Journal of
Parasitology and Vector Biology
Volume 8 Number 8, August 2016
ISSN 2141-2510

Academic Journals

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Journal of Parasitology and Vector Biology

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academicJournals

Vol. 8(8), pp. 74-85, August 2016
DOI: 10.5897/JPVB2016.0242
Article Number: 5EEAB0D59700
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Altitudinal variation in the parasitological and entomological indices of malaria around Mount Cameroon, South West Region of Cameroon

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Received 26 March, 2016; Accepted 27 June, 2016

This study aimed to define the heterogeneity in the parasitological and entomologic indices of malaria transmission from sites of contrasting altitudes in the Mt. Cameroon region. Blood samples were collected by pricking the finger. Thick and thin blood films were prepared and Giemsa-stained. Slides were examined under x100 objective for the identification of asexual and sexual stages of malaria parasites. Quantification of asexual stages and gametocytes was done against 200 WBC and 500 WBC, respectively assuming a WBC count of 8000 leucocytes/µl blood. Mosquitoes were collected by the landing catch method by human bait. All mosquitoes caught were separated into *Anopheles, Culex, Aedes* or *Mansonia*. Man biting rate (MBR), vectorial capacity and entomological inoculation rate (EIR) were calculated using standard formulae. The data generated was analyzed using SPSS version 15. Overall, 876 pupils aged 4-16 years of both sexes were enrolled in this study. The prevalence of asexual stages of malaria was 45.31% while that of sexual stages was 24.69%. Tiko, the locality at the lowest altitude recorded the highest prevalence of malaria while Bonakanda at the highest altitude recorded the lowest and the difference was significant, p=0.01. The geometric mean parasite density (GMPD) of infection was highly heterogeneous amongst the different localities, p=0.02. Age significantly affected the prevalence of malaria, p=0.02. Sex did not affect the prevalence nor the GMPD of malaria infection, p>0.05. *P. falciparum* was found to be the most prevalent *Plasmodium* species infecting children. *An. gambiae* was the most aggressive anopheline species. The highest EIR and vectorial capacity of anophelines was recorded in Tiko. The malaria epidemiology is highly heterogeneous among the different localities. Malaria control programmes should be based on evident spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium* species in a particular area so as not to waste resources which would be of limited effectiveness to the populations at risk.

Key words: Altitudinal variation, parasitological, entomological, indices, malaria, Mount Cameroon region.

INTRODUCTION

Malaria is a disease of great public health concern especially in tropical and sub-tropical areas of the world where 3.3 billion individuals in about 106 countries live at risk. Approximately 200–300 million people worldwide

become infected annually and totally 0.6–1 million individuals lose their lives, most of them children under 5 years of age, pregnant women and immunosuppressed travelers (del Prado et al., 2014; Salmanzadeh et al., 2015; Sumo et al., 2015). The disease is transmitted to people through the bites of infected *Anopheles* mosquitoes. Five known species of *Plasmodium* infect humans: *P. falciparum*, *P. vivax*, P*. ovale*, P*. malariae*, and *P. knowlesi*; with *P. falciparum* being the most dangerous recording the highest rates of complications and mortality.

In Cameroon, the disease is a major public health problem in Cameroon with over 90% Cameroonians being at risk of malaria infection, and ~41% having at least one episode of malaria each year (Mbenda et al., 2014). Prevalence in malaria has been shown to vary spatially and temporally with climatological factors (Lowe et al., 2013), topography (Atieli et al., 2011), altitude (Drakely et al., 2005), availability of breeding sites (Atieli et al., 2011), and level of urbanization (Tatem et al., 2013). Variability in micro geographical factors related to disease prevalence is an important determinant e.g. in localities in malaria endemic regions, some localities have a high malaria burden while others seem to be disease-free (Kleinschmidt et al., 2002).

The criteria previously used to classify the malaria transmission level were based on parasitological and clinical data, splenic index and prevalence of the parasitaemia (Gilles and Warrel, 1993). Entomologic indices especially the entomologic inoculation rates (EIR) are considered key factors when establishing the degree of endemicity or transmission level (Kilama et al., 2014). Thus, an EIR under 1 is typical of a hypoendemic zone and an EIR between 100 and 1000 identifies a holoendemic zone. Integrated parasitological and entomologic studies are required for the identification and analysis of relationships between transmission intensity and malaria disease burden over large areas where heterogeneous malaria prevalence has been documented (Bousema and Baidjoe, 2014). With national malaria control programmes being guided by the Roll Back Malaria Programme of the World Health Organization (WHO), the development of sound control strategies for malaria transmission requires a solid understanding of the vector dynamics and the factors influencing their spatial and temporal distribution (Ngom et al., 2013). Such information would help to develop early warning systems for predicting malaria epidemics and for planning control programmes based on accurate predictions of their likely effects. Moreover, identification of spatial and temporal variations in vector bionomics and transmission within and among sites, on a regional scale provides useful information for designing effective control programmes.

The heterogeneity of malaria transmission has important implications for vector and morbidity control. Understanding the spatial pattern of vector distribution provides opportunities for limited and thus more cost-effective control programmes (Ngom et al., 2013). For example due to large areas affected by epidemic malaria, it is not possible to spray every house with indoor residual insecticides. Knowledge of transmission foci would also lead to a better understanding of spatial distribution of the stability of transmission and the risk of severe disease. This would enable a more rational application of interventions in areas of varying malaria exposure.

In spite of the enormous problem malaria causes in Cameroon (Mbenda et al., 2014), there is a paucity of basic data and lack of understanding of the situation. The Mt. Cameroon region presents a large variability in terms of altitude, climate, level of urbanization, housing type, topography, relative humidity, availability of breeding sites, temperature and rainfall. It has undergone serious environmental modifications over the years owing to the rapid growth in populations, road and house constructions and the agro-industrial activities of the Cameroon Development Corporation (C. D. C.), the largest agricultural scheme in Central Africa. Such modifications may have led to ecological changes that affect the vector population structure and hence malaria transmission in the area.

