

Environmental Factors and Preventive Methods against Malaria Parasite Prevalence in Rural Bomaka and Urban Molyko, Southwest Cameroon

Helen Kuokuo Kimbi^{1*}, Yannick Nana¹, Irene Ngole Sumbele¹, Judith K Anchang-Kimbi¹, Emmaculate Lum¹, Calvin Tonga¹, Malaika Nweboh¹ and Leopold G Lehman²

¹Faculty of Science, Department of Zoology and Animal Physiology, University of Buea, P.O. Box 63, Buea, SWR, Cameroon

²Faculty of Science, Department of Animal Biology, University of Douala, P.O. Box 2701, Douala, Cameroon

Abstract

Malaria remains a major public health problem in Cameroon. This work was aimed at determining the influence of environmental factors and control measures on malaria parasite prevalence, and anaemia in pupils in rural Bomaka and urban Molyko, Southwest Cameroon. A total of 303 pupils (174 and 129 from Bomaka and Molyko, respectively), aged 4-15 years were studied. Information on demographic data, environmental and anti-malarial measures was recorded. Malaria was diagnosed from Giemsa-stained blood smears. Packed Cell Volume (PCV) was determined. The overall malaria parasite prevalence was 33.0%, and children from Bomaka had a significantly higher value (38.51%) than those from Molyko (25.58%). Malaria parasite prevalence was significantly higher in males and highest in children aged ≤ 6 years. Overall location, age and stagnant water were associated with malaria parasite prevalence. Although malaria parasite prevalence was higher in pupils who had bushes around their homes, lived in plank houses, and did not use Insecticide Residual Spraying (IRS), the differences were, however, not significant. The overall prevalence of anaemia was 14.0%, with the ≤ 6 years old pupils having the highest anaemia prevalence. Parasite density was significantly higher in anaemic ((1369, CI=504.25-2511.89) than non-anaemic children ((507, CI=313.74-603.32). A combination of environmental and preventive measures (especially in rural areas) as well as community participation will reduce malaria transmission.

Keywords: Influence; Environmental; Control; Factors; Malaria; Anaemia; Prevalence; Cameroon

Introduction

Malaria transmission is perennial in Southwest Cameroon with seasonal increases during the rainy season, and varies with levels of urbanization [1]. The disease is also a major contributor to anaemia and may have profound consequences on learning and educational achievement of school age children [2]. Some studies have suggested that poor environmental sanitation and housing conditions, as well as lack of appropriate control measures might be significant risk factors for malaria parasite burden [3]. Environmental factors such as the presence of bushes and stagnant water around homes, rainfall, low altitude and high temperatures favor the breeding of malaria vectors, as well as parasite reproduction within them [4], while increased urbanization tends to reduce the rate of Anopheles breeding. The key to addressing the challenge of reducing malaria parasite prevalence is an integrated approach that combines preventative measures, such as Insecticide Treated bed Nets (ITNs), Indoor Residual Spraying (IRS), improved access to effective anti-malarial drugs [5], as well as proper environmental management.

Although malaria continues to put a heavy toll on human life and on fragile economies of many African countries, recent trends in some African countries like Kenya [6], Tanzania [7] and Cameroon [8], indicate that malaria morbidity and mortality is on a decline, as a result of scale up use of ITNs and increased availability of anti-malarial medicines. A combination of environmental manipulation and other control measures have also been shown to reduce the overall malaria incidence and mortality by 50% in Zambia [9].

There is no documented information on the influence of environmental factors and use of preventive measures on the malaria parasite prevalence in inhabitants of rural Bomaka and urban Molyko in Southwest Cameroon. This study was therefore aimed at evaluating the influence of environmental factors, as well as some control

measures on malaria parasite prevalence, density and anemia in school children in these areas. The results of this study will help the National Malaria Control Programme in Cameroon to plan proper management strategies for malaria, while taking into consideration the levels of heterogeneities that exists within the same localities.

Materials and Methods

Study area

The study was carried out at two sites in Southwest Cameroon-Bomaka and Molyko. Bomaka is a new layout (rural) located at an altitude of 507 m above sea level (a.s.l.). The area was formerly a banana plantation owned by the Cameroon Development Corporation (CDC). The land was recently handed over to the natives (Bakweris) of the area, and a lot of construction work is presently taking place in the area. Many houses are surrounded by bushes, cultivated crops and stagnant water. There is a mixture of two major house types-plank and cement bricks. Molyko (600 m a.s.l.) is an urbanized area in the neighborhood of Bomaka, and hosts the first English-speaking university in Cameroon. There are few or no bushes around houses in Molyko, and the majority of houses are constructed with cement bricks. The two sites have an annual rainfall of about 4,000 mm and a relative humidity of over 80%.

