mm. The average maximum temperatures are in the range of 16–26.5°C and the average relative humidity varies from 52% to 67%. *A. arabiensis* and *A. funestus* s.l. are the main drivers of malaria transmission in the area (Muturi et al. 2008). The prevalence of malaria in Mbuinjeru, Kiamachiri, and Murinduko among children 9 years and younger is 0%, 17%, and 54%, respectively (Mutero et al. 2004).

Mbuinjeru, Karima, and Rurumi are within the Mwea Rice Irrigation Scheme, and about 75% of land is under rice cultivation. Farmers in these villages follow a defined rice cropping cycle as determined by the National Irrigation Board (planned rice cultivation). Kiamachiri is ≈5 km away from the rice scheme, and about 20% of the village land is used for rice cultivation. Individual farmers decide their own cropping cycle depending on water availability. Murinduko is ≈15–20 km from the scheme and is generally a non-rice growing village mainly because of its hilly terrain that renders much of the area (about 90%) unsuitable for rice cultivation. Limited rice growing activity (<5% of the total area) is done along one major river valley that runs along the edge of the village.

Mosquito sampling and handling

Anopheline mosquitoes were sampled twice per month between April 2005 and March 2007 by two sampling methods: pyrethrum spray catch (PSC) technique (World

Health Organization 1975) for indoor population and Centers for Disease Control and Prevention (CDC) light traps (J.W. Hock Ltd, Gainesville, FL, USA) for outdoor population. PSC was conducted twice per month in 20 randomly selected houses, and six CDC light traps were operated the night before and the night after PSC. The samples were transported to the laboratory and identified to species using morphologic characteristics (Gillies and Coetzee 1987). All *A. funestus* samples were scored as unfed, blood-fed, semi-gravid, or gravid by visual examination of abdomen under a dissecting microscope and later preserved dry in silica gel. A detailed description of the sampling strategy can be found elsewhere (Muturi et al. 2006).

Sample processing and analysis

The heads and thoraces of all *A. funestus* were tested for *P. falciparum* circumsporozoite proteins by enzyme-linked immunosorbent assay (ELISA; Wirtz et al. 1987). The hind guts of blood-fed samples were tested for human, cattle, goat, chicken, dog, and cat as possible hosts by ELISA (Beier et al. 1988). The legs and wings of each specimen were used for *A. funestus* sibling species identification by rDNA polymerase chain reaction (Koekemoer et al. 2002). Chi-square test was used to determine whether the human blood index for *A. parensis* differed significantly among the three study sites.

Results

In total, 340 out of 362 specimens were successfully characterized into respective species of *A. funestus* complex by PCR (Table 1). *A. parensis* (91.8%), *A. funestus* (6.8%), and *A. leesoni* (1.5%) were the three sibling species identified in the samples. *A. parensis* was the dominant species at all study sites, while 22 of the 23 samples of *A. funestus* identified in this study were from the non-irrigated study site. Of the 66 *A. funestus* s.l. samples collected in Murinduko, 20 (all unfed) were from light trap collections outdoors. These comprised 10 *A. parensis*, six *A. funestus*, and four *A. leesoni*. The remaining samples as well as those from the other study sites were collected indoors. None of the 342 specimens examined for *P. falciparum* circumsporozoite proteins by ELISA tested positive. Because of the rarity in which members of *A. funestus* complex occur in the area, only 94 blood-fed specimens were collected during the study period and tested for host blood meal (Table 2). Including specimens that had mixed blood meals, goat (54.0%), human (47.6%), and bovine (39.7%) were the most common blood-meal sources for *A. parensis*, while the few *A. funestus* s.s. (100.0%) and *A. funestus* s.l. (78.6%) samples had fed mostly on humans. A few specimens were found to have fed on dogs, cats, and chicken. The HBI for *A. parensis* (including specimens with mixed blood meals) in the non-irrigated agro-ecosystem was 0.93 and significantly higher than 0.33 in planned rice agro-ecosystem (χ^2=16.08, *df*=1, *P*<0.01). Samples of *A. parensis* in unplanned rice agro-ecosystem were too few to allow logical comparison of HBI (0.67) with other study sites. Similarly, there were too few samples of *A. funestus* s.s. and *A. funestus* s.l. to facilitate reasonable comparisons of blood-meal hosts in the three study sites. However, *A. funestus* s.l. showed a trend of high HBI in the non-irrigated agro-ecosystem (0.86) than in planned rice agro-ecosystem (0.57). The three blood-fed *A. funestus* were collected in the non-irrigated agro-ecosystem and had human blood meals. Mixed blood feeding was also a common phenomenon (Table 2).

