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ANOPHELES PARENSIS: THE MAIN MEMBER OF THE *ANOPHELES FUNESTUS* SPECIES GROUP FOUND RESTING INSIDE HUMAN DWELLINGS IN MWEA AREA OF CENTRAL KENYA TOWARD THE END OF THE RAINY SEASON

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ABSTRACT. A total of 460 *Anopheles funestus* s.l. was collected inside human dwellings in Mwea, central Kenya. Of the 414 specimens that were positively identified, 1 was *Anopheles leesoni* and the others were *Anopheles parensis.* None of the 373 specimens tested were positive for *Plasmodium falciparum* or *Plasmodium malariae* sporozoites by enzyme-linked immunosorbent assay (ELISA). Of the 139 ELISA blood-meal–positive specimens, 82.01% and 1.44% had fed on cattle and humans, respectively. These results are discussed in the context of implications for malaria control through vector-control strategies.

KEY WORDS *Anopheles funestus* s.l., *Anopheles parensis,* sporozoite enzyme-linked immunosorbent assay, blood-meal enzyme-linked immunosorbent assay, malaria control

INTRODUCTION

Malaria is a major public health concern in most of sub-Saharan Africa, including Kenya, with *Anopheles gambiae* Giles, *Anopheles arabiensis* Patton, and *Anopheles funestus* Giles as the principal vectors. In the Mwea area of Kenya, important vectors include *An. arabiensis, An. funestus,* and *Anopheles pharoensis* Theobald (Mukiama and Mwangi 1989, Ijumba et al. 1990). The *An. funestus* group consists of at least 9 species that are difficult to distinguish based on morphological characteristics (Gillies and De Meillon 1968, Gillies and Coetzee 1987), although some species can be distinguished by egg and larval characteristics. The 9 species are *An. funestus, An. vaneedeni* Gillies and Coetzee, *An. parensis* Gillies, *An. aruni* Sobti, *An. confusus* Evans and Leeson, *An. rivulorum* Leeson, *An. fuscivenosus* Leeson, *An. leesoni* Evans, and *An. brucei* Service. Of these, *An. funestus* is the most anthropophilic and endophilic mosquito and is a major vector of malaria in sub-Saharan African. All the other members of the species group are believed not to be vectors, except for *An. rivulorum,* which was found to be a minor vector in Tanzania (Wilkes et al. 1996). Earlier studies showed that *An. vaneedeni* can be experimentally infected with *Plasmodium falciparum* in the laboratory (De Meillon et al. 1977), but to date no evidence has been found that this species plays a part in malaria transmission. *Anopheles parensis* has been found in Kenya, and it reaches high proportions in villages where residual house-spraying resulted in the elimination of *An. funestus* (Gillies and Furlong 1964), but none was found to be infected with malaria parasites. *Anopheles parensis* also has been found in KwaZulu/Natal, South Africa, resting indoors in formerly sprayed areas (Gillies and De Meillon 1968). More recently, Hargreaves et al. (2000) also found *An. rivulorum, An. leesoni,* and *An. parensis* along with *An. funestus* resting inside pyrethroid-sprayed houses in the same province. However, the blood-meal sources of these specimens were not determined. In the present study, we investigated the composition of specimens of *An. funestus* s.l. collected inside human dwellings in the Mwea area as well as the sporozoite infection rates and the blood-meal sources of the specimens in an attempt to better understand the role of this species group in malaria transmission in the area. This study was prompted by preliminary findings that 15 out of 15 *An. funestus* s.l. collected inside human dwellings in Mwea were *An. parensis* (Koekemoer et al. 2002).

MATERIALS AND METHODS

Study site: Specimens were collected from the Mwea (00°67′S, 37°35′E) area, which is a rice-growing area in central Kenya. The rice fields remain flooded during the growing season, which can extend from June to December. The irrigation canals are slow flowing with vegetation floating on the sides. Several streams also traverse the area. In addition to growing rice, residents of this area also grow vegetables and keep livestock and poultry for subsistence. Animal enclosures are found in very close proximity to the houses (about 10 m away or less). In one of the houses where specimens were collected, the chickens spent the night indoors in a room adjacent to the one where humans slept, with the partitioning wall leaving a 1-ft space to the roof.