***Corresponding author:** Helen Kuokuo Kimbi, Faculty of Science, Department of Zoology and Animal Physiology, University of Buea, Cameroon. Tel: +237 7783 66 03/9403 53 55; Fax: +237 3332 22 72; E-mail: hkimbi@yahoo.co.uk

Received November 27, 2012; **Accepted** December 28, 2012; **Published** January 02, 2013

Citation: Kimbi HK, Nana Y, Sumbele IN, Anchang-Kimbi JK, Lum E, et al. (2013) Environmental Factors and Preventive Methods against Malaria Parasite Prevalence in Rural Bomaka and Urban Molyko, Southwest Cameroon. J Bacteriol Parasitol 4:162. doi:10.4172/2155-9597.1000162

Copyright: © 2013 Kimbi HK et al, This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Temperatures range from 18°C-29°C. There are two seasons, the rainy and the dry seasons, which start from mid-March to October and November to mid-March, respectively.

Study population

Children aged 4-15 years and of both sexes were recruited from Government Schools, Bomaka and Molyko. The purpose and methodology of the survey were explained and planned with the teachers. Informed consent forms were sent to the parents/guardians through the pupils, explaining the purpose and benefits of the study, as well as the safety precautions that had to be taken to ensure the safety of the children. The forms were signed and returned through the children, and selection for participation in the study was based on acceptance of the parents/guardians and willingness of the children to take part in the study.

Study design and ethical considerations

The study was cross-sectional and was carried out for 4 months-March to June, 2011. The investigative methods included the use of questionnaires, clinical evaluation and laboratory methods. A field questionnaire form was administered to the pupils, to obtain important demographic data such as age, sex and area of residence. The questionnaire also sort to obtain information on environmental factors, such as house type, the presence of stagnant water and bushes around the residence, as well as use of preventive methods like use of indoor residual spraying and insecticide treated bed nets. The axillary temperature was determined using a mercury thermometer.

An ethical clearance for the study was obtained from the Ethical Review Committee of the South West Regional Delegation of Public Health, while an administrative clearance was obtained from the South West Delegation of Basic Education, Cameroon.

Collection of blood samples and preparation of blood films

Capillary blood was collected from a finger prick, and thin and thick blood films were prepared by the method of Cheesbrough [10], for the detection and speciation of malaria parasites, respectively. Heparinized capillary tubes were filled with blood for the determination of Packed Cell Volume (PCV).

Staining and microscopic examination of blood films

After fixing thin blood films with absolute methanol, both thick and thin blood films were stained with 10% Giemsa for 20 minutes and examined under the 100X (oil immersion) objectives of a UNICO® light microscope, for the detection and identification of malaria parasites, respectively, using the bench aids of Cheesbrough [10]. Slides were declared negative if no asexual parasites were found after examining 100 high-power fields. Slides were read by two independent parasitologists, and in the case of any disparity, they were read again by a third person. Malaria parasites were counted against 200 leucocytes in thick blood films, and parasite density was expressed as number of parasites per microlitre (µL) of blood, assuming a White Blood Cell (WBC) count of 8,000 leucocytes per µL of blood [10].

Determination of packed cell volume

Blood-filled heparinized capillary tubes were centrifuged for 5 minutes at 10,000 rpm, and the PCV values were read using a microhaematocrit reader. PCV values of <15% were classified as severe anaemia, while values of 15-20% and 21-30% were classified as moderate and mild anaemia, respectively. PCV values of ≥ 31% were considered normal [10].

Statistical analysis

Data was recorded into spread sheets using Microsoft Excel and analyzed with the Statistical Package for Social Sciences (SPSS) version 17. Haemoglobin and patient's ages are normally distributed and therefore, were presented as means ± SDs. Proportions were compared using the chi-square test. Means were compared using independent sample t-test and Fisher's (F-Test). Odds Ratios (OR) was calculated to determine the important factors that were associated with malaria parasite prevalence. Pearson's correlation coefficient was used to evaluate relationship between variables. Significant levels were measured at 95% confidence level, with significant differences recorded at P<0.05.

