IMPACT OF IRRIGATED URBAN AGRICULTURE ON MALARIA TRANSMISSION IN TWO CITIES IN GHANA

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by

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ABSTRACT

In rapidly expanding cities worldwide, urban agriculture is being promoted to increase food security, improve nutritional status and contribute to poverty alleviation. However, there is a concern that urban agriculture, especially when irrigated, could increase urban malaria transmission by providing breeding places for *Anopheles*.

To investigate this, epidemiological and socio-economic baseline surveys were carried out in the two main cities in Ghana, Accra and Kumasi, where communities close to (UA) and far from agriculture (U) were selected. A total of 3525 children (1744 in Accra and 1781 in Kumasi) were enrolled in a house to house survey for malaria parasitaemia, Hb concentration and socio-economic factors. Although overall malaria prevalence was higher in Accra than Kumasi (14.8%, 95% CI 13.1-16.5% and 8.6%, 95%CI 7.3-9.9%, P=0.001), in both cities, malaria prevalence was heterogeneous, ranging from 3-35% between communities. Factors associated with malaria prevalence were low socio-economic status, higher age and anaemia. In Accra, but not in Kumasi, communities near urban agriculture had significantly higher malaria prevalence (OR 1.53, 95%CI 1.10-2.14, P=0.008) and some, but not all, communities showed a significant inverse link between malaria prevalence and distance from agriculture. A second survey in Accra two years later indicated important inter annual variation in malaria prevalence and importance of risk factors. Travel was an independent risk factor likely due to the low malaria prevalence.

Entomological indices were measured by human bait catches (HBC), pyrethrum knockdown catches (PKD) and larval surveys. In Accra man biting rates by HBC were higher in UA than U communities for both *Anopheles* (8.4 in UA and 2.8 in U) and *Culex* (171.4 in UA and 41.7 in U). The annual entomological inoculation rate (EIR) was 19.9 in UA and 6.6 in U communities. Sporozoite infection rate was 0.65% (11/1672) indicating local transmission. Urban *A. gambiae s.s.* were found breeding in water at broken pipes, construction sites and poorly maintained drains. In the urban agricultural sites irrigation wells were the most common breeding sites, although only 6% of wells hosted *Anopheles*.

In a multivariate analysis, agriculture explained only a small proportion of parasitaemia prevalence and it was concluded that vector control might best be directed at adults rather than at breeding sites. In an insecticide-treated bednet trial, a cohort of approximately 250 children in intervention and control areas was followed up at 0, 3 and 6 months after net distribution. After 6 months, there were fewer new cases of malaria and significantly higher scores for nutritional indicators in children under 5 years in the intervention area, than in the control area. Children in the control area living within 300m of households that received nets had significantly higher Hb concentrations and half the chance of being anaemic compared to the children living more than 300m away, suggesting a protective community effect.

Epidemiology of urban malaria is complex and highly heterogenous and as the majority of the African population is moving into the city better insight in risk factors and best options for malaria control is urgently needed.

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GLOSSARY

AMA – Accra Metropolitan Assembly

AR – Attributable Risk

CDC – Centre for Disease Control and Prevention

CI – Confidence Interval

CSIR – Council for Scientific and Industrial Research (Ghana)

DNA – Deoxyribo Nucleic Acid

EHP – Environmental Health Project

EIR – Entomological Inoculation Rate

ELISA – Enzyme-Linked Immunosorbent Assay

GM – Geometric Mean

GPS – Global Positioning System

HAZ – Height for Age Z-score

HBC – Human Bait Catches

HBI – Human blood Index

ITNs – Insecticide-treated bed nets

IVM – Integrated Vector Management

IWMI – International Water Management Institute

KMA – Kumasi Metropolitan Assembly

MBR – Man Biting Rate

MKP – Malaria Knowledge Programme

MoH – Ministry of Health

MUAC – Mid-Upper Arm Circumference

NMCP – National Malaria Control Programme

NS – Not Significant

OPD – Outpatients Department

OR – Odds Ratio

PCR – Polymerase Chain Reaction

PKD – Pyrethrum Knockdown Catches

RUAF – Resource Centre of Urban Agriculture and Forestry

SD – Standard Deviation

SEM – Standard Error of Mean

SIMA – System Wide Initiative on Malaria and Agriculture

WAZ – Weight for Age Z-score

WHZ – Weight for Height Z-score

WHO – World Health Organization

WRI – Water Research Institute of CSIR

Chapter 1

1. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

According to conservative United Nations projections, Africa's population will almost triple by 2050. This boom will happen primarily in urban and peri-urban areas, where the population growth rate of 3.5% is more than three times the rate of the rural population growth and it is estimated that by 2025, over 50% of the African population will live in urban areas (UNPP 2005a). In West Africa the urban population growth rate of 4% is more than four times the rural growth rate and by 2030 the level of urbanization will have reached 58% (UN Habitat 2001). The increasing population will require increased food supply and (peri)-urban agriculture has often been promoted as a means to increase food security in cities and at the same time improve nutrition and contribute to poverty alleviation (Smit *et al.* 1996). However, there is a concern that urban agriculture, especially when irrigated, could result in an increased human health risk. In most cases untreated or partially treated urban wastewater is used to irrigate high value vegetable crops. This poses important health risks to farming communities and consumers of products irrigated with the wastewater. In addition urban agricultural areas can create favourable habitats for insect disease vectors especially malaria mosquitoes (Birley & Lock 1998). This potential risk was highlighted in the e-conference on health impacts of urban and peri-urban agriculture as an area where actual figures are urgently needed (Lock & De Zeeuw 2001). This led to the development of a pilot study (Afrane *et al.* 2004) which continued to my studies, where the main objective was to assess the impact of irrigated urban agriculture on malaria transmission in urban Ghana.

1.2. Malaria

Every year more than one million people die of malaria globally, predominantly children under five years of age. It is estimated that 300-500 million people world-wide are at risk of the disease, of whom 90 percent live in Africa south of the Sahara (Snow *et al.* 1999, Breman 2001). Kleinschmidt *et al.* (2001) estimated that of West Africa's 300 million people, 168 million people live in an area with predicted malaria prevalence above 30%, while 16 million of these live in an area with a predicted

prevalence of more than 70%. There are a number of factors contributing to the high malaria burden in Africa: the majority of cases are caused by *Plasmodium falciparum*, the most severe of the four human malaria parasites, and Africa has the most efficient vector, *Anopheles gambiae s.l.* (Greenwood & Mutabingwa 2002). In Asia, the Entomological Inoculation Rate (EIR, the number of infective bites a person receives) rarely exceeds 5 per year, while in Africa EIRs of over 1000 have been reported (Greenwood & Mutabingwa 2002). Malaria has important economic implication both at households and national level, with malaria endemic countries being poorer and having a lower economic growth rate (Gallup & Sachs 2001, Sachs & Malaney 2002).

Malaria is the most important childhood disease in sub-Saharan Africa and is the principal cause of at least 20% of child deaths in Africa (WHO 2003). Snow *et al.* (2004) estimated that infection with *P. falciparum* more than doubled all-cause child mortality. Several large scale studies have found larger reductions of all cause mortality than was anticipated from a reduction in malaria alone due to the impact of the burden of malaria on chronic anaemia, low birthweight and severity of other childhood illnesses (Snow *et al.* 2004). Malaria burden is highest in children, especially under the age of five, as they have not yet developed immunity. Development of immunity depends on many factors but infection pressure, *i.e.* level of endemicity of malaria is important. As transmission increases severe malaria becomes increasingly concentrated among younger age groups and at high transmission rate is mainly concentrated in infants under the age of one year (Snow & Marsh 2002). Malaria child mortality is similar in areas with high and low transmission intensity and this has led to concerns about the actual impact of large scale control programmes, *i.e.* bednets. on child mortality (Snow & Marsh 1995, Trape & Rogier 1996, Smith *et al.* 2001b); *i.e.* could reduction of malaria due to large scale control shift malaria mortality to the older age groups. However, the analysis of Smith *et al.* (2001b) of all-cause mortality suggests that at very high endemicity, decreasing transmission will reduce child mortality, especially in infants. These data do not suggest that mortality in older children will be increased. Studies in areas of contrasting transmission in Kenya have suggest that malaria morbidity increases among older (school age) children as transmission intensity decreases (Clarke *et al.* 2004) and this could have important implications for school performance and educational attainment.

1.3. Urban malaria

Malaria is in general considered a rural disease as the *Anopheles* mosquitoes that transmit malaria are known to prefer relatively clean water for breeding. Such habitats are usually lacking in the more polluted city environments and previous studies on urbanisation in African cities and mosquitoes have shown that along an urban-rural transect, *Anopheles* mosquito breeding decreases with increasing proximity to urban centres (Trape 1987b, Warren *et al.* 1999, Robert *et al.* 2003).

Previous research on malaria in cities showed in general a low transmission in urban areas compared to rural areas: In Accra, Ghana, urban parasitaemia prevalence was 1.6% but as high as 22% in rural communities (Gardiner *et al.* 1984b). In the Gambia, parasitaemia prevalence in a peri-urban area was only 2% while in rural areas this ranged between 30% and 90% (Lindsay *et al.* 1990). The annual EIR, the estimated number of malaria infected bites per person per year, was 29.2 in the urban areas of Kinshasa, Zaire, but averaged 454.4 in the rural areas (Coene 1993). In Lusaka, Zambia, 2% and 10% of children had malaria parasitaemia in the dry and rainy season respectively while in rural areas this was 10% and 27% respectively (Watts *et al.* 1990). In Brazzaville, Congo, the annual EIR was 22.5, but in the rural areas several hundred infective bites per person per year were reported (Trape 1987a). Robert et al. (2003) showed in their meta-analysis of EIR for sub-Saharan Africa a loose linear negative relationship between mean annual EIR and level of urbanicity. Overall the mean annual EIR increased from 7.1 in city centres, to 45.8 in peri-urban areas and 167.7 in rural areas.

1.3.1. Factors for reduced transmission

Several factors are likely to reduce transmission in urban areas, notably reduced density and reduced longevity of the mosquito (Robert *et al.* 1986, Coene 1993). The reduction in mosquito density is likely to result from fewer suitable breeding sites, due to reduction in open space, and pollution of the restricted number of sites available. *Anopheles* mosquitoes that emerge from polluted habitats are also thought to have reduced longevity (Coene 1993). Other factors such as inter specific competition, micro-climatic conditions in the urban resting habitat, domestic insecticide use *etc.* may also play a role in reduced malaria transmission in cities (Chinery 1984, Coene 1993).

Although the number of breeding sites decreases and becomes gradually different in nature there are reports that *Anopheles* may be adapting to the different breeding conditions, which may increase the risk of transmission. In urban Accra early studies (Chinery 1984) suggested adaptation of *A. gambiae* to breeding in domestic water containers and polluted waters during the process of urbanisation. Adaptation of *A. gambiae* to more polluted water is also suggested by others (Songsore & Mc Granahan 1993, Warren *et al.* 1999) but to date there are no systematic studies available. In a recent study on urban larval ecology in two cities in Kenya (Keating *et al.* 2003), development of breeding sites was linked to urban development as the number of *Anopheles* breeding sites initially increased with increasing household density till a threshold level was reached after which the number of larval sites declined. The majority of breeding sites found by Keating *et al.* (2003) were in unplanned poorly drained areas indicating a link to rapid unplanned urban expansion. In addition most breeding sites were man made and closely linked to urbanization like broken water pipes, construction sites etc.

Reduced vector density will lead to reduced exposure and therefore decreased risk for the inhabitants but in addition, an increase in host choice due to an increase in human population density will already reduce the degree of exposure (Trape & Zoulani 1987b). In addition higher quality of housing in cities and the use of personal protection measures also influences transmission (Lindsay *et al.* 1990). People in urban areas are reported to more often use insecticide spray, mosquito coils and netting in windows and doors and also have more easy access to chemotherapeutics (Vercruysse *et al.* 1983, Gardiner *et al.* 1984b, Lindsay *et al.* 1990, Trape *et al.* 1992). The ready and unregulated access to drugs is thought to have contributed to increased development of parasite resistance (Louis *et al.* 1992, Warren *et al.* 1999) and when combined with the lower transmission in urban areas may delay the onset of effective immunity (Vercruysse *et al.* 1983, Watts *et al.* 1990). In urban Accra, malaria antibody titres were significantly lower, notably in children under 10 years, than in rural areas (Gardiner *et al.* 1984b). Similarly in regions of Brazzaville Congo 63% percent of the children aged 6 and 7 years had no detectable antibodies, while in the rural areas all children over 4 years have antibodies (Trape 1987b). In Lusaka, the Zambia Watts *et al.* (1990) found that 46% of urban children examined had low antibody titres while in the rural area over 97% of children had malaria antibodies.

What remains unclear from previous studies is whether infection is actually acquired within the urban area. Koram *et al.* (1995) showed, using a case control methodology, for a peri-urban area in the Gambia that malaria was associated with residency outside the study area and travel to rural areas. In Lusaka the presence of malarial antibodies was positively associate with travelling outside the main town (Watts *et al.* 1990). Also in Accra people who travelled outside the city had higher parasite rates (Colbourne & Wright 1955). The malaria risk for urban inhabitants travelling outside might even be higher than for rural inhabitants due to the lower immunity related to the low transmission intensity in cities.

1.3.2. Heterogeneity

Heterogeneity in the urban environment means that there is likely to be, spatial variability in transmission. Peripheral areas are often more malarious environments, as these areas are often still very rural in nature (Warren *et al.* 1999). In several cities clear spatial differences were found between different areas of the cities (Robert *et al.* 1986, Sabatinelli *et al.* 1986, Trape & Zoulani 1987b, Akogbeto *et al.* 1992a, Fondjo *et al.* 1992, Manga *et al.* 1993, Robert *et al.* 2003). Trape found in Brazzaville that the number of infective bites ranged from more than 100 per year to less than one every three years. When mapping the different areas, this difference seemed closely linked to the development of the city, *e.g.* the degree of urbanisation, with the older parts showing lower transmission potential (Trape & Zoulani 1987b). Furthermore, those studies that have identified a single or small number of breeding sites have shown a clear gradient of decreasing transmission with increasing distance from the breeding site. In Dakar there was a clear vector density gradient in distance from the main larval breeding place, a marshland; the parasite rate in children in the immediate vicinity of the marsh was on average 3 times higher than at 600 metres away (Trape *et al.* 1992). However, pernicious attacks and mortality need not vary with intensity of transmission (Trape 1987b). A similar gradient of transmission of the main breeding sites was found by Manga *et al.* (1992, 1993) in Yaoundé, Cameroon. Interestingly in this study the gradient was only visible in the entomological parameters and not in the epidemiological ones.

1.3.3. Urban agriculture

Although several studies mention breeding of *Anopheles* in urban agricultural sites (Vercruysse *et al.* 1983, Trape & Zoulani 1987b, Warren *et al.* 1999, El Sayed *et al.* 2000, Keating *et al.* 2003), there are few studies that have investigated the impact of urban agriculture on malaria transmission in cities. One study by Dossou-Yovo *et al.* (1994, 1998) evaluated the impact of rice cultivation on malaria transmission in the town of Bouaké, Ivory Coast. They compared market gardens areas with rice cultivation areas and found that although the biting rates clearly increased in city areas with rice cultivation, mosquito infection levels were lower. The interaction of these two factors on the incidence of morbidity and mortality is however unclear. An interesting observation regarding urban agriculture practices and mosquito breeding was made in Niamey, Niger by Julvez *et al.* (1997), were farmers along a heavily polluted river dug wells to let water infiltrate, using the sand as a filter to treat the water. In these 'clean' wells *Anopheles* were found breeding while they would not breed in the heavily polluted river during the dry season. They further observed that wells used for irrigation contained only first and second instars. This was prescribed to the fact that the permanent disturbance of the water for filling of the watering cans for irrigation would prevent larvae to develop into pupae stage. Robert *et al.* (1998) made a similar observation in the market-garden wells in urban Dakar, Senegal, were they observed that 90% of the *Anopheles* were collected during the rainy season between May and September, when wells are often abandoned. They stated that the farming activities are an essential factor in regulating the mosquito populations in the wells. However, as the presence of larvae in the wells does not coincide with the vector density peaks for Dakar, they suggest the market garden wells might not be the most important mosquito breeding grounds. A recent study in two cities in Kenya found house-hold level farming not to be associated with an increased chance of finding larval breeding sites (Keating *et al.* 2004). A study on the impact of urban agriculture on malaria transmission in Kumasi, showed higher *Anopheles* biting rates in night catches and significant more reported malaria cases in urban areas with agriculture compared to urban areas without agriculture (Afrane *et al.* 2004).

Recently there has been renewed interest in urban malaria as with rapidly developing cities the majority of the population will be living in cities in the future and especially fast unplanned city development might considerable change the epidemiology of urban

malaria (Robert *et al.* 2003, Keiser *et al.* 2004, Omumbo *et al.* 2005, Wang *et al.* 2005b, Hay *et al.* 2005).

1.4. Aims and outline

With increasing numbers of people living in urban areas and urban agriculture being promoted as a means of improving food security (UNDP, 1996), there is an urgent need to establish the risks to human health of (peri) urban agriculture in terms of increased malaria transmission and to develop, if necessary, appropriate measures to reduce this risk. The objective of this study therefore is to investigate and quantify the impact of irrigated urban agriculture on malaria transmission in selected cities in Ghana and identify other urban risk factors for malaria.

This project was carried out under the umbrella of the System Wide Initiative on Malaria and Agriculture (SIMA) as a joined project between the International Water Management Institute and the Liverpool School of Tropical Medicine in close collaboration with the Noguchi Memorial Institute for Medical Research, University of Ghana and the School of Medical Science, Kwame Nukrumah University of Science and Technology, Kumasi.

1.5. Ethical approval

These studies were approved by the ethical review board of the Liverpool School of Tropical Medicine. In addition the Kumasi study was approved by ethical committee of the School of Medical Sciences of the Kwame Nkrumah University of Science and Technology and the Accra work by the ethical and scientific review board of the Noguchi Memorial Institute for Medical Research of the University of Legon, Ghana.

Chapter 2

2. DESCRIPTION OF THE STUDY SITES IN GHANA

The research was carried out in the two main cities of Ghana, Accra and Kumasi (Figure 2-1). Accra is the capital of Ghana and located in the south along the coast, and has an estimated population of 1.6 million (Ghana Statistical Service 2002). Kumasi is located in the central region, is the capital of the Ashanti region, and the second largest city in Ghana with a population of about 1 million (Ghana Statistical Service 2002). In Accra the population is ethnically mixed with the main groups being Akan (42%) and Ga, the traditional inhabitants of Accra (29%) together with Ewe (15%), people from the northern regions (10%) and other tribes (4%). In Kumasi the majority of the population is Akan (78%), with additional people from the northern regions (15%), Ewe (3%), Ga (1%) and other tribes (3%). Annual population growth in Accra is about 3.4% and in Kumasi about 3.1% while the overall growth in Ghana is 2.7% (Ghana Statistical Service 2002). In both cities the majority of the people work in the informal private sector (63 and 67% for Accra and Kumasi respectively) with the 2nd and 3rd most employed by the private formal and the public sector respectively. The illiteracy rate is 17% in Accra and 26% in Kumasi. (Data Population & Housing Census 2000, Ghana Statistical Service) From our baseline study (chapter 3 & 4) we recorded lower illiteracy rates, 11% in Accra and 17% in Kumasi. The majority, about 45%, of respondents had completed junior secondary school (JSS/MLC) Socio-economic score gradually increased with education level (Table 2-1).

Table 2-1 Education level and socio-economic status of respondents in the baseline household surveys in Accra and Kumasi.

	Accra		Kumasi	
education level	**%**	**SE**	**%**	**SE**
illiterate/not attended	10.7	1.10	17.2	1.53
primary school	8.4	1.21	13.9	2.01
junior secondary school (JSS/MLC)	48.8	1.63	44.8	1.93
senior secondary school (SSS/A'level)	22.7	2.07	19.5	2.33
graduate/certificate	9.4	2.33	4.5	2.83

%=Percentage of people who completed the education level, SE =mean socio-economic score for that education level

2.1. Meteorological data

The two cities are located in a different agro-ecological zones (Figure 2-1), Accra in the coastal Savanna zone, where the climate is hot and humid, and Kumasi in the Forest zone, with a semi-humid tropical climate. Rainfall is seasonal in both cities with peak rainfall in April-June and a small rainy season in September-October. Average annual rainfall in Accra is 730 mm and in Kumasi 1488 mm. For Accra, the monthly rainfall pattern for 2002-2004 is shown in Figure 2-2. Total rainfall was 1096, 869 and 625 mm for 2002, 2003 and 2004 respectively. In Accra, average monthly temperature for 2002-2004 was 28.0°C with a minimum temperature of 24.5°C in August and maximum temperature of 31.6°C in March. Monthly average humidity for 2002-2004 was 79.6% ranging from 72% in January-February to 86% in June-July. Monthly figures are given in Appendix 1.

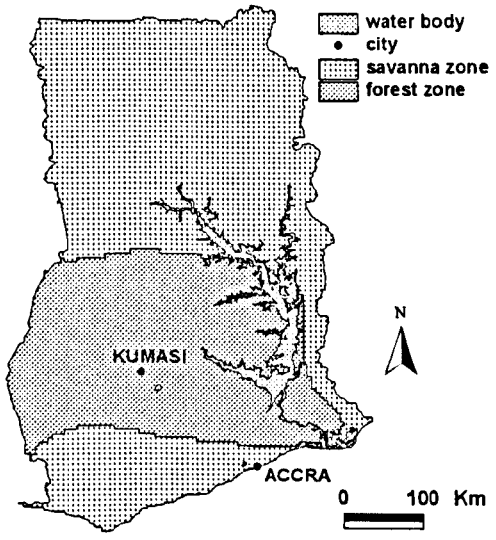

Figure 2-1 Ghana with the ecological zones and location of the two study cities.

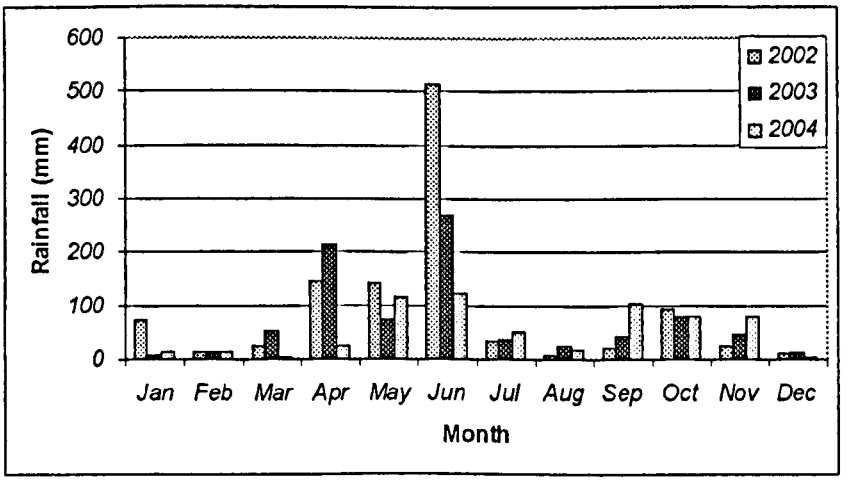

Figure 2-2 Monthly rainfall in Accra, Ghana in 2002-2004 (data meteorological station Water Research Institute (WRI), unpublished)

2.2. Malaria in Ghana

In Ghana, malaria is the main cause of outpatients visits (OPD). Nationwide about 43% of OPD patients are diagnosed with malaria, mainly on clinical symptoms and show a slightly increasing trend in recent years (Figure 2-3). The prevalence is highest in under fives, *e.g.* a prevalence of 56.1% in under fives in 2003 compared to an overall prevalence of 45.2% (MoH, unpublished).

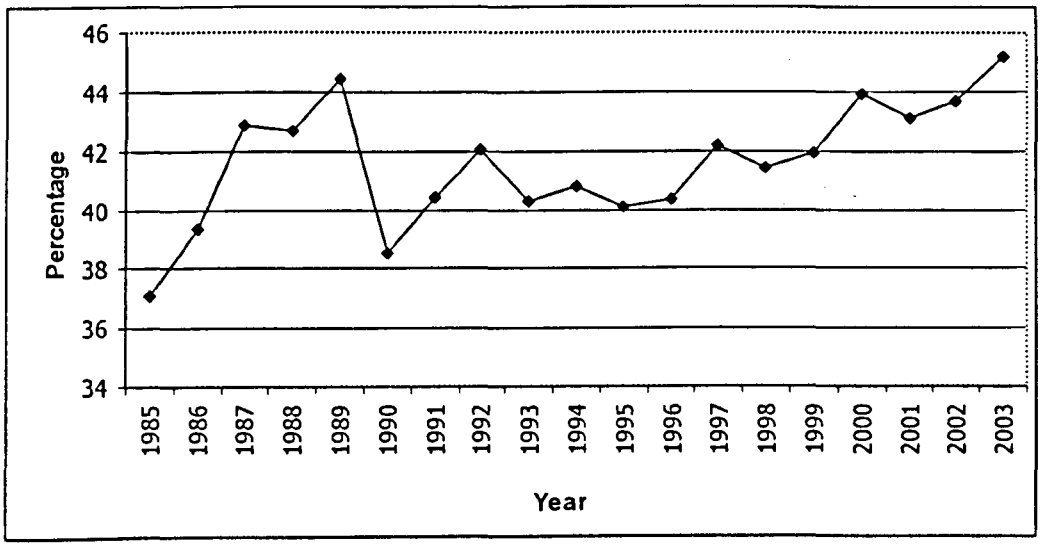

Figure 2-3 Percentage of patients at outpatients department in hospitals in Ghana who are diagnosed with malaria (MoH, unpublished).

A survey on prevalence of *P. falciparum* among 4690 schoolchildren in the three ecological zones in Ghana (Afari *et al.* 1992), showed a malaria prevalence of 35.1% (95%CI 32.3-38.1) in the coastal zone, 39.2% (95%CI 37.3-41.1) in the forest zone and 45.9% (95%CI 43.1-48.6) in the northern savanna zone. With the majority in each zone (83.7-98.6%) being P. *falciparum*. Other *Plasmodium* species present were *P. malariae* and *P. ovale*. A study in 35 villages in the Ashanti region reported prevalence of 50.7 and 49.7% in persons over 2 years old in forest and savanna respectively, with the highest prevalence in the 2-9 year olds (Browne *et al.* 2000). *P. falciparum* prevalence (in single or mixed infections) was 92.4 and 89.2% for the forest and savanna respectively, for *P. ovale* this was 15.5 and 2.6% and for *P. malariae* this was 10.4 and 22.8% respectively. In northern Ghana malaria is very seasonal, depending on the rain and prevalence can reach 80-90% in the rainy season compared to 20-50% in the dry season (Binka *et al.* 1994, Koram *et al.* 2003). In

urban Ghana malaria prevalence has been reported as low. Gardiner *et al.* (1984b) reported in a random sample of households in one community in Accra an overall prevalence of 1.6% (n=413) and 2.5% in under fives (n=199) in 1978.

In Accra reported malaria cases showed little seasonal variation (Figure 2-4), although there seems a slight increase in case numbers after the peak rainfall periods of April-May and September-October. Appendix 2 gives the malaria case figures for the four government polyclinics in our study areas. Total case numbers reported in Accra appear to be low with a maximum of 23,979 cases in July in a population of 1.6 million. This may be due to large number of treatment providers in the city.

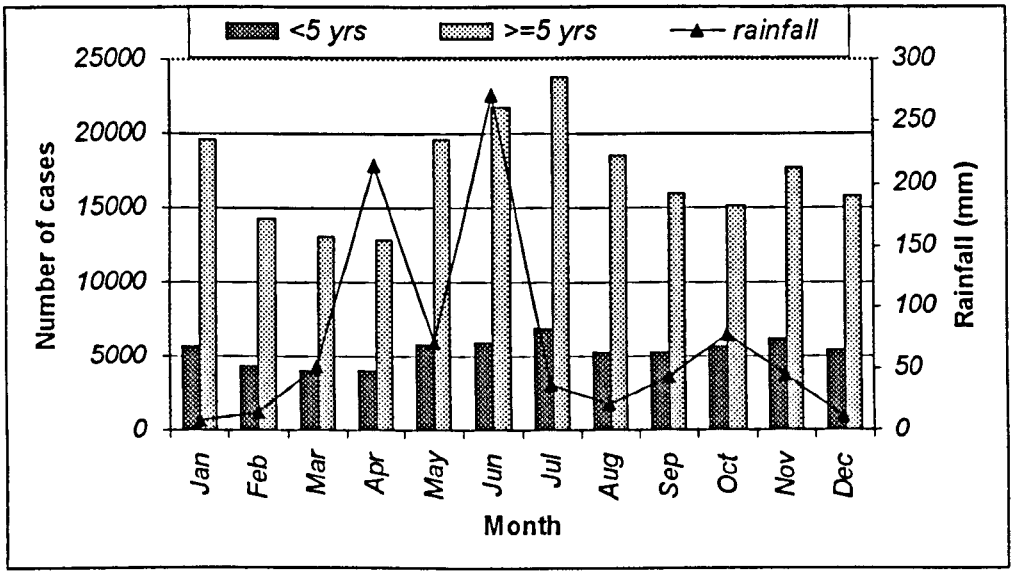

Figure 2-4 Malaria case numbers in under fives and over fives as reported in outpatients department at all clinics in 2003 in Accra (MoH, unpublished); line graph represents rainfall for each month (date WRI, unpublished)

2.3. Malaria Control and Treatment Policy

Ghana endorsed the Abuja Declaration and its Roll Back Malaria Strategy is focused on ITNs, IPTs and improved case management. It targets are to have 60% coverage of pregnant women and children under five sleeping under an ITN, 60% of pregnant women having correct dosage of IPT and 60% of people having access to prompt and effective treatment by 2010 (RBM Ghana, pers, com).

Chloroquine remained the first line treatment for malaria in Ghana during the period of our field study although policy changed mid 2005 to Artesunate-Amodiaquine combination therapy (ACT). In a study on treatment failure in 1988-1990 (n=690) Afari *et al.* (1992) found that 91.1% of cases were sensitive to chloroquine and 8.9% responses classified as resistant to chloroquine at RI (5.1%) and RII (3.8%). The resistance responses were most common in the coastal zone (17.1-22.7%), followed by the savanna zone (8.6-10.0%), and lowest in the forest zone (3.1-6.3%). Chloroquine resistance has rapidly increased and Koram *et al.* (2005) found a chloroquine cure rate of only 25% in 36 children studied in 2003. Koram *et al.* (2005) also tested the efficacy of sulphadoxine/pyrimethamine (SP), amodiaquine+ artesunate (ADQ+ART) combination, and artemether+lumefantrine (Coartem) treatment. Cure rates on day 28 were 60% for SP, 100% for ADQ+ART and 97.5% for coartem.

2.4. Health facilities and malaria control in the study communities

For administrative purposes Accra is divided into six districts termed submetros. Within the Greater Accra Region there are 3 government hospitals, 2 quasi-government hospitals , 5 government polyclinics and 5 government clinics and over 160 private facilities (AMA pers. com 2006). Kumasi has five health submetro's and there are 5 government hospitals, 2 quasi-government hospitals, and the private and public clinics are grouped into the five hospital catchment areas (KMA 2000). In addition to the government health facilities there are many private health facilities in both cities. For example in Kumasi, there are only 60 private health institutions registered in the Metropolis but a rapid assessment carried out in 1998 indicated there are over 100 private institutions in the Metropolis (KMA 2000). In addition to the private facilities there are many dispensaries where people buy over the counter drugs often without prescription (see also 2.5). Biritwum et al. (2000) state that in Accra about 60% of people use private sector to obtain initial malaria treatment. The institutions in the two metropolises follow the government guidelines for malaria treatment (see 2.3). Environmental management to control insects is not generally practiced in AMA (Benneh et al. 1993). Although there are many NGOs and religious organizations active in Accra and Kumasi we are not aware of any specific organizations within the study communities that specifically focus on malaria or

anemia control. Although no government mass treatment programs are being held, treatment programs for helminths carried out by NGOs do exist in the KMA region (Kenny 2005) and most likely also in the AMA region.

2.5. Malaria knowledge and practice in the study communities

Knowledge of carers in Accra and Kumasi about malaria and mosquitoes is given in Table 2-2. The majority (over 90%) of people knew mosquitoes transmit malaria although 1% of people thought you can get malaria from other causes *i.e.* walking in the sun, eating oily or contaminated food. The main symptoms associated with malaria were fever/hot body/hot head. Around 20% of the people (26.6 and 19.5% in Accra and Kumasi respectively, not significant (NS)) reported a household member had malaria recently (in the last two months). Of these, around 75% (76.1 and 76.8% in Accra and Kumasi respectively, NS) obtained treatment from a clinic (in Kumasi 36.5% went to a government and 40.2% to a private clinic, in Accra this information was not recorded). Of the patient attending the clinic, in Accra 33.6% reported to have had a bloodfilm made while in Kumasi this was 16.1% ($P<0.0001$). Around 20% (23.5 and 21.6 in Accra and Kumasi respectively, NS) bought drugs over the counter, while in Kumasi 1.7% did nothing and in Accra 0.4% (NS).

2.6. Vector population

The predominant malaria vectors within Ghana are species of the *Anopheles gambiae* complex although *A. funestus* can also be found. *A. gambiae s.s.* is the major vector and is distributed throughout the country, *A. arabiensis* is confined to the far north of the country, and *A. melas* to localities along the coast (Appawu *et al.* 1994). *A. funestus* is reported through the whole country. The first analysis of chromosomal polymorphism of *A. gambiae s.s.* in Ghana (Appawu *et al.* 1994) showed the presence of the FOREST form in the rain forest localities and the SAVANNA form in the coastal savanna sites. The MOPTI form occurred sympatrically with the SAVANNA form in the northernmost locality. In a later study Yawson *et al.* (2004) reported the occurrence of the molecular M and S forms in sympatry in southern Ghana. The S form predominated throughout its distribution in the coastal savannah, except at one location, while the M form was the only form collected in northern Ghana. In a study in urban Kumasi predominantly *A. gambiae s.l.* were caught in night catches although some *A. funestus* were caught (Afrane *et al.* 2004). In the same study mosquito larvae

found breeding in agricultural areas in the city were all *A. gambiae s.l.* and PCR analyses revealed that all *A. gambiae s.l.* tested were *A. gambiae s.s.* White (1998) found similar results in his study in Kumasi, with a representative subsample (n=80) of *A. gambiae s.l.* caught analyzed as *A. gambiae s.s.*

Table 2-2 Carers knowledge about malaria and mosquitoes in the cities of Accra and Kumasi as obtained in the baseline survey.

Malaria knowledge factors	Both cities n=2204	Accra n=943	Kumasi n=1261	*P*-value*
Symptoms used to identify malaria#				
Fever/hot body/hot head	84.3	82.7	85.6	0.068
Vomiting/nausea	28.4	25.1	30.8	0.003
No appetite	27.9	29.8	26.5	0.086
Lethargic/sleepy/weak	28.3	30.3	26.8	0.069
Headache	10.1	5.9	13.2	<0.001
Yellow eyes	11.6	13.1	10.4	0.045
Diarrhoea	3.3	4.2	2.6	0.035
Cold body/cold sweat	6.4	9.2	4.4	<0.001
Yellow urine	7.5	21.5	15.5	<0.002
Cough/runny nose	2.8	3.3	2.4	0.198
Dizziness	3.2	3.5	3.0	0.523
Body/muscle/joint pain	5.2	6.2	4.4	0.073
Death	0.2	0.2	0.2	0.900
Can't sleep	2.9	2.0	3.6	0.025
Other	0.5	0.6	0.4	0.102
Don't know	1.8	0.7	2.5	0.002
Cause of malaria#				
Mosquito bites	92.3	91.5	92.8	0.257
Dirty environments	2.2	3.7	1.0	<0.001
Other¶	1.2	1.2	1.2	0.949
Don't know	4.7	4.3	4.9	0.513
Whom does malaria affects most				
All	47.8	29.0	62.7	<0.001
Adults	8.2	11.2	5.7	<0.001
Children	41.9	55.6	31.0	<0.001
Don't know/no answer	2.2	4.2	0.6	<0.001
Where do mosquitoes come from#				
Gutters/toilets/stagnant water	71.6	75.7	68.4	<0.001
Dirty environment/refuse	15.9	5.3	24.0	<0.001
Bushes	8.8	17.6	2.1	<0.001
Other	2.5	3.2	2.0	0.086
Don't know/no answer	5.9	4.5	6.9	0.022
What season do mosquitoes mostly bite				
Wet	47.8	52.4	44.3	0.014
Dry	33.1	25.6	38.8	<0.001
All year	18.5	20.6	16.9	0.046
Don't know/no answer	1.7	2.5	1.8	0.548

*p-value is of comparison between Accra and Kumasi of Pearson chi-square test; # multiple responses possible as categories are not mutually exclusive;¶ other causes reported were eating oily food/contaminated food, (walking in) heat/sun, working too hard

2.7. Earlier malaria studies in Accra

The first work carried out on mosquito breeding in Accra was in 1914-1915 (Macfie & Ingram 1916), when a survey was carried out on the domestic mosquitoes of Accra. The predominant species found breeding in compounds were *Culex fatigans* (now called *Culex quinquefaciatus*) and *Stegomyia fasciata* (now called *Aedes aegypti*). Very few *Anopheles* were found breeding, the only species found was *Anopheles costalis* (now *gambiae s.l.*). Interestingly they report that resting catches in one house revealed that the resting fauna did not correspond very well with the breeding sites in the compounds. Several additional species were found resting in the house, *i.e. A. pharoensis, A. funestus* and *Mansonia spp.* In 1952 and 1954 Colbourne and Wright (1955) performed a survey on malaria in the Gold Coast (part of current Ghana) in which Accra was included. From monthly pyrethrum knock down catches they observed seasonality of *Anopheles* linked to rainfall, with densities peaking in June. They investigated three areas, central Accra, intermediate (located towards the periphery of the densely built up areas) and the suburbs. In the centre they found very low densities <1 female *Anopheles* per room year round. In the suburbs highest *Anopheles* densities were found, ranging from <1 in the dry season to 48 female *Anopheles* per room after the rains. The intermediate zone had intermediate densities between centre and suburbs. The sporozoite rate averaged 3.2% and estimates of the annual entomological inoculation rate (EIR) ranged from 0.1 in central Accra to 21 in the suburbs with 2-3 in the intermediate zone. They found perennial breeding of *Anopheles* in earth drains, excavations and domestic waste water collections, which increased in the rainy season due to extension of dry season breeding places and new places in flooded areas, footprints and innumerate puddles. Accompanying prevalence surveys were carried out in central Accra and the suburbs in the dry and rainy season. In central Accra, prevalence in children under 7 years averaged 16% in the dry and 35% in the rainy season, while in suburban Accra this was 51% and 56% respectively. In both areas prevalence peaked in childhood indicating development of immunity although in suburban Accra this peak was reached earlier. Ninety eight percent of positive slides were due to *Plasmodium falicparum*, while 14% contained *P. malariae* alone or in mixed infection and *P. ovale* was found in <1%. Malaria in Accra was classified as meso endemic.

During 1964-1966 extensive control operations were carried out to reduce breeding (Chinery 1968, 1969, 1970). Mechanical methods used consisted of drainage, filling, clearing of vegetation, destruction and burial of breeding receptacles. Chemical methods consisted of application of insecticides and oils occasionally supplemented by aircraft spraying. The main mosquitoes found in Accra, both before and after control, were *Culex pipiens fatigans* (now *C. quinquefasciatus*) and *A. gambiae*, which was present year round but increased breeding linked to rainfall. The control operation reduced overall mosquito breeding by 62% and *A. gambiae* breeding by 73%. A paper by Chinery (1990) comparing adult *Anopheles* densities and breeding indicated that adult densities increased with increased larval breeding and that most adults invade the human habitation closest to the breeding sites.

