

ASSESSMENT OF IMPACTS OF INDOOR RESIDUAL HOUSE SPRAYING ON THE DIVERSITY, ABUNDANCE AND DISTRIBUTION OF HUMAN MALARIA VECTORS IN CHONGWE DISTRICT, ZAMBIA.

Namafente, O.^{*1,2}, Mbata, K.J.², and C. Katongo².

¹Tropical Diseases Research Centre, P.O. Box 71769, Ndola. ²Department of Biological Sciences, School of Natural Sciences, University of Zambia, P.O. Box 32379, Lusaka. .

* Correspondence author: namafenteo@tdrc.org.zm or onamafente@yahoo.co.uk

Accepted 22 November 2013

Abstract

Dominant human malaria vectors in Chongwe District, Zambia, were identified and impacts of Indoor Residual House Spraying (IRHS) on their diversity, abundance and distribution assessed. A case-control type of study design was used in which Chishiko village in the district was the case in point, where houses had been sprayed with DDT insecticide during the 2008-2009 malaria transmission period through a government of Zambia sponsored IRHS programme and Chiota village was the control, where houses had not been sprayed with any insecticide during the same period. Human malaria vector identification was both morphological using mosquito identification taxonomic keys and molecular, for morphologically inseparable mosquito sibling species complexes through use of Polymerase Chain Reaction (PCR) assays. Vector abundance was determined through computations of mosquito mean densities and comparison of these using ANOVA, while the variance: Mean ratio (S^2/\bar{x}) was used to determine vector distribution patterns in the study areas.

*Three endophilic mosquito species were identified from the study areas as *Culex quinquefasciatus* Say 1823, *Anopheles squamosus* Theobald 1901 and a species from the *A. gambiae* complex comprising seven morphologically indistinguishable sibling species. Molecular discrimination of the *A. gambiae* complex species collected from the study areas through PCR revealed that it was *A. arabiensis* Patton 1905 and further, this species was found to be the major vector of malaria in Chongwe District. The Expected Species Total computed for the study areas indicated the mosquito vector abundance to be three in the study areas, while the variance/Mean ratio showed that malaria mosquito vectors were contagiously distributed in Chiota village ($S^2 > \bar{x}$) and that there were no malaria vectors in Chishiko village. The difference in density of *A. arabiensis* between DDT-insecticide-sprayed houses in Chishiko village and the non-sprayed Chiota village houses was significant ($p < 0.05$) indicating that the IRHS programme exerted a positive impact on the diversity, abundance and distribution of human malaria vectors in Chongwe District. But it is also possible that the vectors might have resorted to feeding and resting outside of the sprayed houses. A longitudinal study would be necessary to complement these findings.*

INTRODUCTION

Presently in Zambia, Indoor Residual House Spraying (IRHS) is one of the major interventions employed by the government in its fight against malaria. The aim of IRHS is

to kill off adult female mosquitoes that enter human habitations in search of human blood meals and which in the process of feeding on the blood, transmit malaria to man. This method of malaria vector control involves application of long-acting contact chemical

insecticides such as DDT on walls and roofs of all houses and other types of domestic shelters in given areas. When mosquitoes come into contact with such sprayed surfaces, they pick-up the insecticide on their bodies and eventually die from the toxic effects of the chemical (WHO, 1982). The primary effects of IRHS is to reduce life spans and densities of vector mosquito species in given areas and consequently to reduce the numbers of infective bites by mosquitoes on man, which in turn reduces the incidences of malaria disease in him. Consistent use of IRHS over time and over large areas has been reported to have altered malaria vector distribution and subsequently the epidemiological patterns of malaria disease in countries like Botswana, Namibia, South Africa, Swaziland and Zimbabwe. According to World Health Organization (WHO, 2006), in these countries, the densities of *Anopheles funestus sensu stricto* and *Anopheles gambiae sensu stricto*, the major mosquito vectors of malaria, were reduced to negligible levels through IRHS.

