



Spatio-temporal distribution of mosquitoes and risk of malaria infection in Rwanda



Emmanuel Hakizimana^{a,b}, Corine Karema^{c,d,k}, Dunia Munyakanage^a, John Githure^e, Jean Baptiste Mazarati^f, Jon Eric Tongren^g, Willem Takken^b, Agnes Binagwaho^{h,i,j}, Constantianus J.M. Koenraadt^{b,*}

^a Malaria and Other Parasitic Diseases Division, Rwanda Biomedical Centre (RBC), Ministry of Health, Rwanda

^b Laboratory of Entomology, Wageningen University & Research, PO Box 16, 6700 AA, Wageningen, The Netherlands

^c Swiss Tropical and Public Health Institute, University of Basel, Switzerland

^d University of Basel, Switzerland

^e Abt Associates Inc. Integrated Vector Management Project, MOPDD, Rwanda

^f Biomedical Services Department, RBC, Kigali- Rwanda, Ministry of Health, Rwanda

^g USAID/PMI Office, Kigali, Rwanda

^h Department of Global Health and Social Medicine, Harvard Medical School, Boston, USA

ⁱ Geisel School of Medicine, Dartmouth College, Hanover, USA

^j University of Global Health Equity, Kigali, Rwanda

^k Quality and Equity healthcare, KN 67 ST, Kigali City, Rwanda

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ABSTRACT

To date, the Republic of Rwanda has not systematically reported on distribution, diversity and malaria infectivity rate of mosquito species throughout the country. Therefore, we assessed the spatial and temporal variation of mosquitoes in the domestic environment, as well as the nocturnal biting behavior and infection patterns of the main malaria vectors in Rwanda. For this purpose, mosquitoes were collected monthly from 2010 to 2013 by human landing catches (HLC) and pyrethrum spray collections (PSC) in seven sentinel sites. Mosquitoes were identified using morphological characteristics and PCR. *Plasmodium falciparum* sporozoite infection rates were determined using ELISA. A total of 340,684 mosquitoes was collected by HLC and 73.8% were morphologically identified as culicines and 26.2% as anophelines. Of the latter, 94.3% were *Anopheles gambiae* s.l., 0.4% *Anopheles funestus* and 5.3% other *Anopheles* species. Of *An. gambiae* s.l., *An. arabiensis* and *An. gambiae* s.s. represented 84.4% and 15.6%, respectively. Of all *An. gambiae* s.l. collected indoor and outdoor, the proportion collected indoors was 51.3% in 2010 and 44.9% in 2013. A total of 17,022 mosquitoes was collected by PSC of which 20.5% were *An. gambiae* s.l. and 79.5% were culicines. For the seven sentinel sites, the mean indoor density for *An. gambiae* s.l. varied from 0.0 to 1.0 mosquitoes/house/night. *P. falciparum* infection rates in mosquitoes varied from 0.87 to 4.06%. The entomological inoculation rate (EIR) ranged from 1.0 to 329.8 with an annual average of 99.5 infective bites/person/year. This longitudinal study shows, for the first time, the abundance, species composition, and entomological inoculation rate of malaria mosquitoes collected throughout Rwanda.

1. Introduction

Understanding heterogeneity in human malaria transmission intensity is important for National Malaria Control Programs in order to identify key differences and similarities that could be exploited to distribute valuable resources in a most optimal way. The most common entomological measure of malaria transmission intensity is the entomological inoculation rate (EIR), which is expressed as the number of

infectious bites per person per unit time, usually per year (Tusting et al., 2014). At present, vector control interventions are considered effective tools that are able to interrupt transmission (Shaukat et al., 2010). Unfortunately, both LLINs and IRS are chemical-based and as such, the extensive use of insecticides could have selected for development of resistance and may even lead to changes in feeding and resting behavior (Gatton et al., 2013; Ranson et al., 2011). In addition, *Anopheles* mosquito breeding sites are created through agricultural and economic

* Corresponding author.

E-mail address: sander.koenraadt@wur.nl (C.J.M. Koenraadt).

activities, such as rice cultivation and other water resource management projects that promote the proliferation of malaria vectors (Keiser et al., 2005; Hunter et al., 1982; Imbahale et al., 2011). These diverse ecological settings result in variation in risk of malaria across African countries, but often this variation has not been well characterized (Keiser et al., 2005; Himeidan and Kweka, 2012). To date, the Republic of Rwanda has not reported on distribution, diversity and malaria infectivity rate of mosquito species. Through the support of the Malaria & Other Parasitic Diseases Division (equivalent to National Malaria Control Program – NMCP) of the Ministry of Health, entomological surveys have been carried out in seven study sites since 2010. For the current study, mosquito data were collected monthly from these sites and analyzed to determine mosquito diversity, distribution (vectors and non-vectors) and entomological inoculation rate. We specifically aimed to characterize nocturnal biting patterns (hourly rates) and spatial biting patterns (endophily versus exophily), because these are under selection pressure with the currently used insecticide-based mosquito control interventions (Russell et al., 2011; Killeen, 2014).

