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Characterization of malaria vectors in Huye District, Southern Rwanda

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Abstract

Background: Effective control of malaria requires knowledge of vector species, their feeding and resting behaviour as well as breeding habitats. The objective of this study was to determine malaria vector species abundance and identify their larval habitats in Huye district, southern Rwanda.

Methods: Adult mosquitoes were collected indoors using light trap and pyrethrum spray catch techniques, and outdoors using light traps. Female *Anopheles* mosquitoes were identified to species level by morphological characteristics. Enzyme-linked Immunosorbent Assay (ELISA) was used to screen for *Plasmodium falciparum* circumsporozoite protein and host blood meal sources. *Anopheles* larvae were sampled using dippers and raised into adult mosquitoes which were identified morphologically.

Results: *Anopheles gambiae* sensu lato comprised of 70% of the 567 *Anopheles* collected. Other *Anopheles* species identified were *An. funestus* 4%, *An. squamosus* 16.5%, *An. maculipalpis* 6.5%, *An. ziemanni* 1.7%, *An. pharoensis* 1.2 % and *An. coustani* 0.1%. The majority, 63.5% of the collected mosquitoes were from indoors collections. The overall human blood index was 0.509. The *P. falciparum* circumsporozoite protein was found in 11 mosquitos including 8 *Anopheles gambiae* s.l. and 3 secondary vectors out of the 567 tested. The overall sporozoite rate was 1.9%. A total of 661 *Anopheline* larvae from 22 larval habitats were collected. They comprised of *An. gambiae* s.l. (89%) and *An. ziemanni* (11%). The absolute breeding index was 86.4%. The most common larval habitats were in full sunlight with still water like rice paddies and pools of stagnant water.

Conclusion: These findings show that *Anopheles gambiae* s.l. is the dominant malaria vector in the area with other vectors playing a secondary role in malaria transmission. Malaria interventions need to be strengthened to reduce even further the malaria transmission in the area.

Keywords: malaria, mosquito, composition, larval habitats, Rwanda

Introduction

Malaria remains a leading cause of mortality and morbidity worldwide. In 2013, 90% of global malaria deaths occurred in Africa and about 78% of these deaths were in children under 5 years (WHO, 2014). However, there has been a considerable success in malaria control in the last decade attributed to heightened prevention and control interventions where malaria mortality in Africa has declined by 54% between 2000 and 2013 (Kamau & Mulaya, 2006; WHO, 2013). In Rwanda, malaria related mortality rate in children under-five years old also decreased by 61% between 2000 and 2010 and the prevalence dropped from 2.6% in 2007 to 1.4% in 2010 (USAID/CDC, 2014). In Africa, similarly in Rwanda, *Anopheles funestus* and *Anopheles gambiae* s.l. comprise the major malaria vectors (Sinka *et al*. 2012; World Health Organisation 2014). However, studies have shown that host preference and biting behaviour of the species highly vary across Africa (Tirados 2006). *An. gambiae* s.s. is mainly endophagic and endophilic with few exceptions, whereas *An. arabiensis* displays high variation in these behaviours (Tirados *et al.,* 2006; Coetzee *et al.* 2013). However, in West Africa *An. arabiensis* populations are more anthropophagic, endophilic and endophagic whereas those in the East Africa are more zoophagic and exophilic (Tirados *et al.,* 2006).

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The breeding of *Anopheles* mosquitoes occur in various habitats which can either be man-made or natural, sunny or shaded, permanent or temporal (Machault *et al.* 2009). Interventions targeting the larval stage of mosquitoes offer good alternative tools for management of insecticide resistance and it is more advantageous as it targets immobile stage which occupy minimum habitats compared to adult stages that can disperse very fast over large area (Manguin, 2013). Moreover, recent studies in East Africa have shown that larviciding is a relatively cost-effective intervention (Rahman *et al.*, 2016). Thus knowledge on ecological characteristics of the larvae habitats can help in designing effective vector control interventions (Soleimani-Ahmadi *et al.,* 2013). Nonetheless, there are no studies that have been conducted to characterize Anopheles mosquito larvae habitats in Southern Rwanda.