Use of the IRHS malaria vector control intervention in Zambia goes back to the 1950s during the country's colonial days under British rule. The most effective insecticide for the programme at the time was DDT and the country recorded success in controlling malaria vectors (MoH, 2000). However, because of the negative effects DDT had on non-target organisms, as well as on the environment, the World Health Organization imposed a global ban on its use in the 1970s. Consequently, public IRHS programmes began to decline in Zambia. Malaria cases, on the other hand, began to increase, particularly in the urban areas. Over the past decades, malaria incidence and case fatality rate in Zambia has nearly tripled. In 1976, there were 121.5 cases of malaria per 1000 people and this rose to 398.8 cases per 1000 people in 1998 (NMCC, 1999).

In response to world concerns raised on increasing deaths due to malaria, particularly in the tropical and subtropical countries,

WHO lifted the ban it had imposed on the use of DDT, in 2006, on condition that the insecticide was only to be used in IRHS campaigns against malaria vectors. Prior to its global ban by the WHO, DDT was used for both agricultural and health programmes to control insect pests and disease vectors, respectively. Zambia is one of the countries that reverted to the use of DDT to control malaria vectors. Presently, IRHS programmes are being implemented in 72 districts in the country including Chongwe District, in Lusaka Province, where the programme started in 2005 (NMCC, 2010).

However, despite the IRHS control strategy being implemented in 72 districts in Zambia, as part of the national Integrated Vector Management (IVM), under the Roll Back malaria programme, very little has been done to assess the effects the programme has on the diversity, abundance and distribution of human malaria vectors in the districts. This paper reports on a study conducted to determine firstly, the major human malaria vectors in Chongwe District, Zambia, and secondly, to assess impacts of the IRHS programme in the district, on the human malaria vector diversity, abundance and distribution.

MATERIALS AND METHODS

Study Areas

The study was conducted in Chishiko and Chiota villages in Chongwe District (Longitude, between 28° and 42° E: Latitude, between 15° and 20° S) about 45 km east of the capital city Lusaka, Zambia (Figure 1). The District covers a total area of approximately 10, 500 km² and has a warm tropical climate with seasonal rainfall. Mean annual rainfall at Chongwe town is about 800 mm and is distributed mainly from November to March (wet season), followed by a long dry season that lasts from April to October. Seasonal temperature variations are pronounced to the extent that three distinct seasons can be recognized namely: cool dry (April-July), hot dry (August-October) and

hot wet (November-March). The District is sparsely populated with high concentrations of people at the district centre and in farming areas. According to the national population census, the total population of Chongwe District was approximately 137, 272 people (CSO, 2000). A large proportion of that population (92%) consisted of rural dwellers.

Chiota village is about 10 km east of Chongwe town centre, while Chishiko village is about 3 km to the south of the centre (Figure 1). Chiota village had a population of 2, 488 occupying 333 homesteads, while Chishiko village had a slightly larger population of 2, 688 people, in 448 homesteads (District Planning Unit, 2012).

Subsistence farming (maize, groundnuts),

commercial farming (maize, cotton, sunflower and horticultural plants) and mining and quarrying (mainly for sand) are the major sources of livelihood among the people of the district and villages.

The study involved indoor mosquito sampling in Chishiko village, where houses were sprayed with DDT insecticide during the 2008-2009 malaria transmission period and in Chiota village where houses were not sprayed with any insecticide during the same period.

The study involved indoor mosquito sampling in Chishiko village, where houses were sprayed with DDT insecticide during the 2008-2009 malaria transmission period and in Chiota village where houses were not sprayed with any insecticide during the same period.