Specimen collection: Several groups of scientists

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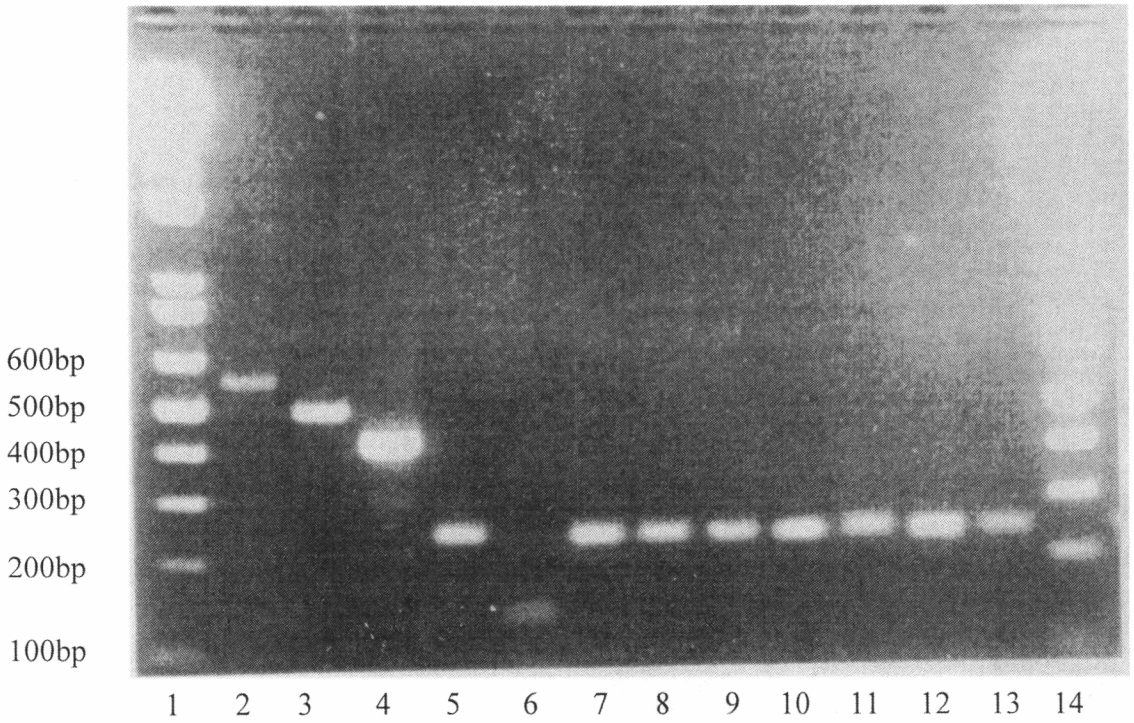

Fig. 1. The species-specific polymerase chain reaction assay for identification of members of the *Anopheles funestus* group. The figure shows amplified fragments. Lane 1: 1 kilobase (kb)-plus DNA ladder; lane 2: *An. vaneedeni* control (587 base pairs [bp]); lane 3: *An. funestus* control (505 bp); lane 4: *An. rivulorum* control (411 bp); lane 5: *An. parensis* control (252 bp); lane 6: *An. leesoni* control (146 bp); lanes 7–13: *An. parensis*; lane 14: 1 kb-plus DNA ladder.

have carried out research in this area and as such the residents have a basic understanding of the fact that mosquitoes are the vectors of malaria. Before carrying out our collections, a brief explanation of the aims of our study was given and a formal request was made for the owners of the houses to allow the field team to collect mosquitoes from their houses. It was emphasized that no one was under obligation to allow the collections. In fact, collections were not made in several houses where such permission was not granted. Collections were made from 30 houses by aspiration between June 12 and 15, 2001, toward the end of the rainy season. During this time, the rice paddies already were flooded in most places. Half-gravid specimens were immediately preserved in Carnoy's fixative (3:1 part ethanol : acetic acid, Sigma, Steinheim, Germany). Bloodfed specimens were held in paper cups and kept under surveillance and preserved in Carnoy's fixative when they became half-gravid. Gravid and unfed specimens were desiccated and stored.

Laboratory procedures: In the laboratory, the heads and thoraces of the bloodfed, half-gravid and gravid specimens were tested for infection with *Plasmodium falciparum* and *Plasmodium malariae* by using the enzyme-linked immunosorbent assay (ELISA) technique of Wirtz et al. (1987). For half-gravid specimens, the ovaries were separated from the blood meal. These blood meals, together with those from fully fed specimens, were tested with the ELISA method as described by Beier et al. (1988) for the source of the blood meal (all ELISA reagents were from Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD). All specimens were tested for cow, cat, dog, human, goat, and chicken as possible hosts. The ovaries were used for polytene chromosome preparation according to Hunt (1973) and all inversions scored according to the nomenclature of Green and Hunt (1980). Fixed inversions were used for species determination; *An. parensis* was distinguished from *An. funestus* s.s. by the fixed inversion c on arm 3, that is, 3c (Green and Hunt 1980). A newly developed rDNA species-specific polymerase chain reaction assay (Koekemoer et al. 2002) also was used for species identification (reagents were from Promega, Madison, WI). This technique uses 2 or 3 legs from each specimen and can be used on all specimens including fully fed, fully gravid, and unfed mosquitoes.