This study was therefore, designed to determine the altitudinal variation in the parasitological and entomological indices of malaria around the Mt. Cameroon region during the rainy season.

MATERIALS AND METHODS

Study localities

Six localities of the Mt. Cameroon region were selected for the study so as to have maximum representation of the area. Geographical data such as the altitude, longitude and latitude of each locality were obtained using a hand-held geographical positioning system (GPS). The geographical data were then entered into ARCVIEW and a map of the study area was drawn. The mean values of climatological factors, that is, rainfall, relative humidity and temperature during the study period (that is, from March-August 2006) were calculated from monthly values recoded by the weather stations owned by the C.D.C. in the different study localities. The housing types in the various localities were noted by numerical observations made by the same research team moving through each study area, to maintain consistency. Based on the housing types and the amount of non-agricultural economic activities in each locality; they were classified either as urban or

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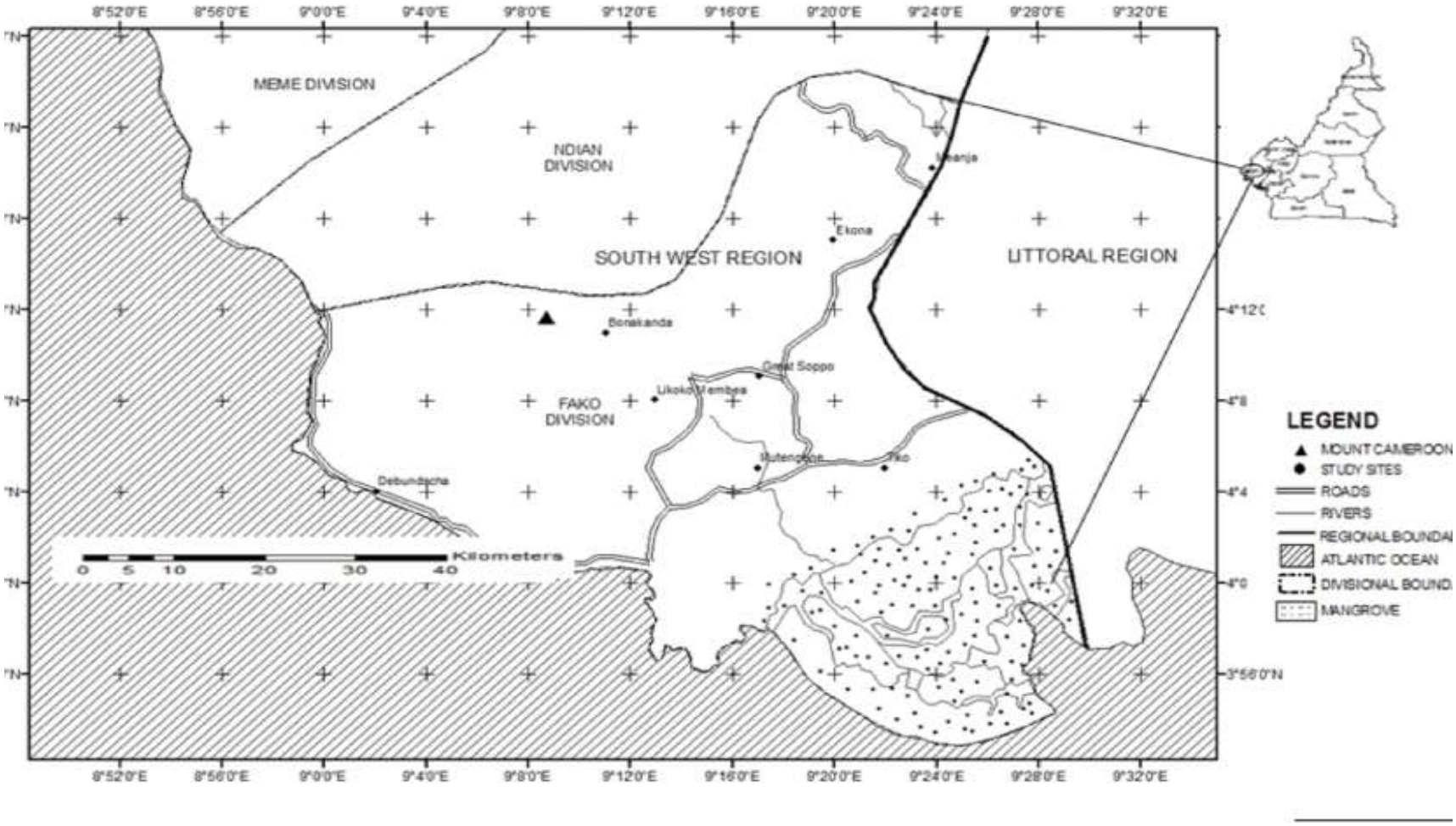

Figure 1. The study of six localities of Mt. Cameroon; Bonakanda (1,197 m a.s.l; 04°11'N; 09°12'E), Likoko Membea (800 m a.s.l; 04°08'N; 09°13'E), Meanja (300 m a.s.l.; 04°18'N; 09°24'E), Mutengene (220 m a.s.l.; 4°05'N; 09°18'E), Debundscha (50 m a.s.l.; 4°04'N; 09°04'E), Tiko (10 m a.s.l; 04°04'N; 09°22'E).

rural. Briefly, the study localities selected were: Bonakanda (1,197 m a.s.l; 04°11'N; 09°12'E), Likoko Membea (800 m a.s.l; 04°08'N; 09°13'E), Meanja (300 m a.s.l.; 04°18'N; 09°24'E), Mutengene (220m a.s.l.; 4°05'N; 09°18'E), Debundscha (50m a.s.l.; 4°04'N; 09°04'E), Tiko (10m a.s.l; 04°04'N; 09°22'E). The different study localities are shown in Figure 1.