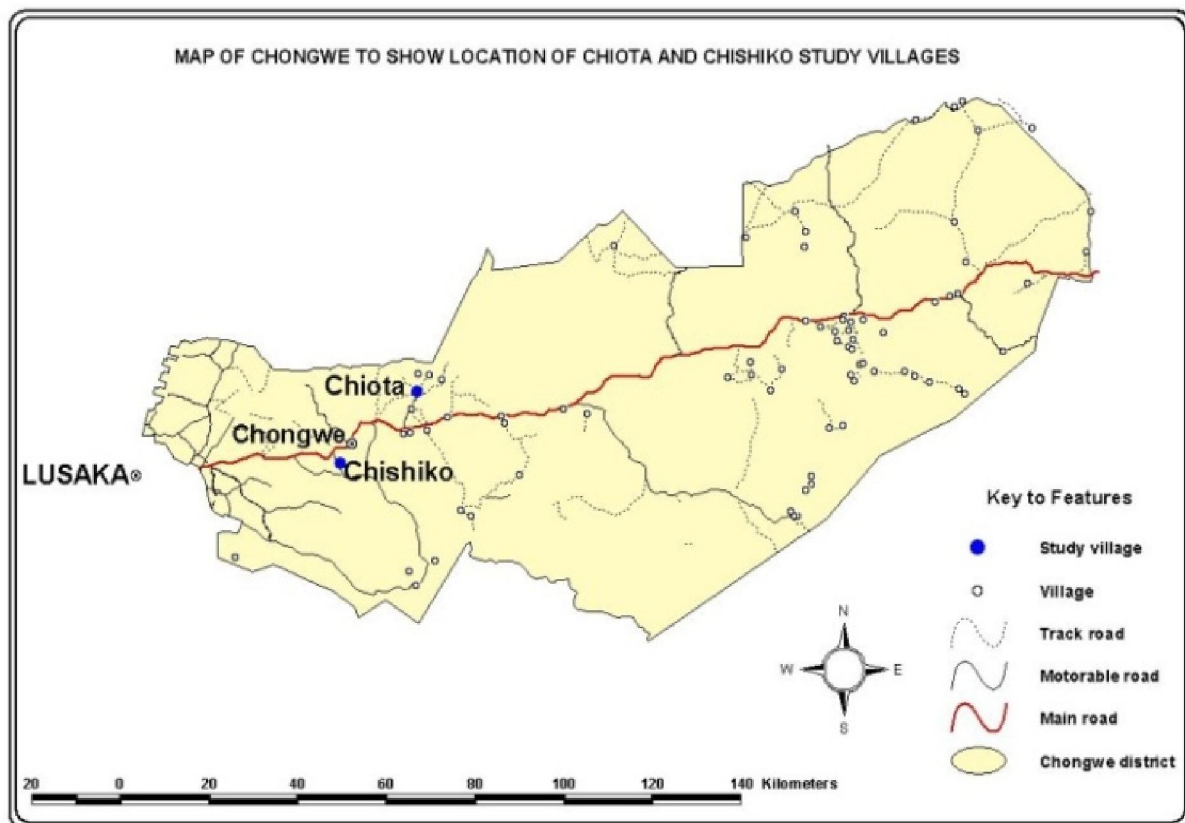


Figure 1. Location of the Study Area (Chiota and Chishiko Villages) in Chongwe District, Zambia (Source: Surveyor-General, Lusaka, 1986).

Sample size

The formula below was used to determine representative samples for insecticide sprayed and non-sprayed houses in Chishiko and Chiota villages, respectively.

$$n = Z^2pq/d^2:$$

Where:

n = sample size

Z = 1.96 at 95% confidence level

p = 94% (proportion of houses that had mosquitoes from a study done by NMCC in the study area)

q = 5% (100-p)

n = Z^2pq/d^2

$$\begin{aligned} \therefore n &= (1.96)^2 \times 94 \times 5 / (5)^2 \\ &= 72.22 \\ &\approx 72 \end{aligned}$$

Thirty-six sprayed and 36 non-sprayed houses (total 72 houses) were required to be sampled for human malaria vectors in the study.