Several studies have classified Rwanda as a malaria endemic country (Guerra *et al.*, 2008; Hay *et al.,* 2009) and Sinka *et al.*, 2010) with *Plasmodium falciparum* annual parasite incidence greater than 0.1 per thousand (Henninger, 2013). Transmission of malaria in the country is related to altitude and microclimate and occurs with two peaks, in May to June and November to December. In addition to the favourable climate, other factors such as proximity to marshlands, irrigation schemes and cross border movement of people influence the transmission especially in the Southern and the Eastern parts of the country (USAID/CDC, 2014). Although different measures for malaria control in Rwanda such as insecticide treated mosquito nets and indoor residual spraying and antimalarial drugs have resulted in significant malaria incidence decline (WHO, 2014), this achievement is fragile as potential for local malaria transmission through existing vectors remains high. Malaria transmission in Rwanda, is increasingly becoming heterogeneous in its distribution as it differs from one village to another due to diversity in type and behaviour of vectors (Okara *et al.,* 2010; Bizimana *et al.,* 2015). Historical evidence shows that an effective vector control programme and reduction of the malaria burden requires detailed knowledge of the vector species and identification of mosquito species in order to separate non-vector species from vectors (Temu *et al.* 2007) . However, information on species distribution in many countries in Africa including Rwanda is not readily available (Temu *et al.*, 2007; Okara *et al.*, 2010). Thus the necessity for more focused studies to identify type and behaviour of vectors is high (Badu *et al.,* 2013). There is dearth of information on the malaria transmission indices in Southern Rwanda. This study was therefore carried out to determine the malaria vector species and their malaria infection rates and to characterize the *Anopheles* breeding habitats in Huye district, southern Rwanda.

Materials and Methods

Study area

The study was conducted in 13 villages of Rukira Cell located in Huye district, Southern Province of Rwanda. The study was conducted during high malaria transmission season in May, 2016. Rukira Cell is made up of 13 villages and covers an area of 10 km^2, with a population of 6,529 persons living in 1,600 households. It is situated in the central plateau with hills of an average altitude of 1,700 m. The annual average temperature is 20°C and rainfall of 1160 mm. Huye district was chosen because of high incidence of malaria in the region (Gahutu *et al.* 2011). The closeness to a marshland, rice field and irrigation practice in the Rukira Cell provides breeding sites of malaria mosquitoes and increased malaria transmission potential.

Larval sampling and Identification

The study area was searched for all potential *Anopheles* larval habitats which were identified through a systematic ground survey. *Anopheles* larvae were collected using standard dipper 350 ml according to the procedures for larval sampling (WHO, 1975). A total of 10 dips were taken per site. In small breeding sites where dippers were not effective, larval collection was done by using plastic pipette. Environmental characteristics of each *Anopheles* breeding habitat were recorded during larval collection. Recorded characteristics of breeding sites included origin (natural or manmade), permanence (permanent or temporary), water current (still or slow flowing) and

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intensity of light (full or partial sunlight). All *Anopheles* larvae collected were placed into plastic bottles labelled with date and type of habitat. All sampled larvae were then transported in a cool box to Sovu Health Centre where they were reared until hatching to adult stage. Emerged adult mosquitoes were identified using a morphology based keys by Gillies & Coetzee (1987).

Adult mosquito sampling and processing

Mosquitoes were collected for 4 weeks in May, 2016 both indoors and outdoors in 39 selected houses from 13 villages of Rukira Cell. Purposive sampling method was applied in order to capture the peripheral and central parts of the village. Three houses in each village were selected from which mosquitoes were collected. For indoor resting adult mosquitoes, collection was done by use of CDC light trap and pyrethrum spray catches (PSC). A light trap with a lid was hung inside houses where children slept, about 1.5 m from the floor at the foot end of the bed. The traps operated by batteries were switched on by the head of household at 18:00hr and off at 06:00hr after tying the string around the neck netting of the mosquito collecting cage (Lines *et al.*, 1991; Mboera, 2005).