Study design

This was a cross-sectional study conducted in March to August2006 carried out during the rainy season in the Mt. Cameroon region. Primary school children from Government Primary schools in the different localities were used as proxy to estimate the parasitological indices because it is generally easy to work with school children. Blood samples for parasitological indices and *Anopheles*

mosquitoes for entomologic indices were collected during the same study period so as to match parasitological and entomologic data. Children aged 4-16 years of both sexes were enrolled into this study following informed parental consent. Only children whose parents consented and they agreed to be pricked voluntarily were enrolled in this study. The children's whose parents consented but they refused to be finger-pricked were not enrolled into this study. Entomologic parameters were measured using the anophelines caught when they land on an individual who acts as bait (Verhulst et al., 2013). This type of collection method has been widely discussed from ethical and technical point of views. Exposing technical staff to infective mosquito bites is ethically unacceptable, even when they are protected by a chemo-prophylactic treatment. On the other hand, differences in human attractiveness, motivation and diligence in the collection work give a certain degree of subjectivity to catching mosquitoes using human bait (World Malaria Report, 2008). Using mechanized collection methods such as light traps solves the aforementioned problems, although there are discrepancies with respect to the quality of the information obtained and its application to determine the EIR i.e. using light traps mosquito species that feed on other animals will be caught and this will give an erroneous value of the mosquito abundance in a particular area.

Ethical considerations

The ethics committee of the Tropical Medicine Research Station, Kumba, South West Region Cameroon, reviewed and approved the study. Authorizations for the study were obtained from the South West Regional Delegations of Public Health and Basic Education. The chiefs and their council members in each locality were visited and sensitized on the benefits of the study. One Primary school in each locality was visited and a series of meetings held with the parents/legal guardians, teachers and head teachers to explain the purpose and methodology of the survey. After these series of meetings, the children were then given the informed consent forms to take home to their parents/legal guardians for signature. Participation for blood sample collection was voluntary and parents had to fill and sign the informed consent form which explained the benefits (that is, those found infected would be treated) of the study. Individuals used as human baits voluntarily accepted to participate in the study. The Research team explained to them what they had to do and each mosquito collector was placed on an anti-malaria chemo-prophylactic treatment before and after mosquito collection.

Parasitological indices of transmission

Blood collection, preparation and staining of blood smears

Prior to blood sample collection demographic information such as the age, sex, temperature, area of residence, and housing type of the children were recorded. Using sterile disposable lancets, finger pricks were performed. Thick and thin blood films were prepared using the method described by World Health Organization (WHO, 2000). The code number of each individual was written on the slide and the blood films were allowed to air dry protected from dust and flies. In the laboratory, the thin films were fixed with absolute methanol for one minute and both thick and thin blood films were stained with 5% Giemsa stain solution for 30 min (Cheesbrough, 2000).

Detection and estimation of parasitaemia of asexual and sexual stages of Plasmodium species

The slides were read under x100 (oil immersion) objective of the microscope for the detection of malaria parasites by an experienced microscopist. A second experienced microscopist, blinded to the first reading, read all thick smears and any discrepancies (positive vs. negative; results that did not match each other; >25% difference in parasite density) were resolved by a third microscopist. Asexual and sexual parasite densities were determined from thick blood smears by counting the number of asexual parasites or sexual parasites per 200 WBCs and 500 leucocytes, respectively, and converted to number of parasites/µl blood assuming a standard WBC count of 8000/µl. A smear was considered negative if no parasites were seen after review of 100 high-powered fields. Thin smears were used to determine the parasite species. The different species of *Plasmodium* were identified using identification charts (WHO, 2000).

Entomological indices of transmission

Mosquito collection

Mosquitoes were collected by the landing catch method by the same work force at all sites during the entire period of collection through standardized human-biting collections. Four human-landing collections (four nights of collection) were carried out each month of the six months of collection in the four sites using a team of collectors made up of eight trained collectors. The activities of the collectors were monitored throughout the night by two supervisors. The first team worked from 6:00pm-12:00am and the second from 12:00am-6:00pm. Two houses were selected per site with one collector being indoors (generally inside the bedroom) and the other outdoors. Collection took place over eight man-nights per station per month with different houses used for subsequent collections. A man-night constitutes a complete night of collection from 6:00pm-6:00am by two collectors (one working from 6:00pm-12: 00am and the other from 12:00am-6:00pm). This gave a total of 16 man-nights per month per site. Tubes were labelled hourly and mosquitoes that came to feed on the collectors were captured using aspirators.

Mosquito species identification

All the mosquitoes collected were frozen, separated into *Anopheles, culex, Aedes* or *Mansonia* species and counted. The anophelines were identified using the morphological key of the Afro-Tropical Region and the *I.R.D. / ORSTOM* software (Gilles and Mellion, 1968; Gilles and Coetzee, 1987; Harvey et al., 1998). The sex and feeding state were recorded and the mosquitoes were then stored in tubes containing silica-gel and cotton wool for future studies.

Man-biting rate (MBR)

Man-biting rate which refers to the average number of bites per person per night by the vectors species (also called the aggressiveness of the species) was calculated directly from landing-catch collections as the average number of *Anopheles* bites experienced by a collector during an entire night of collection.