Mosquito sampling and collection

Selection of the 72 houses to be sampled for mosquitoes from the study area (both sprayed and non-sprayed) was done randomly using a GraphPad random number generator. The 36 houses in Chishiko village were sprayed with DDT (at a recommended rate of 2g/m²) earlier in November 2008, under the national IRHS programme, while those of Chiota village were not. Each house was sampled only once during the study and its location in the village, relative to other houses, marked using a hand-held Global Positioning System (GPS) receiver for later mapping of the study areas. Further, at each selected house, information on the number of people occupying the house and whether or not the occupants had used mosquito nets the previous night was collected through a questionnaire survey.

A day before sampling each house, occupants of the selected houses were requested to prepare their houses for sampling by clearing floor surfaces on which sheets of white cloth would be spread during sampling to collect

mosquitoes knocked down by the space sprayed insecticide in the spray-catch mosquito sampling method used (WHO, 1975). Mosquito sampling in the study areas was done between 31st December, 2008 and 25th January, 2009.

Sampling each selected house for mosquitoes required coordinated activities of two field assistants. One field assistant collected mosquitoes from inside the house, while the other guarded against mosquito escape from the house through house eaves from the outside. The insecticide, Target® was sprayed along the eaves of the house by the field assistants beginning from opposite ends of the house. After spraying, doors and windows of the house were closed for ten minutes. White cloth sheets that were spread on the floor of the house before spraying insecticide in and outside it, were then removed, lifting them by the four corners to the outside daylight for examination for knocked down mosquitoes (WHO, 1975). These mosquitoes were picked using pairs of forceps and were stored separately in dry vials containing Silica gel. Mosquito collections were done in the morning between 04:30 and 08:30 hours (WHO, 1975).

Morphological Identification of Malaria Vectors

Morphological identifications of collected mosquitoes were done using a compound microscope and standard taxonomic identification keys (Gillies and Coetzee, 1987; Service, 1990; Edwards, 1941). All mosquitoes that were tentatively identified as belonging to the *A. gambiae* complex and/or *A. funestus* complex of morphologically indistinguishable sibling species by the morphological methods were subjected to further identification using Polymerase Chain Reaction (PCR) molecular assays.

Molecular Identification of Malaria Vectors

Polymerase Chain Reaction assays to separate sibling species of the *A. gambiae* and/or *A. funestus* complexes, if any, were carried out

according to the protocol described by Scott (Scott *et al.*, 1993). The Chelex method of extracting DNA from whole female mosquito malaria vectors for amplification in the PCR was used. Whole mosquito samples were individually introduced into 1.5 ml microfuge tubes, containing 400 µl of 1 x PBS/ 1% saponin solution. The sample was then crushed mechanically using a bent pipette tip until no body part of the insect was recognisable. The crushed specimen was then left to stand in the solution at room temperature for 20 minutes after which it was spun at 14, 000 revolutions per minute (rpm) for two minutes at 26 °C using an Eppendorf Centrifuge (5417R). The supernatant was then aspirated and discarded, retaining the debris (pellet) in the tube. Thereafter, 400 µl of 1 x PBS was added and the tube spun again at 14,000 rpm for another two minutes at 26 °C. As in the previous step, the supernatant was again aspirated and discarded. Then 25 µl of 20% w/v of Chelex and 75 µl of sterile water (ddH₂O) were added to the tube. The sample tube was then closed and a fine hole pierced in its lid using a hot sterile hypodermic needle. The needle was flamed between piercing different sample tubes to avoid sample cross contamination. The tube contents were then boiled at approximately 100 °C for 10 minutes in a water bath. The fine hole in the lid of the tube was for the purpose of releasing vapour during the boiling step to prevent the lid from popping open. The tubes were then spun at 14, 000 rpm for 1 minute in the centrifuge at 26

°C after which the supernatant was aspirated into sterile vials for storage at -70°C. The extracted DNA in this supernatant was later used for PCR assays while the remaining pellets in the tubes were discarded. Table 1 presents the mosquito primers that were used in the assays.