Results

Baseline characteristics of the study population

Out of a total of 550 informed consent forms given out, only 303 pupils brought back signed forms and were all included in the study. A total of 174 and 129 pupils were from Bomaka and Molyko, respectively. Out of the 303 blood samples collected, the identity numbers of 10 blood samples got missing during transportation from the field to the University of Buea Malaria Research Laboratory. Thus, PCV values were analyzed on 293 samples. The study consisted of more females (189) than males (114), and the majority of pupils examined were in the 7-10 years age group. The overall malaria parasite prevalence in the study population was 33.0%. The prevalence of pyrexia was 26.7%, while that of anaemia was 14.0% (Table 1).

Malaria parasite prevalence and density with respect to sex

The prevalence of malaria parasites was significantly higher ($\chi^2=8.23$, d.f.=1, P=0.01) in males (43%), when compared with females (27%). On the contrary, geometric mean parasite density was higher in females (614.45 parasites/µl of blood, CI=321.55-807.30) than in males (607.66 parasites/µl of blood, CI=355.90-786.85), but the difference was not statistically significant (F=0.0013, d.f.=1, P=0.9709).

Malaria parasite prevalence as affected by environmental factors, control measures, house type, age and location

The prevalence of malaria parasites was higher in pupils who had stagnant water around their homes (42.95%), when compared with those who did not have stagnant water around their homes (23.38%). The presence of stagnant water around homes showed a significant association with the prevalence of malaria parasite (OR=2.47, P=0.0003) as seen in table 2. The highest prevalence of malaria parasites was recorded in pupils in the youngest age group, ≤ 6 years (50.00%), while the lowest was recorded in pupils of the oldest age group, 11-15 years (23.76%). Age was associated with malaria parasite prevalence (OR=0.31, P=0.0012). Those who lived in Bomaka had a significantly higher malaria parasite prevalence (38.51%), when compared with those

Characteristic	Category	Value
	Male	37.6% (114/303)
	Female	62.4% (189/303)
Age Groups (Years)	≤ 6	16.5% (50/303)
	7-10	50.2% (152/303)
	11-15	33.3% (101/303)
Mean Age (Years)		9.29 ± 2.39 (range: 4-15) years
Mean Temperature at Enrolment		37.12 ± 0.55 (range: 35.5-38.7) oC
Mean PCV ± SD (%)		35.85 ± 3.32 (range: 24-48)
Overall Prevalence of Malaria		33.0 % (100/303)
Anaemic Status	Anaemic (PCV<31%)	14% (41/293)

Table 1: Baseline Characteristics of the Study Population.

living in Molyko (25.58%), hence, living in Bomaka was associated with malaria parasite prevalence (OR=1.82, P=0.0180). Although malaria parasite prevalence was higher in pupils who had bushes around their homes, lived in plank houses, and did not use IRS, the differences were however not significant (Table 2). None of these factors was associated with malaria parasite density.

Anaemia status as affected by site, gender and age

The prevalence of anaemia was similar at the study sites ($\chi^2=1.79$, d.f. =1, P=0.18) and in the sexes ($\chi^2=0.82$, d.f =1, P=0.37). The highest prevalence of anaemia (27.3%) was recorded in the ≤ 6 years age group, while the lowest (9.9%) was recorded in the oldest age group, and the difference was statistically significant ($\chi^2=8.02$, P=0.02) as shown in figure 1. Among children with anaemia, the majority were moderately anaemic (80.5%), when compared with the mild anaemic cases (19.5%). No case of severe anaemia was recorded in the study population.

PCV Levels and anaemia status as affected by malaria parasite prevalence and density

Children infected with malaria parasites had a significantly (t= 2.47, P=0.01) lower mean PCV level (35.16 ± 3.52), when compared with malaria negative children (36.17 ± 3.17). More so, a negative correlation (r=-0.20), was observed between PCV levels and malaria parasitaemia, which approached significance ($\chi^2= 7.99$, d.f.=2, P=0.05), as shown in figure 2. Anaemic children had a significantly (F=6.537, d.f.=1, P=0.0122) higher geometric mean malaria parasite density (1369, CI=504.25-2511.89), than non anaemic children (507, CI=313.74-603.32).