Vectorial capacity (C)

It is the capacity of a vector population to transmit malaria in terms of the potential number of secondary inoculations originating per day from an infective person. Vectorial capacity is calculated using the formula

Table 1. Proportion of individuals infected with asexual and sexual stages of *Plasmodium* species in the different localities.

Locality	Altitude (m a.s.l)	Mean temperature (°C)	Relative humidity (%)	Average rainfall (mm)	Number examined	Asexual stages		Sexual stages	
						Number positive (%)	GMPD (range)	Number positive (%)	GMPD (range)
Bonakanda	1197	19.5	80.4	2400	146	18 (12.33)	522.53(120-12000)	1 (5.56)	40.00(40)
Likoko Membea	800	22.5	81.8	2654	107	19 (17.76)	496.14 (120-4200)	1 (5.26)	80.00 (80)
Meanja	300	27.5	85.6	2475	159	99 (62.26)	839.42 (40-29520)	20 (20.20)	62.29 (40-160)
Mutengene	220	27.5	83.1	1854	188	88 (46.81)	700.39(120-16240)	29 (32.95)	0.00 (0-160)
Debundscha	50	27	89.6	11000	75	31 (41.33)	650.29(120-16000)	12 (38.71)	71.59 (40-200)
Tiko	10	27.9	83.1	4524	201	142 (70.65)	656.34(120-48000)	35 (24.65)	0.00 (0-160)
Overall					**876**	**397 (45.31)**	**690.89 (40-48000)**	**98 (24.69)**	**0.00 (0-200)**

ma^2P^n / -lnp

where, m=density of vectors in relation to man,
a=number of blood meals taken on man per vector per day (=human blood index multiplied by 0.5, if a gonotrophic cycle of two days is assumed), p= daily survival probability (or the proportion of vectors surviving per day),
n= incubation period (days) in the vector (Garret-Jones, 1964) which varies with the infective life of the mosquito (P^n / -lnp), the man biting rate (ma), and the feeding habit (a) of the vector,
a= the product of the feeding frequency (0.5) and the human blood index (100%) (Wanji et al., 2003).

Entomological inoculation rate (EIR)

The EIR, a standard measure of transmission intensity, is expressed as the number of infective bites per person per unit time (e.g., daily, monthly, yearly). It is obtained by multiplying the MBR by the proportion of sporozoite positive mosquitoes. Sporozoite positive mosquitoes were determined as previously described (Wirtz et al., 1987).

Data analysis

The prevalence of malaria was determined for each locality, sex and age group. The Chi-square test of heterogeneity was used to assess the differences in prevalence of malaria in the different localities. Intensities were obtained by calculating geometric means and expressed as geometric mean parasite density per locality, sex and age group. Kruskal-Wallis test was used to compare parasite densities in the different categories. The different *Plasmodium* species and their combinations were expressed as proportions. The MBR, C and EIR were calculated as explained above. The chi square test was used to check for significant differences in the MBR, EIR and sporozoite rates for the different localities. The statistical analyses were accomplished using microsoft Excel 2003 and SPSS version 15.0 (SPSS Inc., Chicago) with respect to the locality at the different altitudinal sites. All tests were performed at the 5% significance level.

RESULTS

Demographic information of the study population

The number of pupils sampled were 876. 42.58% (373) were males while 57.42% (503) were females. The proportions of pupils per age group were as follows: 27.28% (239) for the age group 4-8 years; 57.31% (502) for the age group 9-12 years and 15.41% (135) for the age group 13-16 years. The mean age of the study population was 9.84±2.45 years. The mean body temperature recorded by the pupils was 36.61±0.36°C. Table 1 shows the number of pupils examined per locality.

Prevalence and density of Plasmodium species in the study population

The prevalence of asexual stages of *Plasmodium* species in the study population was 45.31% (397) while that of sexual stages was 24.69% (98). The GMPD of asexual stages of *Plasmodium* species was 690.89 parasites/µl blood (40-48000 parasites/µl blood) while that of the sexual stages was 0.00 gametocytes/µl blood (0-200 gametocytes/µl blood). Table 1 shows the prevalence and density of *Plasmodium*.

Prevalence and density of asexual stages of Plasmodium species with respect to locality

The highest prevalence, 70.65% (142) of asexual stages of *Plasmodium* species was recorded in Tiko while the lowest prevalence value, 12.33% (18) was recorded in Bonakanda, and the difference was significant, p=0.01 (Table 1). A higher GMPD value 839.42 parasites/ µl blood (40-29520 parasites/ µl blood) was recorded in Meanja while a lower GMPD value 496.14 parasites/ µl blood (120-4200 parasites/ µl blood) was recorded in Likoko Membea. The GMPD of

Table 2. Prevalence and density of malaria by sex and locality.

Locality	Males			Females			Overall		
	N°. examined	N° positive (%)	GMPD (range)	N°. examined	N°. positive (%)	GMPD (range)	# examined per site	# +ve (%)	GMPD (range)
Bonakanda	83	9 (10.84)	348.63 (120-3360)	63	9 (14.29)	783.15 (120-12000)	146	18 (12.33)	522.53 (120-12000)
Likoko Membea	31	4 (12.90)	491.95 (200-1760)	76	15 (19.34)	497.26 (120-4200)	107	19 (17.76)	496.14 (120-4200)
Meanja	72	42 (58.33)	927.58 (40-29520)	87	57 (65.52)	779.60 (40-20000)	159	99 (62.26)	839.42 (40-29520)
Mutengene	76	30 (39.47)	862.01 (160-5160)	112	58 (51.79)	629.07 (120-16240)	188	88 (46.81)	700.39 (120-16240)
Debundscha	37	16 (43.24)	585.43 (120-2880)	38	15 (39.47)	727.87 (160-16000)	75	31 (41.33)	650.29 (120-16000)
Tiko	74	51 (68.92)	638.14 (120-12000)	127	91 (71.65)	666.76 (120-48000)	201	142 (70.65)	656.34 (120-48000)
Overall	**373**	**152 (40.75)**	**713.04 (40-29520)**	**503**	**245 (48.71)**	**677.49 (40-48000)**	**876**	**397 (45.31)**	**690.89 (40-48000)**

asexual stages of *Plasmodium* species was highly heterogeneous amongst the different localities, p=0.02. Table 1 shows the prevalence and density of asexual stages of *Plasmodium* in the different localities sampled.