Sample DNA amplification: One and half microlitres of the mosquito DNA extract were introduced into each microfuge tube. Then 25 µl of the master mix was added. The tubes were then placed in the PCR instrument for the amplification of the sample DNA. Amplification was conducted through 30 cycles. The initial step involved denaturation of the DNA for two minutes at 94 °C, followed by primer annealing for 30 seconds at 50 °C, then extension for 30 seconds at 72 °C. The successive cycles were done at denaturation for two minutes at 94 °C whereas the final extension step was done for seven minutes at 72 °C (Scott and Collins, 1993). The resulting PCR products were temporarily (20 minutes) stored at -70 °C as the electrophoresis gel was being prepared.

Gel preparation: The agarose gel was prepared by mixing 50 ml of 0.5 x TBE and 2.50 g agarose in a beaker and heat applied in a microwave until the mixture boiled. Then 11.25 µl of ethidium bromide were added and mixed while gel was still molten. The mixture was then poured into a mould and allowed to set at room temperature for approximately 15 minutes.

Table 1. Mosquito DNA primers used in PCR assays

Mosquito Species	Prime Sequence	PCR Product Size
<i>Anopheles gambiae s.s.</i>	5' – GA CTG GTT TGG TCG GCA CGT TT - 3'	390 bp
<i>Anopheles arabiensis</i>	5' – AAG TGT CCT TCT CCA TCC TA - 3'	315 bp

Loading of PCR products: Six microlitres of each of the PCR products were mixed with 4 µl of loading dye on a parafilm using a pipette and then loaded onto the wells on the agarose gel. Wells on the flanks (i.e. well number 1 and 22) were loaded with 1.5 µl of 100 base pair ladder. The rest of the wells in between were loaded in the order of: Negative control (2), *A. gambiae* standard (3), *A. arabiensis* standard (4), samples of *A. arabiensis* from the Macha area (5 and 6), *A. arabiensis* from Zimbabwe (7 and 8), 10 samples from the study area (9 -18), *A. arabiensis* samples from the Macha area (19 and 20), and another sample from the study area (21). Electrophoresis was then performed at 120 Volts and 500 mL Amps for 120 minutes in 0.5 x TBE. The gel was then captured on a camera for visualization of the DNA bands.

DATA ANALYSIS

Mosquito Diversity: The alpha (α) diversity index of 0.5 (from William's nomograph for determining α diversity indices) was used to estimate the expected species total (EST) for the study area. The following formula was used:

$$EST = \alpha X / 1 - X$$

Where:

EST = Expected Species Total
 α = Alpha Index of Biodiversity
 X = Sampling Factor [$X = 1 - e^{-N/\alpha}$]

where $e = 2.7182818$ and, N (in the formula $X = 1 - e^{-N/\alpha}$) = Total number of mosquito species collected in the sample.

Mosquito Abundance: The mean mosquito densities as a measure of abundance were calculated and analysis of variance (ANOVA) performed in STATISTIX version 2.0 for significance of abundance between Chiota and Chishiko Villages. Percent composition for each of the mosquito species were calculated manually.

Mosquito Distribution: Distribution of mosquito species in the study areas was determined by comparing the distribution variance (S^2) and arithmetic mean (\bar{x}) of mosquito numbers (Southwood and Henderson, 1978). The distribution variance was computed manually using the following formula for each of the mosquito species:

$$S^2 = \frac{\sum x^2 - (\sum x)^2 / n}{n - 1}$$

Where;

Σ denotes summation of all factors to the right

x = value of number of mosquitoes per house (mean density)

n = total number of houses

S^2 = distribution variance

Impacts of IRHS on Human Malaria Vector Diversity, Abundance and Distribution:

Analysis of variance (ANOVA) was used to assess impacts of the IRHS programme in Chongwe District on malaria mosquito vector diversity, abundance and distribution. Mean anopheline mosquito densities in DDT sprayed houses in Chishiko village were compared with those of non-sprayed houses in Chiota village.