2.8. Urban agriculture

Several types of urban agriculture can be distinguished in cities, *e.g.* commercial vegetable production, backyard farming, horticulture and rain-fed agriculture of maize, cassava *etc.* The latter occurs mostly in the peri-urban areas but can also be found on fallow land within cities, mainly during the rainy season. Estimates for the area of rainfed agriculture are difficult as the land under cultivation changes every year as farmers utilise any available fallow land. Many people are engaged in backyard farming around their house of plantain, cocoyam *etc.* often to reduce household expenditures. Back yard farms are in principle rain fed but often the household/bath water is used for additional watering.

Commercial vegetable production is the only major type of urban agriculture reliant upon irrigation. The main crops cultivated are lettuce, onion and cabbage. Common sites are along (storm) drains (Figure 2-5D) and streams that are used as a water source. It is also frequent carried out in areas not suitable for building, for example in Accra one of the main agricultural areas, Dzorwulu-Kotobabi, is adjacent to the power plant (Figure 2-5C) under the electricity lines where no houses can be built. Although irrigation is small scale and mostly informal, *e.g.* non-governmental with minimal use of structures, all vegetable farmers irrigate their crops. This is mainly achieved using watering cans (Figure 2-5C,E) although some farmers use a watering hose connected to a piped water supply. As land tenure is often insecure there is little investment in irrigation infrastructure. The most common structures are hand dug wells (Figure

2-5A,G) filled by a drain, stream or hose. Drains and streams are also used directly and often blocked for easy filling of watering cans (Figure 2-5B). In addition to wells and drains farmers also use furrows in between raised beds to irrigate there crops (Figure 2-5F). As commercial vegetable production is irrigated this type of UA is most likely to contribute to creating additional breeding sites in the city in the irrigation structures and on the fields. Therefore the study focused on this type and UA hereafter refers to commercial irrigated vegetable production.

Figure 2-5 Impressions of urban agriculture in Accra (A-E) and Kumasi (F-G). A). hand dug well close to Alajo; B) Blocked drain at Korle Bu; C) Irrigation of onions using water cans at Dzorwulu; D) Farms are often located along drains which are used as a water source; E) Female farmer watering her crops at Dzorwulu; F) Farms behind Kumasi university; G) Shallow well with furrow at farm in Kumasi.

An inventory of irrigated urban agriculture in six West African cities (IWMI, in pres.) showed that the main irrigation system used in open space irrigated urban vegetable production is irrigation with watering cans. Cans are filled in the local stream or from small wells or cisterns, which could form excellent breeding places for anophelines, depending on water quality. Wells can be either dug out wells or cement constructions. Figure 2-6 gives an overview of the main types of irrigation used UA in West Africa, and shows the system components with higher potential risk for malaria vector breeding. As indicated, the risk is mainly at the level of water storage, as this is where open water bodies are provided which could be suitable for breeding, depending upon type of reservoir and water quality. In addition to water quality and storage method, the specific location of UA sites may also affect breeding in some cases. For example, in Kumasi, Ghana, UA is mainly practiced in low-lying areas in the city which have easy access to water, and it could be that such areas in general produce more mosquitoes, regardless of UA (Afrane et al. 2004). On the other hand, in Accra, the main agricultural sites are at places were due to presence of electricity line or other reasons no houses are built. Sites are close to streams that are used as a water source but they could be developed as normal housing areas.

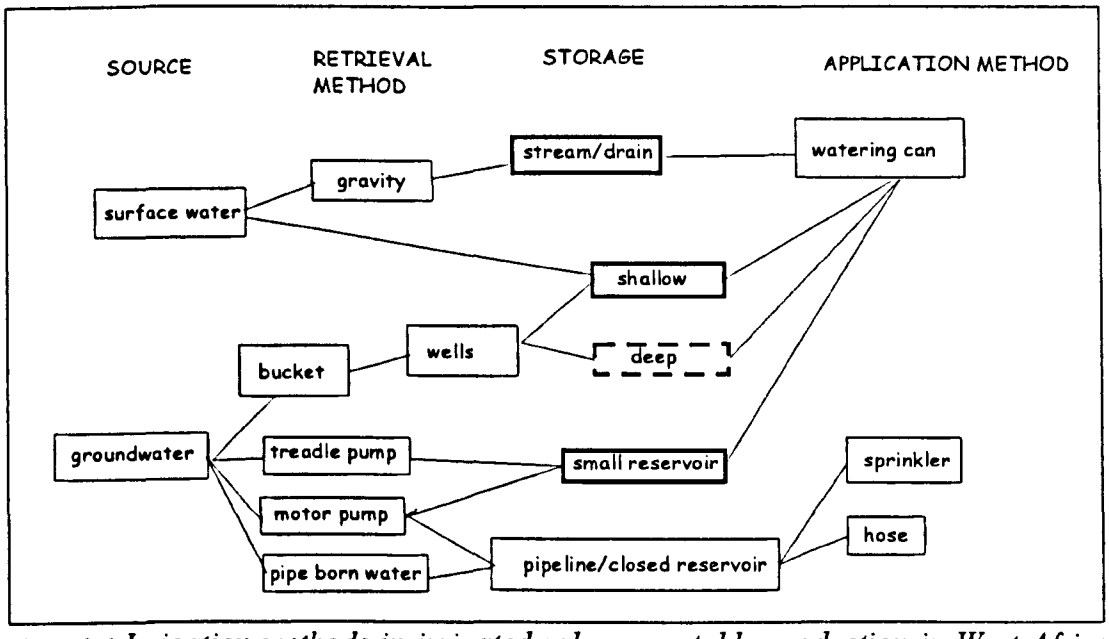

Figure 2-6 *Irrigation methods in irrigated urban vegetable production in West Africa (reproduced from (Klinkenberg and Amerasinghe in press).* Boxes with thick outlines indicate increased possible malaria risk for that level. A dotted outline indicates less risk.

The intensification of crop production in UA often implies high usage of pesticides. This, too, could have an impact on malaria risk. Depending upon the concentrations of pesticides washed from the fields into the wells and other breeding sites this could either retard breeding or facilitate the development of resistance Various farmer practices can affect the possibilities for anopheline breeding. For example, growing fish in the wells could reduce potential anopheline breeding, but irrigation frequency and water level in the wells, among other factors, can also have an effect.

In Accra about 250 ha is under mixed production (staple crops and vegetables) with 50-150ha commercial vegetable cultivation (IWMI & RUAF, unpublished). About two third of the households (67%) are engaged in some form of backyard farming (IWMI, 2000 unpublished). Communities for the study were selected around the three main agricultural sites, Dzorwulu, Kotobabi and Korle Bu farm (Figure 2-6) and the smaller site of Cantonments. Dzorwulu and Kotobabi are adjoining areas around a main drain in central Accra, divided by a main road. The area is located under the main electricity lines and along the railway track and no houses can be built in this area. Irrigation takes places from wells, filled by piped water or channelled in by water from drains. At Korle Bu the farm area is located on the hospital grounds in an area they are developing for construction of health worker accommodation. The wells used for irrigation are all filled with water from drains and part of the fields are irrigated straight from the main drain coming from the hospital, which is blocked at points for easy watering (Figure 2-5B). On some fields, furrows are constructed for irrigation which are filled with drain water. In Cantonments there is a small farming area in the middle of a residential area near a stream which is used for irrigation together with dug wells.

In Kumasi, the total crop area is about 80 hectares with about 40 ha of irrigated vegetables. As in Accra, about two thirds of the households are engaged in backyard farming (IWMI, unpublished). Communities for the study were selected around the three main agricultural areas, around the university, around the airport, and around Manhiya, a large open space in the centre of town (Figure 2-7). In Kumasi hand dug wells are often made in low lying areas where the groundwater table is close to the surface (Figure 2-5G). The UA areas in Kumasi are mostly located in a larger low

lying area of which part is used for commercial vegetable production and hand dug wells are used for irrigation. Wells are less deep then in Accra as the water table is high.

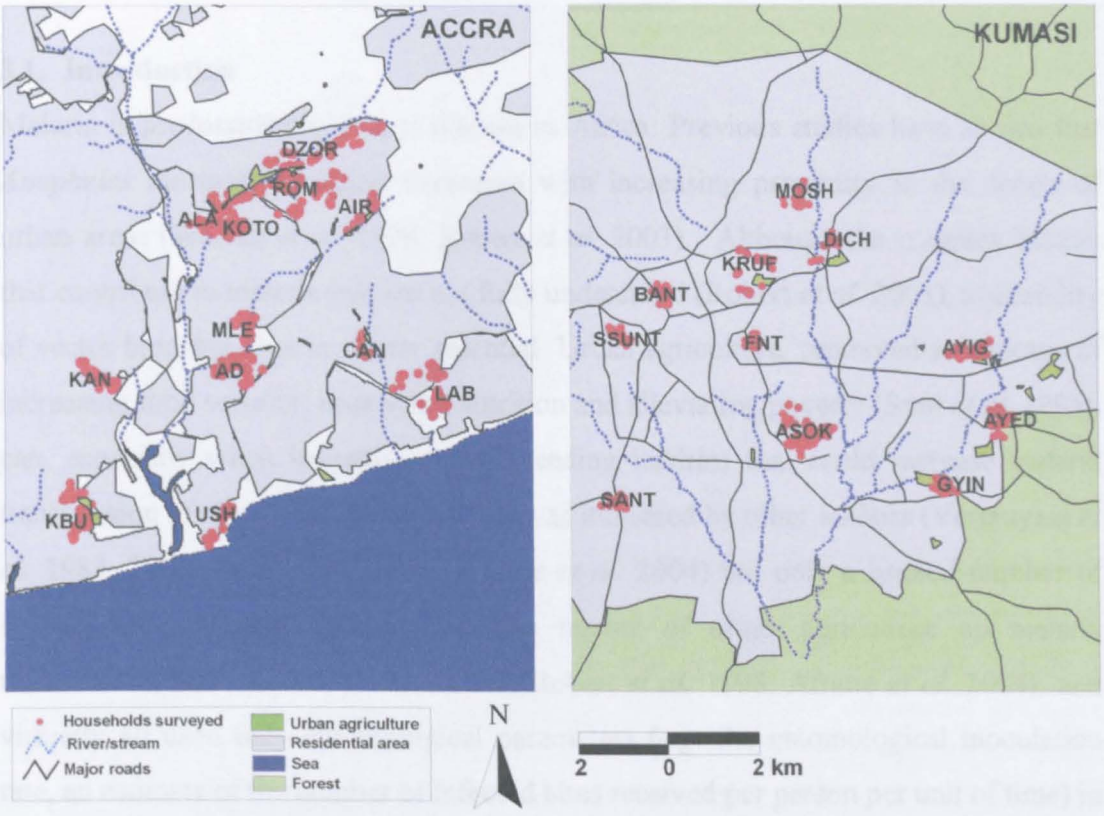

Figure 2-7 Location of areas of urban agriculture and communities selected for the study in Accra (left) and Kumasi (right).

Abbreviations indicate community names:

Accra: AIR =Airport, ALA=Alajo, DZOR =Dzorwulu, KBU =Korle Bu, KOTO =Kotobabi, ROM= Roman Ridge, CANT=Cantonments, MLE=Kokomlemle, AD=Asylum Down, KAN=Kaneshie, LAB= Labonie/La, USH=Ushertown.

Kumasi: ASOK=Asokwa, AYED=Ayeduase, AYIG=Ayigia, BANT=Bantama, DICH=Dichemso, FNT=Fanti New Town, GYIN=Gyiniasi, KURO=Krofrom & Manhiya, MOSH=Moshie Zongo, SAN= Santasi, SSUN=South Suntreso

Chapter 3

3. MALARIA AND IRRIGATED CROPS IN ACCRA[1]

3.1. Introduction

Malaria is predominantly a rural disease in Africa. Previous studies have shown that *Anopheles* mosquito breeding decreases with increasing proximity to the centre of urban areas (Warren *et al.* 1999, Robert *et al.* 2003). Although the complex factors that contribute to malaria risk are not fully understood (Robert *et al.* 2003), availability of vector breeding sites is clearly essential. Urban agriculture, promoted as a means of increasing food security, improving nutrition and alleviating poverty (Smit *et al.* 1996), can, especially when irrigated, create breeding habitats that could increase malaria transmission in cities. This potential risk was indicated by other authors (Vercruysse *et al.* 1983, Trape & Zoulani 1987b, Afrane *et al.* 2004) but only a limited number of studies have attempted to quantify the impact of urban agriculture on malaria transmission (Dossou-Yovo *et al.* 1998, Robert *et al.* 1998, Afrane *et al.* 2004), and virtually all used only entomological parameters (*e.g.* the entomological inoculation rate, an estimate of the number of infected bites received per person per unit of time) in their analyses. Such measures are only proxies of actual malaria risk and no studies have assessed the malaria parasite prevalence, a direct indicator of the malaria burden, in communities with and without urban agriculture. By 2025, an estimated 700 million people will live in urban communities in Africa, which is approximately double the current urban population (UNPP 2005a). With such rapid expansion, identification of the risk factors for urban malaria needs urgent attention (Keiser *et al.* 2004).

3.2. Material and Methods

From October 2002 to January 2003, we investigated malaria parasite prevalence in central Accra, Ghana, in communities bordering irrigated urban agriculture areas and in control communities (defined as sites located >1 km from an urban agricultural

[1] An amended version of this chapter has been published as Klinkenberg E, McCall PJ, Hastings IM, Wilson MD, Amerasinghe FP and Donnelly MJ 2005. Malaria and irrigated crops, Accra, Ghana. *Emerging Infectious Diseases* 11, p 1290-1293

areas, based on the likely appetitive flight distance of female mosquitoes (Service 1997) (Figure 3-1). Communities around the main agricultural sites in Accra were selected and based on them representative control communities in terms of socio-economic status, housing and crowding were selected.

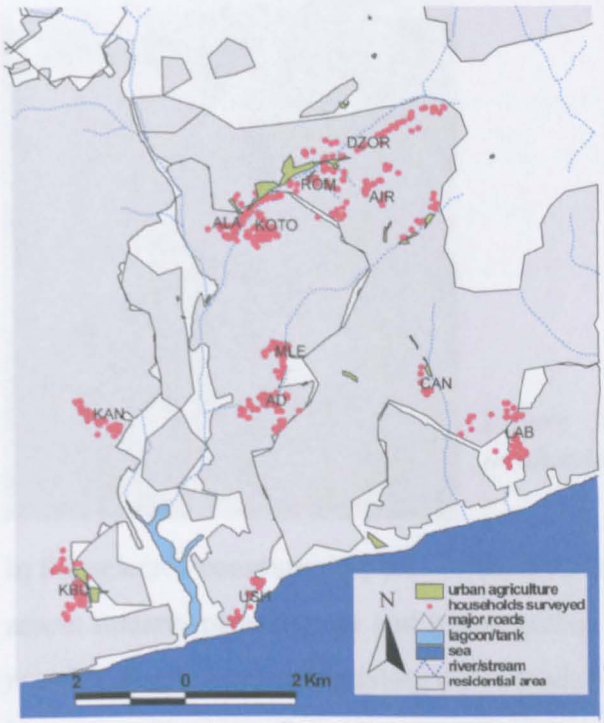

Figure 3-1 Location of urban agriculture sites (UA) and households surveyed within Accra. Communities surveyed were as follows with full name, UA or control(C), number of children sampled, and malaria prevalence given in parenthesis: AIR (Airport, UA, n=77, 19.5%), ALA (Alajo UA, n=166, 15.1%), DZOR (Dzorwulu (UA, n=132, 19.7%), KBU (Korle Bu, UA, n=181, 8.8%), KOTO (Kotobabi, UA, n=219 18.3%), ROM (Roman Ridge, UA, n=105, 22.9%),CANT (Cantonments, UA, n=23, 13.0%), MLE (Kokomlemle (C*,n=160, 20.6%), AD (Asylum Down, C*, n=160, 11.3%), KAN (Kaneshie (U, n=159, 19.5%), LAB (Labonie/LA, U, n=175, 9.7%), USH (Ushertown (C, n=200, 6.5%). Communities marked C* were originally identified as control communities but small UA sites were later identified close to them.

Different types of urban agriculture exist: basic backyard farming in or around the house, cultivation of stable crops such as maize on (temporary) fallow land and cultivation of ornamental plants, mostly along roadsides. An important part of agriculture in the city is commercial cultivation of vegetables, such as lettuce, onion and cabbage (Figure 3-2). These crops are irrigated from wells or streams with watering cans and crops are sometimes cultivated on raised beds with water-filled furrows. Irrigated farming has the greatest potential to create additional breeding sites, and irrigated, open spaced vegetable farming has been linked to higher *Anopheles*

densities in Kumasi, Ghana (Afrane et al. 2004). The study focused on this type of urban agriculture which refers to irrigated, open spaced, commercial vegetable production.

Figure 3-2 Commercial irrigated vegetable production in urban Accra

In the selected communities, we conducted a cross-sectional house-to-house survey to assess malaria parasitaemia and haemoglobin (Hb) concentration in children 6 to 60 months of age. A team consisting of technicians and trained enumerators went house to house to collect the data. Houses were selected arbitrarily and queried regarding the presence of children <5 years of age. For each community, the whole area was covered to account for spatial heterogeneity. If most compounds or houses had children <5 years of age, houses were omitted to obtain the target sample size of 150 children from the community.

Informed consent was obtained from each child's caregiver. Thick and thin bloodfilms were collected and read according to standard World Health Organization protocols. Hb concentrations were assessed by using a blood haemoglobin photometer (Hemocue, Angelholm, Sweden). Children with parasitaemia or Hb concentration <8·0 g/dL were provided free treatment at a local clinic. The epidemiological data were related to proximity to sites of urban agriculture, socio-economic status based on household assets following a World Bank template (Gwatkin *et al.* 2000), and possible confounding factors obtained by questionnaire from the child's caregiver. The location of each house, study site boundaries, landmarks and urban agricultural areas were mapped by using a hand-held global positioning system. For each household in the

urban agricultural communities, the shortest distance to nearest agricultural site was calculated using Arcinfo (ESRI, Redlands, CA, USA). Ethical approval was granted by the Liverpool School of Tropical Medicine and the University of Ghana, Legon. More details on material and methods can be found in Appendix 3.

3.3. Results

A total of 1,757 children from 938 households in 12 different communities were enrolled in the study. Table 3-1 shows the baseline characteristics of the children with *Plasmodium*-positive and -negative slides and Table 3-2 shows the characteristics for the urban agricultural and control communities.

Table 3-1 Summary of variables measured for all children, children with and without malaria parasites, with results of univariate tests (Pearson chi-square/T-test).

	Plasmodium positive bloodslide (n=261)	*Plasmodium* negative bloodslide (n=1496)	p value
Mean Hb in g/dL (SD)*	10·17 (1·62)	10·94 (1·42)	*P*<0·001
Hb<8 g/dL (%)	11·3 (29/257)	3·3 (49/1481)	*P*<0·001
Mean age in months (SD)	36·44 (16·03)	32·92 (17·19)	*P*<0·001
Mean socio-economic score† (SD)	1·42 (0·99)	1·74 (0·98)	*P*<0·001
Male (%)	123 (47·1)	739 (49·4)	(*P*=0.498)
Travelled to village‡ (%)	17 (6·5)	93 (6·2)	(*P*=0.855)
Taken malaria medication in the last 2 weeks§ (%)	63 (24·1)	344 (23·0)	(*P*=0.686)
History of fever§ (%)	64 (24·5)	293 (19·6)	(*P*=0·067)
HH reporting bednet use	89 (34·1)	499 (33·4)	(*P*=0.814)
HH who spray weekly¶	71 (27·2)	435 (29·1)	(*P*=0.537)
HH with netting at windows/doors	208 (79·7)	1282 (85·8)	*P*=0·012
HH without ceiling	77 (29·8)	382 (25·6)	(*P*=0.147)

*Hb=Haemoglobin; SD= standard deviation; HH=Household; †The composite measure of socio-economic status used was the asset factor score of the World Bank for Ghana (www.worldbank.com/hnp); ‡Individuals who had travelled to a rural (potentially malarious) area in the previous 3 weeks; § In the last 48 hours, as reported by the caregiver; ¶Proprietary brands of insecticide aerosols

Of 261 infections detected, 258 were *P. falciparum* alone, 2 were *P. malariae* and 1 was a mixed infection with *P. falciparum* and *P. malariae*. The average Hb concentration was 10·82 g/dL (SD 1.47), and 78 (4.5%) of 1,738 children had moderate-to-severe anaemia (Hb< 8·0 g/dL).

Table 3-2 Summary statistics for variables measured in the study for children in communities near urban agricultural sites and control communities, with results of univariate tests (Pearson chi-square/T-test).

	Children in UA* communities (n= 1223)	Children in control communities (n=534)	p value
Children with plasmodium positive slide (%)	16.4 (200/1223)	11.4 (61/534)	*P*=0.008
Mean Hb in g/dL (SD)	10.93 (1.46)	10.59 (1.46)	*P*<0.001
Hb<8 g/dL (%)	3.4 (41/1215)	5.5 (29/529)	*P*=0.039
Mean age in months (SD)	33.3 (17.1)	33.8 (17.0)	(*P*=0.601)
Mean socio-economic score‡ (SD)	1.78 (0.96)	1.49 (1.02)	*P*<0.001
Travel to village§ (%)	7.9	2.4	*P*<0.001
Taken malaria medication in last 2 weeks¶ (%)	23.5	22.3	(*P*=0.600)
History of fever¶# (%)	21.2	18.2	(*P*=0.155)
HH reporting bednet use (%)	37.7	24.2	*P*<0.001

*UA=irrigated urban agriculture (communities around UA sites);U=control communities (communities at least 1 km from UA); SD= standard deviation; HH=Household; †number of children in UA group is higher, as small plots of agriculture were discovered in two communities originally designated control sites. If these two communities were omitted from the analysis similar results were obtained and significance remained the same except for children with moderate to severe anaemia which lost significance (*P*=0.065) (data not shown); ‡The composite measure of socio-economic status used was the asset factor score of the World Bank for Ghana (www.worldbank.com/hnp); §Individuals who had travelled to a rural (potentially malarious) area in the previous 3 weeks;, ¶as reported by the carer; #in the last 48 hours

Overall malaria parasite prevalence was 14·9% (261/1,757; 6-22% range) and was higher in communities around urban agriculture sites than in control communities (16·5 and 11·4% respectively, odds ratio [OR] 1·53, 95% confidence interval [CI] 1·10-2·14, *P*=0·008). In a univariate analysis (Pearson chi-square for binominal variables and *t* test for continuous variables), Hb concentration (negative association, *P*<0·001), moderate-to-severe anaemia (OR 3·49, 95%CI 1·98-6·11, *P*<0·001), having netting at windows, doors or both (OR 0·65, 95%CI 0·46-0·92, *P*=0·012), socio-economic status (negative association, *P*<0·001), and age (positive association peaking

at ~ 3 years of age, P=0·002) showed significant associations with presence of malaria parasites. Reported bednet use by household was 33% (ranging from 6-53% in different communities) but was not significantly associated with presence of malaria parasites in the blood.

A generalized linear mixed model (GLMM) approach, using a SAS macro (Glimmix 800, SAS Inc., USA) that allowed a logistic link function, was used to investigate the association between putative predictor variables and malaria parasite prevalence. Covariates with P<0·1 in the univariate analysis were entered in the multivariate model. Household was nested within community and both variables were treated as random effects. Age was divided into the following age groups: 6-12, 13-24, 25-36, 37-48 and 49-60 months. Hb concentration was entered as a continuous variable. Malaria parasitaemia was significantly associated with Hb concentration (negative association, P<0·001), age group (positive association, P<0·001), socio-economic status (negative association, P=0·0035). The effect of urban agriculture was marginally below significance (P=0·0647) possibly because of reduced statistical power. Having netting in front of windows or doors was no longer significant (P=0·3638), presumably because presence of nets was associated with a higher socio-economic status (P<0·001). In urban agricultural communities, GLMM analysis with parasitaemia as the outcome, was conducted with age group, distance to an urban agricultural site, socio-economic status and house effects. Hb concentration was omitted because it was likely to be the result of malaria infection and its inclusion could obscure the effect of distance. Two of the districts, Mle (P=0·021) and Kbu (P=0·014) showed decreases in prevalence with distance from an urban agricultural site; the odds of infection were reduced ~50% every 100m from the site. However these results need to be interpreted with caution because it is difficult to detect a putative decrease in prevalence with distance against the noise introduced by small, unidentified, often transitory, breeding sites. Their presence may explain why two sites, Rom (P=0·043) and Dzor (P=0·039), showed a significant increase in prevalence with distance, while two others, Air and Koto, showed a significant effect when distance in 100m intervals was cross tabulated with prevalence (P<0·001; Fishers exact test). Since unidentified breeding sites may also introduce unknown data structuring that cannot be incorporated into GLMM, the probabilities obtained may be lower than is appropriate.

3.4. Discussion

The parasitaemia levels obtained in this study are worrisome because high-density urban African populations are not often considered particularly vulnerable to malaria infection. In other West African urban areas, malaria prevalence rates from 2% to 16% have been reported with large variation between communities (Vercruysse *et al.* 1983, Sabatinelli *et al.* 1986). Recently, several authors focused attention on urban malaria (Robert *et al.* 2003, Keiser *et al.* 2004) and stressed the need to investigate risk factors for urban malaria. In our study, the parasitaemia children were more likely to be anaemic, have a lower socio-economic status and live in a community close to areas of urban agriculture. Since recent travel to a rural area did not affect outcome, local malaria transmission is indicated. Our entomological studies in these study areas (unpub. data) have found *Anopheles gambiae* S form breeding in irrigation water at urban agricultural sites and resting at higher densities in houses in urban agricultural communities. These findings are based on a point prevalence survey in the dry season. Although we continue to obtain data during the wet season, analysis of data indicates that the urban poor in Africa may be at higher risk for malaria than expected and that malaria can no longer be regarded only as a rural phenomenon. This finding is of great concern because in Africa the current urban population growth rate of 3.5% is >3 times the rural population growth rate, and by 2015 a total of 25 countries in sub-Saharan Africa will have urban populations larger than the rural populations (UNPP 2005a). Although levels of transmission in urban areas may be lower than in contiguous rural areas, high population densities and possible lower immunity (Trape & Zoulani 1987b) may result in more disease impact in urban settings. Furthermore, although not the sole cause, irrigated urban agriculture may further increase the risk for malaria by providing suitable breeding sites. Further research on the interaction between type of urban agriculture and vector biology is needed because in most African cities urban farmers irrigate with water from polluted sources that is generally not favoured by malaria vectors, although several studies have reported *Anopheles* breeding in heavily contaminated water (Chinery 1984, Keating *et al.* 2004). The advantages of urban agriculture for alleviating poverty are numerous, but care must be taken that unregulated growth does not compromise its success. Integration of the activities of municipal authorities, agriculturalists, health professionals and communities is essential to reduce the existing burden of malaria and to prevent future increases.

Chapter 4

4. URBAN MALARIA AND ANAEMIA IN CHILDREN: A CROSS-SECTIONAL SURVEY IN TWO CITIES OF GHANA[2].

4.1. Introduction

The urban population in Africa is growing at an annual rate of 3.5%, more than three times that of the rural population, and it is estimated that by 2025, over 50% of the African population will live in urban areas (UNPP 2005b). While many of Africa's health problems are common to both urban and rural environments, the epidemiology of some diseases and the challenges to prevention and control can differ (Knudsen & Slooff 1992, Harpham 1997, McMichael 2000, De Castro *et al.* 2004, Keiser *et al.* 2004, Hay *et al.* 2005). Identifying these challenges and meeting the public health needs of the denizens of towns and cities is becoming increasingly important. Urban malaria typifies this challenge.

Historically, malaria was considered a rural disease in Africa. In highly populated urban areas, suitable *Anopheles* vector breeding sites are scarce, the host population is larger (resulting in lower biting rates) and typically has better access to preventive methods and antimalarial chemotherapy, resulting in a reduction in carrier rates (overview in Warren *et al.* 1999, Robert *et al.* 2003). However with increasing urban immigration, a greater number of people will be at risk of infection, and their arrival may itself alter the epidemiological profile of malaria and increase demands on urban health services. Clearly, detailed information on urban malaria epidemiology is urgently needed.

However, while transmission of malaria does occur in urban areas (Sabatinelli *et al.* 1986, Trape 1987b, Akogbeto *et al.* 1992b, Robert *et al.* 2003, Keiser *et al.* 2004, Klinkenberg *et al.* 2005), levels of malaria transmission and prevalence are lower than in rural areas (Robert *et al.* 2003, Omumbo *et al.* 2005). In urban West Africa, malaria

[2] An amended version of this chapter is published as Klinkenberg E. McCall PJ, Wilson, MD, Akoto AO, Amerasinghe FP, Bates I, Verhoeff FH, Barnish G and Donnelly MJ, 2006. Urban malaria in sub-Saharan Africa:

prevalence rates in children under five years of age are usually less than 15-20%, although large variations occur (Vercruysse *et al.* 1983, Sabatinelli *et al.* 1986, Lindsay *et al.* 1990, Julvez *et al.* 1997, Klinkenberg *et al.* 2005). In Ouagadougou (Burkina Faso) for example, mean malaria prevalence in under-fives was 16.1%, but ranged from 3.0 to 26.4% between communities (Sabatinelli *et al.* 1986). In Cotonou (Benin) prevalence rates in children under 15 years ranged from 51.7 to 63.8% in the central urban area (Akogbeto *et al.* 1992b). Some of this variation will be linked to distance from vector breeding sites (Trape *et al.* 1992, Thompson *et al.* 1997), but this is unlikely to be the sole reason. Differences in the vulnerability of certain communities and individuals associated with differences in access to health care, education and socio-economic status may be equally important. Furthermore as low transmission typically delays the development of immunity, all age groups of a sedentary urban population, rather than just young children and pregnant women alone, would be expected to be at risk of severe complicated malaria.

Despite these challenges, urban malaria could be readily and cost-effectively controlled, if diagnosis and treatment were focussed on the most vulnerable (Donnelly *et al.* 2005). The first step in this process is to identify those communities that are at risk of malaria and determine the factors that predispose them to infection and illness. We carried out cross-sectional surveys in children between 6 and 60 months of age in urban communities in two Ghanaian cities. To estimate the impact of malaria on their haemoglobin levels, we used an attributable fraction calculation. Malaria is an important contributor to anaemia (Newton *et al.* 1997), and severe malarial anaemia can be a major cause of morbidity and mortality (Slutsker *et al.* 1994, Murphy & Breman 2001, Kahigwa *et al.* 2002). Although the contribution of moderate anaemia to child mortality remains unclear (Brabin *et al.* 2001, Ghattas *et al.* 2003), its prevention is vital in reducing anaemia-related morbidity.

4.2. Material and Methods

4.2.1. Study area and population

The study was conducted between October 2002 and February 2003 in the two main cities of Ghana, West Africa. Accra, the capital city, has an estimated population of 1.6

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heterogeneities in malaria prevalence in two cities in Ghana. *Tropical Medicine and International Health* 11 (5), p. 578-588.

million (Ghana Statistical Service 2002) and is located in the coastal savannah zone (average annual rainfall 730 mm). Kumasi, the second largest city with a population of 1.2 million (Ghana Statistical Service 2002), is located in the central forest zone with a semi-humid tropical climate (average annual rainfall 1488 mm). In both cities, peak rainfall occurs between April and June, with a secondary peak between September and October.

4.2.2. Community selection

As the study was fundamentally designed as a cross-sectional survey to investigate the impact of urban agriculture on malaria, communities in the proximity of urban agricultural sites (designated UA; located within 1000m of agriculture) and communities without urban agriculture (U; located at least 1000m from agriculture) were selected in each city: 12 in Accra (7 UA and 5 U) and 11 in Kumasi (5 UA and 6 U) (figure 4-1). Selected communities were comparable in terms of their housing status (e.g. type, construction materials), population density and level of development. More details on urban agriculture have been given in a previous paper (Klinkenberg *et al.* 2005 see also paragraph 2.7). Epidemiological and socio-economic data were collected by cross sectional house-to-house survey in each city, carried out by teams of technicians and trained enumerators. In each community, households were selected by enquiring for the presence of children under five years old and selections were made over the whole geographical area of the community, to avoid potential bias resulting from spatial heterogeneity. The purpose of the study was explained and all children between 6 and 60 months old in each house were enrolled after informed consent had been obtained from their carers. The geographical location of each household was determined using a handheld Global Positioning System (GPS).

4.2.3. Data collection

Age (verified from child health cards when available), sex, antimalarial use in the preceding two weeks, history of fever in the past 48 hrs and travel to a rural area within the preceding three weeks (reported by the carer) were recorded for each child. In each household, data were collected on housing type, protection methods for mosquitoes, education level and a composite measure of socio-economic status based on household assets (following a World Bank template Gwatkin *et al.* 2000).

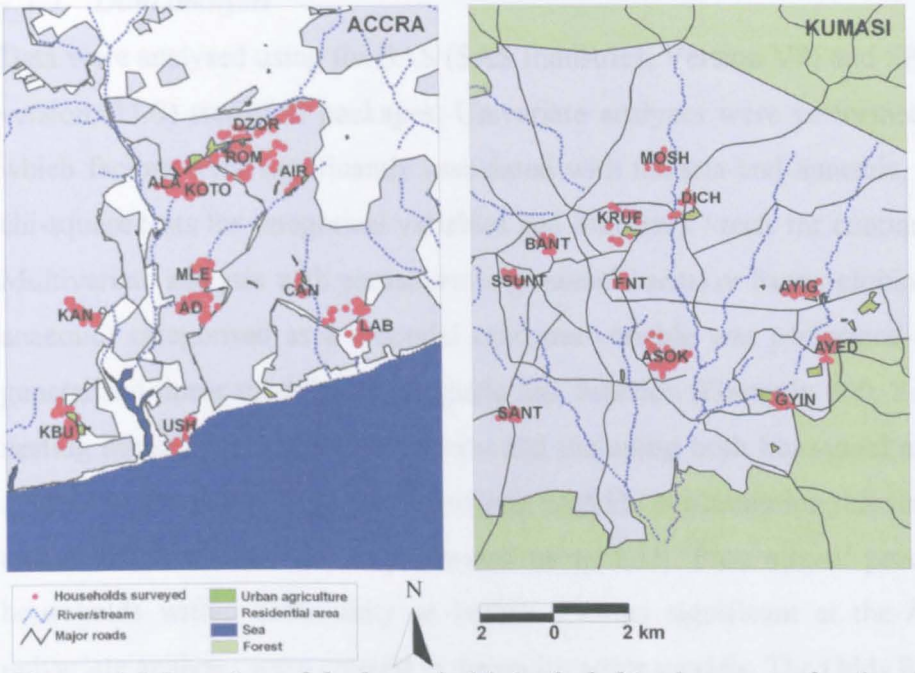

Figure 4-1 Location of the households included in the survey for the city of Accra and Kumasi (details for individual communities are given in table 4-3)

4.2.4. Investigations

Thick and thin blood films were made for each enrolled child, stained with Giemsa stain and examined following standard WHO protocols (Gilles 1993a). Parasite density was estimated by counting the number of fields positive for parasites in 100 high power fields (hpfs). High density parasitaemia was defined as individuals with 100% hpfs positive (Coosemans *et al.* 1994). Quality control of slides in each city was carried out by cross reading 100 slides by the laboratory in the other city. Slides that differed were cross-checked by a third person and scoring was based on the two readings that were in agreement. Haemoglobin (Hb) levels were determined using a blood haemoglobin photometer (HemoCue, Sweden). The HemoCue was validated daily using the manufacturer's cuvette.

Following government guidelines, children with malaria and/or Hb<8.0 g/dL were given a voucher for free treatment at a local government clinic (Accra) or treatment was brought to their homes (Kumasi). Children sampled were apparently healthy (asymptomatic), although carers in some cases reported a history of fever for the child (Table 4-4). More details on material and methods can be found in Appendix 3.

4.2.5. Data analysis

Data were analysed using the SAS (SAS Industries, Version V8) and SPSS (SPSS inc. version, 11.0) statistical packages. Univariate analyses were performed to determine which factors were significantly associated with malaria and anaemia, using Pearson chi-square tests for categorical variables and Student's *t*-tests for continuous variables. Multivariate analysis with parasitaemia (present/absent) or haemoglobin (anaemic/not-anaemic) categorised as a binomial outcome variable was performed using a mixed generalized linear model with a logistic link function (Glimmix 800, SAS Industries), nesting households within community and including both household and community as random variables. Multivariate analysis with Hb concentration (continuous variable) as the outcome variable was performed using SAS 'Proc mixed' procedure, nesting households within community as before. Factors significant at the $P<0.1$ level in univariate analyses were entered in the multivariate models. The Odds Ratios (OR) and coefficients were based on the final model only and include the significant variables unless stated otherwise. The significance level was set at $P<0.05$ for all tests.

4.2.6. Definitions

Parasitaemia: Presence of malaria parasites in blood films from peripheral circulation as counted in 100 high power fields.

High density parasitaemia: parasites present in 100% of high power fields.

Anaemia: Children with Hb<11.0g/dL were classified as anaemic and with Hb<8.0 g/dL as moderately-severely anaemic.

The children were subdivided into five age groups: 6-12, 13-24, 25-36, 37-48 and 49-60 months of age.

Attributable Risk (AR %): $[(n_1m_0 - n_0m_1) / n (n_0+m_0)] * 100$

where n_0 and n_1, respectively denote the numbers of unexposed and exposed diseased subjects (e.g. anaemic children without and with malaria, whereby $n_0+n_1=n$) and m_0 and m_1 the numbers of unexposed and exposed disease-free subjects (e.g. non-anaemic children without and with malaria, whereby $m_0+m_1=m$) (Benichou 2001).

4.2.7. Ethical clearance

Ethical clearance for the study was obtained from the ethical review committees of the Liverpool School of Tropical Medicine, the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon and the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi.

4.3. Results

A total of 3525 children were enrolled in the study: 1744 from Accra (938 households) and 1781 from Kumasi (1215 households). Table 4-1 shows household characteristics of the study families in both cities. Households in both cities were significantly different ($P<0.001$) in all characteristics studied, except household size. Children recruited from the two cities were not significantly different ($P>0.05$) in age and sex ratio (Table 4-2).

Table 4-1 Household characteristics of the study population, overall and by city.

Household characteristic	Accra (n=938)	Kumasi (n=1215)	*between cities*
Average household size (SD)	4.9 (1.8)	4.8 (1.7)	(P=0.479)
% living in compound house (as opposed to self-contained, separate structure or storey building)	81.0	73.7	$P<0.001$
% with access to piped water*	99.5	93.7	$P<0.001$
% with electricity	89.4	96.7	$P<0.001$
% with a bednet	32.8	27.5	$P<0.001$
% with windows/doors nets	85.3	78.3	$P<0.001$
% without a ceiling	26.2	14.8	$P<0.001$
mean socio-economic-score# (SD)	1.73 (0.97)	2.01 (0.83)	$P<0.001$

SD = standard deviation; * either by private or public tap; #based on asset score of the World Bank for Ghana (www.worldbank.com/hnp);

4.3.1. Malaria

There was a marked difference in the prevalence of malaria parasitaemia in the two populations (Table 4-2). In Accra, average malaria prevalence was 14.8% (95%CI 13.1-16.5), ranging from 6-22% by community (Figure 4-1, Table 4-3). In Kumasi, prevalence was significantly lower (P=0.001) at 8.6% (95%CI 7.3-9.9), ranging from

2-33% by community, two of which Dichemso and Moshie Zongo had high parasite prevalences of 15.9 and 32.7% respectively. Excluding these two communities, the malaria prevalence in Kumasi was 5.5% (range 2.5-9.5%).

