FINAL REPORT

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Evaluation of Malaria Vector Susceptibility to Insecticide in After Indoor Residual Spraying in Muleba District Kagera Region

Submitted to

Research Triangle International, Tanzania Office

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1.0 Introduction

Research Triangle International (RTI) has been coordinating and managing pyrethroid based Indoor Residual Spraying activities in Kagera region since 2007. Villages in Muleba district were the first sites in Kagera region to be sprayed. Several rounds of spraying have carried out since then. It is being argued that such large scale continued IRS intervention may lead to selection of resistant mosquito population over time. Therefore, the study being presented in this report was carried out to provide technical support and evidence to RTI on the effect of intervention on response of local malaria transmitting mosquitoes to insecticide being used for IRS in Muleba district. The terms of reference for this undertaking were:

1. To identify sentinel site(s) where adult mosquitoes will be collected and thereafter tested for susceptibility status by bioassay.
2. To provide technical support in the carrying out of susceptibility bioassays to staff of Mwanza Research Centre by involving them in this study.

2.0 Objective

The main objective of this study was to determine the susceptibility status of local *Anopheles gambiae* s.l. and *An. funestus* to pyrethroids: permethrin, deltamethrin, lamdacyhalothrin and organochlorine DDT following several rounds of IRS intervention in Muleba district.

3.0 Starting Insecticide Resistance Surveys

Following remittance of funds from RTI in the second week of April 2010 at the time when there was heavy downfall in Kagera region and information received from focal persons in Mwanza and Muleba that some roads were badly affected, it was deliberated to postpone surveys until when the situation was easing out. Therefore this survey was carried out between 18th May and 29th May 2010

4.0 Identification of sentinel site(s) for adult mosquitoes collections and testing by bioassay

4.1 Identification of sentinel site(s) for adult mosquitoes collections

A search for a site to collect samples of adult mosquitoes by resting catches inside houses did not provide promising numbers to perform the test within a timeframe given. Other collection methods such as exits and pits traps on windows and outdoors respectively were used to increase collections in the first four days without any success (Table 1). After the fourth working day the searches were abandoned.

4.2 Setting up of field insectary for larval rearing and bioassay

This was not part of the terms of reference for the current support but it was necessary for rearing of larvae following failure of first attempt to collect adult mosquitoes and also as a preparation to keeping collected larvae alive to adulthood. In collaboration with local focal persons, two rooms were secured at Kaigala dispensary to provide basic appropriate conditions; one as rearing room and the other for testing adults. The rearing room was provided with gadgets for measuring temperature and relative humidity.

4.3 Identification of sentinel site(s) for mosquito larvae collections

Following failure to collect enough numbers of adult mosquitoes, searches for samples were changed to larval collection. This was carried out in four villages: Katungulu, Ndolage, Rubya and Mulela as indicated in table 1. Collections made in Mulela village were good enough and therefore decided to use these larvae for rearing and further testing. This was towards the end of the planned visit in Muleba, but worse still larvae were developing at a very slow rate. Therefore it was decided to ship them to NIMR Mwanza insectary for proper laboratory rearing condition. Some of the larvae were also transferred to NIMR Amani insectary at Muheza to be reared to adulthood and testing.

4.4 Testing adult mosquitoes on WHO insecticide treated papers

Testing for susceptibility status followed standard WHO procedure (WHO 1998). This involved exposing 1-2 days old adults inside a cylindrical chamber lined with insecticide treated papers

for 60 minutes. The number of knocked down mosquitoes in the test chambers was scored every ten-minutes until 60 minutes. Test and control experiments were run side by side. Batches of not more than 25 female mosquitoes were tested in each test cylinder. Therefore a total of 100 mosquitoes (four replicates of 25) were used for each test and control. After one hour exposure, mosquitoes were transferred to paper-cups and provided with sugar solution under a condition of 26 ± 2°C and 80% relative humidity in the insectary. Mortalities were scored 24 hours later and if control mortality exceed 5%, was corrected Abbott's formula (WHO, 1998). The knockdown times for 50% of the tested mosquitoes were calculated using Polo Plus computer software by LeOra Software (Robertson et al. 2003) for probit and logit analysis (Finney 1971).

4.4.1 WHO insecticide treated papers used for the tests

Two insecticide groups were tested. These included pyrethroid: 0.75% permethrin, 0.05% deltamethrin, 0.5% lamdacyhalothrin and an organochlorine 4% DDT treated papers from WHO reference laboratory in Malaysia.