Discussion

This study investigated the influence of environmental factors, as well as control measures on malaria parasite prevalence, density and anaemia in school children from two Government primary schools, located at sites with different levels of urbanization in the Mount Cameroon Region. The overall prevalence of malaria parasites in the study population was low, when compared to values of similar studies carried out in the Mount Cameroon region [11,12]. This observation might probably reflect the recent improvement in malaria control strategies, especially among the vulnerable groups in Cameroon by the government, consisting of the free distribution of long lasting insecticide treated bed nets, as well as free treatment of malaria among children less than five years of age [8]. This agrees with a report from Kenya [6], which showed that malaria morbidity and mortality was on a decline, as a result of scale up anti-malarial activities.

The study revealed a significant influence of sex on the prevalence of

malaria parasites, with male children having a higher prevalence, when compared with females. This could be due to the fact that males expose their bodies more often than females, especially when the weather is hot, thus, increasing their chances of being bitten by mosquitoes.

The study showed that children in the youngest age group, ≤ 6 years, had significantly high malaria parasite prevalence, when compared to the older ones. This agrees with the report of Bodker et al. [13] who stated that acquired immunity is both exposure- and age-dependent, and the older children are likely to have developed some degree of immunity, as a result of repeated infections.

The study showed a significantly higher prevalence of malaria parasites in school children in Bomaka, when compared to Molyko. Molyko is an urbanized area, while Bomaka is more of a rural area. Some reports have stated that the level of malaria transmission in any area is generally higher in rural than in urban settings [1], due to environmental factors. Generally, it is considered that suitable breeding places are scarce in highly populated urban areas, and this leads to a reduction in the frequency and transmission dynamics of malaria [1]. However, evidence of the adaptation of malaria vectors to the African urban environment has been reported [14]. The finding also suggests that malaria parasite prevalence increases with a decrease in altitude [12,15]. In Africa, lowland generally has a higher malaria transmission, when compared with highland [16]. This is probably due to the fact that temperatures are generally higher at lower altitudes, thus, encouraging a faster growth rate and abundance for the malaria vectors, and consequently, a higher entomological inoculation rate.

The association of Bomaka with malaria parasite prevalence is not surprising, since Bomaka is a 'new layout' with a lot of bushes in many unoccupied plots in-between homes. A lot of farming and house construction works are going on in this area, thus, creating potholes for stagnant water. These probably increase additional breeding sites for malaria vectors. This work was carried out in the rainy season, which probably influenced the presence of breeding sites in the area [17], leading to an increase in vector density, man/vector contact, higher inoculation rates and consequently, higher transmission intensity. Mattys et al. [18] and Klinkenberg et al. [19] reported similar findings in Cote D'Ivoire and Ghana, respectively. Guthmann et al. [20] also reported seasonal malaria during the months of agricultural activity in Peru. Interestingly, Bomaka shares a boundary with Molyko in the Mount Cameroon region and shows such differences in malaria parasite prevalence. Major heterogeneities in malaria transmission [21], and other malarimetric indices [20,22-25] between different areas of the same villages, town and between cities have been reported, and these have been attributed to major differences in access to health structures and a variety of environmental factors.

Characteristic	Category	Malaria parasite		Prevalence	OR	CI	χ^2	p
		Positive	Negative					
House type	Block	36	86	29.51%	Reference			
	Plank	64	117	35.36%	1.31	0.80-2.14	1.13	0.2900
Bushes around the house	No	31	81	27.68%	Reference			
	Yes	69	122	36.13%	1.48	0.89-2.46	2.28	0.1300
Use of ITN	Yes	26	47	35.62%	Reference			
	No	74	156	32.17%	0.86	0.49-1.49	0.30	0.5800
IRS	Yes	17	44	27.87%	Reference			
	No	83	159	34.30%	1.35	0.73-2.51	0.91	0.3400
Presence of water bodies	No	36	118	23.38%	Reference			
	Yes	64	85	42.95%	2.47	1.51-4.05	13.13	0.0003
Age group (years)	6	25	25	50.00%	Reference			
	7-10	51	101	33.55%	0.51	0.26-0.97	4.34	0.0370
	11-15	24	77	23.76%	0.31	0.15-0.64	10.50	0.0012
Location	Molyko	33	96	25.58%	Reference			
	Bomaka	67	107	38.51%	1.82	1.11-3.00	5.60	0.0180

Table 2: Influence of environmental factors, control measures, house type, age, and study site on malaria parasite prevalence between sites.