Table 4-2 Overview of characteristics of the children enrolled in the study per city.

	Accra (n=1744)	Kumasi (n=1781)	*between cities*
Male:Female ratio	0.96	0.99	(*P*=0.680)
mean age in months (SD)	33.5 (17.1)	34.2 (17.4)	(*P*=0.228)
% with malaria parasites	14.8	8.6*	*P*<0.001
% with anaemia (Hb< 11.0 g/dL)	51.0	43.2	*P*<0.001
% with moderate to severe anaemia (Hb < 8.0 g/dL)	4.0	3.1	(*P*=0.137)
mean Hb in g/dL (SD)	10.8 (1.5)	11.1(1.5)	*P*<0.001

SD = standard deviation; Hb= haemoglobin concentration; *There were two communities with prevalence above 10%, i.e. 15.9 and 32.7% without which mean prevalence was 5.5% (range 2.5-9.5%).

Table 4-3 Parasitaemia prevalence and number of children sampled per community in the two cities; locations of communities are shown in Figure 4-1.

City	Code	Community	Type	Number of children sampled	Parasitaemia prevalence (%)
Accra	AIR	Airport	UA	77	19.5
Accra	ALA	Alajo	UA	166	15.1
Accra	DZOR	Dzorwulu	UA	132	19.7
Accra	KBU	Korle Bu	UA	181	8.8
Accra	KOTO	Kotobabi	UA	219	18.3
Accra	ROM	Roman Ridge	UA	105	22.9
Accra	CAN	Cantonments	UA	23	13.0
Accra	MLE*	Kokomlemle	UA/U	160	20.6
Accra	AD*	Asylum Down	UA/U	160	11.3
Accra	KAN	Kaneshie	U	159	19.5
Accra	LAB	Labonie/La	U	175	9.7
Accra	USH	Ushertown	U	200	6.5
Kumasi	ASOK	Asokwa	U	173	2.3
Kumasi	AYED	Ayeduase	UA	173	4.6
Kumasi	AYIG	Ayigia	UA	179	9.5
Kumasi	BANT	Bantama	U	189	4.0
Kumasi	DICH	Dichemso	UA	69	15.9
Kumasi	FNT	Fanti New Town	U	193	5.2
Kumasi	GYIN	Gyiniasi	UA	185	9.2
Kumasi	KRUF	Krofrom & Manhiya	UA	157	3.8
Kumasi	MOSH	Moshie Zongo	U	168	32.7
Kumasi	SANT	Santasi	U	160	4.4
Kumasi	SSUNT	South Suntreso	U	145	3.4

** These communities were originally identified as control communities but small UA sites were later identified close to them.*

Although in Accra, communities near urban agriculture had significantly higher malaria prevalence (16.3 vs. 11.2% in UA and U respectively, P=0.018; chapter 3), this was not observed in Kumasi (7.8 vs. 9.3% in UA and U respectively, P=0.262).

In Accra but not in Kumasi, malaria parasite prevalence increased until the age of four (Figure 4-2). Of the total number of children with malaria parasites, 26.4% had high parasite density, which was comparable in Accra and Kumasi (29.0% [95%CI 23.0-34.9] and 22.7% [95%CI 16.0-29.4] respectively).

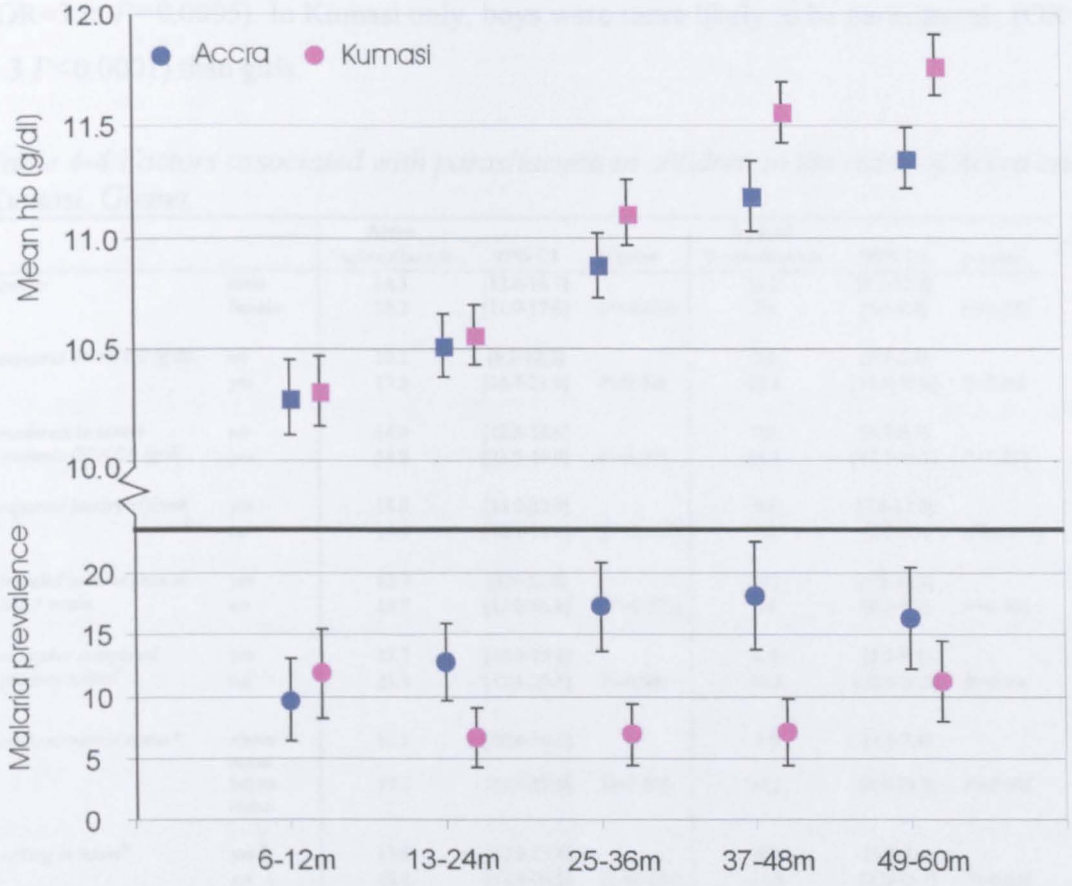

Figure 4-2 Mean haemoglobin concentration (g/dL) and malaria prevalence (%) with 95% confidence intervals in different age groups for the cities of Accra and Kumasi.

Univariate interactions of variables with parasitaemia are presented in Table 4-4 and the results of the subsequent multivariate analysis in Table 4-5. Factors that were significant in both univariate and multivariate analyses were socio-economic status, being anaemic or moderately to severely anaemic, travel to the rural area in the last 3 weeks and gender. The education level of the carer, the use of windows/door nets, the

use of mosquito coils on a daily basis and the presence of a ceiling in the house were significant in univariate models only, possibly because all are associated with socio-economic score.

Multivariate analysis was carried out separately for the two cities as many covariates differed significantly between the two (Table 4-1). In both cities, lower socio-economic status and age-group were significantly associated with the presence of malaria parasites. In Kumasi, but not in Accra, children who travelled to the rural areas in the 3 weeks preceding data collection were more likely to have parasitaemia (OR=7.0; P=0.0005). In Kumasi only, boys were more likely to be parasitaemic (OR= 5.3 P<0.0001) than girls.

Table 4-4 Factors associated with parasitaemia in children in the cities of Accra and Kumasi, Ghana.

		Accra			Kumasi		
		%parasitaemic	95% CI	p-value	%parasitaemic	95% CI	p-value
gender	male	14.3	[12.0-16.7]		10.2	[8.2-12.2]	
	female	15.2	[12.9-17.6]	(P=0.604)	7.1	[5.5-8.8]	P=0.023
anaemia (Hb<11.0 (g/dl)	no	10.1	[8.1-12.1]		5.0	[3.7-6.4]	
	yes	19.3	[16.7-21.9]	P=0.000	13.4	[11.0-15.8]	P=0.000
moderate to severe anaemia (Hb<8.0 (g/dl)	no	14.0	[12.3-15.6]		8.0	[6.7-9.3]	
	yes	34.8	[23.5-46.0]	P=0.000	29.1	[17.1-41.1]	P=0.000
reported history of fever	yes	18.0	[14.0-22.0]		9.8	[7.6-12.0]	
	no	14.0	[12.1-15.8]	(P=0.057)	7.3	[5.7-8.9]	(P=0.077)
traveled to rural area in last 3 weeks	yes	15.7	[8.9-22.6]		14.2	[9.5-18.8]	
	no	14.7	[13.0-16.4]	(P=0.773)	7.6	[6.3-8.9]	P=0.001
caretaker completed primary school#	yes	13.7	[12.0-15.4]		6.8	[5.5-8.1]	
	no	23.3	[17.4-29.3]	P=0.000	16.4	[12.5-20.3]	P=0.000
*socio-economic status**	above mean	12.5	[10.4-14.6]		5.8	[4.1-7.4]	
	below mean	17.2	[14.5-19.8]	P=0.006	11.2	[8.9-13.5]	P=0.000
ceiling in house#	yes	13.9	[12.0-15.8]		8.0	[6.7-9.4]	
	no	16.8	[13.4-20.3]	(P=0.121)	11.9	[8.0-15.7]	P=0.041
household has netting windows/doors	yes	13.9	[12.1-15.7]		8.6	[7.1-10.1]	
	no	19.7	[14.9-24.5]	P=0.015	8.4	[5.6-11.3]	(P=0.919)
household uses mosquito coil everyday	yes	17.8	[14.8-20.8]		5.6	[3.7-7.6]	
	no	13.1	[11.1-15.1]	P=0.008	10.0	[8.3-11.8]	P=0.003

%parasitaemic= percentage of children having parasitaemia in their blood; 95% CI= 95 percent confidence interval; ; p-value are for Pearson Chi-square test, # these variables were not entered simultaneously in the multivariate model with socio-economic status due to their correlation with this parameter; * below average household socio-economic status of the city (calculated from study population) which was 1.73 (SE0.032) and 2.01 (SE0.026) for Accra and Kumasi respectively;

Table 4-5 Logistic multivariate analysis for children with and without parasitaemia for the cities of Accra and Kumasi.

	Accra		Kumasi	
variable	*OR/coefficient†*	*p- value*	*OR/coefficient†*	*p- value*
Age-group 1 (6-12 months) 2 (13-24 months) 3 (25-36 months) 4 (37-48 months) 5 (49-60 months)	OR=0.33 OR=0.41 NS NS ref (OR=1)	<0.0001 <0.0001	OR=3.2 NS NS NS ref (OR=1)	P<0.001
SE status indicator*	-0.5463	<0.0001	-0.6023	P=0.0082
Education level of caretaker#*	NS		NS	
Anaemic (Hb<11)	OR=2.63	<0.0001	NS	
Moderate to severe anaemic (Hb<8.0 g/dL) ¶	OR=6.5	P=0.0001	OR=2.4	P=0.0374
Child taken malaria medication in last 2 weeks	NS		OR=0.24	P=0.0045
Child travelled to the rural area in the last 3 weeks	NS		OR=7.0	P=0.0005
Being male	NS		OR=4.5	P<0.001

NS=not significant, note these variables were not included in the final model; #=entered as continues variable in the model;*either one was added at the same time in the model; ¶ This parameter not entered in model at same time as anaemic; † the Odds Ratio (OR) is given for categorical variables and the coefficient for continuous variables; OR and coefficient are the values when all significant parameters (incl. anaemia) were entered in the model, the models with moderate to severe anaemia (instead of anaemia) slightly changed the estimates (but not the significance) of the other covariates

4.3.2. Anaemia

Haemoglobin levels increased with age in both cities (Figure 4-2). In the younger age group (6-24 months) more than 60% of the children were anaemic. For all age groups, Hb concentration was significantly lower in children with malaria parasitaemia (P<0.001). Children with high-density parasitaemia had significantly lower Hb concentrations than children with low parasitaemia densities (P=0.002, controlled for age) and were more likely to be anaemic (OR 1.91 (95%CI 1.10-3.36) P=0.015).

Again there were marked differences between the two cities. The prevalence of anaemia was significantly higher in Accra than in Kumasi (51.0 vs. 43.2% respectively P<0.001), although the prevalence of moderate to severe anaemia (<8.0g/dL) did not differ significantly between Accra and Kumasi (4.0 and 3.1% respectively). Mean Hb concentration was significantly lower in children in Accra than in Kumasi (10.8 vs. 11.1 g/dL respectively; P<0.001; Figure 4-2). Multivariate analysis showed that in both cities, anaemia was more likely to occur in younger children (P<0.001) with a lower socio-economic score (P<0.008). In both cities, moderately-severely anaemic children were more likely to be parasitaemic (Accra OR=6.5 (P=0.0001); Kumasi OR=2.4 (P=0.0374)). In addition for Accra only, reported history of fever (OR=1.4; P=0.022) was significantly associated with being anaemic.

The attributable risk (AR) of anaemia due to malaria was 5.3 and 5.7% for Accra and Kumasi respectively. The AR of moderate to severe anaemia due to malaria was 23.5 and 22.4% respectively.

4.4. Discussion

The data from these two cities demonstrate that malaria can be a major public health problem in the urban setting and that prevalence can vary markedly between communities and between cities. We found that socio-economic status, anaemia and age were strongly associated with malaria, were common to affected communities in both cities, and are similar to malaria risk factors in rural areas. As these data derive from a point prevalence survey in 23 geographically dispersed communities in two cities, with 3525 children surveyed, they provide a reliable representation of the cross-sectional values for malaria parasitaemia, anaemia and associated factors for the urban environment in Ghana. Moreover, as most other urban malaria and anaemia studies are hospital based and therefore biased towards symptomatic children, these data provide important comparators from an apparently asymptomatic population.

The malaria prevalence rates found in this study are comparable to those recorded 10-20 years earlier in a number of smaller studies in West African cities (Vercruysse *et al.* 1983, Sabatinelli *et al.* 1986, Lindsay *et al.* 1990, Akogbeto *et al.* 1992b, Julvez *et al.* 1997). In the intervening years West African cities have experienced unregulated urban growth resulting in dramatic increases in population size. Our data suggest that this increase in urbanisation will not inevitably lead to less malaria. In fact, this study illustrates that malaria infections can continue to be a serious public health problem in high density population centres, affecting a steadily increasing number of people.

The higher malaria prevalence observed in Accra compared to Kumasi was contrary to expectations, as prevalence studies in contiguous rural areas showed a marginally lower prevalence among 6-15 yr old rural school children in the coastal (Accra) zone (35.1%) compared to forest (Kumasi) zone (39.2%) (Afari *et al.* 1992). In addition, some of the communities that participated in the survey in Kumasi were located more towards the periphery of the urban area (Figure 4-1) where malaria transmission might be expected to be higher than in the more densely populated and polluted central urban environment (Warren *et al.* 1999).

Our data show that anaemia is a common problem in urban Ghana with 47.1% prevalence among the under fives and more than 60% prevalence in children aged up to 24 months. This is within the 39-87% range of anaemia prevalence reported for under fives in Africa (De Maeyer & Adiels-Tegman 1985, Koram *et al.* 2003, Schellenberg *et al.* 2003, Desai *et al.* 2005). Few studies have reported prevalences of anaemia in apparently 'healthy' urban populations. In a cohort study in a small town in Cameroon, Cornet *et al.* (1998) found an anaemia prevalence of 42% in the 6-36 month and 21% in the 37-60 month age groups, which was clearly lower than the prevalences recorded in the large urban centres in this study. Haemoglobin concentrations in our study population were higher than in a rural area of northern Ghana (Koram *et al.* 2003).

The main factors affecting the prevalence of anaemia were age-group and presence of malaria parasites, with highest Odds Ratios for the moderate to severely anaemic children. Additional risk factors that can be important causes of anaemia, such as the presence of geohelminths (Nkhoma 2005), are being investigated in a follow up study. As the Roll Back Malaria programme of the World Health Organization is currently exploring the use of anaemia as a measure of the impact of malaria interventions (Korenromp *et al.* 2004), further details of the risk factors associated with decreased haemoglobin concentrations in urban populations would be beneficial.

The higher anaemia prevalence and the low socio-economic status of children with malaria parasites in these two cities highlights the problem of unregulated urban growth for the health of sub-Saharan African populations. These and other data suggest that the importance of malaria in urban areas is underestimated and that rapid urban growth has the potential to exacerbate the problem (Robert *et al.* 2003, Keiser *et al.* 2004, Donnelly *et al.* 2005). The recent estimate of 200 million people at risk of malaria in urban Africa (Keiser *et al.* 2004) is likely to require an annual upwards revision.

A number of the risk factors associated with malaria parasitaemia and anaemia were different between the two cities. In Accra, anaemia and malaria were linked to socio-economic status and age while in Kumasi, additional risk factors were identified *e.g.* travel to the rural area, consumption of malaria medication in the two weeks preceding

the survey and, surprisingly, being male. Why male had a higher risk is not clear, as gender did not differ significantly between children with parasitaemia that travelled, took malaria medication or reported a history of fever. Furthermore we are not aware of any practices that might have exposed males more, or made females less susceptible, to malaria. In Accra but not in Kumasi, distance to sites of urban agriculture was found to be a risk factor (see Klinkenberg *et al.* 2005) indicating a variable impact. Further studies are needed to quantify and qualify further the risk factors and their possible interactions.

There were wide variations in parasite prevalence observed between communities, in contrast to the relative homogeneity commonly seen in rural areas (Thompson *et al.* 1997, Clarke *et al.* 2002). In Kumasi for example, two areas had a prevalence of infection up to 5 times higher than the other study communities. This variability may reflect the heterogeneous nature of the city, where diverse and often rapidly changing communities and districts present different risks, some of which undoubtedly remain unknown. This intra-city variation, also seen in other urban studies (Sabatinelli *et al.* 1986, Trape 1987b, Fondjo *et al.* 1992, Manga *et al.* 1993, Njama *et al.* 2003), means that in order for interventions to be both cost effective and efficacious, the vulnerability of each urban community must be assessed prior to implementing interventions. As in other studies (Koram *et al.* 1995, Biritwum *et al.* 2000, ter Kuile *et al.* 2003a), the Kumasi community with highest parasitaemia prevalence (Moshie Zongo) showed a negative association between malaria risk and both education and socio-economic score. The community was characterized by a 1.5 times greater number of people with below the average socio-economic status for the study communities and double the mean proportion of people who did not complete primary education. However, urban specific risk factors also emerged: travel to the rural area for example (as seen in earlier (peri-urban) studies; (Watts *et al.* 1990, Koram *et al.* 1995), had an odds ratio of seven and in this community, the number of children travelling was nearly three times the Kumasi average. As this community is comprised predominantly of immigrant ethnic groups originating in northern Ghana, other culturally specific practices and health seeking behaviours, and indeed genetic factors, might potentially have influenced the infection prevalence (Greenwood *et al.* 1987, Modiano *et al.* 1995, Dolo *et al.* 2005). Notably, the Accra population is composed of

many different ethnic groups while in Kumasi the majority of people are of the local Akan group. Clearly, ethnic origin should be taken into account in future studies. The red cell abnormalities present in West Africa should also be considered as these may play a significant role in influencing susceptibility to and clinical outcome of malaria infections (Mockenhaupt *et al.* 2000, Mockenhaupt *et al.* 2004).

Mean haemoglobin in older age groups in Kumasi was significantly higher than in Accra and may have reflected the higher parasitaemia in the older age groups in Accra (Figure 4-2). Attributable risk of anaemia due to malaria was approximately 5% for both cities, indicating that there are other important contributors to anaemia in the urban area. However, with an attributable risk over 20%, malaria was clearly a major contributor to severe anaemia. Interestingly, although the risk factors for malaria showed differences between the cities, the attributable risks were very similar. We found few published attributable risk data for anaemia due to malaria. In a study in pre-school children in Kenya, a relatively high malaria attributable prevalence of anaemia of 14% was calculated (Brooker *et al.* 1999). In primigravid pregnant women in Malawi, attributable risks of 1.8% for anaemia (Hb<11 g/dL) and 14.6% for severe anaemia (Hb<8.0 g/dL) (Verhoeff *et al.* 1999), values comparable to our findings, were recorded.

Malaria in urban areas displays a heterogeneity and complexity that differs in many respects from the rural environment. This has important implications for malaria control. Marked intra-city variation indicates the importance of targeting specific areas or districts and control measures might be expected to differ from area to area depending on risk factors and administrative structure/cultural background of the community. As malaria affects those with lower socio-economic scores and significantly increases the risk of severe anaemia, leading in turn to an impact on long-term health and education, the importance of controlling malaria in urban areas is clear: the most vulnerable, the urban poor, should be prioritized when designing control measures. Early and thorough assessment of the malaria risk pattern in any city will be essential to any integrated urban malaria control programme.

Chapter 5

5. NATURAL HISTORY OF VECTORS AND TRANSMISSION OF MALARIA IN URBAN GHANA

5.1. Introduction

It is typically assumed that urbanization leads to a decrease in malaria because it also results in fewer *Anopheles* breeding sites, better access to treatment and better housing structures (overview in Warren *et al.* 1999). However, there is a concern that rapid unplanned urbanization may not lead to a decrease in malaria transmission in urban areas as a results of declining incomes, poor housing and lack of sanitation (Keiser *et al.* 2004) and urban malaria epidemiology might pose different challenges than can be expected from the rural areas (Robert *et al.* 2003). Urban agriculture, promoted to increase food security and alleviate poverty might, especially when irrigated, further increase malaria risk by creating breeding sites for the *Anopheles* vector (Dossou-Yovo *et al.* 1994, Robert *et al.* 1998, Keating *et al.* 2003, Keating *et al.* 2004). More information on breeding in urban areas can be found in the literature review (paragraph 1.3.3. p19).

An earlier study in Ghana revealed lower *Anopheles* biting rates in sites without urban agriculture compared to sites close to urban agriculture which had biting rates at levels between those in central urban and peri-urban locations (Afrane *et al.* 2004). Moreover, extensive use of pesticides in urban farming might lead to increased development of resistance of *Anopheles* mosquitoes to insecticides. To investigate the impact of urban agriculture on malaria vectors, entomological surveys were carried out in urban Ghana, with a view to collect data on mosquito breeding in an urban setting, to confirm if malaria transmission occurred in urban Accra and to assess resistance status of urban mosquitoes.

5.2. Material and Methods

Entomological surveys were carried out in the same areas as the epidemiological surveys described previously (chapter 3, 4).

5.2.1. Mosquito collections

5.2.1.1. Adult catches - Human Bait Catches (HBC)

From 8 September to 19 December 2003 eight rounds of human bait catches (HBC) were carried out fortnightly at each site in Accra to assess man biting rates, parity rates and nocturnal biting patterns. HBC were carried out in six communities, 3 UA sites (Kotobabi, Dzorwulu and Korle Bu) and 3 U sites (Kaneshie, La and Ushertown) (Figure 3-1, p 35). Two different sites were surveyed per night (one UA and one U). Fixed sampling locations were used at each site. At each site, 2 pairs of catchers were based at two different locations a few houses apart. Pairs of catchers worked in shifts, one group from 18.00-24.00 hrs and one group from 24.00-06.00 hours. Catchers were selected from the local community to facilitate acceptance from residents. Informed consent was obtained from each catcher and malaria prophylaxis was provided free of charge for the catchers. Initially one pair was placed inside and one pair outside but after 1 week it was decided to locate both pairs of catchers outside to minimize collection bias resulting from differences in housing structure and/or the use of preventive methods in the different communities. One coordinator per site supervised collection throughout the night. Mosquitoes were caught from 6pm to 6 am and hourly catches were stored separately. Mosquitoes were caught by a tube when landing on the leg and transferred to a cup with a netting lid following methods described in Service (1993). The catchers were trained to collect landing mosquitoes prior to blood feeding, to minimise any increased risk of malaria transmission. Catches were transported back to the laboratory in the morning for identification and processing (paragraph 5.2.2.).

5.2.1.2. Adult catches – pyrethrum knockdown catches (PKD)

In October 2003 pyrethrum knockdown catches (PKD) were performed in both Accra and Kumasi simultaneously with the HBC in Accra. Three rounds were done in Accra and two in Kumasi. PKDs were done at all the sites of the epidemiological surveys (Figure 4-1, p. 44). Due to the low numbers of mosquitoes caught in the first rounds, more catches were not conducted (see results). In the selected study communities, 15 houses in different parts of the community were selected. PKDs were performed as

described by Service (1993). Briefly, white sheets were spread over the floor of the room after which windows and doors were closed and rooms were sprayed using locally available aerosols ('*Mortein*' brand: Bioallethrin 0.12%, Bioresmethrin 0.08%, Tetramethrin 0.38%, solvent and propellant 99.42%). After 15 minutes mosquitoes were collected from the sheets by mouth aspirators, transferred into paper cups and transported back to the laboratory for identification. Bloodmeals from *Anopheles* mosquitoes were conserved by squashing the abdomen on filter paper and stored over silica gel.

5.2.1.3. Larval collections

To investigate the range of breeding sites where *Anopheles* could be found breeding in urban areas, a larval survey was carried out. Breeding sites were located in both UA and U areas, by searching through the area to identify water bodies with the potential to harbour mosquito larvae. Larvae were collected by the dipping method after Service (1993). If the breeding sites allowed, area wise dipping was done following Amerasinghe *et al.* (1997), making a certain number of dips according to surface area, e.g. 6 dips per square meter for areas < $10m^2$. Habitats were characterised using a standard format for each site, including flora and fauna characteristics, water quality (pH, EC, foul smell, clear or turbid), light conditions (sunlit or shaded), substratum type *etc.* Recording forms used for larval collections are in Appendix 4.

In addition to the characterization of breeding sites in the UA areas as described above, additional longitudinal surveys were carried out to find out the pattern of breeding in the wells used for irrigation. This was done at the three main agricultural sites in Accra where wells are the most common irrigation structure, Dzorwulu farm, Kotobabi Farm and Korle Bu Farm.

Initially in one urban agricultural area (Dzorwulu farm) 15 wells were selected to be measured bi-weekly for larval densities and habitat characteristics as described above. The first results showed that only 1-2 wells were positive at a time, therefore after 7 rounds the protocol was adapted to optimize efforts with results. Every 2 months an inventory was made of all the wells to assess the percentage of wells breeding mosquitoes. This was done by surveying the surface of each well using a small fishing

net after which the net was emptied in a white tray to investigate if mosquito larvae or other fauna were present (Figure 5-1).

Figure 5-1 Examining of wells for presence of mosquito larvae with aid of fishing net at Dzorwulu farm in Accra.

In each well it was noted if the well was positive or negative for mosquito larvae, if positive, number and type of larvae was noted. In addition pH and electrical conductivity (EC) was measured using a battery powered meter with pH and EC electrode (WTW, Germany pH/cond 340i). Habitat characteristics like *e.g.* foul/non-foul smell, substratum type, presence of predators (*i.e.* dragonfly, water beetle, water scorpion etc.) and vegetation (in/around site) were recorded (see Appendix 4 for details of characteristics recorded).

5.2.2. Mosquito identification and processing

Anopheles larvae from the larval collections were reared in the laboratory to adult stage for easier identification. All adult *Anopheles* from the HBC and PKD and reared larvae were identified to species level using a stereomicroscope following the key of Gillies and de Meillon (1968). A sub sample of *A. gambiae s.l.* was identified to species level by polymerase chain reaction (PCR) following Scott et al. (1993). A sub sample of the *A. gambiae s.s.* were identified to molecular form following Fanello *et al.* (2002). More details are given in Appendix 4.

Mosquitoes of the HBC were dissected and ovaries examined to determine parous rates following Detinova (1962). Head and thoraces of these mosquitoes were processed by sandwich ELISA after Wirtz (CDC pers.comm) to assess sporozoite infection level (for protocol see Appendix 6). From 100 randomly selected *A. gambiae s.l.* collected

by HBC, DNA was extracted from the remaining mosquito parts (abdominal remains and legs) using a modified method of Livak (1984) for species identification by PCR (see above). Full protocols for all these methods are given in Appendix 7.

During the PSC surveys bloodmeals were collected from fed *Anopheles* by squashing them onto filter paper and storing them over silica gel, for assessment of the human blood index by direct ELISA after Beier (1988), see Appendix 6. Samples were tested for human, bovine, goat, dog and chicken antibodies. Additional serum added to the testing solution apart from the above mentioned was horse and porcine.

5.2.2.1. Insecticide resistance testing

Both *Culex* and *Anopheles spp.* mosquitoes from the light traps collections (see 7.2.10. p.96) and reared *Anopheles* larvae from the larval surveys were tested for Permethrin susceptibility status (1 hour exposure, 0.75% Permethrin, WHO paper) using the WHO protocol (Onori *et al.* 1993). Up to 20 mosquitoes were exposed for 1 hour in a WHO test tube covered with insecticide treated paper (WHO, 0.75% Permethrin) and allowed to recover for 23 hours after which knock down resistance was scored. During the bednet intervention study (chapter 7) in addition cone tests were performed on insecticide treated nets (PermaNet Vestergaard). Up to 10 mosquitoes were put in a cone on the net for an exposure time of 1 hour after which they were transferred to paper cups and allowed to recover for 24 hours. For both tests types, susceptibility status was scored as resistant when the mosquitoes were still alive after the recovery period and susceptible if they were knocked down and had died.

5.2.3. Statistical analysis

Man biting rates (+1) were log transformed to normalize the data analysed by T-tests or were analyzed by Mann-Whitney U tests. Differences between geometric means were calculated by a two sample *t*-test using the general linear model in SPSS (version 12.0.1) and obtaining the GM ratios from the parameter estimates.

5.3. Results

5.3.1. Species composition

In the HBC a total of 21,801 mosquitoes were collected in 192 man nights, species composition is given in Table 5-1 and Table 5-2. The *A. coustanii* were all caught in

the same night at one site (Dzorwulu). A random subset of 112 of the *A. gambiae s.l.* caught was successfully identified by PCR and all specimens were *A. gambiae s.s.* of which 96 (85.7%) were S-form and 16 (14.3%) were M-form. The results from the PKDs showed a similar species composition (Table 5-1 and Table 5-2), with the majority being *Culex spp.* The average number of mosquitoes caught in Kumasi was half of that in Accra, although the percentage of *Anopheles* caught was significantly higher in Kumasi ($P<0.05$) than Accra and more *A. funestus* were caught.

Table 5-1 Species composition of mosquitoes collected in human bait (HBC) and pyrethrum knockdown catches (PKD) in Accra and Kumasi.

City	Method	#Rounds	# Man nights/ houses	Total # caught	#*Culex*	#*Anopheles*	# *Aedes*	#*Mansonia*
Accra	HBC	8	192	21,801	20,100 (91.8%)	1,648 (7.6%)	111 (0.5%)	32 (0.1%)
Accra	PKD	3	408	4,135	3,915 (94.7%)	153 (3.7%)	67 (1.6%)	0 (0%)
Kumasi	PKD	2	329	1,587	1,395 (87.9%)	170 (10.7%)	17 (1.1%)	5 (0.3%)

#=number; HBC=human bait catch; PKD=pyrethrum knockdown catch

Table 5-2 Species composition of Anopheles collected in human bait (HBC) and pyrethrum knockdown catches (PKD) in Accra and Kumasi.

City	Method	Total # *Anopheles* caught	*A. gambiae s.l.*	*A. funestus*	*A. coustanii*
Accra	HBC	1,648	1,642 (99.6%)	0 (0%)	6 (0.4%)
Accra	PKD	153	146 (95.4%)	7 (4.6%)	0 (0%)
Kumasi	PKD	170	147 (86.5%)	23 (13.5%)	0 (0%)

#=number; HBC=human bait catch; PKD=pyrethrum knockdown catch

5.3.2. Man biting rates (MBR)

Man biting rates derived from the HBC and PKD were different, PKD MBR were much lower (

Table 5-3). Geometric mean (GM) of the daily MBR (hereafter called gmMBR) obtained by HBC was about 3 times higher in UA compared to U communities for *A. gambiae s.l.* (GM Ratio 3.0, 95%CI 1.6-5.9) and 4 times higher for *Culex spp.* (GM Ratio 4.1, 95%CI 2.7-6.2). GmMBR showed variation in the different communities (Table 5-4)), Kotobabi had the highest gmMBR for both *A. gambiae s.l.* as *Culex* spp, while Ushertown showed the lowest number for both. People received from 7 to 67 times as many *Culex* as *Anopheles spp.* bites.

Table 5-3 Geometric mean man biting rate with geometric standard deviation between brackets in Accra and Kumasi as obtained by HBC or PKD, overall and in communities with and without agriculture

	Accra - HBC		Accra-PKD		Kumasi-PKD	
	A. gambiae	*Culex*	*A. gambiae*	*Culex*	*A. gambiae*	*Culex*
UA	8.4 (3.3)	171.4 (1.6)	1.06 (1.24)	1.76 (2.22)	1.06 (1.21)	1.33 (1.88)
U	2.8 (3.0)	41.7 (2.5)	1.02 (1.12)	1.53 (1.90)	1.03 (1.14)	1.18 (1.37)
Overall	4.8 (3.5)	84.5 (2.7)	1.04 (1.20)	1.68 (2.11)	1.05 (1.23)	1.31 (1.75)

HBC=human bait catches; PKD=pyrethrum knockdown catches; UA= community near urban agriculture; U= community without urban agriculture

Table 5-4 Geometric mean man MBR with geometric standard deviation between brackets over 32 man nights of catching for selected communities in Accra and calculated EIR

Community	Type	MBR *Culex spp.*	MBR *A. gambiae s.l.*	EIR *A. gambiae s.l.*
Dzorwulu	UA	137.9 (1.5)	10.2 (2.1)	24.2
Korle Bu	UA	180.0 (1.6)	2.7 (2.1)	6.3
Kotobabi	UA	202.7 (1.6)	22.1 (2.5)	52.5
Kaneshie	U	66.0 (2.1)	9.2 (2.5)	21.8
La	U	55.5 (1.4)	2.1 (1.7)	5.1
Ushertown	U	19.8 (2.8)	1.1 (1.2)	2.6

MBR=man biting rate; EIR= annual entomological inoculation rate; UA= community near urban agriculture; U= community without urban agriculture

5.3.3. Sporozoite rate, parity rate and EIR

A total of 1,672 *Anopheles* from the HBC (including 24 from the trial rounds) were processed for ELISA and 11 (0.65%) were positive for sporozoites. All specimens positive for sporozoites were *A. gambiae s.l.* Seven of the positives were from Kotobabi, two from Korle Bu, one from Kaneshie and one from Dzorwulu. Combining the sporozoite rate and MBR from the human bait catches, this calculates into an annual EIR of 19.9 and 6.6 for UA and U respectively. The EIR for each community is shown in Table 5-4.

A total of 901 mosquitoes were dissected for parity, the overall parity rate was 0.39, and was significantly lower in UA at 0.35 in UA and 0.48 in U (OR 0.59 (95%CI 0.43-0.80), P=0.0004).

5.3.4. *MBR over the survey period*

During the period of HBC from September to December the MBR of *A. gambiae s.l.* increased peaking at the middle of November (Figure 5-2). Increase in *Anopheles* followed a peak in rainfall the month before (Figure 5-3). For *Culex spp.* the biting rate varied over the rounds as well but did not show clear peak densities (data not shown).

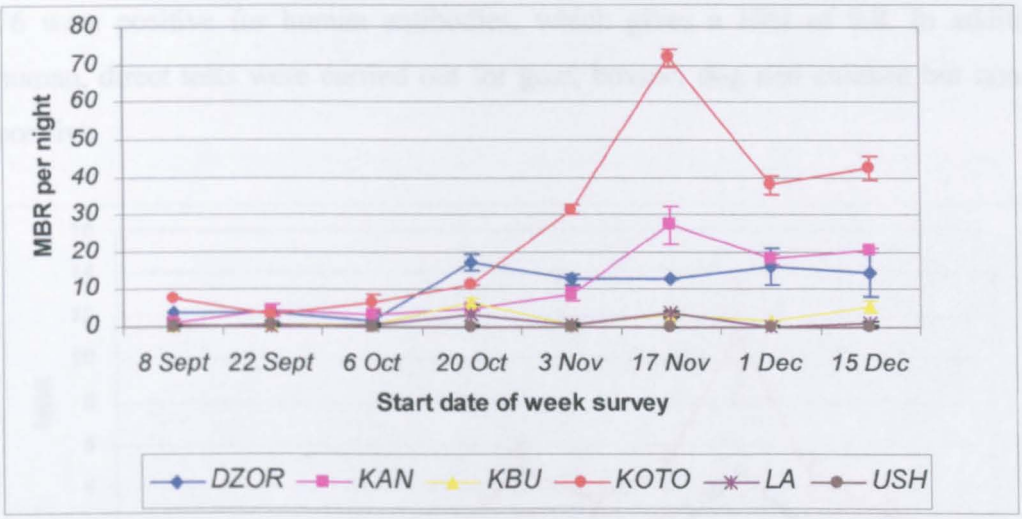

Figure 5-2 Geometric mean man biting rate of 4 man nights over 6 communiies in Accra, for An. gambiae s.l. during 8 rounds of cartching between Septermber and December 2003.

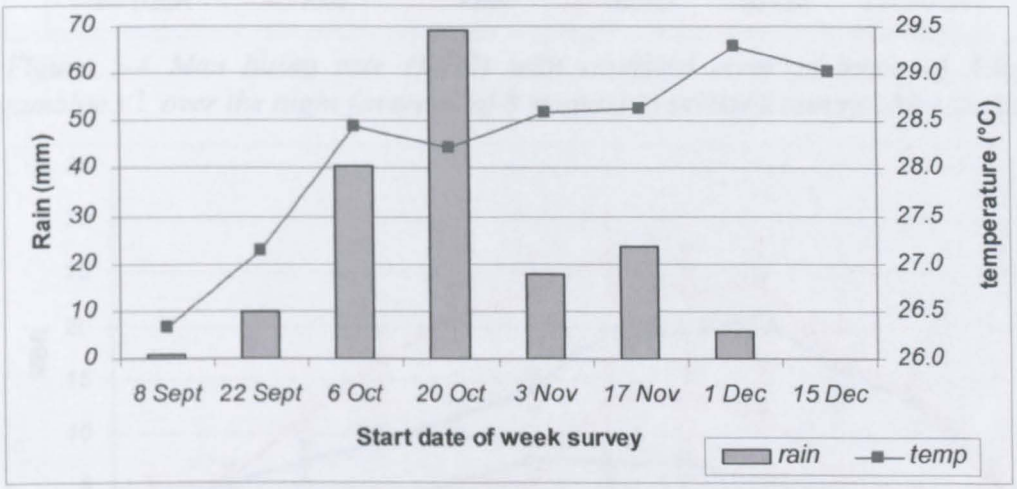

Figure 5-3 Total rainfall and average temperature in the two weeks preceding the start of each round of HBC of figure 5-1 (data WRI, unpublished)

5.3.5. *Nocturnal biting pattern*

Throughout, *A. gambiae s.l.* nocturnal biting peaked at 2.00-3.00 hrs with the majority biting between 23.00 and 5.00 hrs, with a small peak at 23.00-24.00 hours (Figure 5-4). *Culex spp.* also showed increased biting over the night but did not show a marked

peak (Figure 5-5). Biting intensity differed from Sept- Dec but the peak biting hour remained the same.

5.3.6. Human blood index

The human blood index (HBI) was assessed from the spray catches by direct ELISA. A total of 95 bloodmeals was analysed (32 from Accra and 63 from Kumasi) of which 76 were positive for human antibodies, which gives a HBI of 0.8. In addition to human, direct tests were carried out for goat, bovine, dog and chicken but non were positive.