4.5 Interpretation of susceptibility levels in mosquito population

The susceptibility status of field population was based on the decreased mortality rates according to WHO criteria (1998). In this guideline, field mosquitoes showing <80% mortality are regarded as resistant, while mortality of between 80-97% suggests the possibility of resistance requiring confirmation and that of between 98-100% indicates susceptibility.

5.0 Results

Table 2 shows response of *An. gambiae* to standard diagnostic concentrations of insecticides tested on mortality after 24h holding time and estimates of the knockdown time. At the end 24h period all mosquitoes tested succumbed to 100% mortality reflecting their fully susceptibility to all the insecticides tested. However, estimated of KDT_{50} suggested that permethrin elicited a more rapid knockdowns followed by deltamethrin. The KDT_{50} of *An. gambiae* due to Lambdacyhalothrin 0.05% and DDT exposures were similarly twice as long as that of permethrin than deltamethrin.

5.1 Conclusion

Results from this study suggest that population of *An. gambiae* at Mulela village in Muleba district were still highly susceptible to all the pyrethroid and organochlorine tested. However, the late response of *An. gambiae* to knockdown effect of Lambdacyhalothrin calls for concern as this may suggests a certain level of increased tolerance to the insecticide. Therefore a close follow up is suggested in this area to establish if such tolerance may mount to higher levels.

6.0 Recommendation

a. Difficulties experienced in larvae rearing calls for importance of having proper insectary facility in Muleba or Bukoba rather than a makeshift used in this study which had several technical short falls.

b. All tested mosquitoes (dead or survivors) should also be subjected to molecular techniques for speciation of complex population and identification resistance markers if any particularly in Mulela village where KDT50 ratio for Lambdacyhalothrin was found to be elevated than other pyrethroids tested.

References

Finney DJ (1971). *Probit Analysis*. Cambridge University Press, Cambridge.

WHO. (1998). "Test procedures for Insecticide monitoring in malaria vectors, bio-efficacy and persistance of insecticides on treated surfaces: report of the WHO informal consultation." WHO document (WHO/CDS/MAL/98.12).

Table 1. **Schedule of activities for Insecticide resistance survey in Muleba district**

Day	Activity	Remarks
1	Travelling from Tanga to DSM	
2	Flying from DSM to Mwanza	
3	Travelling from Mwanza to Muleba with Chacha Ndege and Samwel Doto	
4	Courtesy call to the DED and DMO	
5	Preparation of sentinel sites and setting of exit traps in Katungulu village	
6	Mosquito resting catches and retrieval of exit traps in Katungulu village	5 mosquitoes collected
7	Retrieval of exit traps, mosquito resting catches, pit traps digging, larvae collection from Ndolage and setting up of field insectary.	4 mosquitoes collected
8	Retrieval of mosquitoes from pit traps and larval collection from Ndorage village	5 mosquitoes collected
9	Retrieval of mosquitoes from pit traps.	4 larvae collected
10	Searching for mosquito breeding sites at Ndorage village.	3 larvae collected
11	Mosquito larval search from breeding sites at Rubya village.	< 5 larvae collected
12	Mosquito larval search from breeding sites at Ndolage village.	
13	Mosquito larval search from breeding sites at Mulela village.	Collected > 10 larvae
14	Mosquito larval search from breeding sites at Mulela village.	Collected good number of larvae
15	Mosquito larval search from breeding sites at Mulela village.	Collected good number of larvae
16	Mosquito larval search from breeding sites at Mulela village.	Collected good number of larvae
17	Transportation of mosquito larvae to NIMR Mwanza insectary for further rearing and testing	
18	Left for DSM with some larvae to Muheza insectary for further rearing and testing	

Table 2. Susceptibility of *Anopheles gambiae* to selected WHO insecticide treated papers

Insecticide treated paper	Species tested	Total Tested	KDT_{50} (95% CI)	% Mortality after 24h holding
Permethrin 0.75%	*An. gambiae*	100	17.7 (16.6-18.6)	100
Deltamethrin 0.05%	*An. gambiae*	100	27.7 (25.9-29.5)	100
Lambdacyhalothrin 0.05%	*An. gambiae*	100	34.3 (32.7-36.0)	100
DDT 4%	*An. gambiae*	100	35.6 (33.9-37.1)	100

KDT_{50} = Time for 50% knockdown in minutes; 95% CI= 95% confidence interval