2. Methods

2.1. Study sites

In Rwanda, malaria is the most important vector-borne disease and a leading cause of morbidity (Karema et al., 2012). The disease is endemic in 19 out of 30 districts, with the main foci in the Eastern and South-Eastern parts of the country where the altitude is generally below 1500 m above sea level, the climate is warmer and where the area is characterized by the presence of marshy plains, rice cultivation and brick-making as economic activity.

The study was conducted in seven out of 12 sites established throughout the country by the NMCP and that are used for routine entomological monitoring. The seven sites were set up in 2010, whereas the other five were established in 2012. In this study, data from the seven study sites are presented (Busoro and Karambi in Southern Province, Rukara and Bukora in Eastern Province, Mashasha in Western Province, Bungwe in Northern Province and Kicukiro in Kigali City (Fig. 1).

2.2. Mosquito collection: human landing catches (HLC)

At each of the seven study sites, three villages were selected and from each village, three houses were randomly selected for mosquito collection during two consecutive nights per month. Thus, a total of nine houses were sampled in each study site per month from January 2010 to December 2013. All houses from which mosquitoes were collected were geocoded using a hand-held Global Position System (GPS). Mosquito biting behavior was assessed by collecting mosquitoes inside and outside each house using human landing catches (HLC) from 6pm to 6am (WHO, 1975, 2013). Two trained volunteers sitting with a torch and an aspirator, one inside the house (at the house entrance) and the other approximately 5–8 m away from the house (AMC, 2009), aspirated mosquitoes that landed on their exposed legs. At each house, the door was usually left open before the sleeping period and closed throughout the sleeping time of house occupants. To correct for differential mosquito attractiveness between the collectors and to avoid fatigue, the collectors took a 10 min break every hour and switched position at midnight. Due to the risk of receiving an infectious bite before aspirating the mosquitoes, the collectors were screened for malaria every month during the survey, and standard malaria treatment was available if needed. None of the collectors was diagnosed with malaria during the survey period. Mosquitoes collected at each hourly interval were placed in separate paper cups pre-labeled with date, time and location of catch and transported to a field laboratory established at the health center of the study site for morphological identification. A 10% sample of the *An. gambiae* s.l. mosquitoes collected was

transported to the central laboratory in Kigali for further processing (see below).

2.3. Mosquito collection: pyrethrum spray collection (PSC)

At each study site, three villages were selected from which five houses (other than those used for HLC) in each village were randomly selected for mosquito collection by pyrethrum spray collection (PSC) for two consecutive days per month (Silver, 2008; Service, 1993). A total of 15 houses was thus sampled in each of the seven sentinel sites. Mosquitoes were collected on a monthly basis between 7am and 11am from January 2010 to December 2013. Each house was prepared for spraying by removing food and water and covering the entire floor and furniture with white cotton sheets. One collector sprayed inside the house with a 400 ml can of non-residual Cypermethrin (BOP[®], Mac Bride International, Manchester, United Kingdom), while the other sprayed the eaves on the outside to stop mosquitoes from escaping. The house was closed for 10 min after which the sheets were moved outside to collect the mosquitoes that were knocked down. Mosquitoes were transferred onto a petri dish lined with moist Whatman filter paper n°4 (110 mm diameter). Mosquitoes from each house were sorted and separated into anophelines and culicines, and pooled in 1.5 ml vials for transportation to the field laboratory at the health center for further processing.

2.4. Mosquito identification

All mosquitoes collected by PSC and HLC were transported to a field laboratory for morphological identification to *An. gambiae* s.l., *An. funestus*, other anophelines and culicines using standard morphological identification keys (Gillies and Coetzee, 1987). Mosquitoes were then pooled per study site and stored in silica gel for transportation to the central laboratory in Kigali for identification of *Plasmodium falciparum* infections and quality control of field mosquito identification. A random sample of 661 *An. gambiae* s.l. from five study sites from 2012 and 2013 were sent to the molecular laboratory of the International Center for Insect Physiology and Ecology (*icipe*), Nairobi, Kenya for molecular characterization using the standard polymerase chain reaction (PCR) assay (Scott et al., 1993).

2.5. Sporozoite rates and entomological inoculation rates

The heads and thorax of 10% of *An. gambiae* s.l. mosquitoes collected by HLC at each site were subjected to sporozoite ELISA to determine their infection rates with *P. falciparum*. A sample with an Optical Density (OD) value above the cut-off (cut-off = 2 x mean OD of 7 negative samples) was considered positive (MR4, 2011). The sporozoite rate was calculated as the number of mosquitoes infected with *P. falciparum* sporozoites divided by the total number of mosquitoes examined. The entomological inoculation rate was obtained by taking the product of human biting rate and sporozoite rate (WHO, 2013).

2.6. Statistical analysis

All data on mosquito collections were entered into Microsoft Excel for the calculation of mean biting densities (number of bites per person per night), the sporozoite index and entomological inoculation rates. Differences in mean biting densities and indoor or outdoor biting behaviour were statistically evaluated using SPSS statistic software, V.20, Chicago, IL, USA. The significance of differences among sentinel sites and years of study (2010–2013) in the proportions of *An. gambiae* s.l. collected indoor or outdoor (endophily) were calculated using Generalized Linear Models with binomial as the distribution and logit as the link function. Hourly biting rates were statistically analyzed by calculating the proportion of mosquitoes biting during the early (6 pm–10 pm) part of the night and comparing differences between years

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