Prevalence and density of sexual stages of Plasmodium species with respect to locality

The highest prevalence, 38.71% (12) of sexual stages was recorded in Debundscha while the lowest value, 5.26% (1) was recorded in Likoko Membea, although the difference was not significant, p=0.25, Table 1. There was no significant difference in the GMPD of sexual stages of Plasmodium species in the different localities, p=0.33.

Table 1 shows the prevalence and density of sexual stages of *Plasmodium* in the different localities sampled.

Prevalence and density of malaria infection in the different localities with respect to sex

A higher prevalence, 48.71% (245) was recorded in females while a lower value, 40.75% (152) was recorded in males, although the difference was not significant, p=0.21. The result of prevalences in the different localities with respect to sex is presented in Table 2. There was no significant difference in GMPD between sexes in the different localities, p=0.51. The result of parasite density in different localities with respect to sex is shown in Table 2.

Prevalence and density of malaria infection in the different localities with respect to age groups

A higher prevalence 56.93% (226) was recorded in the age group 9-12 years while the lower prevalence 15.11% (60) was recorded in the age group 13-16 years. The prevalence of infection was highly heterogeneous between the different age groups in the different localities, p=0.02. Table 3 shows the prevalence of infection in different localities with respect to age groups. There was no significant difference in the GMPD between age groups in the different localities, p=0.83. The result of parasite density in the different age groups is shown in Table 3.

Prevalence of the different Plasmodium species per site

Out of 397 children positive for malaria parasites, 64.99% (258) had *P. falciparum* only, while 14.87% (59) were infected with either one or two the other species. The different species of Plasmodium found in the different study sites are shown in Table 4.

Man biting rate (MBR) of Anopheles species in the different localities

The highest (38.84 b/p/n) MBR of *Anopheles* species was recorded in Mutengene. On the whole *An. gambiae* was found to be the most aggressive species recording very high (32.99 b/p/n) in Tiko. Table 1 shows the different species of *Anopheles* collected from the different localities. *An. funestus* was found to be a major vector only in Mutengene with a MBR of 25.19 b/p/n. *An. hancocki* and *An. nilli* were found to be minor

Table 3. Prevalence and density of malaria parasites in different age groups per locality.

Locality	Total examined per site	Age groups (years)						Total positive per site (%)
		4-8		9-12		13-16		
		N° +ve (%)	GMPD (range)	N° +ve (%)	GMPD (range)	N° +ve (%)	GMPD (range)	
Bonakanda	146	5 (3.42)	1006.38 (200-12000)	10 (6.85)	293.58 (120-1400)	3 (2.05)	1197.63 (800-2440)	18 (12.33)
Likoko Membea	107	3 (2.80)	402.62 (200-4200)	13 (12.15)	473.64 (120-1760)	3 (2.80)	230.76 (160-480)	19 (17.76)
Meanja	159	21 (13.21)	779.75 (120-20000)	60 (37.74)	1089.20 (40-20000)	18 (11.32)	791.99 (40-29520)	99 (62.26)
Mutengene	188	21 (23.86)	676.81 (120-8960)	53 (60.23)	641.21 (120-5160)	14 (15.91)	1029.91 (160-16240)	88 (46.81)
Debundscha	75	13 (17.33)	662.67 (200-16000)	18 (24)	650.67 (120-2880)	0.00	0.00	31 (41.33)
Tiko	201	48 (33.80)	696.68 (120-48000)	72 (50.70)	675.36 (120-12000)	22 (15.49)	524.80 (120-2360)	142 (70.65)
Overall	**876**	**111 (27.96)**	**766.122 (120-48000)**	**226 (56.93)**	**655.64 (40-20000)**	**60 (15.11)**	**695.04 (40-29520)**	**397 (45.31)**

Table 4. Species of *Plasmodium* found at different localities.

Localities (altitude; m a.s.l)	Positive for *Plasmodium* species	No. PF (%)	No. PM (%)	No. PO (%)	No. PFPM (%)	No. PFPMPO (%)	No. PMPO (%)	No. PFPO (%)
Bonakanda (1197)	18	10 (55.56)	4 (22.22)	0 (00.00)	3 (16.67)	1 (5.56)	0 (00.00)	0 (00.00)
Likoko Membea (800)	19	5 (26.32)	5 (26.32)	0 (00.00)	7 (36.84)	2 (10.53)	0 (00.00)	0 (00.00)
Meanja (300)	99	56 (56.57)	16 (16.16)	1 (1.01)	16 (16.16)	9 (9.09)	1 (1.01)	1 (1.01)
Mutengene (220)	88	65 (34.57)	6 (6.82)	0 (00.00)	15 (17.05)	1 (1.14)	0 (00.00)	1 (1.14)
Debundscha (50)	31	18 (58.06)	5 (16.13)	1 (3.23)	3 (9.68)	1 (3.23)	1 (3.23)	1 (3.23)
Tiko (10)	142	104 (73.23)	20 (14.08)	1 (0.70)	11 (7.75)	5 (3.52)	0 (00.00)	1 (0.70)
TOTAL	**397**	**258 (64.99)**	**56 (14.11)**	**3 (0.76)**	**55 (13.85)**	**19 (4.79)**	**2 (0.50)**	**4 (1.01)**

PF=*Plasmodium falciparum*, PM=*Plasmodium malariae*, PO= *Plasmodium ovale.*

vectors at all the localities. Table 5 shows the MBR of all the *Anopheles* species found in the different localities of contrasting altitudes. There was no significant differences in the MBR of the different *Anopheles* species in the different localities, p=0.22.