RESULTS

Mosquito Sampling

A total of 72 houses were sampled for human malaria vectors in Chiota village and Chishiko village of Chongwe District, Zambia. A total of 84 specimens of endophilic mosquitoes were collected from the houses in the study areas of which 18 were human malaria vectors.

Major Human Malaria Vectors of Chongwe District

Two species of endophilic mosquitoes were identified from the study areas using morphological methods. These were; *Culex quinquefasciatus* Patton 1905 and *Anopheles squamosus* Theobald 1901. Eleven (11) of the endophilic mosquito specimens out of a total

of 84 collected from the study areas were only identifiable as *Anopheles* specimens, belonging to the *A. gambiae* complex of sibling species using dichotomous human malaria vector taxonomic identification keys. They were processed further using PCR molecular assays to identify the complex sibling species.

All the 11 *A. gambiae* specimens collected turned out to be only one of the seven sibling species belonging to the *A. gambiae* complex. They were all of *A. arabiensis*. Three endophilic mosquito species were identified as; *C. quinquefasciatus*, *A. squamosus* and *A. arabiensis*. In terms of numbers, *A. arabiensis* was the dominant human malaria vector in the study areas, in Chongwe District.

Mosquito Diversity

The calculated Expected Species Total (EST) for endophilic mosquitoes for the study areas using the alpha (α) index of diversity was three. This tallied with the actual number, three, of endophilic mosquitoes collected from the study areas namely; *C. quinquefasciatus*, *A. squamosus* and *A. arabiensis*.

Mosquito Abundance

Out of the 84 endophilic mosquitoes collected from the study areas, the majority (78.6%) were *C. quinquefasciatus*. The major human malaria vector in the study areas, *A. arabiensis* accounted for 13.1%, and the other anopheline mosquito *A. squamosus*, 8.3%. Thirty four mosquitoes were collected in Chiota village, out of which; 47% were *C. quinquefasciatus*, 32.4 % *Anopheles arabiensis* and 20.6 % *Anopheles squamosus*. There were 50 mosquitoes collected from Chishiko Village all of which (100%) were *C. quinquefasciatus* species. No *Anopheles* mosquitoes were collected in this section of the study area.

Mosquito Distribution

The variance/mean ratio (S^2/\bar{x}) determined for Chiota village was larger than unit i.e. the variance was larger than mean anopheline

mosquito density for the area. This implied that anopheline mosquitoes in the area were contagiously distributed. Since no anopheline mosquitoes were found in Chishiko village the variance/mean ratio was zero and hence, there was no mosquito distribution pattern to be determined for the study area.

Impacts of IRHS on Malaria Vector Diversity, Abundance and Distribution

Anopheline mosquito mean densities varied between Chiota and Chishiko villages. Chiota village had a mean density of 0.50, while that of Chishiko village was zero, since no anopheline mosquitoes were collected from the houses in the village. Analysis of variance of mosquito abundances between the two villages showed that there was a significant difference between the villages, $p = 0.0059$. This implies that the IRHS programme had a positive impact on the malaria mosquito vectors in the study areas.

DISCUSSION

Anopheles gambiae Giles 1902 sensu stricto, *A. arabiensis* and *A. funestus* sensu stricto Giles 1900 are primary vectors of human malaria in sub-Saharan Africa (Gillies and de Meillon, 1968). *A. arabiensis* being the most widespread is found throughout the region except in the equatorial forest-belt (Morlais *et al.*, 2005). The first two are among a group of seven sibling species called *A. gambiae* complex. The third comes from another group of mosquitoes comprising nine sibling species called *A. funestus* complex. While the two complexes are separable morphologically using standard mosquito identification keys, identification of sibling species within each complex is difficult, requiring use of sophisticated molecular assays such as PCR.