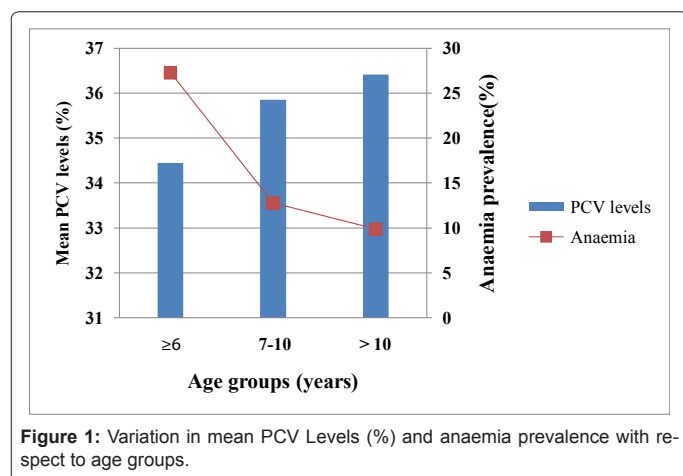


Figure 1: Variation in mean PCV Levels (%) and anaemia prevalence with respect to age groups.

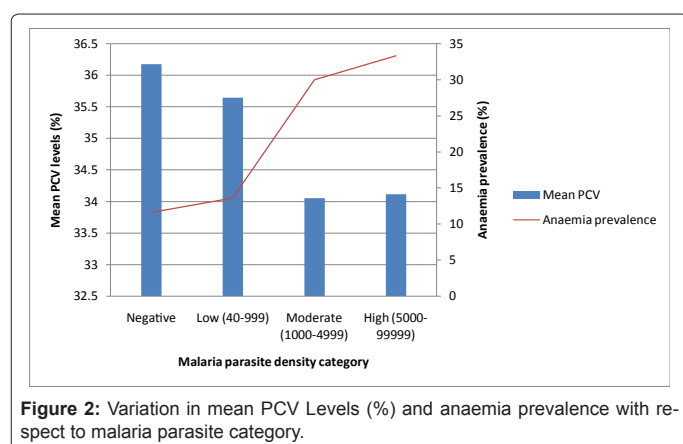


Figure 2: Variation in mean PCV Levels (%) and anaemia prevalence with respect to malaria parasite category.

It is also worth noting that Molyko inhabitants are mostly university students and lecturers who are well educated, and are probably more able to afford protective measures against malaria. It has also been reported that the wealthy and educated households [26], often live in clean environments, and are able to afford better mosquito-bite preventive measures, and good medical attention when afflicted with malaria. The major victims are usually the poor, less privileged and economically downtrodden people, who often have dirty environments, and most often, have no means of acquiring better mosquito-bite preventive measures, and no access to modern treatment.

Stagnant water around residences was associated with significantly higher malaria parasite prevalence, when compared to those who did not have stagnant water around their home. This suggests that the presence of stagnant water around homes constitutes a risk factor that needs to be taken into consideration, when planning control measures against malaria, as malaria vectors need water for the survival of the immature stages.

Overall, there was lower malaria parasite prevalence in the participants that were using IRS, when compared with those not using IRS, although the difference was not significant. The use of IRS normally leads to a reduction in man-vector contact and reduces the transmission of malaria. A comparative study done in Kenya showed that sleeping in a room sprayed with insecticide reduced the risk of infection by 75%, while sleeping under an ITN reduced the risk of infection by 63% [27]. The efficacy of IRS has also been demonstrated in several studies in Peru [20], and Ethiopia [28]. Surprisingly, those using ITNs instead had

a higher prevalence of malaria parasites. Maybe the ITNs were torn and the owners were still exposed to mosquito bites.

Although malaria parasite prevalence was higher in those living in plank than brick houses, the difference was, however, not significant. This agrees with the report of Peterson et al. [16], who did not find any association with malaria incidence and housing quality. It however disagrees with that of Protopopoff et al. [17], who reported that mosquitoes can easily enter houses constructed of poor materials with open eaves (such as plank houses, which usually have crevices on the walls), and these are associated with higher risks of malaria, as mosquitoes can easily enter and remain in the sleeping rooms during the night.