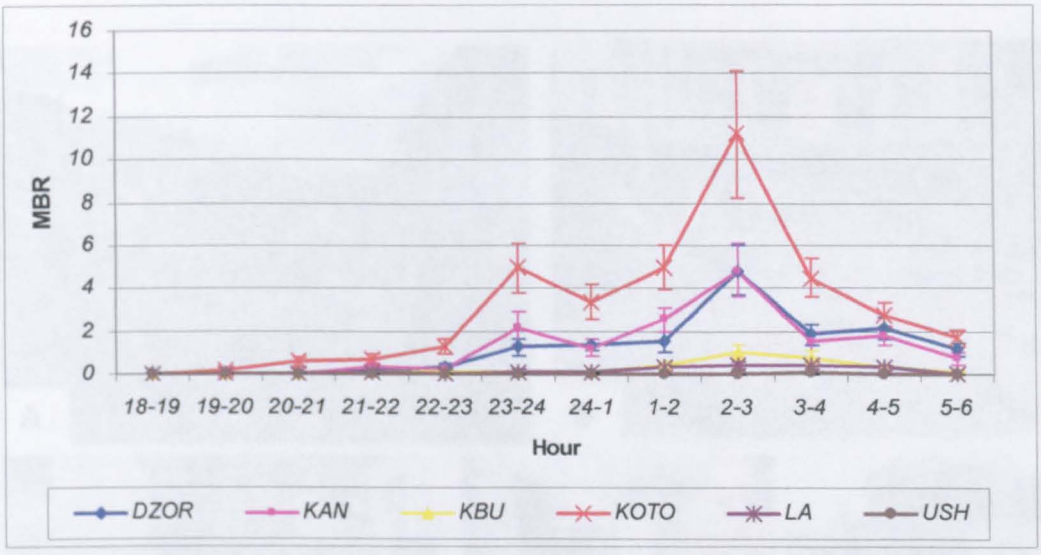

Figure 5-4 Man biting rate (MBR) with standard error of mean of Anopheles gambiae s.l. over the night (average of 8 rounds) in selected communities in Accra.

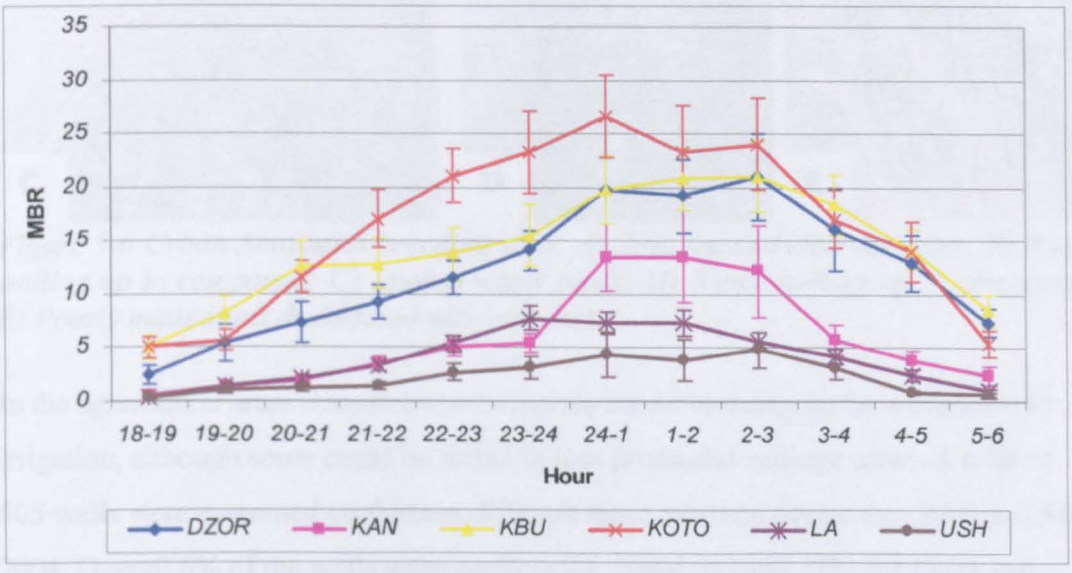

Figure 5-5 Man biting rate (MBR) with standard error of mean of Culex spp. over the night (average of 8 rounds) in selected communities in Accra.

5.3.7. Larval surveys

Anopheles were found breeding in both the agricultural areas and the residential areas. Several larval breeding sites were found in the urban residential communities like broken water pipes, pools at construction sites, areas with 'up-welling' water, poorly maintained drains filled with rain water and garbage and rain pools or flooded areas in low lying areas after heavy rains (Figure 5-6). The first four are typical urban sites although the latter can also be found in rural areas. Open drains were common breeding sites for *Culex spp.* mosquitoes. Table 5-5 gives an overview of the number of potential anopheline breeding sites found positive for *Anopheles* or *Culex* spp.

Figure 5-6 Urban Anopheles breeding sites; A) Area flooded after the rains; B) Water welling up in compound; C) Broken water pipes; D) Water welling up in compound; E) Poorly maintained drain filled with rain water

In the agricultural areas *Anopheles* were mainly found breeding in the wells used for irrigation, although some could be found in foot prints and seepage areas. A total of 505 wells were examined on thirteen different dates between September 2003 and May 2004. Overall 6% of the wells were positive for *Anopheles* and 12% for *Culex spp.* Appendix 8 gives an overview of the data for each survey. In Korle Bu farm 100% of

the wells were filled by drain water, at Kotobabi farm 94% was filled by piped water, 4% by drain water and 2% by a mixture of piped and drain water. At Dzorwulu farm 62% was filled by drain water, 34% by piped and 4% by a mixture of piped and drain water (Figure 5-7). There was a big difference in number of positive wells between the three farm sites (Table 5-6). In Dzorwulu and Kotobabi there were significant more wells positive for *Anopheles* that were filled with piped water than wells filled with drain water (P=0.017 and P=0.046 Pearson Chi-square for Dzorwulu and Kotobabi respectively). Average EC was significantly higher in wells where *Culex* larvae were present (P<0.001) and there were significantly more *Culex* larvae in water with a foul smell (P<0.001) while significantly more *Anopheles* larvae were present in non foul smelling water (P=0.017).

Table 5-5 Overview of number of potential breeding sits found in different residential areas and number found to breed Anopheles and/or Culex spp.

community	#potential breeding sites	# with only Anopheles	# with only Culex	# with Anopheles and Culex
Kotobabi	12	3	2	3
Airport	6	0	1	0
Kaneshie	3	3	0	0
Kokomlemle	7	0	2	1
Roman Ridge	7	1	2	0

From the initial fortnightly survey of 15 wells (see 5.2.1.3.) information on density and mosquito stages was obtained. The average *Anopheles* density in the positive wells was 0.17 larvae per dip and the maximum density found was 0.57 larvae per dip. Of the 63 *Anopheles* found in the wells, 10 were pupae, 22 stage IV, 17 stage III, 2 stage II and 4 stage I, of 8 specimens the stage was not recorded.

Table 5-6 Overview of wells surveyed, number positive for Anopheles and Culex spp. and average pH and EC at the three main farm areas in Accra.

Area	#Wells	# Positive for *Anopheles*	# Positive for *Culex*	pH (SEM)	EC (SEM)
Dzorwulu	370	23 (6.2%)	16 (4.3%)	7.2 (0.09)	726 (68.2)
Kotobabi	59	7 (11.9%)	1 (1.7%)	6.8 (0.07)	519 (61.3)
Korlebu	61	0 (0%)	36 (59.0%)	7.2 (0.08)	1709(55.3)

EC= Electrical conductivity; SEM= standard error of mean.

Figure 5-7 Different wells at urban farms in Accra; A) Well filled by drain water at Korle Bu; B) Newly dug well at Dzorwulu farm; C) Well filled by piped water at Kotobabi farm

5.3.8. Bio assays to determine insecticide susceptibility status

A total of 305 *A. gambiae s.l.* were tested for resistance to Permethrin (1 hr. exposure to 0.75% Permethrin, WHO paper). 157 (51.5%) were resistant, *e.g.* still alive 24hr after recovery from 1 hour exposure. Resistance was 55% (106/194) in females and 46% (51/111) in males. There was no significant difference in resistance between UA and U areas.

5.4. Discussion

The results from the entomological surveys indicated that there was local transmission ongoing in urban Accra as *Anopheles spp.* was found breeding in the city and infected *Anopheles* mosquitoes were found to be resting in houses and that there was an impact of urban agriculture on *Anopheles* and *Culex* densities.

5.4.1. Impact of urban agriculture

The HBCs in Accra revealed significantly higher biting rates for both *A. gambiae s.l.* as well as *Culex spp.* in UA communities compared to U communities. A study in Kumasi, reported similar findings (Afrane *et al.* 2004). In Bouaké, Ivory Coast, Dossou-Yovo (1994, 1998) found increased *Anopheles* biting rates in city areas with rice cultivation, although mosquito infection levels were lower. In Kumasi sporozoite rates did not significantly differ between UA and U but were significantly lower in the rainy season (Afrane *et al.* 2004). In our study in Accra only 11 out of 1672 (0.65%) mosquitoes analyzed were infected and this number was too low to give separate infection rates for UA and U.

A. gambiae s.l. was found breeding in wells used for irrigation although on average just 6% of these wells were breeding *Anopheles* larvae. *Culex spp.* was found breeding in 12% of the wells and in one farm area (Korle Bu) 62% of the wells harboured *Culex spp.* while none harboured *Anopheles spp.* This was linked to the water quality as significant more wells filled with piped than with drain water were found positive for *Anopheles spp.* and less *Anopheles* larvae were found in wells with foul smelling water. *Culex spp.* preferred polluted wells with a higher average electrical conductivity (EC). . It has been suggested that farming activities are an essential factor in regulating mosquito breeding in the well and that frequent irrigation prevents larvae from completing development (Julvez *et al.* 1997, Robert *et al.* 1998). Although our anopheline density and development stage data are minimal, the majority of stages found were III, IV and pupae, this does not seem to confirm the earlier suggestion. Our attempt to follow densities linked to irrigation frequency proved difficult due to the low number of anophelines breeding (see 5.2.1.3).

Monitoring of larval breeding sites and patterns of breeding was difficult because of the transitory nature of the breeding sites and the fact that on several occasions it happened that breeding sites, once discovered and selected for monitoring over time, were demolished by local residents on the second or third visit as people feared a fine from the environmental protection agency (EPA). To assess the contribution of urban agriculture to larval breeding an area based systematic assessment of larval breeding sites and patterns is needed. Recently Keating et al. (2003) suggested a systematic sampling frame for larval studies which was adapted to Accra for follow up studies to better quantify *Anopheles* breeding in agricultural farms compared to breeding in normal urban housing areas

Although irrigation wells formed breeding sites, less than 10% was found to harbour *Anopheles* larvae. However, man biting rates were significantly higher in UA communities than in U communities. *Anopheles* man biting rates obtained from pyrethrum spray catches were very low at around 1 per person per night. These three observations combined could indicate that *Anopheles* are resting outdoors, as outdoor biting rates were much higher than indoor biting rates assessed by PKD, and that the importance of urban agricultural areas may be providing resting sites for mosquitoes and the irrigation wells might not be the most important urban *Anopheles* breeding

sites, as less than 10% was found to harbour *Anopheles* larvae. The latter has been earlier suggested in a study of agricultural wells in Dakar, Senegal (Robert *et al.* 1998), which found that adult resting patterns did not follow larval breeding patterns in the wells. Additional studies using exit traps and assessing outdoor resting behaviour could gain inside in this and confirm if *Anopheles* are biting indoors and resting outside or are biting and resting outside. *A. gambiae s.l.* is in principle known to prefer indoor resting and biting but it is possible that the urban environment has led to changes in its behaviour.

Interestingly there seems to be not just an increased biting for *A. gambiae s.l.*, but also for *Culex spp.* in UA versus U. This might indicate that UA sites provide additional breeding sites for *Culex spp.* as well. Or it could indicate that there are other factors in these communities contributing to the overall higher mosquito presence. As the communities are similar except from the agricultural areas near the UA communities this could point again to the provision of resting sites by the agricultural areas.

Linked to this, the epidemiological work of this project (chapter 3 and 4) revealed significantly higher malaria parasitaemia prevalence in children in UA communities than U communities. Although not all UA communities showed the same pattern, distance to agriculture showed a positive correlation with parasitaemia in some areas while this was inversed or not present in other areas (Chapter 3). The occurrence of irregular transitory small breeding sites in the urban housing areas (see paragraph 5.4.2.) might have obscured the relation with distance to agriculture.

5.4.2. Urban breeding sites and adaptation of A. gambiae s.l.

The larval surveys in Accra revealed breeding of *A. gambiae s.l.* both in the agricultural sites as well as the normal urban housing areas. In the urban housing areas several typical urban breeding sites were found to be breeding *Anopheles spp.* like broken water pipes, construction sites, water welling up in compounds and drains blocked by garbage collecting rain water, all manmade and often temporary in nature. Although *A. gambiae s.l.* is known to prefer relatively clean water for breeding they could sometimes be found breeding in more polluted sites full of garbage where you would not expect them. The breeding of *Anopheles spp.* in polluted water in urban areas has also been reported by others (Chinery 1984, Warren *et al.* 1999, Keating *et*

al. 2004) and could point to an adaptation to more polluted waters either by mutation or via phenotypic plasticity to breed in a wide range of sites if the circumstances require. There are no published results on possible adaptations of *A. gambiae* to more polluted sites but a small common garden experiment carried out in Kumasi (Findlay-Cooper 2005), wherein urban *A. gambiae s.s.* mosquitoes were reared in rural (clean) water and rural *Anopheles* in urban (polluted) water and vice versa, indicated that that median time to pupation was longer for rural larvae in urban water. However this small study requires further follow up to confirm the validity of the results. Chinery (1984) reported *A. gambiae s.l.* to be adapting to breeding in small water containers as he found increasing number of water containers positive for *Anopheles spp.* after comparing surveys between 1912 and 1964. The adaptation of A. *gambiae s.l.* to breed in polluted water or different habitats is clearly an area that needs further studies as this could have important implications for urban malaria epidemiology

5.4.3. Culex spp. biting

The results from the entomological surveys showed that over 95% of mosquitoes found in the urban setting were *Culex spp.* as is common in urban settings (Bang *et al.* 1977, Trape & Zoulani 1987a). *Culex quinquefasciatus* is a known vector for lymphatic filariasis but not in West Africa where *A. gambiae s.l.* is the main vector (Gyapong *et al.* 2002). Malaria is the predominant public health problem with over 40% of outpatient department (OPD) cases attributed to malaria nationwide. Based on the above, in urban Ghana, *Culex* is mainly a nuisance biter with biting rates up to 67 times the *Anopheles* biting rate.

5.4.4. Larval control

Anopheles breeding in cities is very focal and therefore larval control has been suggested as a suitable control method (Warren *et al.* 1999, Robert *et al.* 2003, Caldas de Castro *et al.* 2004, Keiser *et al.* 2004). In the early 20th century larval control was quite successfully applied in many cities but lost favour after malaria eradication campaigns moved to indoor residual spraying during the global malaria eradication campaign launched by the World Health Organisation in 1957. In the 1960s larval control was successfully applied in Accra (Chinery 1968, 1969, 1970 see paragraph 2.6). A recent review of the Dar es Salaam urban malaria control programme concluded that malaria control in urban settings could be achieved by an integration of

vector control for targeting of breeding sites with case management and strong involvement of the community (Caldas de Castro *et al.* 2004). The high nuisance biting of *Culex spp.* could jeopardize the success of a larval control programme, as the majority of mosquitoes are *Culex spp.* and targeting *Anopheles* breeding sites in control measures would have little impact on overall mosquito numbers. Therefore local residents would still experience nuisance biting and would find it difficult to assess the effect of a larval control programme as they do not distinguish different types of mosquitoes and their different roles in disease transmission (chapter 2, Stephens *et al.* 1995). Adding to this challenge is that larval control programmes often require high input from the local community which will be easier to sustain if programmes targeted all mosquitoes. In addition the majority of the breeding sites found in this study were manmade and temporary in nature; the latter aspect alone would be a challenge for larval control as breeding sites are very transitory and locating them would require a constant effort.

In urban malaria control there is a clear role for municipalities and public works departments (Donnelly *et al.* 2005). Proper construction of drains and sewage systems would reduce the amount of open drains proliferating high *Culex spp.* breeding at present. The larval inventory revealed that broken pipes and pools formed at construction sites were major *Anopheles* larval breeding sites in the urban housing areas and are clearly related to outpacing infrastructure in rapid expanding urban areas. This was also stressed by Keating *et al.* (2003, 2004), who found the majority of breeding sites in unplanned poorly drained areas in urban Kenya.

5.4.5. Bednets in urban areas

Bednets could be easily integrated with larval control and could be successfully used for malaria control in the urban setting (chapter 7). Bednets have proven effective in rural areas in Africa (Lengeler 2004). However an education campaign on use should go hand in hand with a distribution programme as usage in Ghana is very low at present and apart from economic constraints residents do not always like to use bednets because of heat or discomfort (Agyepong & Manderson 1999 and this study). Another issue related to bednet use is the emergence of insecticide resistance. In West Africa low resistance to permethrin has been reported for *A. gambiae s.l.* in Ghana and Nigeria (Kristan *et al.* 2003). However for *C. quinquefasciatus* high levels of

resistance to pyrethroids have been reported in West Africa (Chandre *et al.* 1998) and it is suggested that this might negatively impact the success of a bednet control programmes especially in urban areas (Chandre *et al.* 1998) were high nuisance biting is common and people might lose confidence in bednets if *Culex* mosquitoes keep biting. Bio assays carried out during the project showed very high levels of resistance in both *A. gambiae s.l.* as well as *Culex spp.*, surprisingly they were much higher than earlier levels reported in the same locality where 100% susceptibility for *Culex spp.* was recorded in May 2004 (Peppiatt 2004). In other localities in Accra some level of resistance was recorded with corrected mortality levels of 64-83% for *A. gambiae s.l.* with significantly higher levels of the kdr mutation, a genetic marker for knock down resistance, in resistant mosquitoes (Adasi 2001). Further research needs to be done to confirm the susceptibility levels in more localities and look further into the frequency of kdr mutation in urban areas. Fortunately possession of the kdr allele does not make pyrethroid nets immediately useless as experiments showed that kdr homozygote are killed after prolonged contact with nets and few ingest to take a bloodmeal (Chandre *et al.* 2000).

A factor that may add to development of resistance in urban areas could be urban agriculture, which apart from being dependent on a continuous supply of water and nutrients, also uses high inputs of pesticides as crop cultivation is intensive. This high pesticide use in farming could favour selection for resistance to pesticide used in public health (Lines 1987, Herath & Joshi 1989, Diabate *et al.* 2002). In the urban areas the high use of mosquito coils and aerosols could add to this selection pressure. The resistance test carried out in this study did not show a significant difference between UA and U areas however additional studies are needed to investigate this further.

5.4.6. EIR and entomological methods

The overall EIR calculated from the human bait catches in central Accra was 18.4 ranging from 0.9-63 infective bites per person per year in the different communities, with an EIR of 19.9 for UA and 6.6 for U areas. These values are comparable to the mean annual EIRs of 7.1 in the city centres, 45.8 in periurban areas, and 167.7 in rural areas reported by Robert *et al.* (2003) in a review of urban EIRs. However they are lower than the results of Afrane *et al.* (2004) who reported EIRs of 57 and 112.8 for

UA and 1.2 and 18 for U in dry and rainy season respectively (their monthly figures were multiplied by 12 for comparison to our annual figures).

In the rural areas the standard entomological methods are well tested and work well but a modification of established methods might be necessary to make them applicable to the urban environment. The high presence of *Culex spp.* and low sporozoite rates makes the need for extensive numbers of sites or extensive lengths of time to obtain 'sufficient' *Anopheles* numbers. In addition the high variation in housing status results in large variations in collections and calls again for a large number of collection points. In addition the crowdedness and crampedness of rooms/houses in the urban areas makes proper execution of the protocols more challenging.

The results of this study show that there is urban malaria transmission ongoing and that the EIR is increased in urban areas where irrigated farming takes place compared to areas far from agriculture. The combined results of the larval surveys and HBC and PKD indicate that the main role of the agricultural area may be providing resting sites and not breeding sites as such although further research is needed to confirm this. There is a high level of nuisance biting in the urban areas which could impact on perceived success of control programmes and this should be taken into account when planning interventions. Very high levels of resistance to permethrin were reported although it needs to be investigated weather usage of insecticides in agriculture, public health or domestic use provides selection pressure.

Chapter 6

6. ANNUAL VARIATION IN MALARIA PREVALENCE IN SELECTED COMMUNITIES IN ACCRA

6.1. Introduction

The baseline survey in Accra indicated that malaria was associated with older age, lower haemoglobin level and lower socio-economic status. Malaria prevalence was significantly higher in communities around urban agriculture although an inverse relation between distance to agriculture and malaria prevalence was not observed in all communities (chapter 4). These results were based on one large scale point prevalence survey carried out in 12 communities in the beginning of the dry season in 2002. To investigate inter annual variation and consistency of risk factors of malaria prevalence observed, a second epidemiological survey was carried out in 2004 in 8 of the 12 baseline selected communities in Accra in the same period of the year.

6.2. Material and Methods

The study was carried out in the communities of Kaneshie, Ushertown, Korle Bu, Alajo, Roman Ridge, Dzorwulu, Airport and La (Figure 3-1, p.35) between the 5th of October and 26th of November 2004. The only alteration in methodology from the baseline survey (0) was that treatment for children found positive for malaria and/or anaemia was brought directly to the homes. This survey included children up to 72 months. For the comparative analysis only children 6-60 months were included as this was the age group included in the 2002 baseline survey. The 60-72 age group was analyzed separately to see if children in the older age group had higher parasitaemia as was indicated by the results of the baseline survey (chapter 4). Apart from separate analyses the data from both years were combined into one dataset with year as covariate. Statistical analysis for assessment of risk factors was done as before. In addition to separate analyses both data sets were combined into one set to investigate risk factors within the different communities.

All data were double entered and cross checked using Epi Info data compare (Epi info version 3.3.2, CDC, Atlanta). Checks were carried out for extreme or unlikely data by looking at minimum and maximum value for *e.g.* age, Hb, length, weight and socio-

economic score. Before calculating distances, geo-coordinates were cross checked by plotting them on the map and checking that households for each community were located in the community.

6.3. Results

6.3.1. Results second epidemiological survey (2004)

A total of 1694 children were sampled from 852 households. Of 136 parasite positive slides, 135 were *Plasmodium falciparum* and 1 was *P. malariae*. Thirteen children had gametocytes only and were not included in the main analysis. A total of 1469 children from 804 households were included in the comparative analysis (age group 6-60 months). Table 6-1 shows the baseline characteristics of these children with and without malaria parasites and Table 6-2 the characteristics in communities with and without agriculture. The results for the 2002 baseline are given in chapter 3 (Table 3-1 and Table 3-2). In 2004, overall malaria prevalence was 7.8% and did not differ significantly between communities with and without agriculture. The average Hb concentration was 10.47 (95%CI 10.40-10.55). Eighty six (5.9%) of the 1465 children had moderate-to-severe anaemia (Hb< 8.0 g/dL) and 926 (63.2%) were classified as anaemic following the WHO classification (Hb < 11.0 g/dL). The number of anaemic children was significantly higher in the communities without agriculture as compared to communities around agricultural sites (OR 1.74, 95%CI 1.40-2.17, P=0.001). The same pattern was observed for the moderate-to-severe anaemic children (OR 2.30, 95%CI 1.42-3.77, P<0.001). Children who travelled to the rural area in the last 4 weeks had significantly more often malaria parasites (OR 3.55, 95%CI 1.72-7.23, P<0.001). Children who's carers did not complete primary school education had a higher risk of having parasitaemia (OR 1.51, 95%CI 0.99-2.30) and being anaemic (OR 1.36, 95%CI 1.06-1.76, P=0.014). Children with below city average socio-economic score had more chance of being anaemic (OR 1.51, 95%CI 1.21-1.89, P<0.001) and moderately to severe anaemic (OR 1.72, 95%CI 1.06-2.79, P=0.019).

In the multivariate model, malaria was significantly associated with age (positive, P=0.004), Hb (negative, P<0.0001) and travel (OR 27.7, 95% CI 13.1-58.6, P<0.001). Completion of primary school by the carer was not significant.

Table 6-1 Summary of variables measured for children with and without malaria parasites in the 2004 survey with results of univariate significance tests (Pearson chi-square or t test).

Variables	malaria parasites[t] (n=115)	no parasites (n=1354)	p-value
Mean Hb, g/dL (SD)	9.8 (1.74)	10.5 (1.46)	<0.001
% with Hb <8.0 g/dL	15.8 (18/114)	5.0 (68/1351)	<0.001
% with Hb< 11.0 g/dL	76.3 (87/114)	62.1 (839/1351)	0.003
% male	47.8	49.0	(0.814)
Mean age, months (SD)	34.6 (15.0)	31.6 (16.3)	0.055
Mean socio-economic score[#] (SD)	1.54 (0.85)	1.50 (0.88)	(0.764)
% of carers that completed primary school	66.1	74.6	0.046
% that travelled to the rural area in the last 4 weeks	10.4	3.2	<0.001
% that has taken malaria medication in last 2 weeks	30.4	23.5	0.094
% with reported history of fever§	27.0	20.5	(0.100)
% of children that slept under a bednet the previous night	11.3	13.7	(0.477)
% of HH who spray at least weekly[¶]	14.0	17.3	(0.379)
% of HH who use mosquito coils daily[¶]	64.5	57.7	(0.171)
% of HH with netting in front of windows and doors*	92.5	87.4	(0.120)
% of HH without ceiling in the house	30.6	29.2	(0.755)

Hb=haemoglobin; SD=standard deviation; HH=household; t children with only gametocytes were excluded; * netting without large holes; #The composite measure of socio-economic status used was the asset factor score of the World Bank for Ghana (www.worldbank.com/hnp); § In the last 48 hours, as reported by the carer; ¶Proprietary brands of insecticide aerosols/mosquito coils

In multivariate analysis anaemia (Hb<11.0 g/dL) was significantly associated with age (negative, $P<0.001$), malaria (OR 2.26, 95%CI 1.42-3.59, $P=0.011$) and socio-economic score (negative, $P=0.018$). Living in a community without agriculture was marginally significant (OR 1.79, 95%CI 1.08-2.95, $P=0.064$). Moderate-to-severe anaemia (Hb<8.0 g/dL) was significantly associated with age (negative, $P<0.001$), malaria (OR 6.13, 95% CI 3.88-9.67, $P<0.001$), a reported history of fever in the last 48 hours (OR 2.27, 95%CI 1.53-3.336, $P=0.0046$), and if child had taken malaria

medication in the last 2 weeks (OR 1.90, 95%CI 1.30-2.87, P=0.0132). Living in a community without agriculture was not significant (OR 2.51, 95% CI 1.01-6.26, P=0.095).

Table 6-2 Summary of variables measured for children in communities with and without agriculture in the 2004 survey, with results of univariate (Pearson chi-square or t test).

Variables	community without urban agriculture (n=779)	community around urban agriculture (n=690)	p-value
% with malaria parasites	8.5 (66/779)	7.1 (49/690)	(0.329)
Mean Hb, g/dL (SD)	10.3 (1.60)	10.6 (1.43)	<0.001
% with Hb <8.0 g/dL	7.7 (60/778)	3.8 (26/687)	0.001
% with Hb< 11.0 g/dL	69.2 (538/778)	56.5 (388/690)	<0.001
% male	49.8	47.8	(0.448)
Mean age, months (SD)	32.1 (16.0)	31.5 (16.4)	(0.553)
Mean socio-economic score# (SD)	1.41 (0.79)	1.63 (0.95)	<0.001
% of carers that completed primary school	70.8	77.4	0.004
% that travelled to the rural area in the last 4 weeks	4.1	3.3	(0.435)
% that have taken malaria medication in last 2 weeks	20.4	24.8	0.035
% with reported history of fever§	24.1	17.4	0.002
% of children that slept under a bednet the previous night	8.0	19.7	<0.001
% of HH who spray at least weekly¶	15.9	18.2	(0.245)
% of HH who use mosquito coils daily¶	59.7	56.5	(0.234)
% of HH with netting in front of windows and doors*	87.7	87.9	(0.599)
% of HH without ceiling	28.2	30.4	(0.378)

Hb=haemoglobin; SD=standard deviation; HH=household; t children with only gametocytes were excluded; * netting without large holes, if all netting is considered P=0.052; #The composite measure of socio-economic status used was the asset factor score of the World Bank for Ghana (www.worldbank.com/hnp); § In the last 48 hours, as reported by the caregiver; ¶Proprietary brands of insecticide aerosols

6.3.2. Comparison with baseline survey

The age distribution in the children sampled in 2002 and 2004 was similar (Figure 6-1) and there was no difference in gender. Socio-economic score was slightly but significantly lower in the 2004 sample (Table 6-3). In both years socio-economic score

was significantly higher in communities with than without urban agriculture (Table 6-2 and Table 3-2, p.38). There were marked differences in use of preventive measures. Significantly less people reported to have a bednet or use spray in 2004, while more households used mosquito coils and had netting in front of windows and doors (Table 6-3). Significantly less children had travelled to the rural area in the 4 weeks preceding the survey. The number of children that had taken malaria medication in the last 2 weeks or had a reported history of fever in the last 48 hours did not differ between the years.

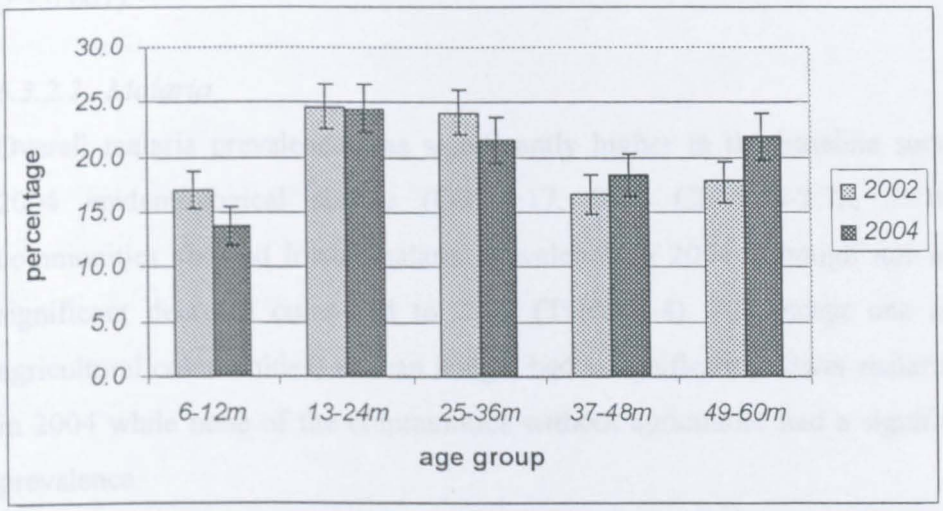

Figure 6-1 Distribution of different age groups with 95% confidence intervals among children sampled in the 2002 and 2004 survey.

Table 6-3 Overview of characteristics of the sample population in the 2002 and 2004 epidemiological surveys.

Variables	2002 survey	2004 survey	p-value
Mean socio-economic score[#] (SD)	1.65 (1.02)	1.51 (0.87)	<0.001
% of carers that completed primary school	88.7	73.9	<0.001
% that travelled to the rural area in the last 4 weeks	6.0	3.7	0.007
% of HH with a bednet	32.3	18.0	<0.001
% of HH who spray at least weekly[¶]	25.6	17.0	<0.001
% of HH who use mosquito coils daily[¶]	36.3	58.2	<0.001
% of HH with netting in front of windows and doors	81.7	90.8	<0.001
% of HH without ceiling in the house	72.8	70.7	0.218

SD=standard deviation; HH=household; #The composite measure of socio-economic status used was the asset factor score of the World Bank for Ghana (**www.worldbank.com/hnp**); ¶Proprietary brands of insecticide aerosols

6.3.2.1. Differences between communities

The age distribution was similar in the communities between the two surveys although there were differences in 3 groups. In La significantly more children in age group 2 were sampled in the first survey (P=0.019). In Roman Ridge more children in age group 5 were sampled in the second survey (P=0.024). In Ushertown significantly more children were sampled in age groups 2 in the second survey (P=0.037). Socio-economic score was significantly lower in the 2004 survey for the communities of Airport, Roman Ridge and La (P<0.001), while it was higher in 2004 for Ushertown (P<0.001).

6.3.2.2. Malaria

Overall malaria prevalence was significantly higher in the baseline survey than the 2004 epidemiological survey (OR 2.17, 95% CI 1.74-2.72, P<0.0001). All communities showed lower malaria prevalence in 2004 although not all showed a significant decrease compared to 2002 (Table 6-4). All except one of the urban agricultural communities, Roman Ridge, had a significantly lower malaria prevalence in 2004 while none of the communities without agriculture had a significantly lower prevalence.

Table 6-4 Overview of children surveyed and malaria prevalence found in the baseline and second epidemiological survey in selected communities in Accra.

		Oct 2002-Jan 2003			Oct-Nov 2004		
Community	**type**	n	cases	prevalence	n	cases	prevalence
Airport Res.[b*]	*UA*	77	15	**19.5**	109	8	**7.3**
Alajo[a]	*UA*	166	25	**15.1**	169	14	**8.3**
Dzorwulu[b]	*UA*	132	26	**19.7**	133	10	**7.5**
Kaneshie[c]	*U*	159	31	**19.5**	322	42	**13.0**
Korle Bu[a]	*UA*	181	16	**8.8**	222	9	**4.1**
Laboni/La[c]	*U*	175	17	**9.7**	198	15	**7.6**
Roman Ridge[c]	*UA*	105	24	**22.9**	57	8	**14.0**
Ushertown[c]	*U*	200	13	**6.5**	259	9	**3.5**
	UA[b]	661	106	**16.0**	641	49	**7.1**
	U[c]	534	61	**11.4**	779	66	**8.5**
	OVERALL[b]	1195	167	**14.0**	1469	115	**7.8**

*Significant difference between the two surveys at a) P<0.01; b) significant at P<0.05; c) not significant; UA = communities around urban agriculture, U=communities without agriculture

Ranking the malaria prevalence of the two years indicated that Roman ridge had the highest prevalence in both years and Korle Bu and Ushertown where number 7 and 8 both years. The others changed order with the biggest shift being Dzorwulu from rank 2 to 5. The rank correlation of prevalence between the two years was 0.695 (Spearman ρ), with only 8 values this is not significant.

Of the children with parasitaemia significantly more children had high density parasitaemia (>5000/µl) in 2002 than 2004 (OR 2.39, 95%CI 1.05-5.53) and the geometric mean parasite density was higher (405.8 and 121.3 in 2002 and 2004 respectively).

6.3.2.3. Anaemia

In contrast to malaria prevalence, overall anaemia prevalence was higher in the 2004 epidemiological survey (OR 1.63, 95%CI 1.34-1.91, $P<0.001$,Table 6-5). Linked to this mean Hb concentration was significantly higher in the 2002 survey (10.81, 95%CI 10.72-10.90, and 10.48, 95%CI 10.40-10.55, in 2002 and 2004 respectively, $P<0.0001$).

Table 6-5 Overview of number of children sampled and prevalence of moderate to severe (Hb< 8.0g/dL) and overall anaemia (Hb<11.0 g/dL) found in the baseline and second epidemiological survey in selected communities in Accra.

		Oct 2002-Jan 2003			Oct-Nov 2004		
community	**type**	n	<8 g/dL	<11 g/dL	n	<8 g/dL	<11 g/dL
Airport Res.[c,c*]	*UA*	75	2.7	48.0	109	3.7	53.2
Alajo[c,a]	*UA*	164	1.8	44.5	169	5.9	62.1
Dzorwulu[c,c]	*UA*	131	2.3	42.7	132	3.0	53.0
Kaneshie[c,c]	*U*	154	11.7	70.1	322	8.7	70.2
Korle Bu[c,c]	*UA*	181	6.1	48.1	221	2.3	54.3
Laboni/La[b,c]	*U*	174	0.6	47.7	197	3.6	56.9
Roman Ridge[c,a]	*UA*	105	1.9	43.8	56	5.4	62.5
Ushertown[c,a]	*U*	201	5.0	59.2	259	9.7	77.2
	UA	656	3.2	45.4	687	3.8	56.5
	U	529	5.5	58.6	778	7.7	69.2
OVERALL		1185	4.0	51.0	1465	5.9	63.2

*Letters indicate significant difference between the two surveys for Hb<8 g/dL and Hb<11 g/dL respectively at a) $P<0.01$; b) significant at $P<0.05$; c) not significant; UA = communities around urban agriculture, U=communities without agriculture

In both years overall anaemia prevalence was significantly higher in communities without urban agriculture (OR 1.70, 95%CI 1.34-2.16, $P<0.001$ in 2002 and OR 1.73, 95%CI 1.34-2.15, $P<0.001$ in 2004). Moderate to severe anaemia did not differ between these groups in 2002 ($P=0.052$) but was significantly higher in 2004 in communities without agriculture (OR 2.12, 95%CI 1.30-3.50, $P=0.001$). This difference disappeared when accounting for data structuring and socio-economic status in multivariate analysis. For Hb as a continuous variable type of community (UA or U) was an independent risk factor, after adjusting for socio-economic status, malaria, age and data structuring ($P=0.0325$), with Hb concentration being on average 0.44 g/dL higher in children living around urban agriculture. The community of Ushertown had the highest levels of anaemia in both surveys and over 75% of children were anaemic in the 2004 survey, with nearly 10% moderate to severe anaemic children (Table 6-5) while malaria prevalence was lowest in this community (Table 6-4).

Ranking the anaemia prevalence indicated that for both anaemia and moderate to severe anaemia the rank orders of both years were not significantly correlated (0.167 and 0.524 for Hb<8 g/dL and Hb<11 g/dL respectively). Several communities shifted their rank but Kaneshie and Ushertown had one of the highest prevalences in both years. For moderately to severe anaemia, number 8 and 6 in 2002 and 7 and 8 in 2004 respectively and number 7 and 8 in both years for overall anaemia.

Malaria prevalence showed a similar pattern among age groups in both surveys (Figure 6-2). Malaria prevalence was significantly lower in age group 25-36 and 37-48 months in the 2004 survey. Prevalence in age group 60-72 months (only surveyed in 2004) was 9.3% (95%CI 5.5-13.5). Haemoglobin concentration was significantly lower ($P<0.01$) in age group 13-24, 24-36 and 48-60 months in the 2004 survey (Figure 6-1). Age groups 2 and 3 were similarly represented in the sample (Figure 6-1) although there were slightly more children of age group 5 in the 2004 sample, but this would have relatively increased the Hb concentration and not decreased. The largest proportion of anaemic children were in the younger age groups (Figure 6-3), with over 60% of children up to 24 months being anaemic. In the older age groups over 40% was anaemic. There was no difference in malaria or anaemia prevalence between the different sexes.

Attributable risk (AR) for anaemia due to malaria was lower in the 2004 survey (5.4% and 1.7% in 2002 and 2004 respectively). AR for moderately to severe anaemia due to malaria was also lower in 2004 (21.8 and 14.3% in 2002 and 2004 respectively).