All three primary vectors of human malaria mentioned above have been reported to occur in Zambia, although their exact geographical distribution patterns are yet to be determined. For instance, Hervy, (Hervy *et al.*, 1998) documented 27 *Anopheles* mosquitoes including *A. arabiensis*, as occurring in

Zambia. *Anopheles gambiae sensu stricto* has been reported to occur in Kitwe, on the Copperbelt, and in rural areas of Kasama in the Northern Province of the country (Gillies and de Meillon, 1968). Kent (2006) observed concentrations of *A. arabiensis* occurring in different parts of the Southern Province of Zambia. *Anopheles funestus sensu stricto*, on the other hand, is said to occur throughout the country (Walter Reed Biosystematic Unit, 2012). Recently, *A. gambiae sensu stricto* and *A. arabiensis* were reported to occur together in same localities in Lusaka urban district (Chanda, 2007). This association between the two members of the *A. gambiae* complex has also been observed in other African countries like Nigeria (White and Rosen, 1973).

Only one of the three primary human malaria vectors i.e., *A. arabiensis*, was identified from Chongwe District in this study. Since the district is only 45 km from Lusaka urban district where both *A. gambiae sensu stricto* and *A. arabiensis* are known to occur together in same localities, it is possible that *A. gambiae sensu stricto*, including *A. funestus sensu stricto*, were also present in Chongwe district but may not have been collected during the present study. It is possible that the vectors may have modified their feeding and resting behaviour in avoidance of the insecticide-sprayed houses. Molineaux and Gramiccia (1980) in a study conducted in Nigeria observed that in areas sprayed with residual insecticide, a significant proportion of both populations of *A. arabiensis* and *A. gambiae sensu stricto* were resting outside the houses. In areas where *A. arabiensis* is abundant, for instance, the species resorts to feeding on cattle and other animals outside human dwellings to avoid the insecticide. Further, even without such insecticide avoidance behaviour, species like *A. funestus sensu stricto* readily bite hosts inside houses but a good portion of them may not remain indoors after feeding. Such species are usually missed when sampling using the spray-catch method adopted for this study. More sampling for principal human malaria vectors is required in the Chongwe district to ascertain

the absence of *A. gambiae sensu stricto* and *A. funestus sensu stricto* in the area.

Indoor Residual House Spraying is one of malaria management interventions employed by Zambia that aims at decreasing population densities of female anopheline mosquitoes, the vectors of human malaria, and consequently, reducing the number of infective bites on man and the incidences of the disease. Other interventions targeting malaria vectors are; use of insecticide treated bed nets and more recently introduced in the country, larviciding. The latter prevents development larvae and pupae of mosquitoes by killing them off outright, consequently preventing emergencies of adults which vector the disease. The biolarvicides used in Zambia are *Bacillus thuringiensis* var. *israelensis* and *B. sphaericus* (Kandyata, 2012).

The findings in this study showed that the IRHS programme in Chongwe District had positive effects on the abundance, diversity and distribution of *A. arabiensis* and *A. squamosus*. However, a follow-up longitudinal study would be necessary to make these findings more conclusive.

ACKNOWLEDGEMENT

The staff of the National Malaria Control Centre of the Ministry of Health of Zambia is thanked for facilitating the mosquito sampling programme for this study. Dr. S. Mharakurwa, Dr. P. E. Thuma, Mr. M. Musapa and the entire staff at the Malaria Institute in Macha, Southern Province, are greatly acknowledged for facilitating the molecular identification of the *Anopheles* mosquitoes. Gratitude is also extended to the leadership and residents of Chiota and Chishiko villages for their cooperation during mosquito sampling from homes in their villages. My field assistants: Mr. K. Kabuswe, Mr. E. Chabu, Mr. Mwaachimota, Mr. L. Malambo, Mr. A. Kandyata and Ms. T. Mwauseya are also very much appreciated for their hard work in support of this study.

Financial support was provided by the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), and the Ministry of Health of Zambia through the National Malaria Control Center (NMCC), Lusaka.