Vectorial capacity (C) for all the different Anopheles species in the different localities

The highest (28.80) vectorial capacity for all the *Anopheles* species observed per locality was recorded in Tiko while the lowest (0.00) was observed in Bonakanda, although the difference in vectorial capacities between the various localities was not significant, p=0.21. Table 5 shows the vectorial capacities of the different *Anopheles* species obtained in different localities.

Entomological inoculation rates (EIR) of the different Anopheles species in the different localities

On the whole, *An. gambiae* recorded the highest EIR than the other *Anopheles* species in all the different localities. The EIR of all *Anopheles* species found and in the different localities is shown in Table 6.

DISCUSSION

The results of this study show that malaria epidemiology in the Mount Cameroon region is unevenly distributed, and exhibits a highly heterogeneous profile. The relative abundance of mosquitoes fluctuates with the altitude (locality).

Table 5. Vectorial capacity and man-biting rates (MBR) of *Anopheles* species at different localities during the study period.

Locality	MBR (b/p/n) of species					Vectorial capacity
	An. funestus	*An. gambiae*	*An. hancocki*	*An. nilli*	Total	
Bonakanda	0	0	0	0	0	0
Likoko Membea	4.38	1.38	4.92	0	10.68	10.06
Meanja	10.71	19.98	0.90	0	31.41	9.03
Mutengene	25.19	4.73	4.92	1.92	38.84	21.04
Debundscha	0	27.20	0	0	27.20	21.20
Tiko	2.54	32.99	0	0.67	36.20	28.80

An.= Anopheles; b/p/n= bites per person per night.

Table 6. Entomological inoculation rates (EIR) per species per locality.

Locality	Species	EIR (infective bites/person/night)
Bonakanda	**---**	---
Likoko Membea	*An. funestus*	0.15
	An. hancocki	0.03
Meanja	*An. funestus*	0.32
	An. gambiae	0.69
	An. hancocki	0.04
Mutengene	*An. funestus*	1.40
	An. gambiae	0.25
	An. hancocki	0.23
	An. nilli	0.14
Debundscha	*An. gambiae*	0.43
Tiko	*An. gambiae*	2.51

Anopheles species were most abundant at the lowest level. Although not linear, the prevalence of malaria increased as one moves from a high altitude to a low altitude. Variations in the prevalence and intensity of malaria transmission can be important in different areas of a region as this information could be very useful in allocating resources for malaria management and control.

There are several vector-related dynamics that could contribute to the associations observed in this study. *Anopheles gambiae* and *An. Funestus* are the major vectors of malaria in this study area and have been found to be infected during the dry and rainy seasons (Jambou et al., 2001). These two species are well known as efficient vectors of malaria in other areas of Africa and Madagascar (Wanji et al., 2009; Nkuo-Akenji et al., 2006). These vectors have been shown to have an uneven distribution within communities of the Mount Cameroon region (Jambou et al., 2001) with areas like Tiko and Meanja having high percentages of the mosquitoes collected than other areas. These differences in the vector population numbers could contribute to the micro-geographic differences found in malarial infection. Altitude is known to be associated with differences in mosquito population and malaria cases on a larger scale (Drakely et al., 2005).

The highest MBR was recorded in Tiko and the least in Bonakanda. Comparatively, the MBR at different localities are a clear reflection of the number of anophelines caught per locality. The fluctuations in MBR are directly related to variation in humidity and temperature conditions at each locality. The results have demonstrated that MBR decreased an increase in altitude implying that an inhabitant in a low altitudinal area is more likely to be exposed to bites by the vector than one of a high altitudinal area. *An. hancocki* and *An. nilli* were found to be minor vectors at all the localities. This agrees with the

findings of Fontenille et al., (2000) who demonstrated that *Anopheles hancocki* is a secondary vector of malaria in Cameroon.

The vectorial capacity also varied with altitude. Since the vectorial capacity is influenced by MBR and life expectancy, *An. gambiae* in Tiko maintained the bulk of aggressive vectors in this area. The high vectorial capacities observed in this study indicate the necessity of introducing vector control measures in the region as one of the strategies of fighting malaria. Such vector control needs to focus on providing an effective personal protection for the most susceptible age groups against vector contact rather than aiming at reducing the potential for transmission at the regional level (Wanji et al., 2003). The most appropriate vector control option in this area could be the use of insecticide treated nets (ITN) as these tools are currently the most effective and practical vector control option in areas where vector densities and vectorial capacities are high (Diallo et al., 1999; Guillet, 2000).

The EIR is a direct product of the man-biting rates and the sporozoite rates, implying that the high EIR recorded directly indicate a high level of bites by infected mosquitoes on man which directly reflects the level of malaria transmission in these low-lying areas. This high EIR calls for serious vector control measures to be undertaken both by the local population and the Government authorities concerned. There is probably the need to use insecticides that will kill the vectors and reduce vector densities. Also, the use of bed nets impregnated with both insecticides and repellents will help to reduce vector density and the number of bites on man. The use of lavicides could also be a good way of reducing vector densities. It is probably of great importance to combine all the control measures against vectors since this will go a long way to reduce human misery from malaria burden.