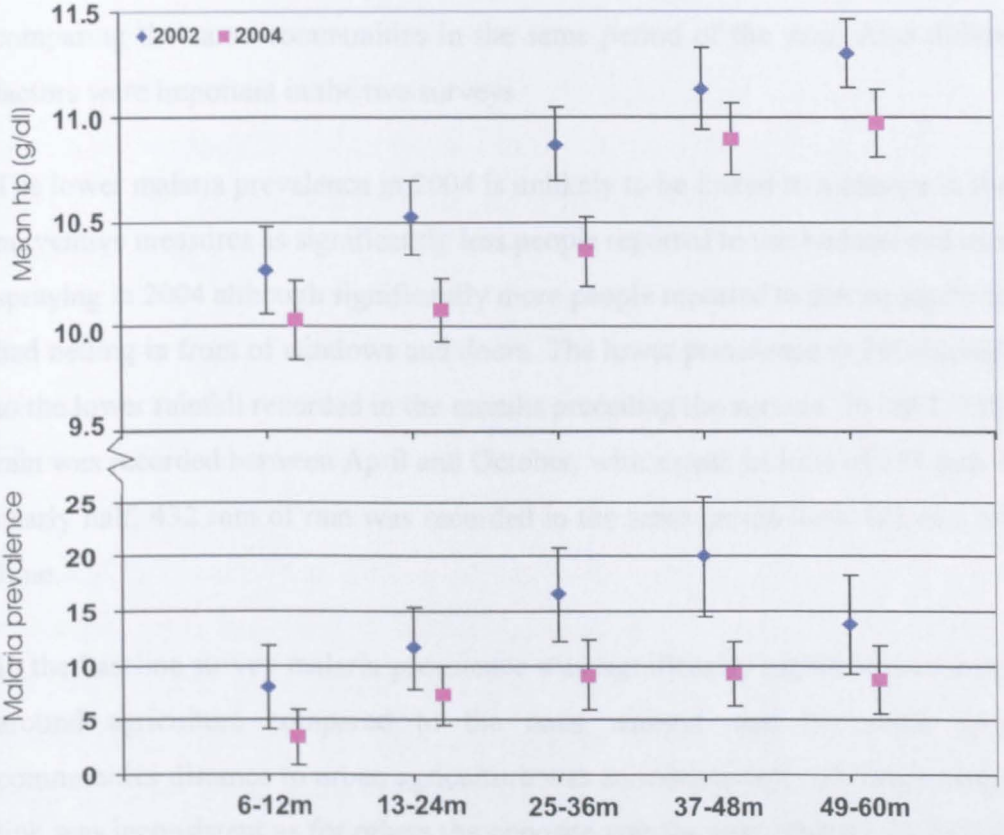

Figure 6-2 Mean haemoglobin concentration (g/dL) and malaria prevalence (%) with 95% confidence intervals in different age groups for the 2002 and 2004 survey.

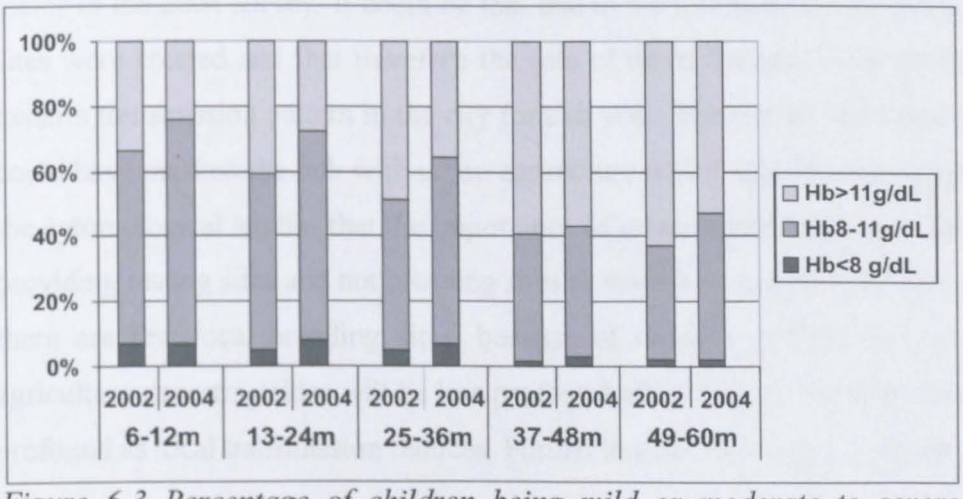

Figure 6-3 Percentage of children being mild or moderate to severe and none anaemic in different categories in the 2002 and 2004 survey.

6.4. Discussion

In studying urban malaria epidemiology annual fluctuations need to be taken into account as in the 2004 epidemiological survey malaria prevalence was significantly lower than in the 2002 baseline survey, while anaemia prevalence was higher when comparing the same communities in the same period of the year. Also different risk factors were important in the two surveys.

The lower malaria prevalence in 2004 is unlikely to be linked to a change in the use of preventive measures as significantly less people reported to use bednets and insecticide spraying in 2004 although significantly more people reported to use mosquito coils and had netting in front of windows and doors. The lower prevalence in 2004 could be due to the lower rainfall recorded in the months preceding the surveys. In 2002, 858 mm of rain was recorded between April and October, with a peak in June of 514 mm. In 2004 nearly half, 432 mm of rain was recorded in the same period with 121 mm of rain in June.

In the baseline survey malaria prevalence was significantly higher in the communities around agriculture compared to the ones without, and for some agricultural communities distance to urban agriculture was an independent risk factor although the link was inconsistent as for others the opposite was the case (chapter 3). In 2004 there was no difference between communities with and without agriculture. On the other hand travel, which was not a risk factor in the baseline survey, was an independent risk factor in the 2004 survey. It could be that due to the low rain less *Anopheles* breeding sites were created and that therefore the role of travel became more profound in the malaria transmission pattern in the city for that year. The overall reduction in breeding could have masked the link with urban agriculture which supports the suggestion form the entomological studies that the importance of urban agricultural areas seem to be in providing resting sites and not breeding sites as such (see chapter 6 for more details). If there are few local breeding sites, because of reduced rainfall, the role of urban agriculture as resting sides will be less profound while the role of travel becomes more profound as local transmission reduces. Further studies are needed to confirm this.

Interestingly anaemia prevalence was nearly 1.5 times higher in 2004, this could be linked to socio-economic status. In the 2004 survey average socio-economic status of

households was lower than in 2002 and a smaller percentage of carers had completed primary education. This could point to a deteriorating socio-economic status in Accra although more information needs to be collected to confirm this. This corresponds to the shift in use of preventive measures as poorer people would have less chance to buy a net or spray but resort to cheap short term tools like mosquito coils instead. Also the higher prevalence of anaemia in 2004 could point to deteriorating socio-economic conditions resulting in poorer health status and lower Hb concentrations resulting in more anaemia. In both years prevalence of anaemia was associated with lower socio-economic status.

Anaemia prevalence, both Hb<11.0 and <8.0 g/dL, was in both years lower in the communities with agriculture and linked to this Hb concentration was significantly higher in these communities. In Kumasi this difference was not visible (chapter 4). Why would people in areas with agriculture have higher haemoglobin levels? Households around urban agriculture had in both years a significantly higher socio-economic status but living near urban agriculture was independently associated with Hb concentration when accounting for socio-economic status. It could be that people around farms eat more vegetables and therefore have a better nutritional status which impacts on Hb concentration but only a small percentage of the people living around the agricultural sites are actual farmers. Of 852 households surveyed in 2004 only 9 reported to be involved in vegetable farming, all in communities around agricultural sites. Only 9 households reported to be involved in backyard farming around their house, of which 7 lived in a UA community. A total of 66 households (7.7%) reported to be involved in farming back in the village, of which 18% lived in communities with agriculture. So farming does not seem to explain the difference. In addition, studies in Accra on livelihoods, food and nutrition (Maxwell *et al.* 2000) found that urban agriculture does not play a large role in household livelihood strategies accounting for <1% of food produced and when taking into account only the households involved in urban farming just 7% (in value terms) is home produced. Further studies on food intake and risk factors for anaemia are needed to investigate what is underlying the higher Hb concentrations in UA communities. The higher mean socio-economic status of the communities around agriculture could have a positive impact on general health status as health is linked to wealth. But other risk factors for anaemia, like i.e. worm

infection could also play a role. However, it is not clear why children in communities without agriculture would have more intestinal helminth infections.

In the 2004 survey, surprisingly, socio-economic status was not significantly associated with malaria and neither was the fact if households had a socio-economic score below city average. Why there was no relation with socio-economic score is unclear, maybe as indicated earlier the low overall prevalence increased the importance of travel as a risk factor which masked other risk factors. A contributing factor could have been reduced statistical power due to the lower malaria prevalence. Socio-economic score was an independent risk factors for anaemia (Hb<11.0 g/dL) in both years.

Two communities, *e.g.* Kaneshie and Ushertown, had the highest number of moderate to severely anaemic children, approaching 10%. In Kaneshie malaria prevalence was one of the highest in both years but in Ushertown malaria prevalence was the lowest in both years. These two communities also had very high overall levels of anaemia (Hb<11.0 g/dL), with over 75% of children being anaemic in Usher town in the 2004 survey and 70% being anaemic at the baseline survey in Kaneshie. The high malaria prevalence in Kaneshie is likely to explain the high anaemia prevalence in this community. As malaria was very low in Ushertown other factors likely underlie the high anaemia prevalence in this community. Analysis of the risk factors per community found that for this community anaemia was apart from age, associated with education level as children who's carer did not completed primary school had double the risk of being anaemic (OR 2.12, 95%CI 1.19-3.76, P=0.011). Prevalence of intestinal worms is a known important contributor to anaemia and this could play a role although it is not clear why the community of Ushertown would have much higher worm infection levels than the other communities. A contributing factor could be that in both years Ushertown had the lowest socio-economic score of all communities surveyed and as mentioned earlier lower socio-economic status was associated with lower haemoglobin levels. At community level this was not found significant but this could be due to the small variation in socio-economic score within the community. In Ushertown the major ethnic group are the Ga's, the traditional inhabitants of Accra, who make up around 80% of the community. Differences in prevalence of haemoglobinopathies between different ethnic groups could have influenced anaemia

prevalence. However, the community of Korle Bu also consisted for about 80% of the Ga ethnic group and in this community anaemia prevalence was not above average.

Nearly all children were asymptomatic but presence of parasitaemia can still have important implications. A study in Kampala, Uganda showed that children with asymptomatic parasitaemia had a significantly higher chance of developing symptomatic malaria within 30 days when compared with children without parasitaemia (Njama-Meya *et al.* 2004). This stresses the need for treatment of all parasitaemia.

The results of the second survey did not confirm the age related pattern that malaria is more prevalent in the older age groups which was observed in the first survey. Again, the low overall prevalence could have masked this. A follow up survey in 2005 in Kumasi which included children up to 9 years of age, found higher (OR 4.1) prevalence in the 5-9 year olds than in the 1-4 year olds (Ronald 2005). In areas of low transmission, like urban areas, malaria morbidity might be higher in the older age groups and it is important to include this group in further surveys (Clarke *et al.* 2004).

Comparing the results of the two large prevalence surveys carried out in Accra indicates that the urban epidemiology is more complex and that different risk factors can be of different importance at different periods. This is important as only good knowledge on prevalent risk factors can lead to a successful integrated control programme. Therefore there is the need to carry out longitudinal studies taking risk factors not investigated in these surveys into account like intestinal helminth infections, dietary intake characteristics, and performing an additional socio-economic assessment to assess the general health status of especially the urban poor.

Chapter 7

7. BEDNET INTERVENTION STUDY

7.1. Introduction

Malaria control can be done at different points in the transmission cycle, measures targeted at the human host, *i.e.* that prevent the malaria parasite from developing, or at the mosquito vector, *i.e.* that prevent mosquitoes from feeding on humans or measures directly targeted at the adult or larval stages, either by destroying their breeding sites or killing of the adults or larvae by chemical or biological control (after Onori *et al.* 1993).

For the urban areas larval control is often mentioned as a potentially successful method as breeding is very focal (Robert *et al.* 2003, Caldas de Castro *et al.* 2004, Keating *et al.* 2004). Larval control can be done by environmental management by either permanent or temporary modification, by larviciding using different species of microbial pesticides *i.e. Bacillus spp.*, or by use of larvivorus fish. On a small scale and in specific settings environmental management/larval control has been successful (Matsuno *et al.* 1999, Shililu 2001, Utziger *et al.* 2001, Keiser *et al.* 2005, Mekonnen *et al.* 2005) but few programs have been conducted on a large scale. In two cities in Uganda a community based control program focusing on environmental management was carried out with successful reduction in number of breeding sites, decrease in number of adult mosquitoes and malaria prevalence (Lindsay *et al.* 2004). At present several large scale trials are underway (Donnelly *et al.* 2005), the results of which will help for planning future application and integration of this method within integrated vector management (IVM) (Killeen *et al.* 2002).

Bednets have been widely used for personal protection against mosquitoes, and latterly malaria, for centuries. Application of insecticides to the net increases its efficacy enormously, as it does not only form a barrier but also kills the mosquitoes, resulting in a greater degree of protection. A Cochrane review by Lengeler (2004) showed that insecticide treated nets (ITNs) reduced the incidence of uncomplicated malarial episodes by 50-62%. Protective efficacy (PE) of ITNs for severe malaria was 45% (95% CI 20 to 63) and ITN use improved the average haemoglobin level in children by

1.7% packed cell volume. About 5.5 lives (95% CI 3.39 to 7.67) can be saved each year for every 1000 children protected with ITNs.

All large scale bednet trials to date have been conducted in rural areas (Binka *et al.* 1996, McElroy *et al.* 2001, Binka *et al.* 2002, Lengeler 2004). With increasing urbanisation more people are moving into the urban areas and although efficacy of ITNs as such has been proven, different habitual factors of urban residents might affect their efficacy in the urban setting, *i.e.* people might go to bed later (Moyou-Somo *et al.* 1995), nuisance biting of *Culex spp.* might make bednets more readily acceptable (Coene 1991) or due to heat or crowded housing people might be more reluctant to use nets. Also, urban areas are known to be slightly warmer than surrounding rural areas (Tereshchenko & Filonov 2001, Jonsson 2004). Such different habits could account for different efficacy of bednets in the urban setting. Few trials have been done on the impact of bednets in the urban setting. A small study (n=40) in a small town in Cameroon found a significant reduction in overall malaria prevalence due to the nets although not in the rainy season. A cross sectional surveys on ITN use in urban Blantyre, Malawi, found that children under five not sleeping under a net, had double the risk of having malaria parasitaemia (Holtz *et al.* 2002).

Apart from an impact on malaria, control measures also impact on related factors like anaemia (Lengeler 2004). The Roll Back Malaria programme of the World Health Organization is currently exploring the use of anaemia as a measure of the impact of malaria interventions (Korenromp *et al.* 2004). Results of the Asembo bay trial in Kenya also show an effect of the bednet intervention on the nutritional indicator weight for age score (ter Kuile *et al.* 2003a, ter Kuile *et al.* 2003b). Nutritional status could be another proxy to demonstrate impact of intervention measures.

The cross sectional epidemiological surveys (chapter 3, 4 & 6) and the entomological studies (chapter 5) indicated transmission is ongoing in urban Ghana. Infective *Anopheles spp.* were caught in urban Accra and there was no association with travel in the baseline survey (chapter 4). The results of the epidemiological baseline survey showed only in some communities significant associations between urban agriculture (UA) and parasitaemia (chapter 3) and the general high prevalence found in the urban areas suggested that the most effective overall control tool would likely be bednets with their documented community effect (Lines *et al.* 1987, Hii *et al.* 2001, Maxwell *et*

al. 2002, Gimnig *et al.* 2003, Hawley *et al.* 2003a) rather than a specific intervention targeted directly at UA. The entomological studies (chapter 5) suggested that *Anopheles* may be resting outdoors which is important for the choice of intervention. Larval control could be added in certain areas although the transitory nature of most breeding sites would pose a big challenge to continuous effort and strong community involvement.

To address the above issues we conducted epidemiological and entomological studies linked to a bednet trial in urban Accra to demonstrate transmission in the urban setting and efficacy of interventions, as well as to assess efficacy of bednets in urban settings and look at the impact of bednets on outcome variables other than malaria, *e.g.* haemoglobin concentrations and nutritional status indicators obtained from antropomorphy. The work is a prelude to integrated management strategies as after introduction of nets we can readily design additional intervention studies using other control strategies, *i.e.* larval control through environmental management, and look at additional effects of these on malaria transmission indicators.

7.2. Material and methods

7.2.1. Study design

Two areas were selected for the study, an intervention and control area. The study was designed as a cohort intervention study with follow up at months 0, 3 and 6 after net distribution. Before the start of the intervention an area wide pre-intervention survey was carried out in a similar fashion to the epidemiological and socio-economic baseline study (chapter 4 & 5). To assess if there was an impact of the nets on mosquito populations light traps were set fortnightly for the period of one year during the intervention study.

7.2.2. Selection of study sites

Accra was chosen as the city for the intervention as it had significantly higher malaria prevalence than Kumasi in the baseline survey (chapter 4). In addition the UA areas in Accra were all located in the central urban zone. Based on high prevalence rates in the epidemiological baseline survey, access to the community, neighbouring location and similarity in housing structure and population density, Kotobabi and Alajo in central Accra were selected (Figure 3-1, p.35). The communities are homogeneous and closely

grouped around the biggest agricultural area in the city with low income households, mostly in compound houses with 8-15 households per compound and a few interspaced self-contained houses (Figure 7-1). We feel they are representative of rapidly growing urban communities where development is outpacing city infrastructure such as sanitation and urban planning. Malaria parasite prevalence is high, 18.3% and 15.1% in children between 6 months and 5 years (October/November 2002, chapter 3).

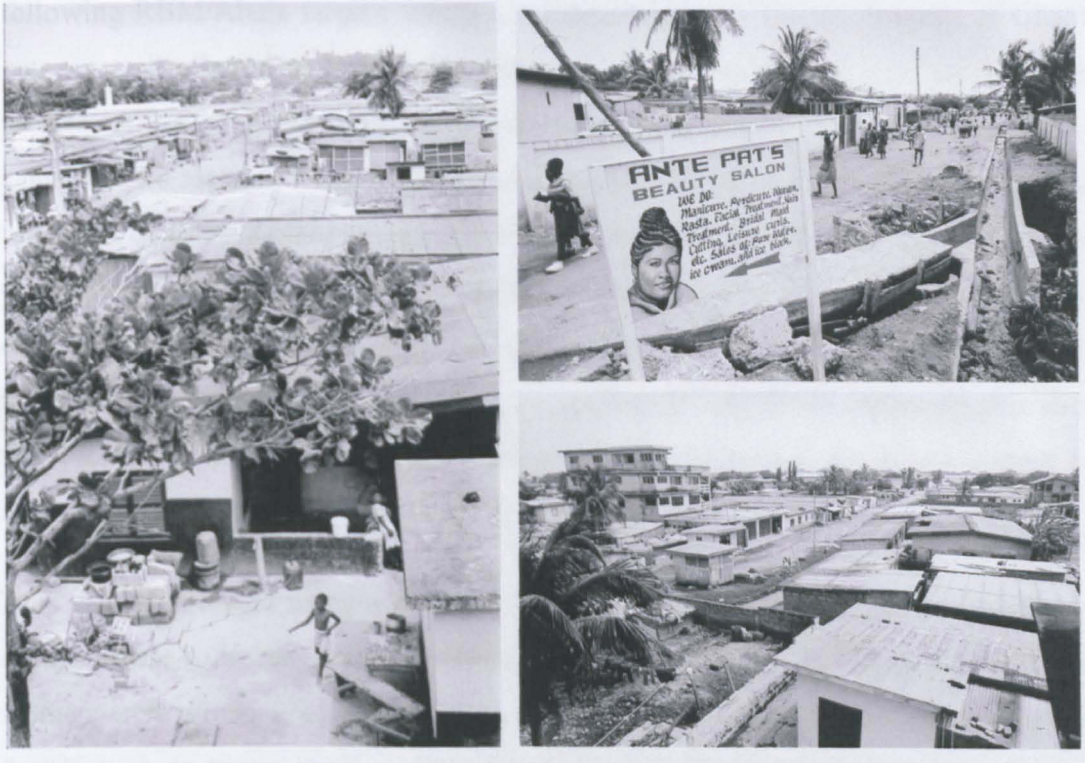

Figure 7-1 Impressions of Kotobabi and Alajo in Accra (photos courtesy Dr. PJ McCall)

Kotobabi has an estimated population of about 23,485 with about 5000 households (Accra Metropolitan Assembly (AMA) unpublished) and an average of 4.9 members per household with about 61 households per hectare (data from this study). For Alajo there was no exact population estimate available but it has on average 5.5 members per household and about 35 households per hectare (data from this study).

7.2.3. Sample size

Using Win Episcope (version 2.0) we calculated a cohort sample size of 200 with power 90% and 95% confidence level. This calculation was based on the mean Hb concentration in our current non parasitaemic population of 10.8 g/dL, with an

expected improvement to 11.3g/dL with a standard deviation of 1.5 (difference between parasitaemic and non-parasitaemic children in baseline survey 0.8 g/dL). Calculations based on malaria prevalence reductions gave similar figures, assuming malaria prevalence to be reduced to below 10% (from 18%). As people might be lost to follow up we aimed at getting a cohort sample size of 250 children in each group.

7.2.4. Identification of vulnerable groups and net distribution system

The groups most at risk for malaria are children and pregnant women, therefore, following RBM/Abuja targets, which are subscribed to by the government of Ghana, nets were distributed to households with children under 10 and/or pregnant women present. Although RBM targets children under 5 we choose the cut off at under 10 years for two reasons. First, we targeted a net coverage of at least 60% of households to try and establish a community wide mass protection effect as found in earlier studies (Lines *et al.* 1987, Hii *et al.* 2001, Maxwell *et al.* 2002, Gimnig *et al.* 2003, Hawley *et al.* 2003a). Second, we have observed that malaria infections were most prevalent in the upper age groups of our study population (chapter 3 & 6). Therefore by extending the distribution to these age groups we anticipated to have a significant impact on disease prevalence in these older children.

Nets were distributed using a voucher system. Vouchers were distributed in the area during house-to-house visits, households with children under 10 and/or pregnant women were given a voucher and an information sheet (Appendix 9). Household details such as house number, GPS location, child health card number, number of children and household composition were recorded and the purpose of the distribution programme was explained and the day and location of the net distribution announced. A duplicate of each voucher was made using carbon paper. One copy was retained by the enumerator and one by the household. Nets were distributed from a central location in the community and ahead of the distribution day announcements were made in the community from a van with loudspeakers. On the net distribution day people brought their vouchers and their children's birth cards for cross-check, the copy voucher was collected and attached to the original. After they signed for consent to enrol in the programme, they were given their net (Figure 7-2) and an information sheet plus instructions how to deploy the net (Appendix 10). Households with three or more children were given two nets. The information in the sheet was also verbally explained

to each person collecting a net, specifically the instruction to leave the net out for 24 hours before putting it up to minimise irritation from produced volatiles that may lead to skin irritation.

In the intervention area, nets were distributed on 20th and 21st May 2004 just before the onset of the long rainy season. The control area received nets on 17th December 2004, except the children enrolled in the control cohort who received their net on the last day of the cohort follow up (24-26 November 2004).

Figure 7-2 Bednet handout in the communities of Kotobabi and Alajo in Accra.

7.2.5. Cohort study

The cohort of children was randomly selected from the intervention area using the household composition information recorded on the vouchers (Appendix 9). Each child under five years was assigned a number and from these numbers the cohort of children was randomly selected. In the control area this could not be done as vouchers were distributed only 6 months later, therefore a random selection was made from the children enrolled in the pre-intervention study as the control area was completely covered in the pre-intervention study. In both areas 250 children were selected. On months 0, 3 and 6 the cohort of children was seen in a mobile clinic by a trained paediatrician (Dr. Onwona-Agyeman). During the mobile clinic visit malaria parasitaemia, haemoglobin level and anthropomorphic measures were taken from each child and a questionnaire on bednet usage, travel to the rural area and socio-economic status was taken (Appendix 11). Informed consent was obtained from each child's guardian for enrolment in the study and an information sheet provided (Appendix 12). Malaria parasitaemia and haemoglobin levels were measured and analyzed in the same

way as described previous for the epidemiological survey (0) and were taken by a trained technician from the Noguchi Memorial Institute for Medical research. Anthropomorphic measures, height, weight and mid-upper arm circumference (MUAC) were measured by the paediatrician, as well as temperature and spleen size. For children under two years of age the supine (lying) length was measured with the aid of a measuring board (Figure 7-3A). Standing height was measured for children older than 2 years using a fixed hanging measuring tape (Figure 7-3D). Height was measured in centimetres to the nearest 0.5 cm. Weight was measured using a sitting scale for children under 2 years of age (Figure 7-3B) and a standing scale for children over 2 years (Figure 7-3C). If children would not sit individually in the scale the child was measured together with the mother and the weight of the mother subtracted, this happened in 2 or 3 occasions. For measuring weight the child's shoes and any outer clothing were removed. Weight was measured in kg to the nearest 0.1 kg. MUAC was measured to the nearest 0.1 cm using a MUAC measuring band (Figure 7-3E).

Temperature was measured in the child's arm pit using a digital thermometer. Spleen size was measured both in cm and following Hackett scale (Gilles 1993b). On the first round of the cohort mobile clinic the first 100 children in each area were screened for possible presence of haematuria by analysing urine with a colour indicator strip for presence of blood. On round 0 and 6 months in addition stool samples were obtained for each child and examined for geohelminths. Stool sample containers were distributed in advance. All children positive for geohelminths or malaria or with Hb < 8.0 g/dL were treated. Children testing positive for malaria parasites were given a course of Amodiaquine and children with Hb<8.0 g/dL were given a course of iron supplementation (ferric sodium acetate (Ferrostrane) 34mg elemental iron/5ml) and multivitamins. Children testing positive for geohelminths were given a single dose of Albendazole (adjusted to weight). In the field 10% formal saline was added to the stool and the sample shaken thoroughly. Analysis of stool samples was done using the volumetric dilution method as described by Ashford *et al.* (1992). The protocol is given in Appendix 13.

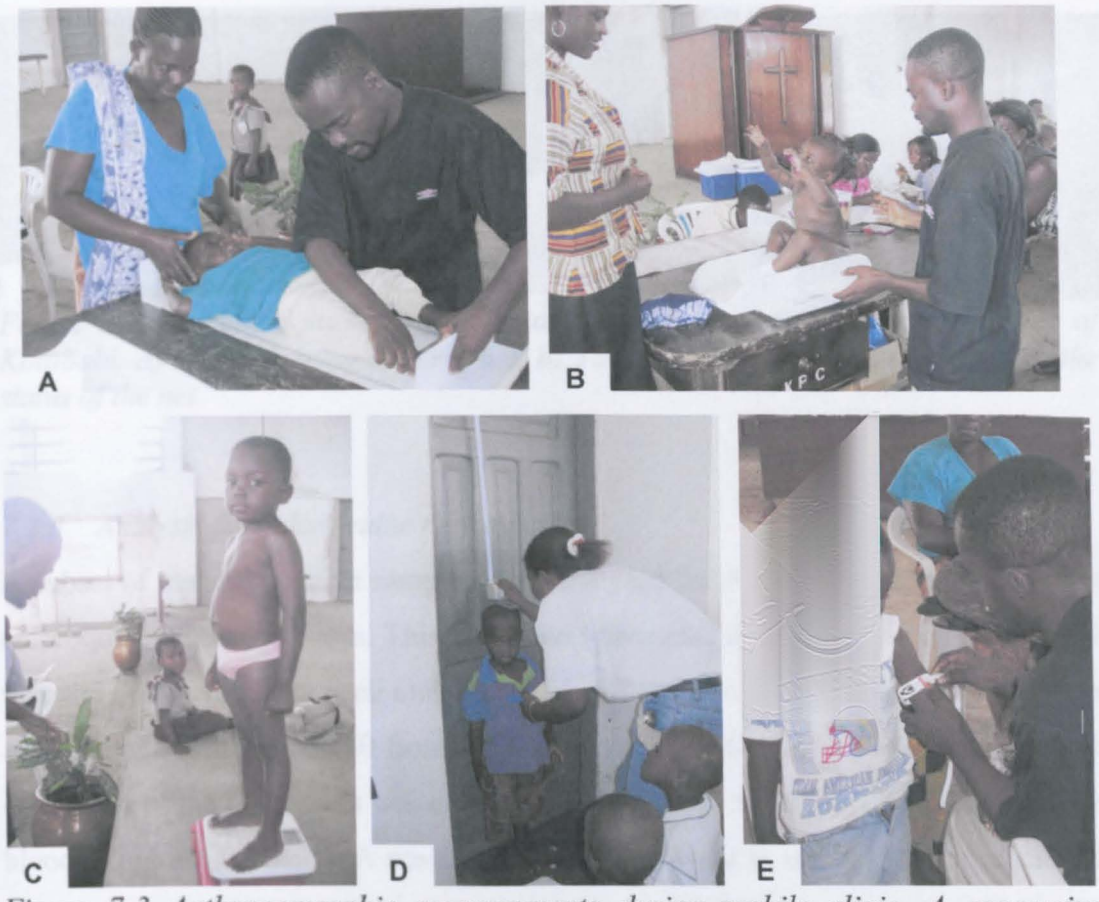

Figure 7-3 Anthropomorphic measurements during mobile clinic. A. measuring supine length; B measuring weight for child under 2 years; C measuring weigh for child over 2 years; D measuring standing height; E measuring mid-upper arm circumference

7.2.6. Nutritional status

Indicators for nutritional status, e.g. height for age (HA), weight for height (WH) and weight for age (WA) score were calculated using the nutrition extension of the Epi-info software (version 3.3.2, CDC, Atlanta) with the WHO 1978 reference curve. Children with a Z-score <-2 for HA were classified as stunted, and for WH Z score<-2 as wasted and WA Z-score<-2 as underweight.

7.2.7. Bednet compliance

Usage of nets was assessed two times. Both communities were surveyed (Figure 7-4after distribution to assess how many people had hung their net and if there were problems putting it up (questionnaire in Appendix 14). A second survey was performed in Kotobabi after 6 months to see if there was a change in net use.

Figure 7-4 Household survey on compliance to bednet usage in the community of Kotobabi. A) Child sleeping under a net, B) Taking a questionnaire, C) Checking the status of the net.

7.2.8. Analysis for chloroquine resistance

A subset of malaria positive samples were analysed for *Plasmodium* species type and chloroquine resistance status. This was done from spots of blood dried on filter paper that were collected from each child enrolled. All samples from the cohort study were analysed and a subset of 180 from the baseline survey (chapter 3 & 4), 95 from each city. DNA of the bloodspots was extracted using the QIAGEN kit (250 QIAamp DNA blood mini kit, QIAGEN). A nested PCR was performed to identify which species of plasmodium was present using an adapted protocol of Snounou *et al.* (1999). The protocol is given in Appendix 15. The same samples were tested for presence of *pfcrt* T76, a marker for chloroquine resistance following Djimde *et al.* (2001a, 2001b). A mutation specific nested PCR strategy was used to detect the two alleles of *pfcrt* T76. The protocol is given in Appendix 16.

7.2.9. Data entry and statistical analysis

All data were double entered and cross checked using Epi Info data compare (Epi info version 3.3.2, CDC, Atlanta). Checks were carried out for extreme or unlikely data by looking at minimum and maximum value for *e.g.* age, Hb, length, weight and socio-economic score. Before calculating distances, coordinates were cross checked by plotting them on the map and checking that households were located in the community. Z-scores were calculated using the Nutstats programme of Epi Info. Using Epi Info's inbuilt flagging system, data were checked for recording errors and extreme values. Two points were omitted because of impossible data (a height of 151.8 cm at 49.3 months and a weight of 20 kg at an age 21.9 months) which is likely due to reporting/collection error.

Height and weight measurements were crosschecked for unlikely measurements by analyzing the difference in height and weight between the different rounds. Some increments seemed unlikely, *e.g.* an increase in height of more than 10cm in 3 months, and might have been due to measuring/reporting errors. Proportional height and weight increments were calculated and where these were more than three standard deviations off the mean value, increments were classified as unlikely and the respective measurements omitted from analysis. This was done separately for boys and girls.

Statistical analysis of the data from the pre-intervention cross sectional survey was carried out in a similar fashion as the earlier epidemiological studies (chapter 3 & 4). The data from the three rounds of the cohort study were analysed using the Proc Mixed procedure in SAS (version 8.02) with a repeated measure statement with unstructured covariance structure. Covariance structure was selected based on the covariance matrix and the best fitting model, *i.e.* lowest -2 Res Log likelihood and Akaike's information criterion (AIC) values. Household was included as a random effect. The dependent variable was tested for effect of round, area and the interaction of round and area. Models with height, weight, WHZ score and Hb as dependent were adjusted for age and sex. Models with HAZ and WAZ score as dependent were only adjusted for sex as age is embedded in the variable. Nutritional scores were also adjusted for Hb concentration. For the dichotomous variables, *i.e.* presence/absence of malaria parasitaemia and Hb concentration above/below a cut off value, a generalized linear mixed model (GLMM) approach, using a SAS macro (Glimmix 800, SAS Inc., Cary, NC, USA) that allowed a logistic link function was run with a repeated measure statement. The same GLMM approach (without repeated measure statement) was used to investigate the association between putative predictor variables and malaria parasite prevalence at round 3. In both cases household was included as a random effect.

To investigate if there was a protective effect of the bednets distributed in Kotobabi on the children in Alajo living close to Kotobabi a GIS approach was used. Based on previous findings in Kenya (Hawley *et al.* 2003a) a protective range of 300m was assumed. Using Arcview (version 3.1), all children in the Alajo cohort living within 300m of a household in Kotobabi who received a net were selected. Then it was tested if there were differences in malaria, anaemia and haemoglobin concentration within or outside the boundary zone. In addition the distance for each cohort household to the

border dividing Alajo and Kotobabi, a drain, was calculated and the relation between this distance, called the net line distance, with malaria and anaemia prevalence and Hb concentration was investigated.

7.2.10. Adult catches - Light traps

During the bednet intervention light traps were set in the two intervention areas to assess if there was an impact of the nets on the mosquito population in the area. A total of sixteen light traps, eight in each area, were set fortnightly for the period of one year. This commenced in both areas in June 2004 just after the bed nets were distributed in the intervention area and continued until July 2005. The nets in the control area where handed out on 17th December 2004. Light traps were hung next to untreated nets (that were provided to the residents free of charge) and run overnight when residents were sleeping following Service (1993). For each room it was noted how many people had slept there in the previous night. The collection reservoir of the trap was closed in the morning before the battery was turned off and collected by the field team in the early morning. Upon return to the laboratory mosquitoes were transferred to paper cups and provided with sugar water. Collections were examined on the day of collection. Gravid or fed female mosquitoes were taken out and transferred to separate paper cups for egg laying. On the bottom of each paper cup a circle of moist filter paper was put and the gravid mosquito carefully transferred into the cup and provided with sugar water. All mosquitoes in the collections were counted and numbers recorded, *Anopheles* were identified to species level and status (unfed, fed, half-gravid, gravid, and male) and stored over silica for later analysis. Other mosquitoes were identified to genus level and scored as e.g. *Culex*, *Aedes spp.* distinguishing between males and females.

Figure 7-5 Set up of a light trap in a house in Kotobabi, Accra

Mosquito numbers were compared using non parametric Mann Whitney U tests as also after log distribution variance was not homogenous due to the large variation in numbers caught.

7.3. Results

7.3.1. Pre-intervention survey

A total of 1031 children were enrolled in the pre-intervention survey, 517 children from 371 households in Kotobabi, the intervention area and 514 children, of 328 households in Alajo, the control area. In both areas the majority of the population lived in compound houses although in Alajo significantly more people live in wooden structures (Table 7-1). In the two communities 47.0% of households had a socio-economic score below the city average score as obtained from the baseline survey (chapter 3), which did not differ between communities (Table 7-1) or from the baseline survey in 2002 (45.2%). Socio-economic status did not differ between the areas although more carers had completed primary school in Kotobabi (Table 7-1). Average household size was similar in both areas at about 4.8 members per household. The majority of the people were from the Akan ethnic group, other groups were Ewe, Ga and Northerners (all tribes from the northern region of Ghana combined). In Kotobabi there were significantly more Ga people while in Alajo there were significantly more people from the northern regions (Table 7-1). Overall malaria prevalence was 9.1% (93/1024) and did not differ between intervention and control area (Table 7-2). Overall 53.5% of the children were anaemic (Hb<11.0 g/dL) and 4.8% were moderately to severely anaemic (Hb<8.0 g/dL). In the control area significantly more children had travelled to the rural area in the preceding month and taken malaria medication in the last 2 weeks (Table 7-2).

Reported number of households with a bednet was higher in Alajo than in Kotobabi (Table 7-1) and in Alajo more children were reported to have slept under a bednet the previous night (Table 7-2). There was no difference in use of other protective measures between the areas, over 90% of houses had netting in front of windows and doors, about 80% used mosquito coils at a daily basis and nearly 60% used mosquito spray at least weekly. Nearly 75% of the people perceived malaria to be a problem for the

household and nearly 90% found mosquitoes a problem. Over 90% of people knew mosquitoes transmit malaria, and this was significantly higher in Alajo than Kotobabi. About 25% of household said somebody in the household had had malaria in the last 2 months, this did not differ between communities.

Table 7-1 Overview of household characteristics in Alajo (control) and Kotobabi (intervention) area as obtained during the pre-intervention survey.

	Alajo	Kotobabi	overall	p-value
Ethnic group				
Akan	49.7	47.0	48.3	
Ewe	29.7	31.9	30.9	
Ga	2.5	9.1	6.0	*P*=0.0003
Northerner	17.1	10.8	13.8	*P*=0.019
other	0.9	1.1	1.0	
Household characteristics				
average household size (SEM)	4.7 (0.103)	4.8 (0.094)	4.8 (0.069)	*P*=0.717
*% living in compound house**	85.9	94.5	88.9	*P*=0.019
*%living in wooden structure**	8.2	3	5.5	*P*=0.0023
% with electricity	95.1	94.3	94.7	*P*=0.645
mean socio-economic-score# (SEM)	1.62 (0.041)	1.68 (0.041)	1.65 (0.029)	*P*=0.329
%below city average	48.1	43.2	45.5	*P*=0.190
%without ceiling	48.1	24.0	35.3	*P*=0.000
%completed primary school	55.2	75.5	66.0	*P*=0.000
Mosquito protection				
% with window/door nets	90.1	91.6	90.9	*P*=0.501
% with bednet	22.0	9.2	15.2	*P*=0.000
% of bednets impregnated	28.4	10.0	22.7	*P*=0.046
% that uses coils at least daily	44.2	42.4	43.2	*P*=0.648
% that spray at least weekly	23.9	30.5	27.3	*P*=0.057
Malaria perception				
% finding malaria a problem for the household	72.8	74.7	73.8	*P*=0.593
%know mosquitoes transmit malaria	96.3	90.5	93.2	*P*=0.003
% reporting mosquitoes to be a problem	91.1	86.6	88.7	*P*=0.064
%with a malaria case in the household in the last 2 months	24.8	27.5	26.2	*P*=0.422

SEM = standard error of mean; *as opposed to compound, self-contained, erected structure/ uncompleted building or storey building; # based on asset score of the World Bank for Ghana (www.worldbank.com/lnp);

Multivariate analysis revealed malaria parasitaemia to be associated with age (positive, P=0.014) and socio-economic status (negative, P=0.026) Although in univariate analysis malaria parasitaemia were significantly associated with Hb<8.0 g/dL (OR 3.11 (1.44-6.59), P=0.003), travel to the rural area (OR 2.38 (1.38-4.07) P=0.0006) and carer not completed primary education (OR 1.90 (1.21-2.98) P=0.003) this was not significant in multivariate analysis accounting for confounders and data structuring.

When taking the child slept under a net the previous night as the dependent variable, socio-economic score (negative, P=0.032), age (negative, P<0.001) and Hb concentration (negative, P< 0.001) were significantly associated with sleeping under a net the previous night. Gender was approaching significance with boys more likely than girls to have slept under a bednet the previous night (OR 11.0 (6.9-17.5), P=0.063).