The significantly high prevalence of anaemia recorded in children aged 6 years and below, agrees with findings of Ekvall et al. [29], who observed that anaemia was determined by cumulated *P. falciparum* parasite densities in Tanzanian children. Sumbele et al. [30] reported similar findings.

Malaria parasite status had a significant impact on PCV levels, while anaemic children showed a significantly high parasite density. These could be due to the destruction of the red blood cells by the parasites, as they feed on haemoglobin of red blood cells, accelerated removal of both parasitized and non-parasitized red blood cells, as well as depressed and ineffective erythropoiesis, hence, reducing haemoglobin levels leading to anaemia [31]. More so, a negative correlation was observed between PCV levels and malaria parasitaemia, which approached significance.

The limitation of this study is that data was not collected on the vector population and man-biting habits of the population. The household size and presence of other animals in homes, which serve as alternative sources of blood meals for vectors was not also assessed.

Due to the heterogeneities that exist between village, town and city centers, it is necessary to target malaria control interventions in specific urban and rural populations and areas. Therefore, detailed information on malaria indices from these areas can help in planning effective control measures and allocation of health resources. Our study also indicates that if malaria control targets stagnant water around homes, in combination with other preventive measures (especially in rural areas), the breeding sites for malaria vectors will be eliminated and transmission will decrease. Therefore, community participation is very necessary for such a programme to succeed.

Acknowledgements

We are grateful to all the parents/guardians for their consent and the children who participated in this study.

References

1. Kimbi HK, Lum E, Wanji S, Mbuh JV, Ndamukong-Nyanga L, et al. (2012) Co-infections of asymptomatic malaria and soil-transmitted helminths in school children in localities with different levels of urbanization in the Mount Cameroon Region. *J Bacteriol Parasitol* 3: 134.
2. Laloo D, Olukoya P, Olliaro P (2006) Malaria in adolescence: burden of disease, consequences and opportunities for intervention. *Lancet Infect Dis* 6: 780-793.
3. Nkuo-Akenji T, Ntonifor NN, Ndikum MB, Abongwa EL, Nkwescheu A, et al. (2006) Environmental factors affecting malaria parasite prevalence in rural Bolifamba, South West Cameroon. *Afr J Health Sci* 13: 40-46.
4. Messina JP, Taylor SM, Meshnick SR, Linke AM, Tshetu AK, et al. (2011) Population, behavioural and environmental drivers of malaria prevalence in the Democratic Republic of Congo. *Malar J* 10: 161.
5. Kokwaro G (2009) Ongoing challenges in the management of malaria. *Malar J* 8: S2.