Tiko, had the highest prevalence of malarial infection and this fact is proven by the high vectorial capacity recorded at this locality. This is probably due to the fact that children living in this locality had more skin surface area exposure to mosquito bites and are consequently more exposed to malaria transmission. Localities of higher altitudes tended to be very hilly with little or no flat points where water could settle and form a pool. The increasing rate of malaria infection at low altitudinal localities could also be as a result proximity to mosquito breeding sites. As most C.D.C plantations are located in these sites, residences located closer to these agricultural fields might result in a greater local mosquito density. A recent study carried out in some localities of this region demonstrated that there was a marked dominance of temporary breeding sites over permanent breeding sites in low altitudinal localities (van Der Hoek et al., 2003), and the distribution of breeding sites could be influenced by the topography of the area. Temporal breeding sites in these areas were observed to be found around houses and these breeding sites were found to harbour more *An. gambiae* species than the other *Anopheles* species (Klinkenberg et al., 2004). Most of these temporal breeding sites were found in lower altitudinal localities (Tiko and Meanja) where the terrain is fairly flat and could allow water to stand, whereas the relief of Bonakanda (1197 m a.s.l.) is hilly and very sloppy and prevents water from standing. Alternatively, the heterogeneity observed in the study could be related to differences in the level of urbanization and housing type of the human population.

Human-vector contact is influenced to a great extent by housing type, housing and roofing material, house location, gradient, surrounding drainage and cleanliness of immediate environment Klinkenberg et al., 2004; Uarpham, 1997; Kreuels et al., 2008). Generally, it was observed that most of the houses in the area sampled in Tiko were built from wood and most of these houses have holes and crevices on their walls with no form of screens either on the doors or windows. Tiko, could is considered as a semi-urban locality. Generally, studies (Cohen et al., 2008) have shown that when compared with urban areas, mothers living in rural or semi-urban communities have lower vaccine coverage, poorer physical access to health services and lower use of insecticide-treated bed nets (ITNS), lack of screens on doors and windows. Hence, there is always an increase in the transmission of vector-borne diseases such as malaria in such areas (Cohen et al., 2008). Tiko is a low lying area and such topography will allow water to stand whereas that of Bonakanda is hilly and prevents water from standing. Topography derived wetness indices have been shown to be associated with household-level malaria (Ganser and Wisely, 2013).

The high prevalence of malaria observed in Tiko, a low altitudinal locality could be attributed to the fact that African cities in general are complex dynamic structures. Western definitions emphasize characteristics that differentiate between urban, semi-urban and rural areas, including land use patterns, increased density of households, differences in housing material, access to public transport, access to utility services and, access to social services. Many cities in sub-Saharan Africa do not meet these characteristics as in many towns vegetation still remains (Klinkenberg et al., 2008). This fact coupled with urban farming, often provides ample aquatic habitat for mosquitoes. Physical deterioration (broken or blocked water drains, potholes, rubbish , tyres, new construction activities for example excavation, building construction and irrigational schemes) and increase in human activity may increase opportunities for mosquitoes through the enhancement of shallow bodies of water and through an increase in the number of artificial water collection reservoirs. These urban agricultural areas have been shown to be associated with higher risk of malaria transmission (Klinkenberg et al., 2008). These characteristics support observations in studies carried out in Dar es Salaam, Tanzania (Dongus, 2001) which

confirmed that urban agriculture creates suitable breeding grounds for malaria vectors, although surprisingly the level of endemicity observed was very low. There is some evidence that anopheline species may be adapting to urban ecosystems. Chinery (1984, 1990) observed some adaptation of *Anopheles gambiae s. s.* to urban aquatic habitats, such as water filled domestic containers and polluted water habitats created as a result of urbanization in Accra, Ghana. In a recently urbanized area of Kenya, Khaemba et al. (1994) concluded that *An. gambiae* showed a strong preference for man-made temporary sites over permanent aquatic habitats in the rainy season, although dams and swamps remained the preferred sites during the dry season.

A notable finding in this study is the demonstration that altitude is one of the main factors that affect significantly the variability of malaria endemicity in the Mt. Cameroon region. Altitude is known to define the ecology of an area and thus malaria transmission (Bødker et al., 2003; Maxwell et al., 2003). While altitude has long been recognized as an important factor determining malaria endemicity, it is those transmission factors which are directly or indirectly affected by altitude that are of epidemiological significance, rather than altitude *per se*. Probably most important of these is environmental temperature, which has been shown to affect malaria transmission by acting as a limiting factor on the development of the *Anopheles* mosquitoes (Cox et al., 1999). Humidity is also suitable for transmission because it affects the survival rate of mosquitoes. If the average monthly relative humidity is below 60%, it is believed that the life of the mosquito is so shortened that there is no malaria transmission (Dhiman et al., 2003).

Generally, it was observed that the mean intensities of malaria infection varied greatly between localities and the difference was significant. The great heterogeneity observed in the mean intensities of malaria infection is probably due to the fact that parasite density distribution is not uniform among pupils in the different localities. Generally, younger children (4-8 years) were found to have higher parasite counts than their older school mates, although none presented with clinical signs or symptoms. It is generally known that immunity to malaria infection builds up with multiple exposures to the infection. Thus, as children get older they have probably had several attacks thereby having some partial immunity which aids in reducing the parasite load and suppressing clinical manifestations of the disease. The high prevalence rate of *P. falciparum* observed in the study population is in conformity with earlier reports from Africa in general and some parts of the Mt. Cameroon region which described *P. falciparum* as the most common cause of malaria infection (Kimbi et al., 2005). Most cases of malaria in the study area are therefore caused by *P. falciparum*, the most fatal of all the *Plasmodium* species.

Finally, it remains to be seen if this observed pattern of malaria transmission is the same in other areas in Cameroon. The Mount Cameron region has many unique environmental, socio-economical, and geographical aspects, and one might expect to find different trends in different regions. More important is the identification of high risk areas in the region which underscores the importance of tailoring a malaria control programme to meet local needs.