Table 7-2 Overview of characteristics of the children enrolled in the pre-intervention survey

	control area (Alajo)	intervention area (Kotobabi)	overall	p-value
number of children	514	517	1031	
% male	49.6	53.0	51.3	P=0.277
% with malaria parasites	9.4	8.8	9.1	P=0.729
% with Hb<11 g/dL	56.3	50.8	53.5	P=0.081
% with Hb<8 g/.dl	4.7	4.9	4.8	P=0.850
% that took malaria medication in last 2 weeks	28.0	19.8	23.9	P=0.002
% with reported history of fever in last 48 hrs	24.7	21.3	23.0	P=0.196
% that travelled to rural area in last 4 weeks	17.7	9.3	13.5	P=0.000
% that slept under a bednet the previous night	15.6	4.3	9.9	P=0.000

SEM = standard error of mean; Hb= haemoglobin concentration;

7.3.2. Cohort baseline characteristics

A total of 498 children were enrolled in the cohort at baseline (round 1), of which 487 qualified for the study. Carers had upon enrolment reported their child to be under 5 years but when later shown their child health card some actually were over 5 years of age. There were about 25 children that were just over 60 months, therefore we extended the age to 72 months. A total of 11 children were excluded from analysis: from 5 children the date of birth could not be verified, 3 children were just under 6 months and 3 were over 72 months. Figure 7-6 gives an overview of the children in each round of the cohort follow up. Figure 7-7 gives the geographical locations of the households of the children enrolled in the cohort and the net distribution zone. A total of 2426 nets were distributed in Kotobabi and 2020 nets six months later in Alajo. Using the 2002 population figure for Kotobabi with an annual urban growth rate of 3.4%, there were about 25,109 people in 2004, in about 5231 households, taking average of 4.8 members per household of the pre-intervention survey. We distributed 2177 vouchers (to households with children under 10 and/or pregnant women) of which 2062 households collected 1 or more nets. This calculates into a net coverage of 39% and on average 17.7 nets were distributed per hectare. We assessed number of households in 10 grids of 1 hectare and found on average of 61 HH per hectare in 85 housing structures. Using these figures a coverage of 24% can be calculated. Therefore the actual coverage was probably in between 24 and 39%.

Cohort tree	Control	Intervention
Enrolled	248	239
Round 2	224	208
Round 3	215	196
Summary	431 children 2 rounds 385 children 3 rounds	

Figure 7-6 Overview of children enrolled in the cohort and re visits in subsequent rounds.

Table 7-3 gives the baseline characteristics of the children enrolled in the cohort at baseline. There was no difference in gender. Division of age groups was similar between the intervention and control area. There were more children in the intervention areas in the 6-12 and 60-72 months age group but this was not significant after Bonferoni correction for multiple comparisons.

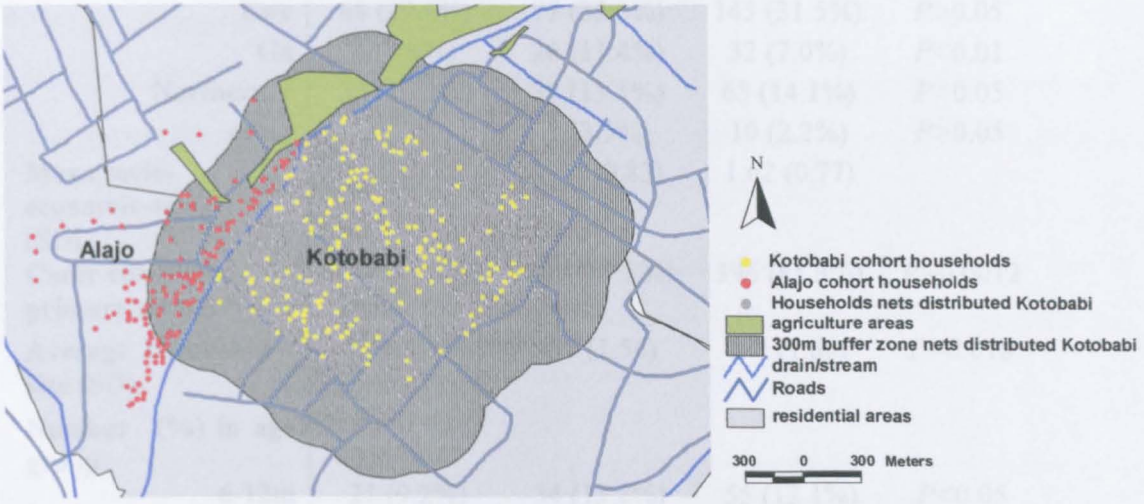

Figure 7-7 Location of cohort households and net distribution zone in the intervention area.

Socio-economic status was similar in both areas but significantly more carers in the intervention area had completed primary school (Table 7-3). A total of 51 (20.2%) of the control households reported to have a bednet of which 19 (37.3%) were impregnated. Significantly more carers in the control area reported to use mosquito coils at a daily basis (48.4 vs. 29.0%, P=0.000). There was no significant difference in use of spray (at a weekly basis; 21.3 and 18.3% in control and intervention respectively) or having netting in front of windows and doors (89.1 vs. 90.3% in control and intervention respectively).

There were no significant differences (P>0.05) in the number of children eating meat but slightly more children were reported to eat vegetables in the intervention area than in the control area at enrolment. The average age of children being breastfed was 12.5 (SEM 0.42) months and did not differ between the two areas. At enrolment in both areas a urine sample of the first 100 children was analyzed for possible haematuria but none were found positive for traces of blood indicating no infection with *Schistosomia haemotabium*. At enrolment four children had an enlarged spleen, two in Kotobabi and two in Alajo.

Table 7-3 Characteristics of children enrolled in the cohort at baseline

	control area	intervention area	overall	p-value
Number of children	248	239	487	
Number male (%)	121 (48.8%)	128 (53.3%)	250 (51.0%)	P=0.293
Number (%) in ethnic group				
Akan	120 (51.9%)	88 (38.4%)	208 (45.2%)	P<0.01
Ewe	68 (29.4%)	77 (33.6%)	145 (31.5%)	P>0.05
Ga	6 (2.6%)	26 (11.4%)	32 (7.0%)	P<0.01
Northerner	35 (15.2%)	30 (13.1%)	65 (14.1%)	P>0.05
other	2 (.9%)	8 (3.5%)	10 (2.2%)	P>0.05
Mean socio-economic-score# (SD)	1.60 (0.70)	1.64 (0.83)	1.62 (0.77)	
Carer completed primary school	187 (75.7%)	209 (87.1%)	396 (81.3%)	P=0.0012
Average household size (SD)	5.0 (1.79)	4.7 (1.56)	4.8 (1.69)	P=0.018
Number (%) in age group				
6-12m	21 (9.2%)	34 (15.2%)	55 (12.1%)	P<0.05
13-24m	53 (22.3%)	55 (25.8%)	109 (24.0%)	P>0.05
25-36m	54 (22.3%)	52 (24.0%)	105 (23.1%)	P>0.05
37-48m	65 (27.3%)	43 (19.8%)	108 (23.7%)	P>0.05
49-59m	47 (18.9%)	34 (15.2%)	78 (17.1%)	P>0.05
60-72m	8 (3.2%)	21 (8.8%)	29 (6.0%)	P<0.05

SD = standard deviation; # based on asset score of the World Bank for Ghana (www.worldbank.com/hnp);

7.3.3. Impact of the intervention

7.3.3.1. Malaria

At enrolment and round 2 there was no difference in malaria prevalence in the two areas (Table 7-4). At round 3 there were 15 new cases in the control area and 7 in the intervention area (P=0.170). In the intervention area 3 out of 7 cases were in the 60-72 month age group. This age group was under represented in the control area (Table 7-3) and if this group was omitted, there were significantly more new cases in the control area than in the intervention area (OR 2.98, 95%CI 0.95-11.43, P=0.036). Children in the intervention area who reported not to use the net at all were excluded from this analysis, which were 8 children at round 2 and 14 children at round 3. At round 3 malaria was significantly associated with travel (OR 4.27, 95%CI 1.41-12.70; P=0.002).

Table 7-4 Overview of malaria prevalence in the three rounds of the cohort.

	control	intervention	overall	p-value
round 1	13/248(5.2%)	7/239 (2.9%)	20/487 (4.1%)	0.198
round 2	8/223 (3.6%)	10/201 (5.0%)	18/434 (4.1%)	0.474
round 3	15/215 (7.0%)	7/183 (3.8%)	22/398 (5.8%)	0.170

Note: children who were reported not to use the net at all were excluded from analysis

7.3.3.2. Anaemia

Average Hb concentration did not differ between intervention and control for any of the age groups and there was no difference in either moderate-severe (Hb<8.0 g/dL) or mild (Hb< 11.0 g/dL) anaemia at any of the rounds (Figure 7-8).

At round 1, attributable risk (AR) for anaemia due malaria was 3.6% and due to geohelminth infection 1.6%. For moderate to severe anaemia the AR for anaemia due to malaria was 11.5% and for geohelminth 1.5%.

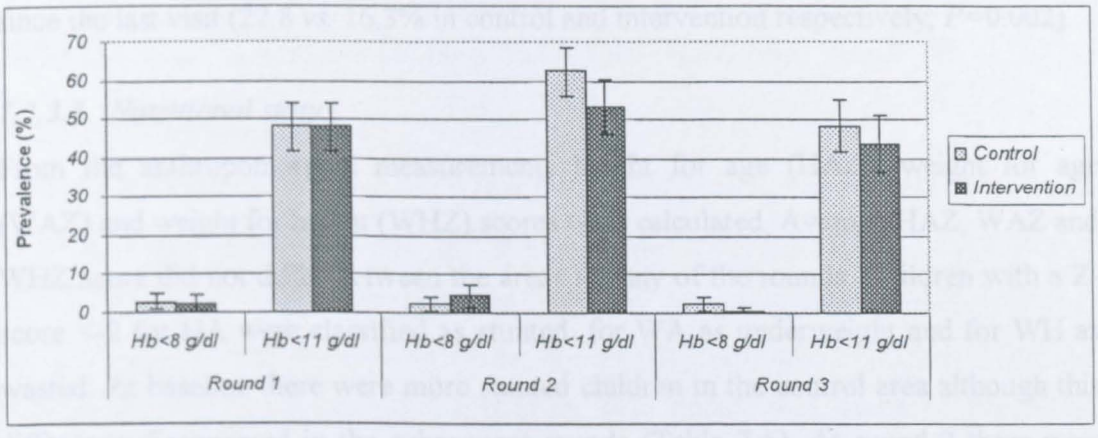

Figure 7-8 Prevalence of moderate to severe (Hb<8 g/dL) and any anaemia (Hb<11.0 g/dL) in each round of the cohort.

7.3.3.3. Geohelminths

Prevalence of geohelminths analyzed from stool samples was significantly higher at round 3 in the control as compared to the intervention area (Table 7-5). At baseline there was no difference in prevalence. At round 1 there were 65 cases, 39 (60%) were *Ascaris*, 19 (29%) *Hookworm*, 3 (5%) mixed *Ascaris* and *Hookworm*, 2 (3%) *Taenia*, 1 (1.5%) *Trichuris* and 1 (1.5%) *Strongyloides* infection. There was no difference in prevalence of any of the geohelminth species between the control and intervention areas. At round 3 of the 8 infections, 4 were *Ascaris*, 1 *Hookworm*, 1 *Trichuris* and 2 *Strongyloides*.

Table 7-5 Percentage of positive stool samples in intervention and control area at baseline and round 3.

	control	intervention	overall	p-value
round 1	37/245 (15.1%)	28/216 (13.0%)	65/461 (14.1%)	0.51
round 3	8/208 (3.8%)	0/156 (0.0%)	8/364 (2.2%)	0.013

Positive stool samples contained Ascaris, hookworm, Strongyloides, Trichuris or Taenia.

7.3.3.4. Fever and unspecified illness

At round 3, but not at the other rounds, significantly more children in the control compared to the intervention area were reported to have been ill since the last visit (OR 1.88 (1.25-2.85), P=0.002). Fever as symptom for health facility visit since the last visit was significantly higher in the control than the intervention area (OR 2.31, 95%CI 1.52-3.51, P<0.001). There was no difference in number of children who reported with fever (axillary temperature ≥37.5°C) at the day of the visit in any of the rounds. At round 3 significantly more children in the control area had taken malaria medication since the last visit (27.8 *vs.* 16.3% in control and intervention respectively, P=0.002).

7.3.3.5. Nutritional status

From the anthropomorphic measurements height for age (HAZ), weight for age (WAZ) and weight for height (WHZ) scores were calculated. Average HAZ, WAZ and WHZ score did not differ between the areas for any of the rounds. Children with a Z-score <-2 for HA were classified as stunted, for WA as underweight and for WH as wasted. At baseline there were more stunted children in the control area although this difference disappeared in the subsequent rounds (Table 7-6). At round 2 there were significantly more wasted children in the intervention area. This was mainly due to a reduction in wasted children in the control area. Average relative weight increase (proportional by weight at round 1) was significantly higher in the intervention than the control from round 1 to 3 and from round 2 to 3 (P<0.01). Increase in relative height was not significant at any of the rounds.

Looking at the data at enrolment, children who were reported to eat meat had on average a Hb concentration which was 0.33 g/dL higher (P=0.029) than those who did not. Those who ate vegetables had on average a Hb concentration that was 0.41 g/dL higher (P=0.003) than those who did not. Values were adjusted for age and sex. Children reported to eat vegetables also had significantly higher WAZ, WHZ and HAZ

scores (on average 0.62 (P<0.001) for WAZ, 0.58 (P<0.001) for WHZ and 0.32 (P=0.02) for HAZ).

Table 7-6 Overview of stunted (HAZ<-2), underweight (WAZ<-2) and wasted (WHZ<-2) children in the different rounds of the cohort plus significance for difference between areas (Pearson's Chi-square)

	control area		intervention area		overall		p-value
HAZ<-2	n		n		n		
round 1	238	26 (10.9%)	232	11 (4.7%)	476	37 (7.8%)	0.011
round 2	199	21 (10.6%)	159	12 (7.5%)	358	33 (9.2%)	0.329
round 3	195	19 (9.7%)	159	14 (8.8%)	354	33 (9.3%)	0.763
WAZ<-2							
round 1	246	30 (12.2%)	242	35 (14.5%)	489	65 (13.3%)	0.408
round 2	201	16 (8.0%)	167	21 (12.6%)	369	37 (10.0%)	0.132
round 3	195	21 (10.8%)	145	18 (12.4%)	335	39 (11.6%)	0.998
WHZ<-2							
round 1	236	35 (14.8%)	232	34 (14.7%)	468	69 (14.7%)	0.957
round 2	198	10 (5.1%)	157	29 (18.5%)	355	39 (11.0%)	<0.001
round 3	195	14 (7.2%)	158	15 (9.5%)	353	29 (8.2%)	0.431

HAZ=height-for-age Z-score; WAZ=weight-for-age Z-score; WHZ=weigh-for-height Z –score

7.3.3.6. Multivariate analysis

Multivariate analysis of malaria at round 3 indicated presence of malaria parasitaemia to be significant associated with age group (negative, P=0.038), Hb<11.0 g/dL (OR 5.96 (2.0-17.0), P=0.009), travel to the rural area since the last visit (OR 3.96 (1.4-11.0), P=0.011) and distance to urban agriculture (negative, P=0.046). Area, *e.g.* control or intervention, was not significant and neither was usage of the bednet (children in the control area who were reported using the net were scored as using a net). At round 3, having been ill since the last visit was significantly associated with area (control area OR 1.64 (1.1-2.5), P=0.02) and travel to the rural area since the last visit (OR 2.2 (1.2-4.1), P=0.0098).

The continuous variables were analysed using repeated measure analysis. The dependent variables weight (P=0.0054), WAZ score (P=0.0162), HAZ score (P=0.0132) and WHZ score (P=0.0002) score showed significant differences over the rounds per area after adjustment for sex, socio-economic score, presence of malaria parasites and data structuring. Weight and WHZ score were also adjusted for age. Height and Hb concentration did not show a significant interaction effect. Table 7-7 gives an overview of the result from the final models, the table presents the mean

values for each group using the average age. Appendix 17 gives the model parameter estimates for each of the dependent variables. For all of them the value improved or remained the same for the intervention area well it deteriorated for the control area, the differences were mostly visible at round 3. There was no significant effect of the interaction of round and area on the binominal dependent variables, presence of malaria parasitaemia and being anaemic.

Table 7-7 Least square means per round of proc mixed repeated measure analysis of significant variables in the intervention and control area.

area/round	Control area			Intervention area		
parameter	Round 1	Round 2	Round 3	Round 1	Round 2	Round 3
weight	13.03	13.13	12.99	13.10	13.18	13.22
HAZ	-0.335	-0.454	-0.475	-0.089	-0.211	-0.339
WAZ	-0.909	-0.831	-0.912	-0.843	-0.812	-0.796
WHZ	-0.787	-0.595	-0.708	-0.904	-0.770	-0.682

7.3.4. Protective effect

Hb concentration was significantly higher in the children in Alajo living within 300m of a net in Kotobabi as compared to children who lived >300m from the net distribution zone at round 3 (P=0.016) and approaching significance at round 2 (P=0.054). At round 1 there was no difference (

Figure 7-9). The number of anaemic children was significantly lower at round 2 in the zone <300m of Kotobabi (OR 2.01, 95%CI 1.09-3.72, P=0.017), while at round 3 this was marginal significant (P=0.055). Malaria parasitaemia and moderately to severe anaemia did not show a significant difference between the two groups of households, possibly due to the low overall case numbers.

To investigate this effect further the relation between the distance to the netline in the control and intervention area was analysed (see paragraph *7.2.9.*). In the control area, there was a significant positive association of anaemia prevalence with distance to the border of the net area at round 2 (P=0.037) and round 3 (P=0.0064) when adjusted for socio-economic status, age and sex of the child. At round 1 this distance was not significant. In the intervention area there was no significant association between netline distance and anaemia at any of the rounds. For malaria and moderately to severe anaemia this association could not be found, possibly due to the low case numbers. Haemoglobin concentration showed the same impact as there was a significant negative association between Hb concentration and netline distance at

round 3 for the control area (P=0.0048) when adjusting for sex (P=0.0461), age group (positive, P<0.0001) and socio-economic status (P=0.1061) With Hb concentration reducing 0.12 g/dL every 100 m. This was not significant at round 2 (P=0.0592) and round 1 (P=0.0941) or in the intervention area.

Figure 7-10 shows Hb concentrations at 200m intervals from the netline for the intervention and the control area at each round.

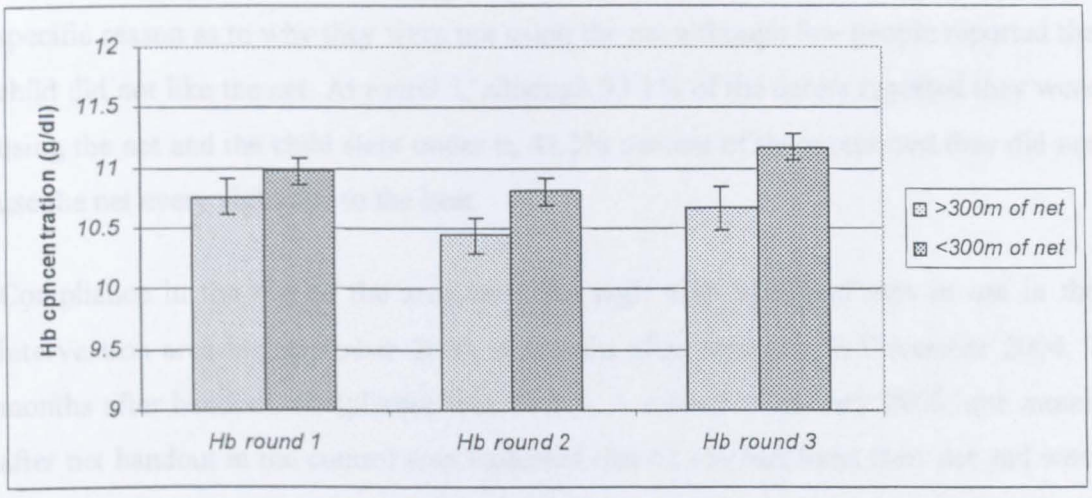

Figure 7-9 Haemoglobin concentration in children of the control (Alajo) cohort living <300m or >300m from a household with a net distributed in Kotobabi area.

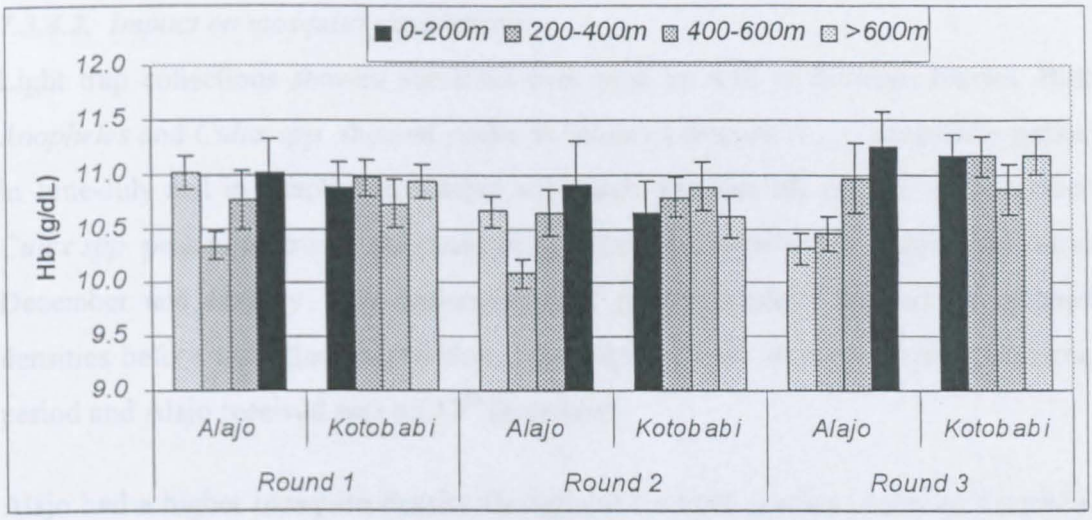

Figure 7-10 Average haemoglobin concentration with standard error of mean in 200m interval from the borderline dividing the intervention (Kotobabi) and control (Alajo) area.

7.3.4.1. Compliance

Overall compliance to using the net in the cohort was high at 95.0%. At round 2, carers of 7 children of the intervention area (3.4%) reported that their child did not sleep under the net at all, some of them reported they could not fix the net or had no bed to hang it above. In these cases additional instructions were given on how to use the net. At round 3 carers of 13 children (6.7%) reported they were not sleeping under the net at all, 10 of whom did sleep under the net at round 2. Most people could not give specific reason as to why they were not using the net although few people reported the child did not like the net. At round 3, although 93.3% of the carers reported they were using the net and the child slept under it, 41.2% percent of them reported they did not use the net every night due to the heat.

Compliance in the rest of the area was also high with 81.9% of nets in use in the intervention area in September 2004, 4 months after handout. In December 2004, 7 months after handout, compliance was 74.7%. A survey in January 2005, one month after net handout in the control area indicated that 61.4% had fixed their net and were using it. Many people reported to take down the net every morning and fix it again in the evening as they have only one room that they use as living room and as bedroom.

7.3.4.2. Impact on mosquito populations

Light trap collections showed variation over time as well as between houses. Both *Anopheles* and *Culex spp.* showed peaks in densities (Figure 7-11). *Anopheles* peaked in June-July and in October-November with high densities till the end of December. *Culex spp.* peaked following the peaks in densities of *Anopheles*, in August and end of December and January. The distinctive peak pattern made it difficult to compare densities before and after intervention. Kotobabi had nets throughout the monitoring period and Alajo received nets on 17th December.

Alajo had a higher mosquito density throughout for both species. Average *Anopheles* density per person was slightly lower in Alajo after nets were introduced (mean rank before 223.1; and after 201.5, P=0.056 Npar test), while for *Culex* this was higher (mean rank before 198.0; and after 230.2, P=0.007, Npar test). Although this should be interpreted with care as two peaks in vector density occurred before handing out of the nets in this area and only one after. The peaks in *Anopheles* density are likely linked to rainfall (Figure 7-11 bottom graph). *Anopheles* density was significantly positively

correlated with rainfall with a two week lag (Pearson r =0.567 P=0.01). *Culex* density was significantly negatively correlated with rain two weeks preceding collection (Pearsons r = -0.520, P=0.01).

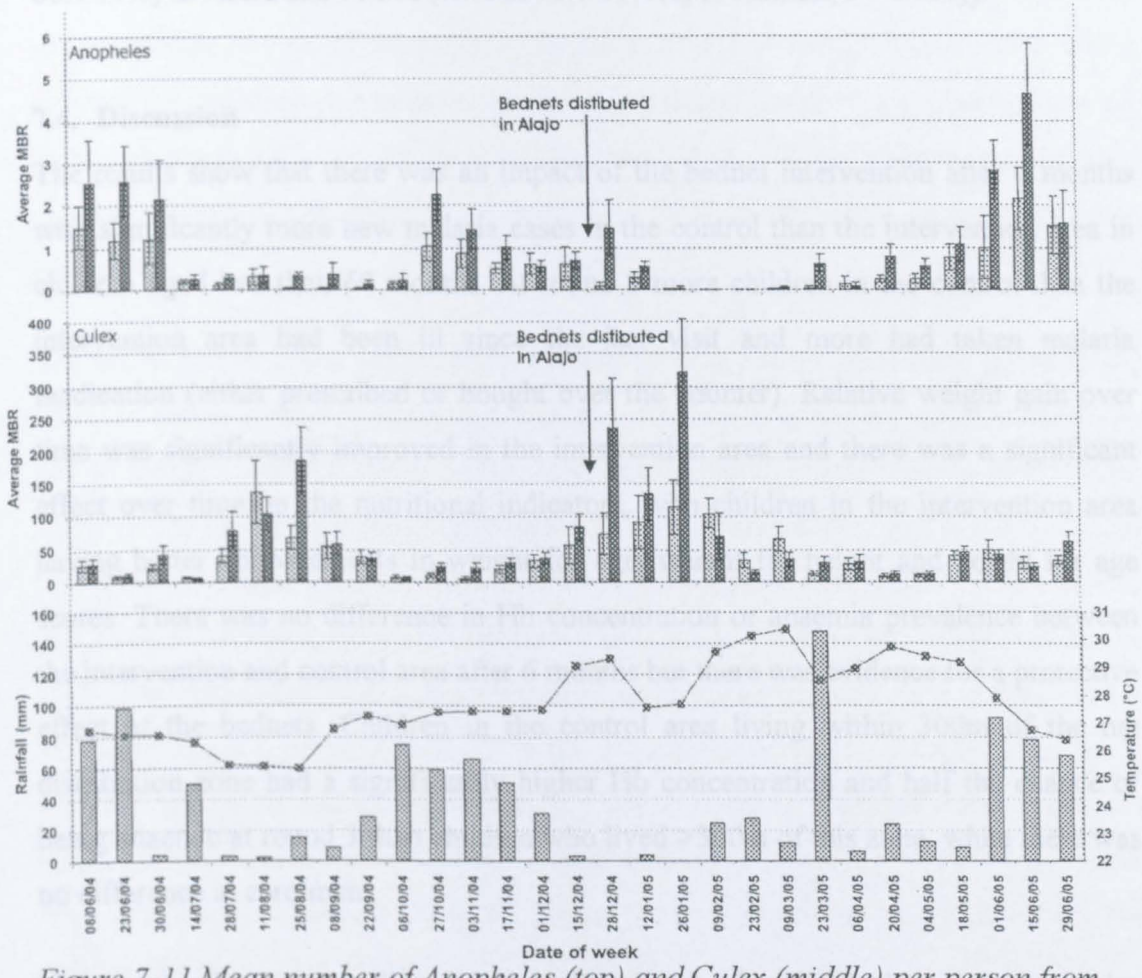

Figure 7-11 Mean number of Anopheles (top) and Culex (middle) per person from light trap catches over the period of one year in the intervention (Kotobabi) and control (Alajo) area in Accra. Bottom graph shows the meteorological parameters total rainfall and average temperature (in two weeks preceding collection, data Water Research Institute, Accra, unpublished).

7.3.5. Chloroquine resistance

A selection of the malaria positive cases from the baseline survey in Accra and Kumasi and all cases from the cohort were analyzed for *Plasmodium* species and presence of the *pfcrt* T76 codon, a marker for chloroquine resistance.

Of 201 samples successfully analysed, 196 (97.5%) were *P. falciparum*, 3 (1.5%) *P. ovale*, 1 (0.5%) a co-infection of *P. falciparum* and *malariae* and 1 (0.5%) a co-infection of *P. falciparum, malariae* and *ovale*. Of the 220 samples successfully tested,

181 (82.3%) were classified as resistant (R), 29 (13.2%) were wild type (S) and 10 (4.5%) were mixed infections of R and S. When comparing the figures of the baseline surveys, chloroquine resistance was higher in Accra than in Kumasi (90.0% (95%CI 86.4-97.7) in Accra and 76.5% (95%CI 71.7-89.4%) in Kumasi, P=0.030)).

7.4. Discussion

The results show that there was an impact of the bednet intervention after 6 months with significantly more new malaria cases in the control than the intervention area in children aged less than 60 months. At round 3 more children in the control than the intervention area had been ill since the last visit and more had taken malaria medication (either prescribed or bought over the counter). Relative weight gain over time was significantly improved in the intervention area and there was a significant effect over time on the nutritional indicators, with children in the intervention area having better improvements in weight for age, weight for height and height for age scores. There was no difference in Hb concentration or anaemia prevalence between the intervention and control area after 6 months but there was evidence for a protective effect of the bednets. Children in the control area living within 300m of the net distribution zone had a significantly higher Hb concentration and half the chance of being anaemic at round 3 than children who lived >300m of this zone, while there was no difference at enrolment.

The intervention was monitored for only 6 months after which the control area also received nets. The short period might be responsible for the fact that no differences of Hb concentration could be observed between intervention and control area. Intervention studies in rural areas have shown an impact on malaria morbidity indicators anaemia and Hb measured at 14 and 22 months after distribution of the nets (ter Kuile *et al.* 2003b). Another factor contributing to the reduced impact could be that although compliance was high at 95%, 41% of the carers reported at round 3 that they did not use the net every night because of heat. In the baseline studies, heat or inconvenience was given as an important second reason for not having a net, although the main reason given by Accra residents in this study was economically related, not having money to buy a net. The inconsistency in sleeping under the net could have reduced the protective effect. In the Asembo Bay trial in Kenya adherence was also mentioned as an important factor in the success of a bednet control programme

(Hawley *et al.* 2003b). In addition twenty percent of control households reported to have a bednet and this conservative factor could also have played a role in the reduced effect. However, when taking this into account in multivariate analysis ownership of a bednet was not significant. In addition malaria prevalence was much lower than found in the baseline surveys of this project (chapter 3 & 4). This low incidence substantially reduced the statistical power as the study was designed based on a prevalence around 18% which was 4.1% at enrolment of the cohort. The reduced malaria prevalence is probably linked to the much lower rainfall in 2004 as compared to previous years (chapter 6).

Malaria at round 3 was significantly associated with age group, haemoglobin level, travel to the rural area since the last visit and distance to urban agriculture. Area was not an independent risk factor and neither was bednet use. The latter is likely to be partly due to the reduced statistical power as indicated above. Having been ill since the last visit was independently associated with area at round 3. As suggested in chapter 6, travel might have become more important due to the low overall malaria prevalence in 2004. The association with distance to urban agriculture was also found in the baseline survey in Accra (chapter 3) but not in the subsequent survey in 2004 (chapter 6). A recent rapid urban malaria appraisal in Ouagadougou in Burkina Faso, found no association of malaria with a visit to the rural area in the preceding 90 days but malaria was significantly associated with having an urban agricultural land/garden near the compound (Wang *et al.* 2005a)

A large trial in Kenya reported a protective effect for people not using a net but living within 300 meter of a compound with a net due to a mass effect on the mosquito populations (Gimnig *et al.* 2003, Hawley *et al.* 2003a). In this study a similar effect was observed, a correlation between distance to the net distribution area in the control area and anaemia prevalence and Hb concentration. Children in the control area living within 300m of a household in the intervention area that had a net, had higher Hb concentrations and half the chance of being anaemic compared to children that lived more than 300m from this zone. This finding is important as it indicates that the protective effect earlier found in studies in rural areas (Lines *et al.* 1987, Hii *et al.* 2001, Smith *et al.* 2001a) is also present in the urban area. Interestingly the net coverage was relatively low at around 30% household coverage. In rural areas strong

community effects have been observed at coverage of over 50% (Hawley *et al.* 2003a). It could be that due to the higher density of people and lower density of anophelines in the urban areas a lower net coverage is needed to establish a community effect. The protective effect has important implications for policymakers as it stresses the importance of high net coverage to reach additional beneficial effects for the whole community (Hawley *et al.* 2003b).

Repeated measures analysis showed an impact of the intervention on average change in WAZ, HAZ and WHZ score. This was also observed in a large scale trial in Kenya (ter Kuile *et al.* 2003a) and stresses again the importance of reducing malaria and anaemia for child development.

To investigate an impact of the bednets on the mosquito population, light traps were set fortnightly in intervention and control areas. Although there was a difference before and after introduction of the nets in Alajo visible, with slightly lower *Anopheles* and slightly higher *Culex* densities after distribution, there was large seasonal variation with two distinct peaks before and one after distribution and this likely explains the difference. The peaks in densities are correlated with the peaks in rainfall, for *Anopheles* there is a positive correlation with rainfall 4 weeks before the survey. For *Culex* there is a negative correlation with rainfall in the two weeks preceding collection. Interestingly *Culex* densities seem to peak just after *Anopheles* densities. An explanation for this could be that during the rains *Anopheles* breeding sites are established while *Culex* breeding sites, *i.e.* drains are flushed (hence the negative correlation) and later *Culex* breeding sites are re-established or that water stagnating from the rains gets polluted. Earlier studies have found a mass effect of large scale net distribution on mosquito populations (Mbogo *et al.* 1996, Gimnig *et al.* 2003). These studies were all in rural areas so that could be of importance although there was a protective effect on anaemia visible. Therefore it is likely that the large variation between houses masked the effect on the mosquito population. High levels of resistance in especially *Culex spp.* could also have contributed (for a discussion on this see chapter 6).

Analysis of a subset of malaria positive slides showed that there was a very high prevalence of chloroquine resistance at 81.25% with 4.5% co-infection of resistance and wildtype. Resistance was slightly but significantly higher in Accra than in Kumasi.

This is in concordance with earlier studies by Afari *et al.* (1992) who also found higher levels of chloroquine resistance in the coastal savanna than the forest zone. High levels of chloroquine resistance are amongst others linked to in-proper use of anti malaria drugs. Previously it has been linked to urbanization, with increased prevalence of resistance in urban areas (Warren *et al.* 1999). In both Accra and Kumasi between 20-25% of people who recently (last 2 months) had a malaria case in their household reported to have bought over the counter drugs without prescription. High levels of chloroquine resistance have been previously reported in Ghana. Ehrhardt *et al.* (2003) found in a primary health care facility in northern Ghana that in 84% of *P. falciparum* cases the *pfcrt* T 76 codon was present. Koram *et al.* (2005) found in a study in 2003 a chloroquine cure rate of only 25% in 36 children studied. During the time of this study chloroquine was still the first line treatment in Ghana but mid 2005 Ghana changed from chloroquine to Artesunate-Amodiaquine combination therapy (ACT) as first line treatment. Household spend on average 43,000 Ghanaian cedis (4.8 US$) for prescribed malaria medication and 13,000 (1.4 US$) on over the counter malaria medication per episode (data this study). Cost of drugs will be an important factor in the decision making for parents to go for treatment and with increased costs of new ACT drugs accurate diagnosis is important. Misdiagnosis of malaria is a serious problem everywhere, but in areas of low malaria endemicity, like urban areas, presumptive treatment of all fevers as malaria can result in over 75% of cases being misdiagnosed as malaria (Amexo *et al.* 2004).

The intervention study showed that bednets have an impact on malaria and other malaria related outcome variables like anaemia and nutritional status indicators in urban Accra although a longer follow up period would probably have clearer established the impact. The highly mobile urban population makes cohort follow ups difficult in the urban setting and requires an increased sample size. Although compliance is high in urban areas people do not use their net every night when temperatures are high which likely reduces the efficacy of the nets. A protective community effect of high net coverage was observed in the urban area. This combined with the positive effect for child development stresses the need for Ghana to upscale its current low bednet use to reach beneficial health and economic impacts.

Chapter 8

8. GENERAL DISCUSSION AND CONCLUSIONS

This study assessed the impact of various risk factors, particularly urban agriculture, on malaria transmission in urban Ghana. While the findings contribute directly to an increased understanding of urban malaria in Ghana, the results are also relevant to the broader epidemiological picture of urban malaria in Africa as a whole.

8.1. Local transmission

The combined findings support the hypothesis that local malaria transmission is ongoing in urban Ghana. First, *Anopheles gambiae s.l.* were found breeding in the central urban areas and analysis of collections from Accra revealed *P. falciparum* infected females. In addition in the 2002 epidemiological baseline survey in Accra there was no link with travel to the rural area in the previous 4 weeks, suggesting local transmission. However, in Kumasi and in the 2004 epidemiological survey in Accra there was an association with travel, which was likely due to the low malaria prevalence in these surveys. When there is low local transmission the impact of travel seems more profound. The intervention study with bednets in two communities in Accra supported the hypothesis that local transmission contributes to the overall burden of malaria as the intervention had an impact on malaria and related indicators, *e.g.* Hb concentration and nutritional indicators, after 6 months.

8.2. Impact of urban agriculture

The combined results of the epidemiological and entomological surveys suggest that urban agriculture may contribute to malaria transmission. It has the potential to create breeding sites and it was associated with increased man biting rates and it had the potential to increase malaria prevalence in surrounding communities.

In the 2002 epidemiological baseline survey in Accra malaria prevalence was significantly higher (OR 1.53) in children in communities near urban agriculture (UA) than in children in communities far from agriculture (U). However, only in some communities was there a significant inverse link between distance to agriculture and malaria prevalence. That there are other important risk factors for malaria is revealed by those communities far from agriculture with very high malaria prevalence.

However the impact of UA was not consistent over time and the importance of UA as a risk factor varied between cities as in the second epidemiological survey in Accra in 2004 and in the 2002 baseline survey in Kumasi there was no difference in malaria prevalence between UA and U communities. In addition other risk factors became more important with different transmission pressure. Due to the unexpected low prevalence in the 2004 survey reduced power could have affected the results, therefore additional surveys are needed to confirm the patterns observed.

Interestingly, anaemia prevalence in Accra was lower and Hb concentration higher in UA compared to U communities in both years. Although in multivariate analysis, after accounting for data structuring, survey, age, malaria and socio-economic status anaemia prevalence was not significant (P=0.054), but the impact of UA on Hb concentration was significant (P=0.018). Farming linked to improved diet is unlikely to explain this difference, as only a small percentage of people in the communities around UA are farmers. Further investigations are needed into the reason behind the higher Hb concentration in UA communities compared to U communities. Geohelminth infection could play a role but it is not clear why children in communities around urban agriculture would have different geohelminth infections rates.