6. Okiro EA, Hay SI, Gikandi PW, Sharif SK, Noor AM, et al. (2007) The decline in paediatric malaria admissions on the coast of Kenya. *Malar J* 6: 151.
7. Mmbando BP, Vestergaard LS, Kitua AY, Lemnge MM, Theander TG, et al. (2010) A progressive declining in the burden of malaria in north-eastern Tanzania. *Malar J* 9: 216.
8. Kimbi HK, Keka FC, Nyabeyeu HN, Ajeagah HU, Tonga CF, et al. (2012) An update of asymptomatic falciparum malaria in school children in Muea, Southwest Cameroon. *J Bacteriol Parasitol* 3: 154.
9. Utzinger J, Tozan Y, Singer BH (2001) Efficacy and cost effectiveness of environment management for malaria control. *Trop Med Int Health* 6: 677-687.
10. Cheesbrough M (2004) *District Laboratory Practice in Tropical Countries*. (2nd edn), Cambridge University Press, United Kingdom.
11. Kimbi HK, Awah NW, Ndamukong KJN, Mbuh JV (2005) Malaria infection and its consequences in school children. *East Afr Med J* 82: 92-97.
12. Achidi EA, Apinjoh TO, Mbuwne E, Besingi R, Yafi C, et al. (2008) Febrile status, malarial parasitaemia and gastro-intestinal helminthiasis in school children resident at different altitudes, in south-western Cameroon. *Ann Trop Med Parasitol* 102: 103-118.
13. Bodker R, Msangeni HA, Kisinza W, Lindsay SW (2006) Relationship between the intensity of exposure to malaria parasites and infection in the Usambara Mountains, Tanzania. *Am J Trop Med Hyg* 74: 716-723.
14. Awolola TS, Oduola AO, Obansa JB, Chukwura NJ, Unyimadu JP (2007) *Anopheles gambiae* s. s breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *J Vector Borne Dis* 44: 241-244.
15. Kimbi HK, Tetteh KK, Polley SD, Conway DJ (2004) Cross-sectional study of specific antibodies to a polymorphic *Plasmodium falciparum* antigen and of parasite antigen genotypes in school children on the slope of Mount Cameroon. *Trans R Soc Trop Med Hyg* 98: 284-289.
16. Peterson I, Borrell LN, El-Sadr W, Teklehaimanot A (2009) Individual and household level factor associated with malaria incidence in a highland region of Ethiopia: A multilevel analysis. *Am J Trop Med Hyg* 80: 103-111.
17. Protopopoff N, Van Bortel W, Speybroeck N, Van Geertruyden JP, Baza D, et al. (2009) Ranking malaria risk factors to guide malaria control efforts in African highlands. *PLoS one* 4: e8022.
18. Matthys B, Vounatsou P, Raso G, Tschannen AB, Becket EG, et al. (2006) Urban farming and malaria risk factors in a medium-sized town in Cote d'Ivoire. *Am J Trop Med Hyg* 75: 1223-1231.
19. Klinkenberg E, McCall PJ, Hastings IM, Wilson MD, Amerasinghe FP, et al. (2005) Malaria and irrigated crops, Accra, Ghana. *Emerg Infect Dis* 11: 1290-1293.
20. Guthmann JP, Hall AJ, Jaffar S, Palacios A, Lines J, et al. (2001) Environmental risk factors for clinical malaria: a case-control study in the Grau region of Peru. *Trans R Soc Trop Med Hyg* 95: 577-583.
21. Robert V, Macintyre K, Keating J, Trape JF, Duchemin JB, et al. (2003) Malaria transmission in urban sub-Saharan Africa. *Am J Trop Med Hyg* 68: 169-176.
22. Keiser J, Utzinger J, Caldas de Castro M, Smith TA, Tanner M, et al. (2004) Urbanization in sub-saharan Africa and implication for malaria control. *Am J Trop Med Hyg* 71: 118 -127.
23. Sabatinelli G, Bosman A, Lamizana L, Rossi P (1986) Prevalence of malaria in Ouagadougou and the surrounding rural environment during the period of maximal transmission. *Parasitologia* 28: 17-31.
24. Dabire E (1990) Morbidité et mortalité palustres au sein de la pathologie febrile dans e Service de pédiatrie de l'hôpital de Yalgado ouédraogo. Université de Ouagadougou.
25. Wang SJ, Lengeler C, Smith TA, Vounatsou P, Diadie DA, et al. (2005) Rapid urban malaria appraisal (RUMA) I: epidemiology of urban malaria in Ouagadougou. *Malar J* 4: 43.
26. Macintyre K, Keating J, Sosler S, Kibe L, Mbogo CM, et al. (2002) Examining the determinants of mosquito-avoidance practices in two Kenyan cities. *Malar J* 1: 1-14.
27. Guyatt HL, Corlett SK, Robinson TP, Ochola SA, Snow RW (2002) Malaria prevention in highland Kenya: indoor residual house spraying vs insecticide-treated bed nets. *Trop Med Int Health* 7: 298-303.
28. Bekele D, Belyhun Y, Petros B, Deressa W (2012) Assessment of the effect of insecticide-treated nets and indoor residual spraying for malaria control in three rural kebeles of Adami Tulu District, South Central Ethiopia. *Malar J* 11: 127.
29. Ekvall H, Arese P, Turrini F, Ayi K, Mannu F, et al. (2001) Acute haemolysis in childhood falciparum malaria. *Trans R Soc Trop Med Hyg* 95: 611-617.
30. Sumble IUN, Nkuo-Akenji T, Samje M, Ndzeize T, Ngwa EM, et al. (2010). Haematological changes and recovery associated with treated and untreated *P. falciparum* infection in children in the Mount Cameroon Region. *J Clin Med Res* 2: 143-151.
31. Tarimo DS (2007) Appraisal on the prevalence of malaria and anaemia in pregnancy and factors influencing uptake of intermittent preventive therapy with sulfadoxine-pyrimethamine in Kibaha district Tanzania. *East Afr J Public Health* 4: 80-83.