RESULTS

Of the 460 specimens collected, 413 were positively identified as *An. parensis* by either the species-specific PCR assay (Fig. 1) or by the cytotax-

Table 1. Percentages of the different and mixed blood meals for the *Anopheles parensis* that were positive for the blood-meal enzyme-linked immunosorbent assay.

Host	No. positive	% positive
Cow	114	82.01
Cat	9	6.47
Dog	7	5.04
Human	2	1.44
Goat	1	0.72
Chicken	0	0.00
Mixed hosts		
Cow/cat	1	0.72
Cow/dog	2	1.44
Cat/dog	3	2.16
Total	139	100

onomic technique (Green and Hunt 1980), and 1 specimen was identified as *An. leesoni* by the species-specific PCR assay. A total of 47 specimens was identified by using both methods. These 47 were fixed for inversions 2c, 2g, and 2f. No inversion differences between *An. parensis,* the *An. leesoni* specimen, and *An. funestus* were found on chromosomal arms 4 and 5. All 373 specimens tested were negative for both *P. falciparum* and *P. malariae* sporozoite by ELISAs. When using the blood-meal ELISA, the source of the blood meal could only be identified in 139 of 187 specimens (Table 1). The cow was the most preferred host, with 82.01% of all positive specimens having fed on this host, and only 1.44% of all positive specimens had fed on humans. No specimen was found positive for chicken blood. The proportion of specimens that had fed on more than 1 host was relatively low.

DISCUSSION

About 10% of the specimens could not be identified by either the cytotaxonomic technique (because they were at the wrong ovarian developmental stage) or by PCR. The lack of amplification in the PCR may be due to degradation of DNA associated with problems in storage or problems in the DNA extraction process. Additionally, the PCR that was used identifies only 5 of the most commonly found members of the *An. funestus* group namely, *An. funestus* s.l., *An. vaneedeni, An. parensis, An. rivulorum,* and *An. leesoni.* The specimens that failed to amplify possibly belong to 1 of the remaining 4 members of the group. Indeed, *An. confusus* has been found in Kenya (Gillies and De Meillon 1968).

The negative blood-meal results in 25.67% of the specimens tested may have been due to the small size of the blood meal in some specimens or because the mosquitoes had fed on a host for which tests were not conducted, for example, donkeys and pigs, which also are found in this area.

The specimens that were scored for chromosomal inversions were fixed for 2cgf. However, Green and Hunt (1980) found inversion 2f to float in studies that included a small sample from Kenya, but whether this inversion actually was found in the sample from Kenya is not clear.

Results from the present study confirm earlier studies that suggested that *An. parensis* does not play a role in malaria transmission. The human blood index for this species was extremely low (1.44%) and no mosquitoes were found to be infected with malaria parasites. Previous studies have indicated the nonvector status of this species (Gillies and De Meillon 1968).

Earlier studies carried out in the Mwea area suggested that *An. funestus,* along with *An. arabiensis,* was a major vector of malaria during the rainy season. This was attributed to relatively high sporozoite infection rates, although the human biting index was low and none of the *An. funestus* collected tested positive for human blood in the blood-meal ELISA (Ijumba et al. 1990). However, in a different study in the same area, *An. funestus* was not found to be a major vector of malaria, primarily because of the comparatively very low numbers collected, although the sporozite infection rates within the group were high (Mukiama and Mwangi 1989). Therefore, considering sporozoite infection rates, the earlier studies appear to be in discord with our findings. However, both studies did not distinguish between the different members of this species group. This is most likely due to the fact that the only available methods for identification at the time, namely morphological and cytotaxonomic identification, are time-consuming and cumbersome. The difficulty in morphological identification of *An. parensis* (Gillies and Coetzee 1987) stems from the fact that characteristics in the adult males are used, necessitating the rearing of field-collected specimens in the insectary. This process is time-consuming, more so for this group of species, which has proven difficult to colonize, and often results in high proportions of specimens not being identified. In addition, considerable overlap exists in the characteristics used. On the other hand, the cytotaxonomic method (Hunt 1973, Green and Hunt 1980) requires that specimens be at the half-gravid stage and therefore unfed, fully fed, fully gravid, and male specimens cannot be used. This technique also requires some degree of expertise. The recent development of PCR to differentiate between members of this group (Koekemoer et al. 2002) allows the identification of specimens with just minimal amounts of DNA such as 2 or 3 dried mosquito legs, which makes identification of all life stages and ovarian developmental stages possible.