The entomological studies supported our findings of an impact of urban agriculture revealing higher man biting rates in UA than U communities for both *Anopheles* and *Culex*. *Anopheles spp.* were found breeding at farms sites although only 6% of the agricultural wells were harbouring *Anopheles* and also in the normal housing areas breeding sites could be found. Due to the low percentage of wells being positive the creation of breeding sites might not completely explain the impact of UA. In addition, we observed the very low indoor man biting rates (obtained by PKD) when compared to outdoor biting rates (obtained by HBC), this combined with the breeding capacity of the farm areas could suggest that apart from providing breeding sites, urban agriculture might be important in providing resting sites for the mosquito population. This hypothesis is supported by the fact that in the 2004 survey, when malaria prevalence was much lower due to lower rainfall, there was no difference between UA and U communities in malaria prevalence. As breeding was reduced due to reduced rainfall, there were less mosquitoes around and the use of farm areas as resting sites might not have been profound. Therefore link with agriculture was likely masked by the reduced

breeding due to reduced rainfall. The resting hypothesis was also supported by the observation that the community of Korle Bu showed an inverse relation between distance to UA and malaria prevalence while no *Anopheles spp.* but only *Culex spp.* were found breeding in the drain water filled wells in Korle Bu farm. However this resting hypothesis needs to be investigated further using for example window exit traps to confirm if *Anopheles* are biting indoors and resting outside or are biting and resting outside. The first is more likely as the peak biting time was around 2-3 am when people are in general asleep inside their houses. In addition it should be investigated if the crops or the surrounding vegetation, e.g. grasses, shrubs, trees, are used as resting sites.

What also needs to be taken into account is that the urban agricultural sites are ecologically distinct from the surrounding rural areas due to the cultivation of crops and greenness of the areas. Furthermore in Kumasi the farms are located in low lying areas with low water tables and the specific location of farm sites could also add to the increased mosquito presence in these areas and that it could be that it is not the farming as such which creates the risk but the greenness or rural character of the farm areas. This aspect needs further investigation, although the design of such a study might prove difficult as the setting of UA is constrained by the availability of suitable areas.

Although only about 6% of the wells were found breeding *Anopheles spp.* and more extensive studies are needed, water quality of the agricultural wells seemed important in determining breeding capacity as significantly more *Anopheles* were found in wells filled with piped water and in wells without foul smelling water as compared to wells with a foul smell. *Culex spp.* preferred wells with a higher average electrical conductivity and with a foul smell as opposed to a non foul smell.

There is a need to carry out longitudinal studies to further investigate the effect of annual variation on risk factors and malaria prevalence. In addition similar studies should be conducted in other cities to confirm the association with urban agriculture found in this study and to confirm if similar risk factors are observed in other urban settings.

8.3. Risk factors for malaria and anaemia

Apart from the observed association with agriculture (see 8.2), several other potential risk factors were identified. Malaria prevalence was associated with socio-economic status (negative), age (positive) and Hb concentration (negative). In Kumasi and the 2004 Accra survey, travel was an independent risk factor. In addition, in Kumasi males had a higher risk for malaria (OR 4.5), while children that had taken malaria medication in the last 2 weeks had a lower risk (OR 0.24). Risk factors for anaemia were low socio-economic status and younger age. In addition malaria parasitaemia was a risk factor for moderately to severe anaemia. We calculated the attributable risk for anaemia due to malaria which ranged from 1.7 to 5.7% in the three surveys. For moderately to severe anaemia this ranged from 14.3 to 23.5%. This confirms that also in the urban setting malaria is an important contributor to moderate to severe anaemia.

Lower socio-economic status was an independent risk factor for both malaria parasitaemia as well as anaemia indicating that specifically the urban poor are vulnerable. With rapid urbanization this group is likely to quickly expand and control efforts should be targeted at this and other vulnerable groups like pregnant women and children. In the poorest community in Accra more than 75% of the children were anaemic and 10% moderately to severe anaemic. The intervention study had an impact on nutritional indicators highlighting the potential benefits of anti-malaria interventions on child development.

The results of the epidemiological baseline survey in Accra suggested that older children may be at higher risk. A similar study by Ronald (2005) in Kumasi found similar evidence as 5-9 year olds had higher malaria infection risk than 0-4 year olds (OR 4.1). Higher risk in the older age groups has important implication for control as the vulnerable child groups targeted by malaria control programmes are under fives, in the urban setting this might have to be expanded till 10 years.

The bednet intervention showed an impact on malaria prevalence, frequency of falling ill and nutritional indicators after 6 months and showed the ready acceptance and usage of ITNs by the population. The unexpected low malaria prevalence in the intervention study year severely reduced the power of the study and further studies are needed to confirm the findings. An apparent community effect was observed as children in the control area living within 300m of a household in the intervention area with a bednet

had a higher Hb concentration and half the chance of being anaemic as children living more than 300m from a household with a bednet. This observed community effect has previously only been observed in the rural setting (Hii *et al.* 2001, Hawley *et al.* 2003a) at bednet coverage rates around 60-80% and not at the lower rate of around 30% household coverage at which we observed the community effect in this study. It could be that due to the higher population density in urban areas, a community effect is observed at lower coverage levels although further studies are needed to confirm this. The community effect stresses the need for wide scale use of bednets to enhance benefits for the whole urban community. We observed the apparent community effect by targeting only specific vulnerable groups, e.g. pregnant women and children under 10 years old. Although further studies are needed to confirm the observed findings, if a community effect can be created by only targeting these groups this would have important beneficial implications for malaria control policies and programs.

8.4. Limitations of the study

The study revealed a number of risk factors for urban malaria in Ghana. How broadly applicable to West Africa or Sub Saharan Africa these finding are needs to be further investigated. The fixed location of the agricultural sites and the fact that there are only a few main agricultural sites limited our selection of study communities and sites could not be randomly selected but were linked to the fixed location of the farms. As there was little previous information on urban malaria prevalence we adopted a baseline inventory using cross-sectional surveys in under five year olds. As a lot of information on the areas is now available it would be good to map all the houses and do randomized selection of households and children in future longitudinal surveys. Such studies should be extended to include older age groups as our results indicate that in the low urban transmission zone they might also bear a substantial part of the malaria burden.

For the interventions study we choose for logistic reasons to follow two groups of 250 randomly selected children in two selected communities instead of following a birth cohort. A randomized control trial might have been preferable but the neighbouring location of our two intervention areas had the advantage of very comparable local conditions and gave us the opportunity to assess presence of a community effect of the nets. However this effect together with the reduced power due to the low malaria

prevalence in 2004, made it more difficult to observe significant differences between the control and intervention areas. We had only 6 months for follow up studies, a longer period would have helped to assess longer term implications of net use, compliance to nets and effectiveness of the nets over time.

8.5. Policy implications

This study highlighted urban risk factors and details on the impact of urban agriculture on malaria transmission for two Ghanaian cities. The results show that there was an impact of urban agriculture on malaria although this was not consistent in both cities and in both years of survey. This implies that based on the present results, municipalities should not ban agriculture for its potential to increase malaria. Although there was a significant impact in the 2002 survey in Accra, the additional risk due to agriculture was only small and there were communities without agriculture that also had very high malaria prevalence. Therefore municipalities should focus on control methods with proven overall impact, like e.g. ITNs instead of targeting only farm areas. Our intervention study showed that ITNs are a readily accepted tool for control with a demonstrated community effect. Larval control could be added in certain areas, although breeding sites are transitory and a large and consistent community effort would be needed. For the farm areas additional measures could be taken to minimize breeding in the irrigation structures and further studies are needed to investigate the role of the farm areas in providing resting sites. Urban farming also has clear proven benefits in terms of food security and provision of jobs and livelihoods, therefore farming should be promoted but care should be taken to minimize any potential anopheline breeding; farmer field schools could assist in this.

The current rapid urbanization process makes insight into risk factors for urban malaria transmission urgent as there is rapid, often unplanned, expansion and this could alter urban malaria epidemiology. Our study contributed to this by highlighting several risk factors and indicated that the urban poor are at highest risk. Our findings further suggest that urban malaria transmission can be readily ameliorated by proven control techniques that are readily accepted like ITNs which despite lower prevalence and therefore problems of statistical power had a demonstrable effect. The impact of urban agriculture on malaria transmission was inconsistent and additional studies are needed to investigate this further and establish the impact in other African cities.

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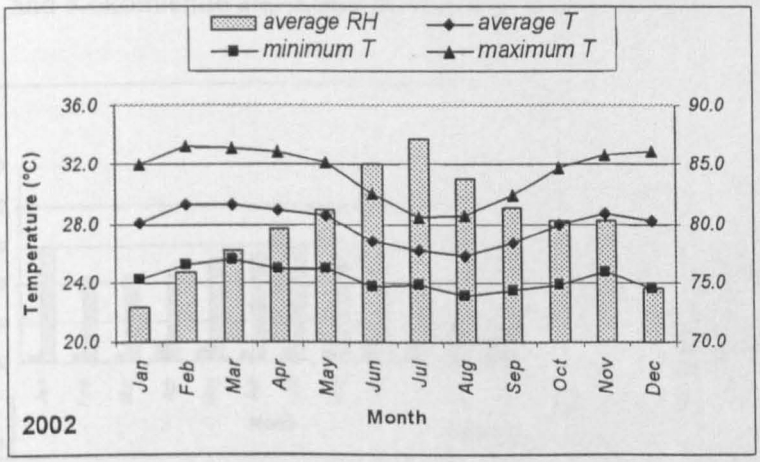
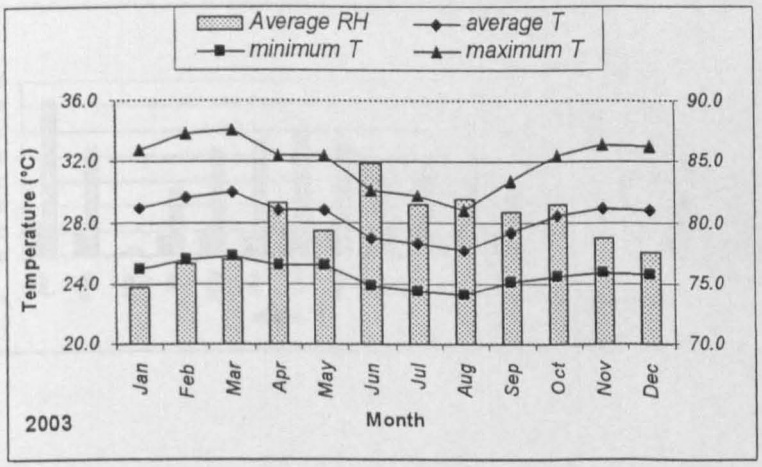
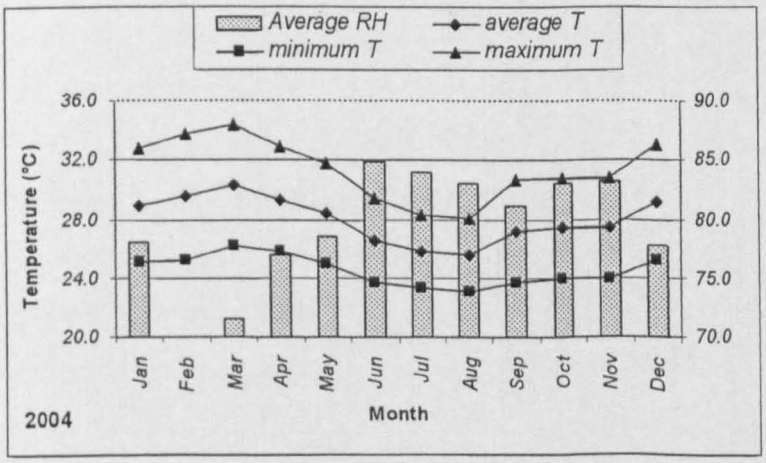
APPENDICES

Appendix 1. Monthly temperatures and humidity for 2002-2004

Monthly average, minimum and maximum temperatures and relative humidity for the years 2002-2004 as reported at Water Research Institute meteorological station (WRI, unpublished).

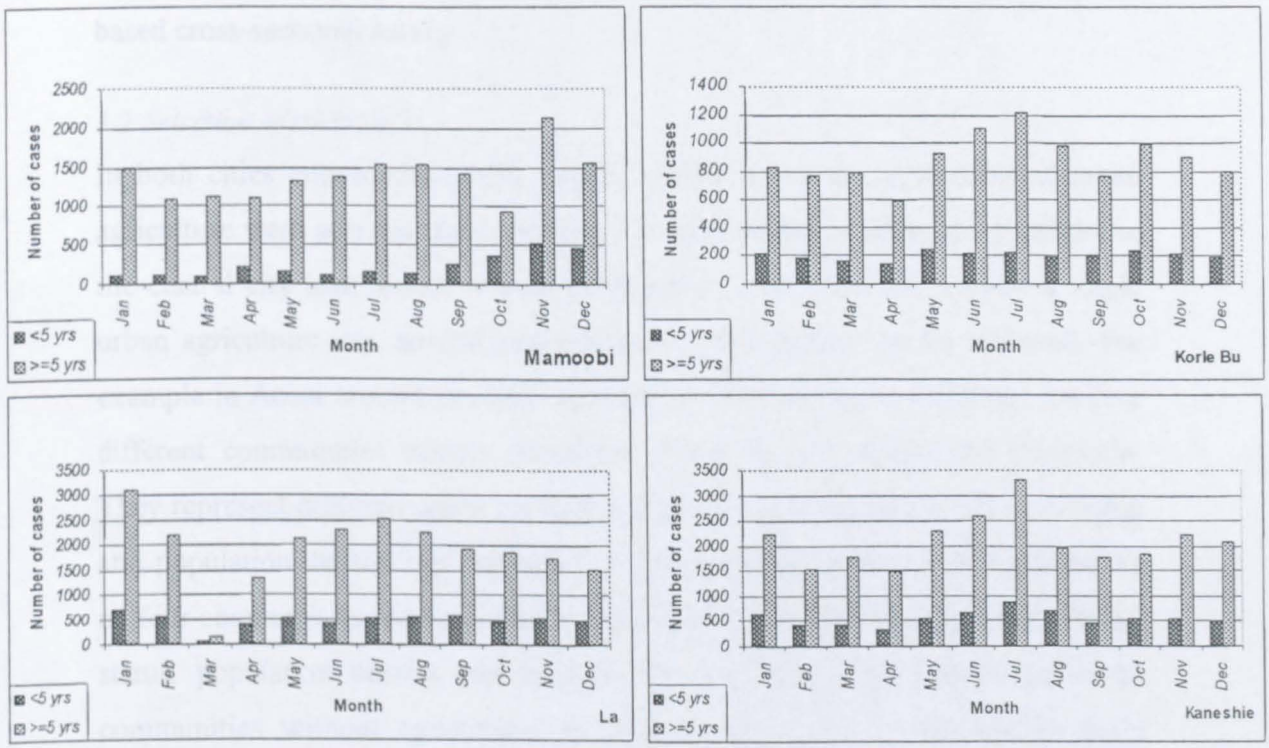






Appendix 2. Monthly malaria cases in Accra in 2003.

Monthly malaria cases in under and over fives reported in Mamoobi, Korle Bu, La and Kaneshie government polyclinic in Accra in 2003. The health area of La polyclinic covers the study site of La/Laboni, Kaneshie polyclinic covers Kaneshie, Mamoobi covers Kotobabi, Alajo, Airport, Roman Ridge, Dzorwulu and Kokomlemle and Korle Bu covers Ushertown and Korle Bu.



Appendix 3. Material and methods epidemiological baseline study

3.1. Design

There were no recent prevalence data available for urban Ghana, so an initial epidemiological and socio-economic baseline study was carried out to assess the malaria prevalence and investigate risk factors and malaria knowledge and practice in selected communities (with and without agriculture) in the two major cities of Ghana, Accra and Kumasi. This first phase was designed as a community based cross-sectional survey.

3.2. Selection of study sites

In both cities communities with varying proximity to the main sites of urban agriculture were selected. Each city has a number of major areas of agriculture in the central city area around which communities were selected. Around a single urban agriculture site, several administrative communities can be clustered. For example in Accra around the main agriculture area (see figure 3.1) there are four different communities namely Kotobabi, Alajo, Roman Ridge and Dzorwulu. They represent different urban settings in terms of socio-economic status, housing and population density representative of typical communities in Accra therefore all four communities were included. Communities comparable in terms of housing status, population density and level of development, were selected as urban communities without agriculture. In Accra a total of 12 communities were selected, 7 communities around urban agriculture (UA) and 5 far from agriculture (U). In Kumasi a total of 11 communities were selected, 5 UA and 6 U (figure 3.1). Our community names may not fully correspond with the official administrative areas as houses for UA communities were selected within 1000m of UA, based on the likely maximum flight distance of female mosquitoes (Service 1997), and therefore not the total area of the community was always included. U communities were located at least 1000m from agriculture. Figure 3.2 present a flow diagram of the process of selection of communities and household in the two selected cities.

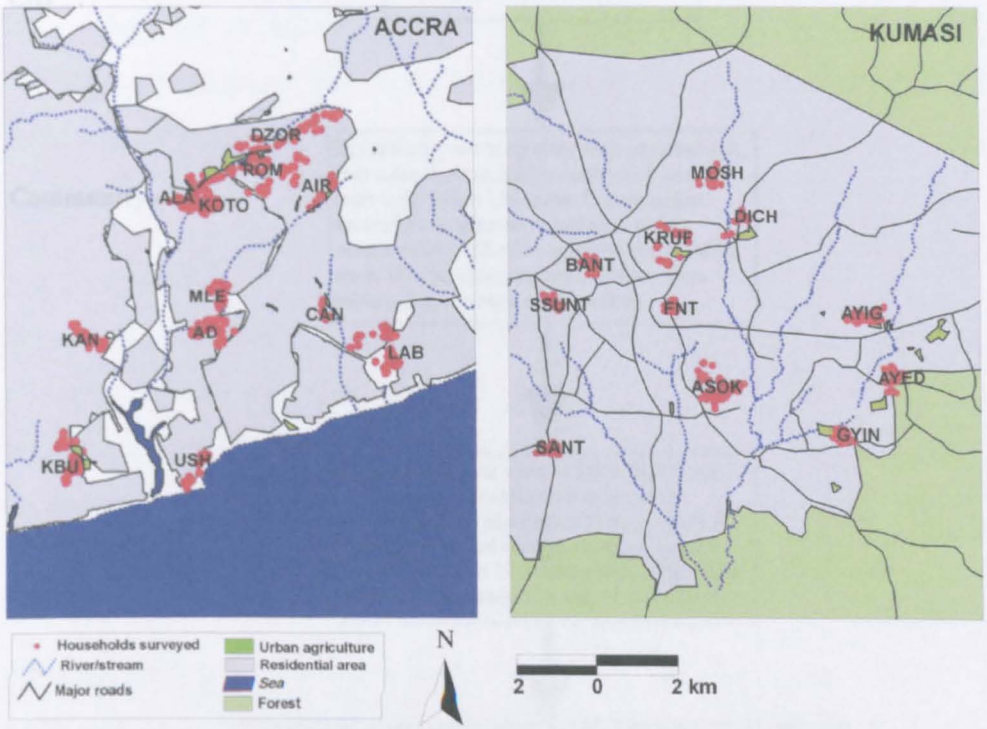
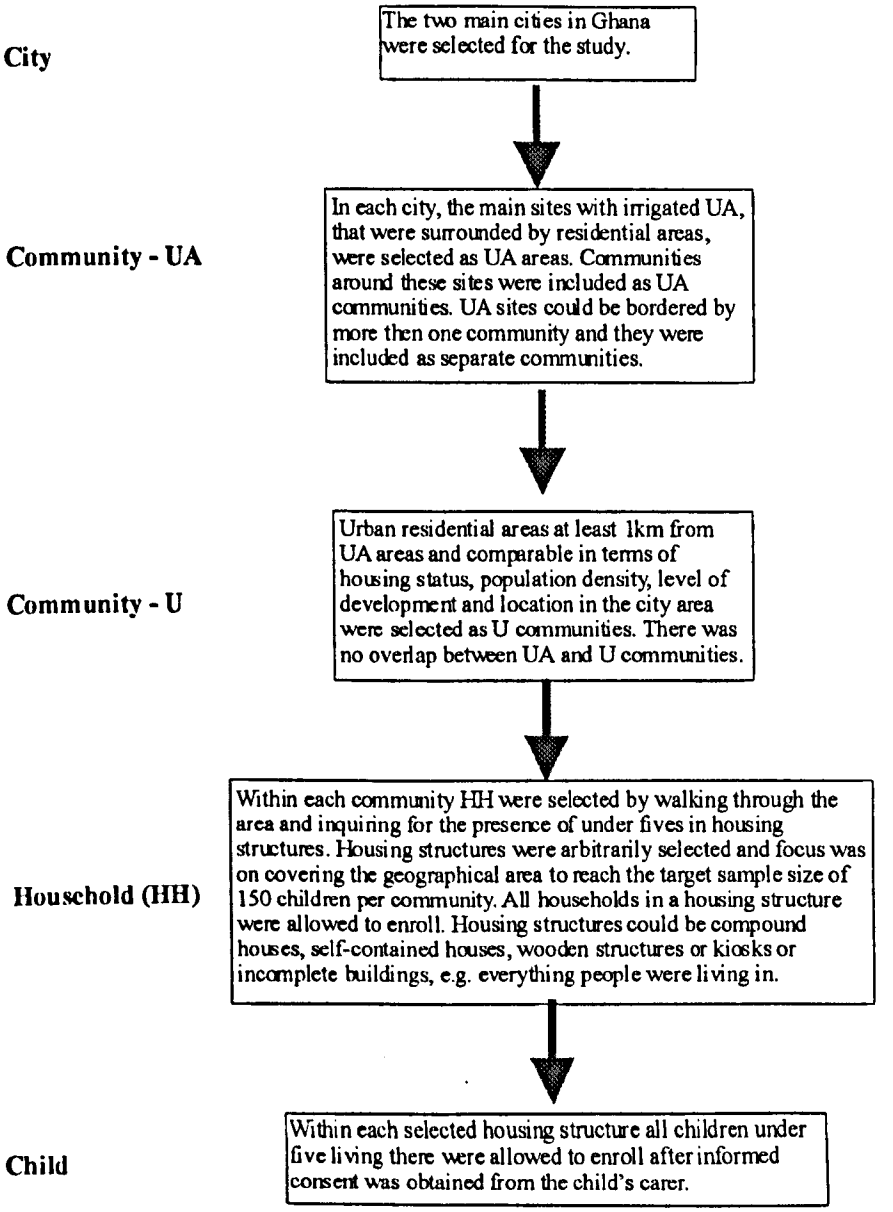


Figure 3.1 Location of the households included in the survey for the city of Accra and Kumasi (Details for individual communities are given in table 3.1)

Table 3.1 Details of communities sampled in the two cities.

City	Code	Community	Type
Accra	AIR	Airport	UA
Accra	ALA	Alajo	UA
Accra	DZOR	Dzorwulu	UA
Accra	KBU	Korle Bu	UA
Accra	KOTO	Kotobabi	UA
Accra	ROM	Roman Ridge	UA
Accra	CAN	Cantonments	UA
Accra	MLE*	Kokomlemle	UA/U
Accra	AD*	Asylum Down	UA/U
Accra	KAN	Kaneshie	U
Accra	LAB	Labonie/La	U
Accra	USH	Ushertown	U

City	Code	Community	Type
Kumasi	ASOK	Asokwa	U
Kumasi	AYED	Ayeduase	UA
Kumasi	AYIG	Ayigia	UA
Kumasi	BANT	Bantama	U
Kumasi	DICH	Dichemso	UA
Kumasi	FNT	Fanti New Town	U
Kumasi	GYIN	Gyiniasi	UA
Kumasi	KRUF	Krofrom & Manhiya	UA
Kumasi	MOSH	Moshie Zongo	U
Kumasi	SANT	Santasi	U
Kumasi	SSUNT	South Suntreso	U



UA=(irrigated) urban agriculture; U=urban (control), e.g. Without UA; HH=household

Figure 3.2 Flow diagram of the process of selection of communities and household in the two selected cities

3.3. Timing of study

The epidemiological and socio-economic baseline study was carried out between 21 October 2002 and 23 January 2003 in Accra and between 14 December 2002 and 13 February 2003 in Kumasi. The study started slightly later in Kumasi due to a delay in obtaining the ethical clearance.

In Accra a second epidemiological survey was carried out in 8 of the 12 selected communities namely Kaneshie, Ushertown, Korle Bu, Alajo, Roman Ridge,

Dzorwulu, Airport and La, between 5 October and 26 November 2004 to investigate consistency and seasonality in malaria prevalence patterns and risk factors found in the first survey. The results of this second survey are discussed in chapter 6.

3.4. Sample size

Winepiscope (version 2.0) was used to determine sample size. The design focused on communities with and without agriculture therefore they were the two population groups investigated. However as different communities were investigated per group and different households in each community, the data are structured and this has to be taken into account. Therefore the calculated sample size was doubled to account for data structuring. Gardiner *et al* (1984a) reported for the community of Jamestown in Accra a malaria parasite prevalence rate of 1.6% and Afari *et al* (1992) reported a 18% prevalence rate in the community of Nima, Accra. Based on this and research in urban Ouagoadougou in the neighbouring country of Burkina Faso (Sabatinelli *et al.* 1986) and other African cities (Vercruysse *et al.* 1983, Trape 1987b) prevalence rates of around 10-15% for urban areas were expected. We anticipated an increased risk in communities around agriculture sites and estimated rates of about 20-25% for these areas. With a confidence interval of 95% and power of 90% this leads to a sample size of about 333 persons per group, multiply by two for data structuring yields a sample size of 666 per group. As the prevalence was an estimate and smaller difference in prevalence between UA and U would clearly increase the sample size, we targeted for a sample size of 750 per group, totalling 1500 children per city. With about 10 communities per city (5 in each group) the target sample size was about 150 children per community. Children from 6 months to five years were included in the study, as over five years of age the effect of acquired immunity will influence parasitaemia rates and under 6 months of age maternal antibodies would also influence parasiteamia rates (Snow & Gilles 2002). An important part of the study was to capture the children in their home environment to assess characteristics of their housing and geographical location therefore house to house visits were performed and not a school based survey.

3.5. Household Selection

Accurate census data do not exist for the study communities. Therefore in each community, households were selected by inquiring for the presence of children under five years old and emphasis was made on covering the whole geographical area of the community, to limit potential bias resulting from spatial heterogeneity. The target sample size was 150 children per community and if every house had children under the age of five not all compounds were surveyed as the focus was on geographical coverage of the selected area. Houses can be single structures or compound houses where more households live together often sharing toilet and water facilities. When a compound house was visited, all households in the compound were allowed to enrol to avoid bias resulting from sampling the most keen to enrol households and in each household all children under five were allowed to enrol to avoid bias resulting from parental selection.

For the communities around urban agriculture we aimed to sample houses within 500 m of the urban agricultural sites. This proved difficult in practice as building is poorly regulated. The average distance from urban agriculture of households sampled was 520m (SD 399) and 470m (SD 183) for UA households, and 2264m (SD 280) and 2306m (SD 964) for control households in Accra and Kumasi respectively. There was no overlap between sites. The range of distances sampled allowed for gradient analysis to investigate the impact of distance from agriculture on malaria prevalence.

3.6. Household visits

Access to the community was through their elected representative, the assembly man. On the first days of the survey a local community person often joined the field team. In each house visited the objective of the survey was clearly explained in the local language. Before a child was enrolled informed consent by signature or thumbprint was obtained from the main caretaker who had to be older than 15 years of age. From each child enrolled in the study a blood film was made by finger prick (see below) and haemoglobin level (see below) was measured (see figure 3.3). Following government guidelines, children with malaria and/or Hb<8.0 g/dL were given a voucher for free treatment at a local government clinic

(Accra) or treatment was brought to their homes (Kumasi). Age (verified from child health cards when available), sex, antimalarial use in the preceding two weeks, history of fever in the past 48 hrs and travel to a rural area within the preceding three weeks (reported by the carer) were recorded for each child. To obtain information on socio-economic and housing characteristics, malaria knowledge and prevention measures, in each household a questionnaire was conducted with the main caretaker in the local language (see 3.9). Socio-economic status was adapted from a Worldbank study for Ghana based on asset scores (www.worldbank.com/hnp). In each house the geographical co-ordinates in latitude longitude were taken with a handheld global positioning system (GPS, Magellan, UK).

Before the survey started enumerators were trained in taking the questionnaire. The questionnaire was written in English and was, depending on the language of the enrolled household, translated by the enumerators into e.g. Twi, Ga, Ewe the main local languages in the study area. Trained technicians from the Noguchi Memorial Institute for Medical research and the Komfo Anokye Teaching hospital took the bloodfilms and measured haemoglobin concentration.

All bloodfilms were taken following the standard WHO protocol as detailed by Gilles (1993b). Before taking the bloodfilm each slide was labelled with a child specific identification number, consisting of area, household number and child e.g. KOTO-1-c1 for the area of Kotababi, household 1, child 1 and KOTO-1-c2 for the second child in the same household. Slides were stained in the laboratory using Giemsa stain after return from the field. Slides were read within 2 days by trained microscopists and the results communicated to the field team for follow up. Slides of children with suspected malaria at the time of sampling from reported history of illness/fever by the mother where marked and read first by the microscopist for rapid follow up. If children were ill during the house visit parents were advised to take the child to hospital. Parasite density was estimated by counting the number of fields positive for parasites in 100 high power fields (hpfs). High density parasitaemia was defined as individuals with 100% hpfs positive (Coosemans *et al.* 1994). Haemoglobin concentrations were measured using a HEMOCUE device

(Hemocoue, Sweden). A drop of blood was applied to a cuvette which was then read in the Hemocue and the results noted. The Hemocues were calibrated each day and were cleaned at the end and start of each survey. Quality control of slides in each city was carried out by cross reading of 100 slides by the laboratory in the other city. Slides that differed were cross-checked by a third person and the two most similar readings were taken as result.

Figure 3.3 Technicians taking malaria blood films in different communities in Accra, Ghana. A. Taking of bloodfilm by technician. B. Measuring haemoglobin using the hemocue, C. Taking of bloodfilm and interview of carer, D Labelling slides to prepare for taking of bloodfilms.

3.7. Mapping of agricultural areas

There was no accurate map from the UA areas therefore all sites were mapped using a handheld GPS (Magellan, UK). Mapping was done by walking around the perimeters of the agricultural sites and taking GPS points at every change in circumference boundary. Points were taken in latitude longitude with WGS84 datum. All points were plotted using Arcview (version 3.1). By connecting individual points using a polygon shape tool, shapefiles for each UA area were

created. Arcinfo (version 7.2.1) was used to calculate the shortest distance from each household to the nearest UA area. By creating buffers of different distances, e.g. 300, 500, 750 & 1000m, different groups of households were selected that where within a specific range of the UA areas (figure 3.4).

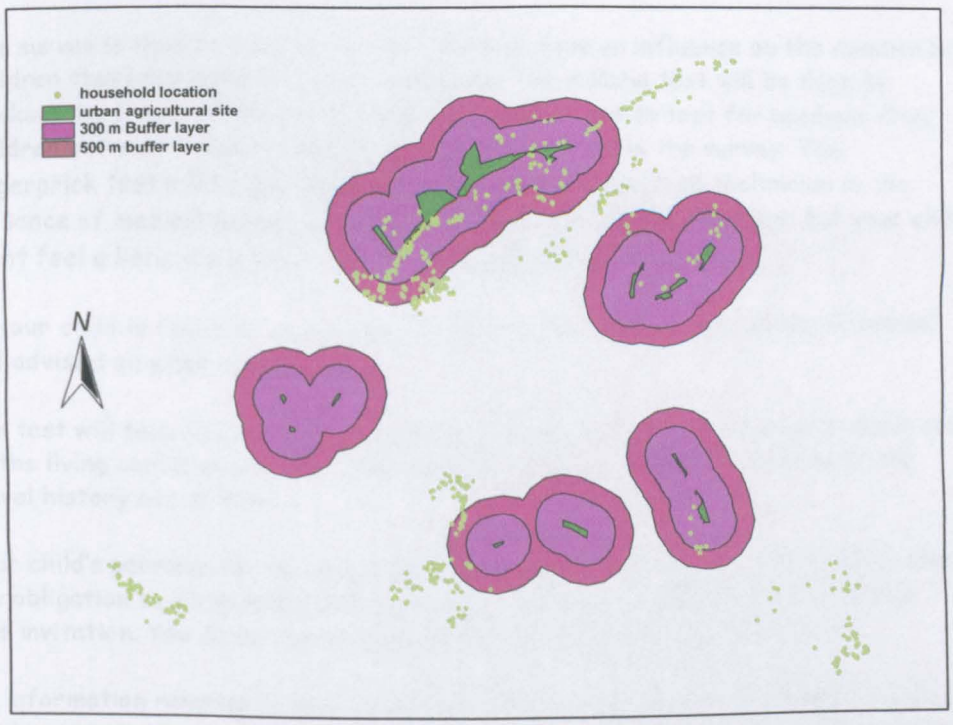


Figure 3.4 Map of part of UA areas and households enrolled in the study in Accra with buffers of 500 and 300m around UA

3.8. Information sheet, consent form and questionnaire

The following pages give the information sheet, consent form and questionnaire as used in the survey.

URBAN MALARIA STUDY ACCRA - INFORMATION SHEET

Your child is being invited to participate in a research on urban malaria in Accra. The study is carried out by the Noguchi Memorial Institute of Medical Research (NMIMR) of University of Legon, Accra and the International Water Management Institute (IWMI) of the CSIR Campus, Accra in collaboration with the Liverpool School of Tropical Medicine

The survey is done to investigate which factors have an influence on the number of children that have malaria in your community. The malaria test will be done by pricking the finger of the child to make a bloodfilm and to test for anaemia. Only children between 6 months and 5 years will be included in the survey. The fingerprick test will be performed at your home by a qualified technician in the presence of medical personnel. The tests carry little risk of infection but your child might feel a little discomfort as the finger is pricked.

If your child is found to be anaemic or positive for malaria you will be informed and advised on what steps to take.

The test will take about 15 minutes and in addition you will be asked some questions on the living conditions of the child, usage of mosquito preventive measures and travel history out of town.

Your child's participation in the programme is entirely voluntary. You are not under any obligation to allow your child to participate and you have the right to refuse this invitation. You do not have the obligation to answer all questions asked.

All information relating to your child's participation will be kept confidential and will not be revealed to anyone except were required by law and regulations. The address and child name will be only used to be able to inform you if your child would be found to have malaria or anaemia. Your child's identity will not be revealed in any reports or publications resulting from this study. Dr. Wilson of Noguchi Memorial Institute and Ms Klinkenberg of IWMI or other members of the team will be available for questions or clarifications regarding the study.

Contact details:
Dr. Wilson, Noguchi Memorial Institute, tel. 021-500374
Ms. Klinkenberg, IWMI, tel. 021-784753

URBAN MALARIA STUDY
CERTIFICATION OF VERBAL INFORMED CONSENT

I .....

of .....

hereby certify that the contents of the information sheet have been communicated to:

.....

of..... HH no.

I also certify that the volunteer has fully understood the contents of the document and has willingly given consent to participate in the research.

Signature..... Signature/Thumbprint.....

Date: Date:

(Study coordinator) (head of household/caretaker)

QUESTIONNARE - BASELINE MALARIA SURVEY ACCRA- GHANA

(Code for CLOSED questions: 1 = Yes; 2 = No; 8 = Don't know; 9 = No answer).

Date .....interviewer: ID No |__|__|__|__|__|__|__|__| (type code, HH id)

GPS_CODE: N W

---

1. How many people sleep in this HH? | | hhmemb
How many children are between 0 and 5 years of age? | | hhmeu5
How many children are between 0 and 10 years of age? | | hhme10

2. a. Housing type? Compound [ ] self-contained [ ] storey building [ ]

Wall type mud [ ] brick-blocks [ ] wood [ ]
Roof tiles [ ] sheets [ ] slates [ ] thatched [ ]
Ceiling yes [ ] no [ ]
Floor cement [ ] wood [ ] earth [ ]
Flooring cover nothing [ ] tiles-terazo [ ] carpet [ ] vinyl [ ] others, specify

b. How many bedrooms do you have in your house? | | hhrooms

c. What is the source of drinking water for your household?
Piped water [ ] private well [ ] public Well [ ] public tap [ ] public borehole [ ] tanker/truck [ ] river/stream [ ] Others (specify).....

d. What toilet facility do you use?
Part 1. Private [ ] public [ ] shared [ ]

Part 2. WC [ ] pit latrine [ ] VIP latrine[ ] bucket latrine [ ] Free range [ ]
Others, specify.....

3. Do you have any of the following items?
(Code Income ranking: 0=High; 1=Middle ; 2=Low) | | Income

Item	Present yes/no
Electricity	
Bicycle	
Motorcycle	
Radio	
Television	
Car	
Fan	
Fridge	
AC	

b) Do you have a domestic worker not related to the head of the household?
(1=Yes; 2=No; 8- Don't know) | | Dom

4. Have you ever attended school?
If YES, what was the last grade you completed? | | Edu

(0=Not attended/illiterate; 1=primary; 2=JSS/MLC; 3=SSS/A'level; 4=graduate/certificate)

5. Is malaria a problem for your household? (1=Yes; 2=No; 8- Don't know) | |
Mal

6. How can you get malaria?.....

7. Mentioned mosquitoes (1=Yes; 2=No; 9=No answer) | | *Mos*

8. Whom does it affect most? (1=All; 2=Adults; 3=Child; 8=Don't Know; 9=No | |
Affect

9.How do you know someone has malaria?

	Hot head		Weakness	
	Hot body		Lethargic	
	Diarrhoea		Muscle pain	
Code for these:	Dizziness		Joint pain	
1 = Mentioned	Cough/chest		Head ache	
2 = Not mentioned	Coma		Sleepy	
	Death		Can't sleep	
	Vomiting		Body pain	
	Yellow eyes		Cold sweat	
	Constipation		No appetite	
	Other		Don't know	
	Fever		yellow urine	

10 Can you prevent yourself (or your household) from getting malaria? | |

if yes, how?

MOSQUITOES:

11. Where do mosquitoes come from?.....| | Breed

12. What time of day do they mostly bite? (1=Day; 2=Night; 3=All day) | | BiteD

13. What time of year do they mostly bite? (1=Wet; 2=Dry; 3=All year) | | BiteY

14. Do you know of any other diseases transmitted by mosquitoes?

15. Do you sit outside during the evenings | | Outside

16. Are mosquitoes a problem for your household? (1=Yes; 2=No; 8- Don't know) | | Mprob

17. Do you protect yourself from mosquitoes? (1=Yes; 2=No; 8- Don't know) | | Prot

a. If NO ask why not? [ ] not necessary [ ] expensive [ ]inconvenient [ ] don't know how
Other, specify.....

b. If YES fill table:

Trap doors/ netting for windows		All or only bedroom?	
Bednet		Who sleeps under?	
	Please ask to see net, status, impregnated		
Coils		how often used and how many	
Burn herbs		How often?	
Spray		How often?	
Others		Specify	

Check if any of above mentioned are in the house.
(1=Yes; 2=No; 9=Not checked. | | *Check*

18. IF NO USE OF BEDNET ASK (check 17a NO or in table no use of net!)
b. Why do you not use a bednet?

c. If mentioned costs in b. please ask: Are there apart from costs other reasons for not using bednets?

d. Do you know where you can obtain a bednet? Where?

e.. If you were given a bednet, who would sleep under it?

19. Have you, or anyone in your house, had malaria recently? | | Rmal

b. If Yes, How do you know it was malaria? | | Howmal
[ ] symptoms tell [ ] doctors judgement [ ] other, specify

c. What did you do? [ ] to clinic [ ] bought drugs [ ]nothing [ ] other, please specify

d. If to clinic was a bloodfilm made? (yes/no) | | BF

e. If drugs what did you take? | | Drug
(1=Chloroquine; 2=Fansidar; 3= Paracetamol; 4=Artesunate; 5=Other, specify 3 None; 8=Don't know)

f. Where did you got the drugs?

On doctors receipt at clinic/pharmacy [ ] At pharmacy without receipt [ ] | | DrugP

Others, specify.....

Area HH-id Plot no.

To be filled per child:

Child id no. Child name (full):

Age: (checked from card yes/no) sex:

Did the child receive malaria treatment in the last 2 weeks?

Does the child have a history of fever?

Did the child travel to the village in the last 3 weeks?