REFERENCES

Central Statistical Office (CSO), 2000. National Census of Population. Lusaka, Zambia.

Chanda, E., 2007. The transmission attributes of peri-urban malaria in Lusaka, Zambia. University of Zambia, MSc dissertation.

District Planning Unit, Chongwe, 2012. Chongwe District profile. <http://www.lga.zambia.org.zm>. Accessed on 14th April 2012.

Edwards, F.W., 1941. Mosquitoes of the Ethiopian Region III-Culicine Adults and Pupae. The Oxford University Press.

Gillies, M.T and M. Coetzee, 1987. Supplement of the *Anopheles* of Africa South of Sahara (Afro-tropical Region). Johannesburg, Republic of South Africa: The South Africa Institute of Medical Research.

Gillies, M.T. and B. de Meillon, 1968. The Anophelinae of Africa south of the Sahara. Johannesburg, Republic of South Africa: The South Africa Institute of Medical Research.

Hervy, J-P., G. LeGoff, B. Geoffroy, J-P. Herve, L. Manga and J. Brunhes, 1998. The Anopheline Mosquitoes of the Afro-tropical region; An Identification and Training Software. Montpellier, Cedex 1.

Kandyata, A., 2012. Effects of *Bacillus thuringiensis* var. israelensis and *Bacillus sphaericus* larvicides on mosquito abundance, diversity and distribution in selected areas of Lusaka urban district, Zambia. M.Sc. dissertation, University of Zambia.

Kent, R. J., 2006. The Mosquitoes of Macha, Zambia. Baltimore Publishers. 575 pp.

Ministry of Health (MoH), 2000. Malaria Situation Analysis Zambia. Lusaka (Unpublished).

Molineaux, L. and G. Gramiccia, 1980. The Garki Project. Research on the Epidemiology and Control of malaria in the Sudan savanna of West Africa. World Health Organization. Geneva. 311p.

Morlais, I., R. Girod, R. Hunt, F. Simard and D. Fontenille, 2005. "Population Structure of *Anopheles arabiensis* on La Reunion Island, Indian Ocean". American Journal Tropical Medicine and Hygiene 73(6), 1077-1082.

National Malaria Control Centre (NMCC), 1999. Guidelines for the Diagnosis and Treatment of Malaria in Zambia. Lusaka (Unpublished).

National Malaria Control Centre (NMCC), 2010. Performance Review. Lusaka (Unpublished).

Scott, J. A., W.G. Brogdon and F. H. Collins, 1993. "Identification of single specimens of the *Anopheles gambiae* complex by polymerase chain reaction". American Journal Tropical Medicine and Hygiene. 49(4), 520-529.

Service, M. W., 1990. Handbook of Afrotropical Toxorhynchitinae and Culicine mosquitoes, excepting *Aedes* and *Culex*. Cromwell Road, London SW7 5BD.

Southwood, T.R.E. and P.A. Henderson, 1978. Ecological methods. Blackwell Science Publishers.

Surveyor-General, 1986. Survey department, government of the Republic of Zambia

Walter Reed Biosystematics Unit, 2012. Systematic catalog of Culicidae. <http://www.mosquitocatalog.org/default.aspx>

. Viewed on 20th April 2012.

White, G. B and P. Rosen, 1973. "Comparative studies of sibling species of the *Anopheles gambiae* Giles complex (Dipt. Culicidae). II. Ecology of species A and B in savanna around Kaduna, Nigeria, during transmission from wet to dry season". Bulletin of Entomology. 62, 613-625.

World Health Organization (WHO), 1975. Manual in Practical Entomology in Malaria.

Part II: Vector Bionomics and Organization of Anti-Malaria activities. 191pp.

World Health Organization (WHO), 2006. Malaria Fact Sheet. World Health Organization, Geneva.

World Health Organization (WHO), 1982. Environmental Management for Mosquito Control. Geneva. 283pp.