The differences between our findings and those of the aforementioned studies may be reconciled by assuming that *An. funestus* s.s. constituted only a small proportion of the total *An. funestus* s.l. collections in the 2 studies but because of the high

vectorial potential associated with *An. funestus* s.s., the overall infection rates for the group were significantly high. The low human biting index and the absence of mosquitoes that had fed on humans in the study by Ijumba et al. (1990) attest to this possibility. If this is truly the case, we are then left to explain the circumstances that could have led to the apparently total absence of *An. funestus* s.s. in our study. The proportions of *An. funestus* s.s. possibly remain low but fluctuate to zero or near-zero numbers sometimes during the year and our sampling possibly coincided with such a time. Although no organized or coordinated insecticide programs for health and agriculture exist in the study area, the use by the local communities of insecticides in aerosols or mosquito coils and use of pesticides in growing rice is widespread. This possibly has lead to the eradication of *An. funestus* s.s. and the increase of populations of *An. parensis.* Earlier studies showed that the proportions of *An. parensis* peaked in areas where *An. funestus* had been eliminated through the use of insecticides (Gillies and De Meillon 1968).

Our findings have implications for the control of malaria through vector-control strategies. Because the main member of the *An. funestus* group of mosquitoes found in the study area appears not to have a role in the transmission of malaria, vector-control efforts directed at this species will be a waste of time and resources. This information is valuable in the rational choice of vector-control methods, especially in sub-Saharan Africa where resources are often limited. Our findings also underscore the importance of proper and complete identification of species in any vector-control effort.

However, the finding of *An. parensis* resting indoors after apparently feeding outdoors, raises the question of its possible involvement in malaria transmission. The fact that a small percentage (1.44%) had fed on humans is suggestive of a possible minor role in transmission. It remains to be investigated, through larger-scale studies, whether *An. parensis* does indeed play even a minor role in malaria transmission and how the population structure of the *An. funestus* species group of mosquitoes changes during different seasons in this area and elsewhere where malaria is a health concern.

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REFERENCES CITED

Beier JC, Perkins PV, Wirtz RA, Koros J, Diggs D, Gargan TP II, Koech DK. 1988. Blood meal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J Med Entomol* 25:9–16.

De Meillon B, Van Eeden GJ, Coetzee L, Coetzee M, Meiswinkel R, Du Toit LN, Hansford CF. 1977. Observations on a species of the *Anopheles funestus* subgroup, a suspected exophilic vector of malaria parasites in northeastern Transvaal, South Africa. *Mosq News* 37: 657–661.

Gillies MT, Coetzee M. 1987. *A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region)* Publication 55. Johannesburg, South Africa: South Africa Institute for Medical Research.

Gillies MT, De Meillon B. 1968. *The Anophelinae of Africa south of the Sahara* Publication 54. Johannesburg, South Africa: South African Institute for Medical Research.

Gillies MT, Furlong M. 1964. An investigation into the behaviour of *Anopheles parensis* Gillies at Malindi on the Kenyan coast. *Bull Entomol Res* 55:1.

Green CA, Hunt RH. 1980. Interpretation of variation in ovarian polytene chromosomes of *Anopheles funestus* Giles, *A. parensis* Gillies and *A. aruni*? *Genetica* 51: 187–195.

Hargreaves K, Koekemoer LL, Brooke BD, Hunt RH, Mthembu J, Coetzee M. 2000. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Med Vet Entomol* 14:181–189.

Hunt RH. 1973. A cytological technique for the study of *Anopheline gambiae* complex. *Parassitologia* 15:137–139.

Ijumba JN, Mwangi RW, Beier JC. 1990. Malaria transmission potential of *Anopheles* mosquitoes in the Mwea–Tebere irrigation scheme, Kenya. *Med Vet Entomol* 4:425–432.

Koekemoer LL, Kamau L, Hunt RH, Coetzee M. 2002. A cocktail polymerase chain reaction (PCR) assay to identify the *Anopheles funestus* (Diptera: Culicidae) group. *Am J Trop Med Hyg* 66:804–811.

Mukiama TK, Mwangi RM. 1989. Seasonal population changes and malaria transmission potential of *Anopheles pharoensis* and the minor anophelines in Mwea Irrigation Scheme, Kenya. *Acta Trop* 46:181–189.

Wilkes TJ, Matola YG, Charlwood JD. 1996. *Anopheles rivulorum,* a vector of human malaria in Africa. *Med Vet Entomol* 10:108–110.

Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG. 1987. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* for ELISA development. *Bull WHO* 65:39–45.