Discussion

A. parensis, *A. funestus*, and *A. leesoni* were the three sibling species of *A. funestus* complex identified in the study area. *A. parensis* was the dominant species in the three study sites, whereas 96% of all *A. funestus* occurred in the non-irrigated site. Previous studies in the same area had shown that *A. parensis* and *A. leesoni* are the two species of *funestus* complex occurring within the rice scheme, the former constituting 99.8% of the samples (Kamau et al. 2003a; Kamau et al. 2003b). Approximately 6% of the

Table 1 Members of the *Anopheles funestus* complex identified in the study area

	Village	No. of specimens	*A. funestus*	*A. parensis*	*A. leesoni*
Planned rice agro-ecosystem	Mbuinjeru	29	0 (0.0)	29 (100.0)	0 (0.0)
	Karima	206	1 (0.5)	205 (99.5)	0 (0.0)
	Rurumi	14	0 (0.0)	14 (100.0)	0 (0.0)
	Total	249	1 (0.4)	248 (99.6)	0 (0.0)
Unplanned rice agro-ecosystem	Kiamachiri	25	0 (0.0)	24 (96.0)	1(4.0)
Non-irrigated agro-ecosystem	Murinduko	66	22 (33.3)	40 (60.6)	4 (6.1)
	All sites	340	23 (6.8)	312 (91.8)	5 (1.5)

Table 2 Blood-meal hosts of three sibling species of *A. funestus* complex in Mwea, Kenya

	A. parensis			*A. funestus s.s.*			*A. funestus s.l.*		
Host	Planned	Unplanned	Non-irrigated	Planned	Unplanned	Non-irrigated	Planned	Unplanned	Non-irrigated
Human	6 (13.0)	1 (33.3)	12 (85.7)	–	–	2 (66.7)	2 (28.6)	–	14 (66.7)
Bovine	7 (15.2)			–	–	–	–	–	1 (4.8)
Goat	8 (17.4)	1 (33.3)	1 (7.1)	–	–	–	–	–	1 (4.8)
Dog	–	–	–	–	–	–	–	–	–
Chicken	–	–	–	–	–	–	–	–	–
Goat/bovine	14 (30.4)	–	–	–	–	–	3 (42.9)	–	–
Human/goat	7 (15.2)	1 (33.3)	–	–	–	–	2 (28.6)	–	1 (4.8)
Human/bovine	1 (2.2)	–	–	–	–	1 (33.3)	–	–	1 (4.8)
Human/dog	–	–	–	–	–	–	–	–	1 (4.8)
Human/chicken	–	–	1 (7.1)	–	–	–	–	–	–
Bovine/goat	–	–	–	–	–	–	–	–	1 (4.8)
Bovine/chicken	1 (2.2)	–	–	–	–	–	–	–	–
Bovine/chicken/goat	1 (2.2)	–	–	–	–	–	–	–	–
Human/dog/goat	1 (2.2)	–	–	–	–	–	–	–	–
Human/dog/chicken	–	–	–	–	–	–	–	–	1 (4.8)
Total	46	3	14	–	–	3	7	–	21

Dash (–) indicate that no species was represented in that category

specimens morphologically recorded as *A. funestus* s.l. by two independent experts could not be identified by PCR. Possible explanations for lack of amplification in PCR may include misidentification of some specimens, DNA degradation due to problems during storage of samples, or DNA extraction process as well as presence of other members of *A. funestus* complex that could not be identified in this study (Kamau et al. 2003a; Temu et al. 2007). The PCR method used in this study identifies five of the nine members of the *funestus* complex: *A. funestus s.s*, *A. parensis*, *A. rivulorum*, *A. leesoni*, and *A. vaneedeni*.