HB level: BF taken (check) Whatman paper taken (check)

Remarks:

Child id no. Child name (full):

Age: (checked from card yes/no) sex:

Did the child receive malaria treatment in the last 2 weeks?

Does the child have a history of fever?

Did the child travel to the village in the last 3 weeks?

HB level: BF taken (check) Whatman paper taken (check)

Remarks:

Child id no. Child name (full):

Age: (checked from card yes/no) sex:

Did the child receive malaria treatment in the last 2 weeks?

Does the child have a history of fever?

Did the child travel to the village in the last 3 weeks?

HB level: BF taken (check) Whatman paper taken (check)

Remarks:

Name field technician:

Appendix 4. Forms larval survey

Larval surveys – agric wells -ACCRA

DATE		COM		well no	

GPS	N		W	

LIGHT	Exposed	Shaded	Exp-shaded

WATER QUALITY	C	T	F

FLOWING	STANDIN

Water level	high	low

Water supply	Pipe	Furrow	drain	

TIME		EC				pH				TEMP			

VEGETATION:	In site	Around site	Non
SIDES WELL	Plain Earth	vegetation	

FAUNA	backswimmer	Water beetle	Dragonfly	Water scorpion

MOSQUITO	Y	N	*ANOPHELES*	Y	N	*CULEX*	Y	N	*AEDES*	Y	N
NO. DIPS			number			number			number		
Species (to be filled after identification)											

REMARKS:

Larval inventory survey -ACCRA

DATE		COM		Site no	

GPS	N		W	

Habitat characterisation (short description):

NATURE	Temp	perm		Man made	natural
AREA	(m2)				

VEGETATION:	In site	Around site	Non
SUBSTRATUM	Earth	cement	Other, specify

LIGHT	Exposed	Shaded	Remark:

WATER QUALITY	C	T	Foul: YES./NO	FLOWING	STANDING

Remark:

TIME		EC				pH				TEMP			

FAUNA:

MOSQUITO	Y	N	*ANOPHELES*	Y	N	*CULEX*	Y	N	*AEDES*	Y	N
NO. DIPS			number			number			number		
Species (to be filled after identification)											

REMARKS:

Appendix 5. Protocol for *Anopheles* species identification by PCR

After Scott *et al.* (1993)

Mastermix (25µl reaction per tube):

DNA template	1.0µl
10x buffer	3.0µl
2.5mM DNTP	1.0µl
10mM Primers	1.0µl each (UN, GA, AR, ME)
Taq	0.125µl
distH2O	15.875 µl

PCR conditions

94°C, 5 min.
30 cycles [94°C, 30 sec.; 50°C, 30 sec.; 72°c, 30 sec.]
72°C, 10 min.
4°C, forever

Specificity	PrimerSequence (5'-3')	Tm (°C)
Universal	UNGTG TGC CCC TTC CTC GAT GT	58.3
A. gambiae	GACTG GTT TGG TCG GCA CGT TT	59.3
A. melas/merus	METGA CCA ACC CAC TCC CTT GA	57.2
A. arabiensis	ARAAG TGT CCT TCT CCA TCC TA	47.4

Species	Fragment size (basepair)
A. gambiae	390
A. melas	466
A. arabiensis	315

Appendix 6. ELISA protocol and solutions

Sandwich ELISA for sporozoite infection rate

Protocol after Wirtz (CDC pers.com) adapted from (Burkot et al. 1984, Wirtz et al. 1985).

Head and thoraxes of mosquitoes where grinded in 50µl BB:IG-630 and pestles rinsed with 200µl BB making a total volume of 250µl. Samples were stored at -20°C till processed. ELISA plates (96 micro-wells, Nuncon, Denmark), were coated with 50µl of Mab-capture and plates where incubated for 0.5hrs at room temperature or overnight in at 4°C. Plates were aspirated and 200µl of BB was added per well and the plate was incubated for 2 hrs at room temperature. Plates were aspirated and 50µl of mosquito triturate was added in each well and one positive and one negative control was added to each plate. Plates were incubated at room temperature for 1 hour after which plates were washed twice with PBS-Tw20 after which 50µl of Mab-peroxidase was added to each well and plates where incubated for an hour at room temperature. Plates were washed three times with PBS-Tw20 and 100µl of peroxidase substrate solution. Plates were read 30 minutes after adding substrate in an ELISA plate reader at 405nm.

Bloodmeal identification by direct ELISA

Protocol after Beier (1988)

Abdomens of fed mosquitoes were squashed on filter paper, labeled and stored on silica gel. In the lab the area encompassing the squash was cut out of the filter paper and placed in a labeled tube to which 500µl of PBS was added and left overnight at 4°C to elude the blood. The next day 50µl of insect extract was added to the wells of 96 well plate (96 micro-wells, Nuncon, Denmark). The plate was covered and hold for 3 hours at room temperature. The plate was washed 3 times with PBS-Tw20 and 50µl of anti-host IgG (gamma antibody, e.g. human (SIGMA, UK) diluted 1:250 with BB was added and incubated for 1 hour at room temperature. The BB mixture also contained serum of all potential hosts in the area (goat, chicken, horse, bovine, porcine, dog, (SIGMA, UK) in a 1:500 dilution. Apart from human test were also carried out for dog, goat, chicken and bovine, for each test the BB solution contained the IgG (1:250) of the potential host being tested with serum (1:500) of all other potential hosts. After the 1 hour incubation plates were washed with 3 times with PBS-Tw20and 100µl of premixed peroxidase substrate was added per well and plated were read visually after 1 hour. Positive and negative controls were added to each plate.

ELISA solutions

A) Phosphate Buffered Saline (PBS), pH 7.4
Dissolve 5 tablets of PBS (Sigma, UK) in 1000 ml of distilled water. Store at 4°C, shelf life is 2 weeks.

B) Blocking Buffer (BB): for 1 liter (Store at 4°C, shelf life is 1 week).
10.0 gm BSA (1%) + 5.0 gm Casein (0.5%) + 1000.0 ml PBS, pH 7.4
+ 0.20 ml Phenol Red (stock solution 1gm/ml)

C) Blocking Buffer: Igepal CA-630 (BB:IG-630)
To 5ml of BB add 25µl of Igepal CA-630 (Sigma I3021), mix well. Store at 4°C, shelf life is 1 week.

D) PBS-Tw20 (washing solution)
Add 500µl of Tween 20 to 1000ml of PBS, mix well. Store at 4°C, shelf life is 2 weeks.

E) Substrate solution (KPL, USA)
Mix ABTS (solution A) and hydrogen peroxidase (solution B) 1 :1 approximately one hour before use.

Pf capture
To make up stock lyophillized Mab (CDC, Atlanta) is dissolved in diluent (1:1mizture of distilled water and glycerol) to give stock solution of 0.5µg/µl. For one plate 40 µl stock is mixed in 5µl of PBS

Pf peroxidase
To make up stock lyophillized Mab (CDC, Atlanta) is dissolved in diluent (1:1mizture of distilled water and glycerol) to give stock solution of 0.5µg/µl. For one plate 10 µl stock is mixed in 5µl of BB

Positive controls
Vial I: Add 500µl BB to lyophilized positive control (5µg) to give 1µg/100µl BB (01.µg or 100ng/10µlBB)
Vial II: transfer 10µl (100 ng) Vial I to 990 µl BB for 1000pg/10µl
Vial III: transfer 10µl (1000pg) vial II to 490 µl BB to yield 100gp/50µl BB

Positive control curve
Dilute 1:1 with BB for positive control curves for quantitative conformational retesting of 100 (vial III), 50, 25, 12, 6, 3, and 1.5pg/50µl BB. E.g. put 100 µl of vial III in well A, transfer 50µl to well B, add 50µl of BB, transfer 50µl to well C, add 50µl of BB, transfer 50µl to well D etc. Run standard curve in triplicate (well 2A-4A to 2H-4H) as well as 8 negative control mosquitoes (well 1a-1H). Freeze the stock solution and vials I and II for continued use.

Appendix 7. LIVAK buffer extraction protocol

LIVAK GRIND BUFFER:

1.6 ml 5M NaCl

5.48 g sucrose

1.57 g Tris

10.16 ml o.5M EDTA

2.5 ml 20% SDS

Bring volume to 100 ml (with dist H2o), filter sterilize. Store 5ml aliquots at -20°C. After thawing aliquot keep at 4°C for no more than 2 weeks; heat in water batch and mix before each use to re-dissolve precipitate.

1. Grind mosquito in 100µl preheated grind buffer in 1.5 ml eppendorf. Or, to maximize yield grind in 50µl, then rinse pestle with a further 50µl (100µl total in tube). Transfer immediately to 65°C.
2. Incubate at 65°C approx. 30 min. Microfuge briefly to collect condensation.
3. Add 14µl 8M K-acetate (to final concentration of 1M). Mix
4. Incubate on ice approx. 30 min.
5. Centrifuge 20min at 4°C. Transfer supernatant to new 1.5 ml eppendorf; be careful not to transfer any debris. If desired re-spin 20 min and transfer supernatant to new tube.
6. Add 200 µl ice cold ethanol. Mix and spin 15 min at 4°C.
7. Remove and discard supernatant, Add 100µl 70% ice cold ethanol being careful not to dislodge the pellet. Spin 5 min at 4°C.
8. Pipet off supernatant as before and put tubes upside down on blue paper on bench for 30 min.
9. Suspend pellet in 100µl water, incubate at 65°C for 10 minutes (If there is little material suspend in 50µl).
10. Put in freezer till PCR is run, or in fridge if used immediately.

Appendix 8. Larval surveys in agricultural wells.

Overview of the larval surveys carried out in the urban agricultural wells in the three main farm areas in Accra.

date	area	#wells	# positive for *Anopheles*	# positive for *Culex*	% positive for *Anopheles*	% positive for *Culex*
Sep-03	Dzorwulu	45	8	3	17.8	6.7
22Sep-03	Dzorwulu*	15	1	0	6.7	0.0
2-3Dec-03	Dzorwulu	70	7	8	10.0	11.4
04-Dec-03	Kotobabi	34	3	1	8.8	2.9
09-Dec-03	Korlebu	25	0	19	0.0	76.0
19-20Feb-04	Dzorwulu	72	2	1	2.8	1.4
23-24Feb-04	Kotobabi	25	4	0	16.0	0.0
04-Mar-04	Korlebu	36	0	17	0.0	47.2
21/04/2004	Dzorwulu	38	0	3	0.0	7.9
28/04/2004	Dzorwulu	38	1	3	2.6	7.9
03/05/2004	Dzorwulu	37	2	0	5.4	0.0
10/05/2004	Dzorwulu	35	1	0	2.9	0.0
17/05/2004	Dzorwulu	35	2	0	5.7	0.0

*these wells were followed for several weeks but only 1-2 were positive each time so survey was abandoned and switch to broader inventory

Appendix 9. Bednet voucher and information sheet

NMIMR/IWMI Urban Malaria Project - BEDNET Voucher

Voucher. No

Child Id Card no

GPS:

If surveyed HH id (com code- number):

Name carer/mother:

Address (house number/street)

How many people sleep in the HH?

Category	male	female	total
0-5 yrs old			
6-10 yrs old			
11-14 yrs old			
>15 yrs old			

Category: [ ] child under 5
[ ] child 6- 10 [ ] pregnant women

Do you use a bednet at present? [ ] yes [ ] no

Date/Signature:

IWMI-Ms Klinkenberg

PLEASE KEEP THIS VOUCHER IN ORDER TO COLLECT YOUR FREE BEDNET ON MAY 20TH, 2004 (LOCATION TO BE ANNOUNCED)

NO VOUCHER, NO NET!

BEDNET INTERVENTION KOTOBABI - INFORMATION SHEET

Your household is being invited to participate in a research project on urban malaria in Accra. The Noguchi Memorial Institute of Medical Research (NMIMR) of University of Legon, Accra and the International Water Management Institute (IWMI) of the CSIR Campus, Accra is carrying out the study in collaboration with the Liverpool School of Tropical Medicine with approval of the Ghana Roll Back Malaria programme.

This is a follow up of the survey carried out last year in your area in which children were tested for malaria and anaemia. We investigated several factors affecting malaria and to reduce the number of children having malaria parasites in their blood we want to introduce bednets for groups at high risk. Bednets will protect people sleeping under them from getting bitten by mosquitoes and therefore from getting malaria.

Children under 10 and pregnant women are at high risk of getting malaria and they will be provided with a bednet free of charge. The groups at risk will be given a voucher with which they can obtain a bednet at a set date and place announced by the assemblyman. When bednets are given you will be instructed how to use the net. The nets will stay property of the project and we will come and check if you use them and we will test the children for malaria again.

The malaria test will be done by pricking the finger of the child to make a bloodfilm and to test for anaemia. Only children between 6 months and 5 years will be tested for malaria. The fingerprick test will be performed at your home by a qualified technician in the presence of medical personnel. The tests carry little risk of infection but your child might feel a little discomfort as the finger is pricked. If your child is found to be anaemic or positive for malaria you will be given a voucher to get the child treated for free at the clinic. The test will take about 15 minutes and in addition you will be asked some questions on the living conditions of the child, usage of mosquito preventive measures and out of town travel history.

Your participation in the programme is entirely voluntary. You are not under any obligation to participate and you have the right to refuse this invitation.

All information relating to your participation will be kept confidential and will not be revealed to anyone except were required by law and regulations. The address and name will be only used to relocate the house during the research project. Your identity will not be revealed in any reports or publications resulting from this study.

Ms Klinkenberg of IWMI and Dr. Wilson of Noguchi Memorial Institute or other members of the team will be available for questions or clarifications regarding the study. If there are any problems please contact us or contact the assemblyman.

Contact details:
Ms. Klinkenberg, IWMI, tel. 021-784753/024-4201337
Dr. Wilson, Noguchi Memorial Institute, tel. 021-500374

Appendix 10. Distribution day information and net instruction sheet

BEDNET DISTRIBUTION - INFORMATION SHEET

As part of a research project on urban malaria in Accra, your household has been invited to participate in a bednet intervention trial in Kotobabi. The Noguchi Memorial Institute of Medical Research (NMIMR) of University of Legon, Accra and the International Water Management Institute (IWMI) of the CSIR Campus, Accra are carrying out the study in collaboration with the Liverpool School of Tropical Medicine, with the approval of the Ghana Roll Back Malaria programme and your Assembly man.

This project is part of the previous work done in your area on malaria and anaemia. in the last study, we investigated factors affecting malaria. To investigate this more and to try to reduce the number of children having malaria parasites in their blood we want to give bednets for people at high risk, like you are. Bednets will protect people sleeping under them from getting bitten by mosquitoes and therefore reduce the risk of getting malaria. Children under 10 and pregnant women are at greater risk of getting malaria and they will be provided with a bednet free of charge.

As you are either a household with children under 10 or pregnant woman you were given a voucher to collect your free net. The nets will be given to you and by accepting it, you will be enrolled in the research programme. This means we might come and ask questions to check if you are using the net, who is sleeping under the net, etc. We will also ask you some questions about your experience with the net and/or test your child(ren) for malaria and anaemia. If your child is selected for the malaria test and your child is found to be anaemic or positive for malaria you will be given a voucher to get the child treated for free at Mamobi polyclinic. The test will take about 15 minutes and in addition you will be asked some questions on the living conditions of the child, usage of mosquito preventive measures and your recent out-of-town travel history.

Attached is information on how to use the net. Also, today at this distribution point, we will show you how to hand and use the net. As children and pregnant women are at high risk the net is provided for them and they must sleep under the net.

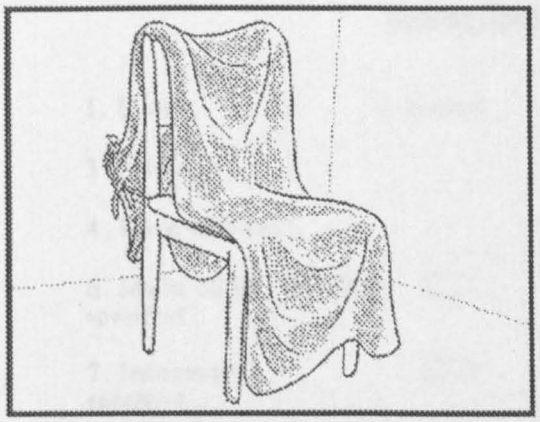
Your participation in the programme is entirely voluntary. You are not under any obligation to participate and you have the right to refuse this invitation and to withdraw at any time.

All information relating to your participation will be kept confidential and will not be revealed to anyone except where required by law and regulations. The address and name will be only used to relocate the house during the research project. Your identity will not be revealed in any reports or publications resulting from this study.

Ms Klinkenberg of IWMI and Dr. Wilson of Noguchi Memorial Institute or other members of the team will be available for questions or clarifications regarding the study and the use of nets. If there are any problems please contact us or contact the assemblyman.

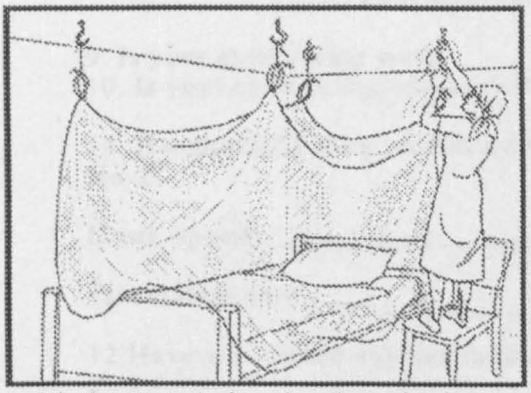
Contact details:
Ms. Klinkenberg, IWMI, tel. 021-784753/024-4201337
Dr. Wilson, Noguchi Memorial Institute, tel. 021-500374

BEDNET INSTRUCTION SHEET

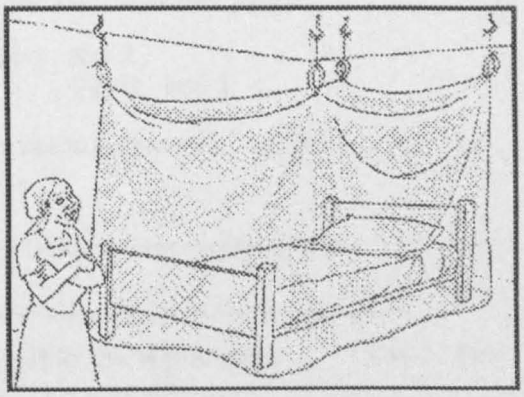


English: Unpack and leave out your net for 24 Hours before hanging it over your bed.

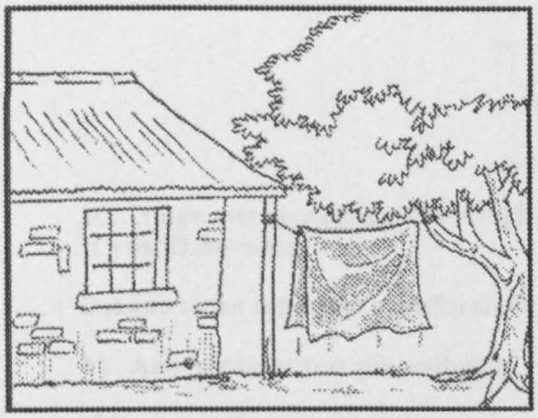
English: Tie the ropes provided onto the four loops at the corner of the net.



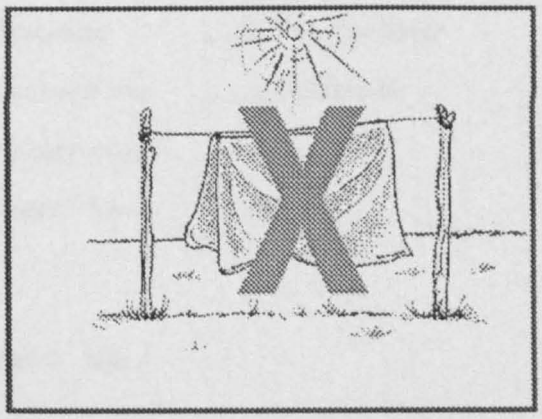
English: Place the the hooks on the ceiling and tie the strings onto the hook.



English: The net should hang low enough to touch the ground, or to enable you to tuck it in.



English: After washing, hang out your PermaNet to dry in the shade.



English: DO NOT hang out your PermaNet under direct sunlight.

Your net should be retreated after 12 washes!

Appendix 11. Cohort questionnaire

Urban Malaria Questionnaire

1. Date: 2. Name: ID#:

3. Address:

4. Date of birth: 5. Sex: male -1 / female-2

6. Main child caretaker: ☐ Mother-1 Father-2 Older siblings-3 Other. specify-4

7. Informant: ☐ Mother-1 Father-2 Older siblings-3 Other. specify-4

8. Is your child still being breast fed? Yes-1 No-2
If no, what milk does he/she get: (formula/cow)

9. Is your child eating meat? Yes-1 No-2
10. Is your child eating vegetables? Yes-1 No-2

11. Has the child been vaccinated within the last month? a) Yes-1 2) No-2

If yes, specify: _____

vaccination date: _____

12.Have you visited any health facility within the last month? Yes-1 No-2
9. If yes, a) When:
b) Why? _____Fever _____Cough
_____Vomiting _____Ear discharge
_____Convulsions _____Diarrhoea
_____Bloody urine

10. Diagnosis recorded from weighing card: Yes-1 No-2
If yes, Diagnosis:

Diagnosis as reported by informant:

11. Any medications prescribed? Yes-1 No-2

if Yes specify:
(specifically ask for antimalarials)

12. Did you buy any over the counter medications in the past two weeks? Yes-1 No-2
If Yes, specify (specifically ask for antimalarials):

13.Did the child travel to the village in the last month? _ Yes-1, No-2.

14. General examination

M.U.A.C. (cm)____ Wt. (gm) Ht. (cm)

Spleen size (cm)_____ Temp. (^{0}C) _____

15. Exclude from study if yes to any of the ff:
Hb< 8.0g/dl
Any chronic disease or disability? If yes, specify

Currently on any treatment? If yes, specify:

16. Actions at month 0 ☐ Thick and thin blood smears done

3 ☐ Hb (g/dl)

☐ Blood spot on filter paper

6 ☐ Stool for microscopy

☐ Urine

Remarks:

Appendix 12. Information sheet cohort

COHORT STUDY - INFORMATION SHEET - KOTOBABI

Your child has been invited to participate in a cohort study which is part of the bednet intervention trial in Kotobabi. The Noguchi Memorial Institute of Medical Research (NMIMR) of University of Legon, Accra and the International Water Management Institute (IWMI) of the CSIR Campus, Accra are carrying out the study in collaboration with the Liverpool School of Tropical Medicine with approval of the Ghana Roll Back Malaria programme and your Assembly man.

This project is part of the previous work done in your area on malaria and anaemia. To investigate all factors related to malaria and anaemia we selected 200 children in Kotobabi to be part of a follow up group. Your child is one of the selected children.

You will be asked to come to a mobile clinic close to your house (exact location to be announced) to have your child tested for malaria, anaemia and worm infection in their stool. If your child is found positive for malaria, anaemia or worms, the child will receive free treatment at the clinic. In addition you will be asked some questions on the child's health and food consumption and the height and weight of your child will be measured. You will need to bring the child's weighing card along to record details on the child. The test will be carried out by qualified technicians in the presence of a children's doctor. To follow what happens over time, we will examine your child now, at the beginning of the study, again in 3 months and finally in 6 months time at the end of the study.

Your participation in the programme is entirely voluntary. You are not under any obligation to participate and you have the right to refuse this invitation and to withdraw at any time.

All information relating to your participation will be kept confidential and will not be revealed to anyone except where required by law and regulations. The address and name will be only used to relocate the house during the research project. Your identity will not be revealed in any reports or publications resulting from this study.

Ms Klinkenberg of IWMI and Dr. Wilson of Noguchi Memorial Institute or other members of the team will be available for questions or clarifications regarding the study and the use of nets. If there are any problems please contact us or contact the assemblyman.

Contact details:
Ms. Klinkenberg, IWMI, tel. 021-784753/024-4201337
Dr. Wilson, Noguchi Memorial Institute, tel. 021-500374

Appendix 13. Volumetric dilution method for counting eggs in stool

After Ashford et al. 1992

1) In the field, shake the stool thoroughly in an excess of 10% formal saline (preferably hot).

2) In the laboratory, shake and decant slurry (sufficient to produce about 1 ml deposit) into a a 15ml graduated centrifuge tube. Make up to 15ml with water.

3) Spin x2000rpm for two minutes.

4) Measure deposit, discard supernatant, make up 10x volum of deposit with water.

5) Mix well and transfer 0.1ml to a slide.

6) Cover with a $22mm^2$ cover slipe; count eggs.

7) The count, x100, equals the number of eggs per ml of packed faeces.

Appendix 14. Questionnaire for bednet compliance

QUESTIONNAIRE NET COMPLIANCE STUDY IWMI-NMIMR

Date **voucher_id** *(read from list)* **House no**

Net number *(read from label)* Number of nets received:

BY QUESTION TO MAIN CARETAKER *(Please circle the answers)*

1. Are you using your net? Yes No
If not, why not? *(check if net still present or if they gave it away/sold it)*

2. How often do you sleep under the net? [every night] [not all nights]

If not every night why not? *(describe reason)*

4. When did you put up the net? *(#days after handout)*

5. Did you have any difficulties putting the net up? Yes No
If yes, define problems:

6a. Who sleeps under the net?
(please fill number sleeping under the net for each category; please note P or O for Permanet or Oth net and fill who are sleeping under all nets in HH)

	P/O	Child<5	child6-10	Youth (11-16 yrs)	Female adult *Non-pregnant*	*pregnant*	Male adult
Net 1							
Net 2							
Net 3							

P=PERMAN ET (the distributed net); O=other net (additional nets they might have)

6b. If the children are not under the net, please ask why the children are not sleeping under the net? *(please inform they are not obliged to use the net, it is there own choice, but we advice them to sleep under it, especially the children/pregnant women as they are at highest risk)*

6c. Do you use any other net apart from the one distributed? Yes No
If yes *(please add them to the table)*,

how many? *(state number)*

are they treated with insecticide? Yes No

7. Do you feel comfortable sleeping under the net? Yes No

If yes why?

If no, why not?

8. Did you travel to the village in the last 4 weeks? Yes No
If yes, did you take the net along/or did you sleep under another net there?
(Please note that in general malaria risk is higher in the rural area so it is advisable to sleep under a net when travelling to the village)
[took net along] [slept under other net]

9. Did you wash your net? Yes No
(Please inform that that they only need to wash 1-2 times (or less) a year and that the net should be retreated after 12 washes)

10. Do you use any other mosquito protection methods? Yes No

Trap doors/ netting for windows	Status good [complete] [small holes] [large holes]	All or only bedroom?
Coils	how often [every night] [1xweek] [1xmonth] [less]	how many
Spray	How often? [every night] [1xweek] [1xmonth] [less]	
Burn herbs	How often? [every night] [1xweek] [1xmonth] [less]	
Others	Specify	

Additional remarks *(please note any issue/remark people raise about the nets, e.g. I don't like the color, it is too complicated, mosquitoes are not dying, I like it, it was nice and warm when it was cold etc.)*:

PLEASE ASK IF YOU CAN SEE THE NET AND HOW THEY FIXED IT, (Please note that when they received the net they in principle agreed to take part in related surveys as was explained in the information sheet and for which they signed consent...)

BY OBSERVATION

1. Is the net hanging yes no (verify and check!)

2. Is the net hanging correctly yes no *(please check if net hang so it can well be tucked in)*

If no, describe problem

3. Are there any holes in nets yes no if yes, holes: *small* *medium* *large*

Remarks on net hanging: (note if any special structure made, if net is hanged permanently etc).

Appendix 15. Protocol for *Plasmodium* species identification by PCR

Adapted from Snounou et al. (1999)

PCR round 1 was the same for all samples, PCR round 2 was run four times with species specific primers for each plasmodium species. The product of the second PCR was run on a 2% agarose gel containing ethidium bromide. *Plasmodium falciparum* shows a band at 205 base pair, *P. ovale* at 800 basepair, *P. malariae* at 144 basepair and *P. vivax* at 120 basepair. Positive controls for each species as well as negative control were added to each PCR run. A second negative control was added to the second PCR.

Mastermix PCR round1
(25µl Reactions)

DNA	1.5 µl
Buffer 10X	2.5 µl
dNTPs (10µM)	1.0 µl
rPLU5 (5µM)	1.0 µl
rPLU6 (5µM)	1.0 µl
Taq	0.2 µl
dH_20	17.8 µl

Conditions:
Step 1) 95°C for 5 mins
Step 2) 57°C for 2 mins
Step 3) 72°C for 2 mins
Step 4) 94°C for 1 min
Cycle 2-4 for 24 cycles
Step 5) 57°C for 2 mins
Step 6) 72°C for 5 mins
Step 7) 4°C pause

Mastermix PCR round2
(25µl Reactions, 1µl of DNA (1 in 10 dilution of PCR product from first round)

PCR product 1	1 µl (diluted 1:20 from first round)
Buffer 10X	2.5 µl
dNTPs (10µM)	1.0 µl
primers (5µM)	1.0 µl (each so 8 primers=8µl)
Taq	0.2 µl
dH_20	12.3 µl

Same conditions as PCR round 1 but 30 cycles.

Primers

rPLU5	CCTGTTGTTGCCTTAAACTTC
rPLU6	TTAAAATTGTTGCAGTTAAAACG
FAL-1	TTAAACTGGTTTGGGAAAACCAAATATATT
FAL-2	ACACAATGAACTCAATCATGACTACCCGTC
MAL-1	ATAACATAGTTGTACGTTAAGAATAACCGC
MAL-2	AAAATTCCCATGCATAAAAAATTATACAAA
VIV-1	CGCTTCTAGCTTAATCCACATAACTGATAC
VIV-2	ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA
OV-1	ATCTCTTTTGCTATTTTTTAGTATTGGAGA
OV-2	GGAAAAGGACACATTAATTGTATCCTAGTG

Appendix 16. Protocol for nested PCR for the *pfcrt* T76 codon

Adapted from Djimde (2001a, 2001b)

A mutation specific nested PCR strategy was used to detect the two alleles of pfcrt codon 76. First, flanking primers CRTP1 and CRTP2 were used to amplify a 537 base pair region around the mutation K76T. The second PCR was performed with internal primers CRTD1 and CRTD2 flanking the K76T mutation to amplify the 145 base pair region. After the PCRs, 5 ml of the 145 base pair amplicon from the secondary PCR was digested with 0.5 units of the restriction enzyme *Apo*1 overnight at 50°C. The digestion reaction was then analyzed by electrophoresis on a 2% agarose gel containing ethidium bromide. *Apo*1 digests the sensitive type allele, resulting in a size reduction of 34 base pairs, but does not digest the resistant allele. Positive and negative controls were included at all steps of experiment.

Mastermix:

PCR 1	PCR2	Digestion
2µl DNA	2µl PCR 1 product	5µl PCR2 product
2.5µl 10x buffer	2.5µl 10x buffer	0.05µl Enzyme
2µl DNTP 2.5mM	2µl DNTP 2.5mM	2µl NE buffer 3
0.5µl P1 primer	1µl D1 primer	0.2µl 100x BSA
0.5µl P2 primer	1µl D2 primer	12.75µl dH2O
0.2µl Taq	0.2µl Taq	
17.3µl dH2O	16.3µl dH2O	

Conditions:	PCR 1 (°C, time)	PCR2 (°C, time)
Initial Denaturation	94, 3 min	95, 5 min
Denaturation	94, 30 sec	92, 30 sec
Annealing	56, 30 sec	48, 30 sec
Extention	60 1 min	65, 30 sec
Final Extention	60, 3 min	65 3 min
No. cycles	40	25-30

Digestion Conditions: overnight at 50°C

Primers:

CRTP1:CCG TTA ATA ATA AAT ACA CGC AG
CRTP2: CGG ATG TTA CAA AAC TAT AGT TAC C
CRTD1: TGTGCTCATGTGTTTAAACTT
CRTD2: CAAAACTATAGTTACCAATTTTG

Appendix 17. Model parameters of repeated measure analysis

DEPENDENT VARIABLE WEIGHT

Solution for Fixed Effects

Effect	area	sex	mal	round	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept					6.8202	0.2888	441	23.61	<.0001
mal			0		-0.02482	0.07112	748	-0.35	0.7272
mal			1		0	.	.	.	.
assetsc					0.3061	0.1032	748	2.97	0.0031
area	ac				-0.2231	0.1682	748	-1.33	0.1850
area	kc				0	.	.	.	.
round				1	-0.1144	0.05641	748	-2.03	0.0429
round				2	-0.03825	0.04218	748	-0.91	0.3647
round				3	0	.	.	.	.
area*round	ac			1	0.1560	0.06730	748	2.32	0.0207
area*round	ac			2	0.1747	0.05413	748	3.23	0.0013
area*round	ac			3	0	.	.	.	.
area*round	kc			1	0	.	.	.	.
area*round	kc			2	0	.	.	.	.
area*round	kc			3	0	.	.	.	.
age					0.1675	0.004560	748	36.72	<.0001
sex		female			-0.5657	0.1522	748	-3.72	0.0002
sex		male			0	.	.	.	.

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mal	1	748	0.12	0.7272
assetsc	1	748	8.79	0.0031
area	1	748	0.50	0.4811
round	2	748	5.59	0.0039
area*round	2	748	5.26	0.0054
age	1	748	1348.71	<.0001
sex	1	748	13.82	0.0002

Least Squares Means

Effect	area	round	Estimate	Standard Error	DF	t Value	Pr > |t|
area*round	ac	1	13.0336	0.1188	748	109.73	<.0001
area*round	ac	2	13.1285	0.1196	748	109.78	<.0001
area*round	ac	3	12.9921	0.1239	748	104.87	<.0001
area*round	kc	1	13.1008	0.1186	748	110.42	<.0001
area*round	kc	2	13.1769	0.1185	748	111.19	<.0001
area*round	kc	3	13.2152	0.1238	748	106.77	<.0001

DEPENDENT VARIABLE WEIGHT FOR AGE SCORE

Solution for Fixed Effects

Effect	area	sex	mal	round	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept					-1.2561	0.1625	441	-7.73	<.0001
mal			0		-0.02503	0.04726	747	-0.53	0.5966
mal			1		0	.	.	.	.
hb					0.01973	0.008742	747	2.26	0.0243
assetsc					0.1578	0.06173	747	2.56	0.0108
area	ac				-0.1167	0.09621	747	-1.21	0.2256
area	kc				0	.	.	.	.
round				1	-0.04752	0.03538	747	-1.34	0.1797
round				2	-0.01629	0.02563	747	-0.64	0.5251
round				3	0	.	.	.	.
area*round	ac			1	0.05100	0.04779	747	1.07	0.2863
area*round	ac			2	0.09739	0.03428	747	2.84	0.0046
area*round	ac			3	0	.	.	.	.
area*round	kc			1	0	.	.	.	.
area*round	kc			2	0	.	.	.	.
area*round	kc			3	0	.	.	.	.
sex		female			0.002715	0.08985	747	0.03	0.9759
sex		male			0	.	.	.	.

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mal	1	747	0.28	0.5966
hb	1	747	5.10	0.0243
assetsc	1	747	6.53	0.0108
area	1	747	0.50	0.4802
round	2	747	5.16	0.0059
area*round	2	747	4.43	0.0123
sex	1	747	0.00	0.9759

Least Squares Means

Effect	area	round	Estimate	Standard Error	DF	t Value	Pr > |t|
area*round	ac	1	-0.9087	0.07491	747	-12.13	<.0001
area*round	ac	2	-0.8311	0.07199	747	-11.54	<.0001
area*round	ac	3	-0.9122	0.07102	747	-12.84	<.0001
area*round	kc	1	-0.8430	0.07441	747	-11.33	<.0001
area*round	kc	2	-0.8118	0.07127	747	-11.39	<.0001
area*round	kc	3	-0.7955	0.07152	747	-11.12	<.0001

DEPENDENT VARIABLE WEIGHT FOR HEIGHT SCORE

Solution for Fixed Effects

Effect	area	sex	mal	round	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept					-1.1548	0.1930	437	-5.98	<.0001
mal			0		-0.06510	0.06749	725	-0.96	0.3350
mal			1		0	.	.	.	.
hb					0.02908	0.01248	725	2.33	0.0201
assetsc					0.1246	0.06185	725	2.01	0.0443
area	ac				-0.02612	0.09738	725	-0.27	0.7886
area	kc				0	.	.	.	.
round				1	-0.2222	0.04937	725	-4.50	<.0001
round				2	-0.08831	0.03601	725	-2.45	0.0144
round				3	0	.	.	.	.
area*round	ac			1	0.1434	0.06681	725	2.15	0.0322
area*round	ac			2	0.2009	0.04812	725	4.17	<.0001
area*round	ac			3	0	.	.	.	.
area*round	kc			1	0	.	.	.	.
area*round	kc			2	0	.	.	.	.
area*round	kc			3	0	.	.	.	.
sex		female			-0.02502	0.09096	725	-0.28	0.7833
sex		male			0	.	.	.	.

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mal	1	725	0.93	0.3350
hb	1	725	5.43	0.0201
assetsc	1	725	4.06	0.0443
area	1	725	0.86	0.3539
round	2	725	16.60	<.0001
area*round	2	725	8.73	0.0002
sex	1	725	0.08	0.7833

Least Squares Means

Effect	area	round	Estimate	Standard Error	DF	t Value	Pr > |t|
area*round	ac	1	-0.7865	0.08144	725	-9.66	<.0001
area*round	ac	2	-0.5951	0.07692	725	-7.74	<.0001
area*round	ac	3	-0.7077	0.07429	725	-9.53	<.0001
area*round	kc	1	-0.9038	0.08124	725	-11.12	<.0001
area*round	kc	2	-0.7698	0.07677	725	-10.03	<.0001
area*round	kc	3	-0.6815	0.07609	725	-8.96	<.0001

DEPENDENT VARIABLE HEIGHT FOR AGE SCORE

Solution for Fixed Effects

Effect	area	sex	mal	round	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept					-0.6451	0.2054	437	-3.14	0.0018
mal			0		0.02721	0.05415	731	0.50	0.6155
mal			1		0	.	.	.	.
hb					0.006081	0.01002	731	0.61	0.5440
assetsc					0.09856	0.08131	731	1.21	0.2258
area	ac				-0.1363	0.1277	731	-1.07	0.2863
area	kc				0	.	.	.	.
round				1	0.2501	0.04975	731	5.03	<.0001
round				2	0.1279	0.02725	731	4.69	<.0001
round				3	0	.	.	.	.
area*round	ac			1	-0.1094	0.06713	731	-1.63	0.1036
area*round	ac			2	-0.1064	0.03657	731	-2.91	0.0037
area*round	ac			3	0	.	.	.	.
area*round	kc			1	0	.	.	.	.
area*round	kc			2	0	.	.	.	.
area*round	kc			3	0	.	.	.	.
sex		female			0.1331	0.1179	731	1.13	0.2595
sex		male			0	.	.	.	.

The Mixed Procedure

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
mal	1	731	0.25	0.6155
hb	1	731	0.37	0.5440
assetsc	1	731	1.47	0.2258
area	1	731	2.64	0.1043
round	2	731	17.34	<.0001
area*round	2	731	4.24	0.0148
sex	1	731	1.27	0.2595

Least Squares Means

Effect	area	round	Estimate	Standard Error	DF	t Value	Pr > |t|
area*round	ac	1	-0.3345	0.09887	731	-3.38	0.0008
area*round	ac	2	-0.4537	0.09789	731	-4.63	<.0001
area*round	ac	3	-0.4752	0.09333	731	-5.09	<.0001
area*round	kc	1	-0.08874	0.09755	731	-0.91	0.3632
area*round	kc	2	-0.2109	0.09706	731	-2.17	0.0301
area*round	kc	3	-0.3388	0.09364	731	-3.62	0.0003