For PSC, members of the household were informed of the technique prior to spraying. All items were covered completely and white sheets were spread on floor of the rooms and all the windows, doors and other exits were closed. The rooms were sprayed using pyrethrum insecticide by two operators between 06:00hrs and 08:00hrs; one inside and another outside in opposite directions. After 10 minutes all knocked down mosquitoes were collected and placed in labelled petri dishes lined with moist filter paper (WHO, 1975).

For outdoor adult mosquitoes, collection was done by use of CDC light trap with a lid hung outside on the eaves of the houses and cattle sheds about 1.5m from the floor from 18:00hr to 06:00hr. All collected mosquitoes were placed in petri-dishes labelled with date, method of collection, place of collection and number of the house. Then transported to the Rwanda Biomedical Centre, Malaria and Other Parasitic Diseases Laboratory for further analysis.

Laboratory processes

In the laboratory, female *Anopheles* mosquitoes were morphologically identified to species level by using morphological identification keys (Gillies & Coetzee, 1987). The heads and thoraces were used for *Plasmodium falciparum* sporozoite detection by Enzyme Linked Immunosorbent Assay (ELISA). The blood fed abdomens were tested for sources of blood meal by ELISA. All adult female *Anopheles* species were tested for *P. falciparum* circumsporozoite protein (PCSP) by ELISA (Benedict 2007). Each fed female *Anopheles* mosquito was tested for source of blood meal as described by Barid *et al*. (2002). Blood samples collected from local butcher houses and human blood samples taken by finger-prick was used as positive controls. PBS was used as negative control in the assay

Data analysis

The sporozoite Index was calculated as the proportion of female mosquitoes carrying sporozoite in the head and thorax among the tested female Anopheles mosquitoes. Entomological Inoculation Rates (EIR) was calculated as number of fed female *Anopheles* mosquitoes caught by PSC/number of human occupants who spent the night in the sprayed house) × (number of human fed mosquitoes / number of mosquitoes tested for human blood meal) × (number of sporozoite positive ELISAs / number of mosquitoes tested) (WHO, 2003). The human blood index was determined as the proportion of mosquitoes that had fed on humans out of the total number tested. The human-biting rates (the number of mosquito bites per person per night), was calculated by dividing the total number of blood-fed and half-gravid mosquitoes caught in PSC by the number of persons sleeping in the house the night preceding the collection.

Data were analysed using IBM SPSS software version 22. Descriptive statistics using frequencies, proportions, means and to establish the association between collection method, place of collection, blood meal and *Anopheles* species and infectivity of mosquitoes with sporozoite. The threshold for statistical significance was set at $p \leq 0.05$.

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Ethical considerations

Ethical clearance for this study was obtained from Kenyatta National Hospital /University of Nairobi Ethics and Research Committee and the Institution Review Board of University of Rwanda. Permission to conduct research in the area was sought from the administration of the study area. Informed consent was obtained from the head or representative of each household before mosquito collection.

Results

Mosquito species composition and abundance

A total of 567 mosquitoes were collected and were identified into An. *gambiae* s.l. 395 (69.7%), *An. funestus* 23 (4.1%), *An. squamosus* 94 (16.6%), *An. maculipalpis* 37 (6.5%), *An. ziemanni* 10 (1.8), *An. pharoensis* 7 (1.2%) and *An. coustani* 1 (0.2%). The majority, 63.5% (360) were collected indoors. Of these indoor collected mosquitoes, 51% (184) were from indoor resting catches and 49% (176) were from light traps. Outdoor collected mosquitoes accounted for 36.5% (207) of the total mosquito collection (Table1).