Sympatric occurrence of members of *A. funestus* complex in Africa is not uncommon. Studies across 10 isolated study sites in Kenya (Kamau et al. 2003b) and in coastal Tanzania (Temu et al. 2007) demonstrated that up to four sibling species of *A. funestus* complex, *A. funestus*, *A. parensis*, *A. rivulorum*, and *A. leesoni* occur in sympatry. In the latter study, *A. funestus* was the dominant species, whereas in the former, species distribution and abundance varied across the study sites. For instance, *A. parensis* was the dominant species in Mwea and Baringo, Kenya, while *A. funestus* was dominant in western Kenya (Kamau et al. 2003b). Such observations highlight the importance of correct identification of members of *funestus* complex to malaria control programs.

Very few samples of blood-fed *A. funestus* s.l. were caught in the current study, significantly reducing the statistical power of our results. Because this species occurs in relatively low numbers than the main malaria vector, *A. arabiensis* (Muturi et al. 2006), we believe the current data, though insufficient for detailed conclusions, provide a general picture of blood-meal hosts for *A. funestus* group. *A. parensis* fed mostly on goat (54.0%), human (47.6%), and bovine (39.7%) while the three blood-fed *A. funestus* samples had human blood meals. The highly anthropophilic nature of *A. parensis* in the study area contradicts previous findings that this species is zoophilic (Kamau et al. 2003a). Nothing much can be said about *A. funestus* because of the small sample size, but detection of human blood meals in the three *A. funestus* samples suggests that this species is anthropophilic. The indoor presence of zoophilic *A. parensis* is indicative of exophagic and endophilic behavior.

In our previous studies, we reported that anthropophily by *A. funestus* s.l. was significantly higher in adjacent non-irrigated areas than within the rice scheme (Muriu et al. 2008; Muturi et al. 2008). The HBI for *A. parensis* and *A. funestus* s.l. in the current study followed this pattern, confirming that land use has significant impact on blood feeding behavior of members of *A. funestus* complex. Several reasons are believed to account for reduced anthropophily of malaria vectors in irrigated rice agro-ecosystems. These include wide-scale use of bednets as a result of higher mosquito densities (Mutero et al. 2004) especially *A. gambiae* s.l. and *Culex quinquefasciatus*, large numbers of cattle that cause a shift from human to animal feeding, as well as economic empowerment created by rice cultivation, enabling communities to invest on mosquito-bite protection measures (Ijumba and Lindsay 2001).

The role of the three members of *funestus* group in malaria transmission in the study area remains questionable. All *A. parensis* and *A. funestus* samples that were tested for *P. falciparum* cirmsporozoite proteins by ELISA were negative. Previous studies that did not distinguish between the different members of this species found *A. funestus* s.l. to be an important malaria vector in the area (Ijumba et al. 1990). Further studies indicated that this species was more abundant and an important malaria vector in neighboring non-irrigated villages than within the scheme (Muturi et al. 2008). Because *A. parensis* was found to be zoophilic and non-vector within the scheme (Kamau et al. 2003a), it was expected that *A. funestus* would be more common in surrounding non-irrigated villages than within the scheme. Unfortunately, very few samples were identified as *A. funestus* to permit evaluation of this prediction. The trend was however clear because 96% of *A. funestus* were from the non-irrigated. A recent study using PCR found the three species identified in this study to be positive for *P. falciparum* in coastal Tanzania (Temu et al. 2007). These findings together with reports of sporozoite positive *A. funestus* s.l. in the study area (Mukiama and Mwangi 1989; Ijumba et al. 1990; Muturi et al. 2008) suggest that the three species could be playing an unknown role in malaria transmission in similar areas. Further studies are needed to establish this role as well as ecology of the three sibling species.

One limitation of the current study is that samples were collected in three study sites in planned rice agro-ecosystem compared to one study site in other agro-ecosystems. The study sites within the planned rice agro-ecosystem did not show any disparity in mosquito species composition, feeding behavior, and sporozoite rates. We assumed that sites outside the scheme would have similar characteristics and effects on these parameters and would provide a broad perspective on the influence of land use on malaria transmission by *A. funestus* complex.

In conclusion, *A. parensis*, *A. funestus*, and *A. leesoni* were the three sibling species occurring in the study area. The results revealed that agricultural practices have significant effect on distribution and blood feeding patterns of *A. funestus* complex. We were unable to link any of the three species with malaria transmission in the study area. However, the increasing proportion of *A. funestus* and the high frequency of human–vector contact in the non-irrigated agro-ecosystem may partly explain why these areas are associated with greater risk of malaria transmission by *A. funestus* complex compared to irrigated agro-ecosystems.

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