Conclusion

This study has shown that the parasitological and entomologic indices of malaria transmission have a heterogeneous pattern in the mount Cameroon region. Tiko, a low altitudinal area recorded the highest prevalence (70.65%) of malaria while Bonakanda the highest altitudinal area recorded the lowest (12.33%). The highest mean intensity of malaria (839.42 parasites/µl blood) was recorded in Meanja whereas Likoko Membea recorded the lowest (496.14 parasites/µl blood). *An. gambiae* was the most aggressive species recording very high number of bites per person per night. *An. funestus* was found to be a major vector only in one site while *An. hancocki* and *An. nilli* were found to be minor vectors at all sites. The vectorial capacity of the anophelines caught varied with altitude. An. gambiae recorded the highest EIR and EIR varied with altitude. Altitude is one of the factors that is a proxy to malaria heterogeneity and hence malaria epidemiology. Malaria control programmess should be based on evident spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium* species in a particular area so as not to waste resources which would be of limited effectiveness to the populations at risk. It is expected that this information will assist public health practitioners to determine where greatest needs lie for more intensively focused malaria control activities in the mount Cameroon region thereby curbing the burden of the disease in the region.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank the parents/legal guardians who willingly consented to their children participating in this study, the staff of the schools where blood samples were collected, the local chiefs for maximum cooperation and all the mosquito collectors for volunteering to carry out this work. The authors are grateful to the people who willingly accepted that the research team should use their houses for mosquito

collection. We are very grateful to Dr. Robert Wirtz of the Malaria Research and Reference Reagent Resource Centre (MR4) for providing reagents for ELISA–CSP. This study received financial assistance from the Research Foundation for Tropical Diseases and Environment (REFOTDE).

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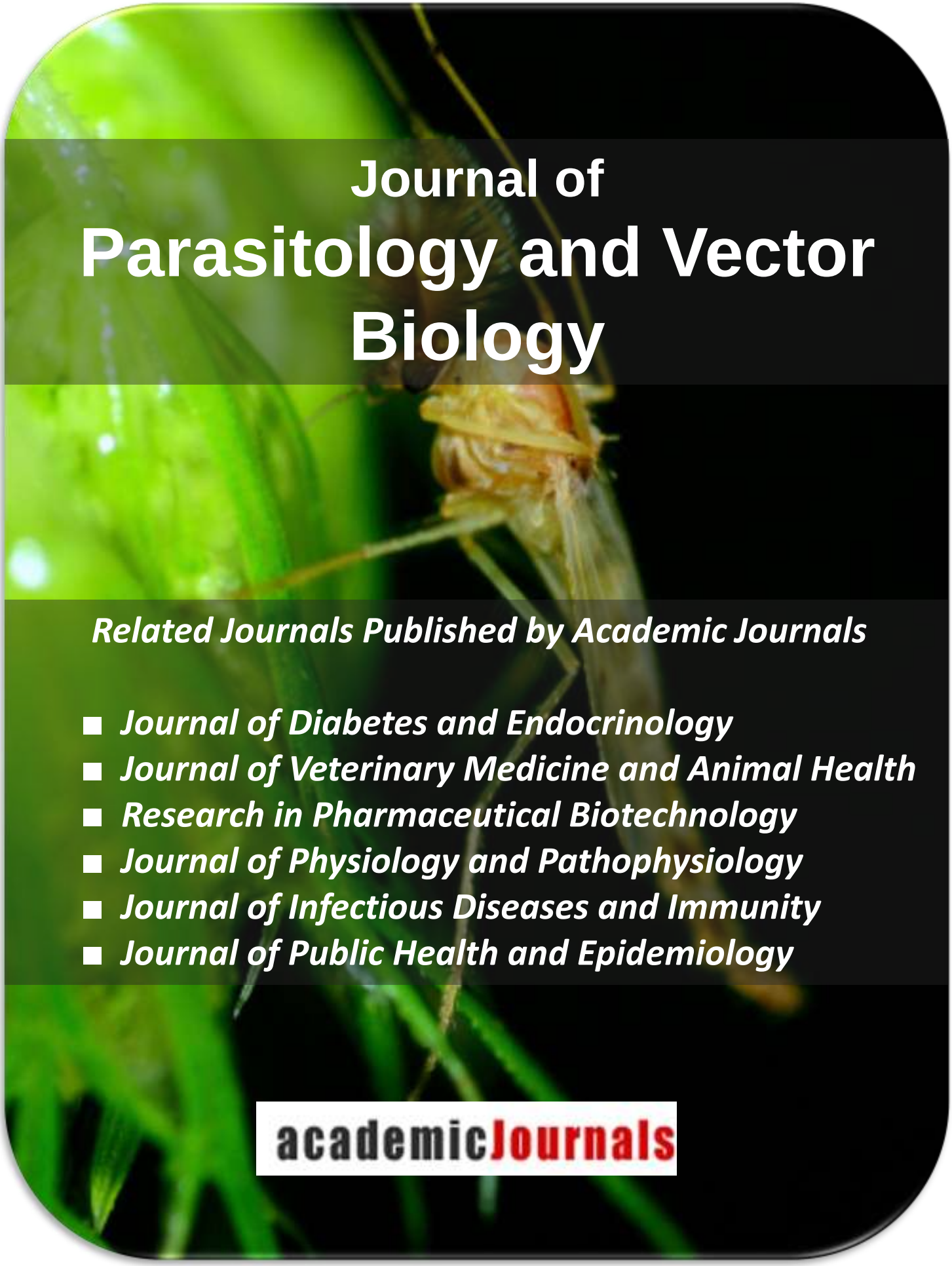

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