Table 1: Mosquito abundance by collection technique, blood meal status and species composition

Variable	Results	Total N (%)	*An. gambiae* s.l. N (%)	Others species N (%)
Collection technique	PSC	184(32.5)	169(91.8)	15(8.2)
	Light trap	383(67.5)	226(59.0)	157(41.0)
Place of collection	Indoors	360(63.5)	319(88.6)	41(11.4)
	Outdoors	207(36.5)	76(36.7)	131(63.3)
Blood meal	Fed	159(28.0)	107(67.3)	52(32.7)
	Unfed	408(72.0)	288(70.6)	120(29.4)
Mosquito species	*An. gambiae s.l.*	395(69.7)		
	An. funestus	23(4.1)		
	An. ziemanni	10(1.8)		
	An. maculipalpis	37(6.5)		
	An. squamosus	94(16.6)		
	An. pharoensis	7(1.2)		
	An. coustani	1(0.2)		

Key: PSC= pyrethrum spray catch

Generally, majority (67.5%; n=383) of *Anopheles* mosquitoes were collected by light trap (LT) technique. Of these, 39.8% (226) were *An. gambiae* s.l. Pyrethrum spray catches (PSC) collected 32.5% (184) of the mosquitoes and 29.9% of these were *An. gambiae s.l*. Only a very few *An. ziemanni*10% (1), *An. maculipalpis* 3% (1), *An. squamosus* 1% (1) were collected by PSC and none of the *An. pharoensis* and *An. coustani* was collected by PSC (Table2).

Table 2: Anopheles species composition by collection technique

Species	Method of collection		Total
	Pyrethrum spray	Light trap	
An. gambiae s.l.	169(29.9)	226(39.8)	395(69.7)
An. funestus	12(2.1)	11(2)	23(4.1)
An. ziemanni	1(0.2)	9(1.6)	10(1.8)
An. maculipalpis	1(0.2)	36(6.3)	37(6.5)
An. squamosus	1(0.2)	93(16.4)	94(16.6)
An. pharoensis	0(0)	7(1.2)	7(1.2)
An. coustani	0(0)	1(0.2)	1(0.2)

Human biting rate, sporozoite rate and the entomologic inoculation rate

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Table 3 shows the *Plasmodium falciparum* sporozoite rates for *Anopheles* species obtained using sporozoite ELISA. Of 567 mosquitoes tested for the *P. falciparum* CSP antigen by sandwich ELISA, *P. falciparum* CSP antigen was detected in 8 (2%) *An. gambiae* s.l. 1 (1.1%) *An. squamosus*, 1(10%) *An. ziemanni* and 1 (2.7%) *An. maculipalpis*. No infections were detected in *An. funestus*, *An. pharoensis* and *An. coustani*. Almost all positive mosquitoes 10 (90.9%) were from indoor resting collections (5 from LT and 5 were from PSC). Only 1 (9.1%) was from outdoors collection. The overall sporozoite rate of *Anopheles* species was 1.9%. The overall human biting rate (HBR) was 0.988 bites per person per night while the entomological inoculation rate (EIR) was 7.068 infective bites per person per year during the study period.

Table 3. *Plasmodium falciparum* Sporozoite rate for *Anopheles* species

Variable	Total	Positive sporozoites n(%)	Chi square (P-value)
Method of collection			
Pyrethrum Spray Catches	184	5(2.7%)	0.352
Light Trap	383	6(1.6%)	
Place of collection			
Indoors	360	10(2.8%)	0.048
Outdoors	207	1(0.5%)	
Blood meal			
Fed	159	7(4.4%)	0.008
Unfed	408	4(1.0%)	
Species			
An. gambiae s.l.	395	8(2.0%)	0.605
An. funestus	23	0(0.0%)	
An. ziemanni	10	1(10.0%)	
An. maculipalpis	37	1(2.7%)	
An. squamosus	94	1(1.1%)	
An. pharoensis	7	0(0.0%)	
An. coustani	1	0(0.0%)	
***An. gambiae* s.l. versus other species**			
An. gambiae s.l.	395	8(2.0%)	0.823
Others	172	3(1.7%)	

*****Significant at p≤*0.05*

Blood meal identification

ELISA analysis of 159 blood meals from Anopheles mosquitoes showed high preference for humans than animals. The overall human blood index was 0.509 (Table 4). The blood meal analysis showed that a total of 69 (64.5%) *An. gambiae* s.l. had fed on human blood, 15 (14.0%) had fed on cattle blood, nine (8.4%) had fed on goats, three (2.8%) on both cattle and goats and 11 (10.3%) had fed on unknown hosts. Seven (70.0%) *An. funestus* had fed on humans while 3 (30.0%) had fed on unknown host. Among *An. ziemanni*, 3 (75.0%) fed on human blood while only 1 (25.0%) had fed on unknown host. For *An. maculipalpis,* three (60.0%) had fed on cattle blood and other two (40.0%) had fed on unknown hosts. *An. squamosus* showed wide host range where two (6.2%) had fed on human, eight (25.0%) on cattle blood, ten (31.3%) had fed on goat blood, eight (25.0%) had fed on both cattle and goat blood and four (12.5%) on unknown host. None of the *An. pharoensis* or *An. coustani* was found to be fed. More blood fed *Anopheles* were sampled indoors 108 (67.9%) than outdoors 51 (32.1%) (Table4).

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Table 4. Blood meal sources for *Anopheles* collected indoors and outdoors

Species	Place of collection	No. Tested	Source of blood meal (n=159)				
			Human, n(%)	Bovine, n(%)	Goat, n(%)	Bovine and goat, n(%)	Unknown host, n(%)
An. gambiae s.l.	Indoors	94	69(73.4)	10(10.6)	7(7.4)	3(3.2)	5(5.3)
	Outdoors	13	0(0.0)	5(38.5)	2(15.4)	0(0.0)	6(46.2)
	Total	107	69(64.5)	15(14.0)	9(8.4)	3(2.8)	11(10.3)
An. funestus	Indoors	10	7(70.0)	0(0.0)	0(0.0)	0(0.0)	3(30.0)
	Outdoors	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Total	10	7(70.0)	0(0.0)	0(0.0)	0(0.0)	3(30.0)
An. ziemanni	Indoors	3	3(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Outdoors	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)
	Total	4	3(75.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)
An. maculipalpis	Indoors	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)
	Outdoors	5	0(0.0)	3(60.0)	0(0.0)	0(0.0)	2(40.0)
	Total	6	0(0.0)	3(60.0)	0(0.0)	0(0.0)	2(40.0)
An. squamosus	Indoors	0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Outdoors	32	2(6.3)	8(25.0)	10(31.3)	8(25.0)	4(12.5)
	Total	32	2(6.3)	8(25.0)	10(31.3)	8(25.0)	4(12.5)

Environmental characteristics of larval habitats

Twenty-two different potential breeding habitats were investigated. Of this, 19 (86%) were found to be positive for *Anopheles* larvae. A total of 661 Anopheles larvae were collected and raised to adults. Emerged adults were identified *An. gambiae* s.l. (89%) and *An. ziemanni* (11%).

Table 5: Characteristics of larvae habitats (N=22)

Variable	Characteristic	Total habitats, n (%)	No. of habitats with larvae
Type of habitat	Rice paddy	6(27.3)	6
	Irrigation channel	3(13.6)	2
	Ground pool	5(22.7)	5
	Stagnant water	3(13.6)	3
	Crashed pot/ other containers	5(22.7)	3
Breeding status	Permanent	10(45.5)	9
	Temporal	12(54.5)	10
Water current	Still	18(81.8)	16
	Slow flowing	4(18.2)	3
Light intensity	Full sunlight	19(86.4)	16
	Partial	3(13.6)	3
Origin of breeding site	Natural	8(36.4)	8
	Man made	14(63.6)	11

Discussion

An. gambiae s.l. was the most abundant and major malaria vector in Rukira district. Similar findings have been reported from elsewhere in in Rwanda (Okara *et al.* 2010). Mulambalah *et al*. (2011) made a similar observation in Kenya. In the current study the abundance of *An. gambiae* s.l. in Rukira is likely to be due to the availability of the favourable larval habitats consisting mostly of rice fields and marshlands. The observation of lower sporozoite infection rates in *An. funestus, An. ziemanni,*

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An. squamous, An. maculipalpis, *An. pharoensis* and *An. coustani* indicate that the species are likely to play a secondary role in malaria transmission. Similar findings have been reported in Kenya, Zambia and Rwanda (Kamau & Mulaya 2006; Christen M. 2011; President's Malaria Initiative 2015). There was a significant difference in the number of *Anopheles* species sampled indoors and outdoors. Higher numbers of *An. gambiae s.l.* and *An. funestus* were endophilic, which is in agreement with other studies showing that despite the use of ITNs the majority of these species remain endophilic. In contrary, some studies have reported gradual increase in exophily and exophagy among *An. gambiae* s.l. (Sokhna *et al.*, 2013). *An. ziemanni, An. maculipalpis, An. squamosus, An. pharoensis, An. coustani* were more exophilic. A similar observation has been reported from Ethiopia (Kenea *at al.*, 2016).

Most blood fed *Anopheles* mosquitoes were predominantly collected indoors than outdoors. The majority of *An. gambiae* s.l. were anthropophilic, indicated by the higher human blood index. Both *An. gambiae s.l.* and *An. funestus* showed both higher endophilic and anthropophagic behaviour despite the high coverage of insecticide-treated nets use by people in the study area (PMI 2014).This is similar to reports from Kenya (Mwangangi *et al*., 2003) and in Nigeria (Oyewole *et al.*, 2005). Contrary to our findings, studies in western Kenya reported unusually high frequency of animal blood meals in the major malaria vector *An. gambiae s.s.,* (Ndenga *et al*. 2016). The reason could be that animals fed mosquitoes might have fed outside and chose to rest indoors.

Among secondary malaria vectors, *An. ziemanni* showed anthropophagic behaviour. Similar findings have also been reported in Kenya (Kamau & Mulaya 2006).This finding suggests that *An. ziemanni* may play an important role in malaria transmission in the area. Although *An. squamosus* in this study showed zoophagic behaviour, a study in Zambia reported high anthropophagic tendencies of *An. squamosus* (Christen M. 2011) demonstrating what can be called regional variations in some *Anopheles* adaptive behaviour due to local circumstances. We report an overall *P. falciparum* sporozoite rate of 1.9%. The higher positivity rate in our study may be explained by the fact that mosquito collection was done during the peak of malaria season. The higher *P. falciparum* sporozoite rate for *An. gambiae* s.l. indicate that the mosquito is the dominant vector of malaria in the area. Another recent study has also reported that the most efficient vectors of human malaria in Rwanda are *An. gambiae s.l*. and *An. funestus* (PMI, 2015). Secondary malaria vectors showed a sporozoite rate of 1.7% which indicating to play a significant role in malaria transmission. *An. ziemanni* has already been reported to be an important local malaria vector in Cameroun (Tabue *et al.*, 2014).

During this study, two species of *Anopheles* larvae were collected, *An. gambiae* s.l. and *An. ziemanni*. The reasons why we did not find any breeding sites for the other mosquito species may be that during the survey some potential mosquito breeding habitats could not be identified or it may the species were too few in number to be detected. Results of this study revealed that rice paddies and stagnant water were important mosquito breeding habitats. These were man-made, open to sunlight and with still water making the environment suitable for the development of anopheline mosquitoes. Similar observation has been made in Ethiopia (Kenea *et al*. 2011).

A notable limitation of this study is that molecular identification of *Anopheles gambiae* s.l. mosquitoes at sibling level could not performed, hence specific species could not be determined. The female *Anopheles* mosquitoes were only tested for *P. falciparum* CSP so infection with other *Plasmodium* species could not be determined. In addition, chemical parameters of *Anopheles* larval habitats were not analysed which could have affected larvae abundance and distribution. Despite the limitations, this study provides baseline information on malaria vectors in southern Rwanda.

In conclusion, this study has shown that *Anopheles gambiae* s.l. is the dominant malaria vector in Huye district in Southern Rwanda. The infection rate among other Anopheles species indicate their important role in local malaria transmission. We recommend the indoor residual spray intervention to reduce the observed high endophilic behaviour of vectors. The findings of this study are useful for the planning of control strategies for malaria vectors in Southern Rwanda.

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Competition of interests

There was no competition of interests.

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Authors' contribution

CN, MM, LK, DM, KJN designed the study and contributed to data collection. RC, MH analysed the data. CN wrote the manuscript with contributions of the other authors. All the authors read and approved the final version of the manuscript.

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