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MOLECULAR CHARACTERIZATION OF MALARIA VECTORS IN ORASHI REGION OF RIVERS STATE, NIGERIA

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ABSTRACT: *A monthly entomological survey was carried out in five rural communities; (Oboburu, Obite, Omoku, Erema and Ahoadah) all in Orashi region of Rivers State in Niger Delta zone of Nigeria for a period of six months. The aim of this survey was to carry out molecular characterization of Malaria vectors present in the study area. In each of the selected villages, mosquitoes were sampled from ten houses by pyrethrum spray collection method (PSC). The collected mosquitoes were sorted morphologically and identified using standard dichotomous taxonomy keys followed by preservation for molecular genotyping. DNA extraction was done using Collins method 1987 where each mosquito was treated individually. The PCR results of the study showed that, Anopheles specie present in the area of study was predominantly An. gambiae sensu stricto (s.s). The result of this study revealed that malaria vectors are predominant in the study area. Adequate preventive measure should be kept in place in other to reduce the heavy transmission of malaria in the study area.*

KEYWORDS: Molecular Characterization, Malaria Vectors, Mosquito, DNA Extraction, Genotypes, Rivers State, Nigeria

INTRODUCTION

In genetic terms, characterization refers to the detection of variation as a result of differences in either DNA sequences or specific genes or modifying factors. Standard characterization and evaluation of accessions may be routinely carried out by using different methods, including traditional practices such as the use of descriptor lists of morphological characters. Due to its nature, genetic characterization clearly offers an enhanced power for detecting diversity (including genotypes and genes) that exceeds that of traditional methods. Likewise, genetic characterization with molecular technologies offers greater power of detection than do phenotypic methods (e.g. isozymes). This is because molecular methods reveal differences in genotypes, that is, in the ultimate level of variation embodied by the DNA sequences of an individual and uninfluenced by environment.

Plasmodium *falciparum* dispersal is all over Africa, as a result of human migration, and adaptation of the parasite to several different indigenous anopheles' species (Gaspar *et al.,* 2016). Malaria is widespread in over 106 countries and is accountable for over 225 million medical cases and 781,000 deaths annually (WHO, 2010) There are five species of Plasmodium that can infect humans. The mortality rate of the disease caused by *Plasmodium falciparum* is high whereas *Plasmodium vivax, Plasmodium. ovale, and Plasmodium malariae* generally cause a less severe form of malaria. The species *P. knowlesi* has been shown to causes illness in humans (Caraballo, 2014). The parasites are transmitted by the

vector's bites from its saliva into a person's blood stream. The parasites travel to the liver where they mature and reproduce (WHO, 2014).

LITERATURE REVIEW

Molecular characterization also helps determine the breeding behavior of species, individual reproductive success and the existence of gene flow, that is, the movement of alleles within and between populations of the same or related species, and its consequences (Papa *et a.,l* 2003). Molecular data improve or even allow the elucidation of phylogeny, and provide the basic knowledge for understanding taxonomy, domestication and evolution (Nwakanma et al 2003). As a result, information from molecular markers or DNA sequences offers a good basis for better conservation approaches. Management of germplasm established in a collection (usually a field, seed or in vitro gene bank) comprises several activities. Markers were also helpful in examining genetic identities and relationships of Malus accessions (Hokanson *et al.,* 1998). Eight primer pairs unambiguously differentiated 52 of 66 genotypes in a study that calculated the probability of any two genotypes being similar at all loci analyzed as being about 1 in 1,000 million. The results not only discriminated among the genotypes, but were also shown to be useful for designing strategies for the collection and in situ conservation of wild Malus species.

Distribution of Malaria Vectors

Anopheles gambiae complex is the major vector species with the sibling species *An. gambiae* s.s. and *An. arabiensis* Paton being the most important within the complex (White, 1974). *Anopheles gambiae* s.s. dominates or is the only species in humid coastal and lacustrine areas, while *An. arabiensis* has been found to predominate in dry and semi-arid areas (Mnzava and Kilama, 1986).

Generally, both male and female mosquitoes feed on nectar and plant juices, but in many species the mouthparts of the females are adapted for piercing the skin of animal hosts and sucking their blood as ectopaqrasite. In many species, the female needs to obtain nutrients from a blood meal before it can produce eggs, whereas in many other species, it can produce more eggs after a blood meal. A mosquito has a variety of ways of finding its prey, including chemical, visual, and heat sensors.(*Freudenrich, and Craig 2001)* Both plant materials and blood are useful sources of energy in the form of sugars, and blood also supplies more concentrated nutrients, such as lipids, but the most important function of blood meals is to obtain proteins as materials for egg production.

Diseases Caused by Mosquitoes

Diseases transmitted by mosquitoes includes malaria, dengue fever, west-nile virus, chikungunya, yellow fever, flariasis, tularemia, dirofilariasis, Japanes encephalitis, saint loius encephalitis, western equine encephalitis, eastern equine encephalitis, Venezuela equine encephalitis, Ross river fever, Harman forest fever.

MATERIALS AND METHOD

Indoors Mosquito's Collection

A cross sectional survey of study was conducted in September, October, November, December of 2017 and March and April 2018. In each village¸ traditional style houses (mud walls with thatched roofs and brick houses) were selected. Household equipment's were removed to enable the spreading of the white sheet collection material.

Occupants of the rooms were evacuated at 6 am in the morning followed by the spreading of white sheets on the floor. Ventilation areas of the rooms that may serve as escape route to the mosquitoes like windows, doors and cracked walls were closed. RAID insecticide was used to spray in the air and around the edges of the room to knock down flying and hanging mosquitoes; the room is left for a period of 10 – 15 minutes for complete impact on the mosquitoes.

Using a pair of forceps, the mosquitoes were picked up and transferred into Petri-dishes for safe keeping before being transported to the laboratory Mosquitoes were sorted based on morphology and identified by using dichotomous Leopoldo M. Rueda 2014 taxonomic keys. *Anopheles* mosquitoes were preserved in labeled Eppendorf tubes with silica gel according to method by (Gillies and De Meillon, 1968). For molecular analysis. All other genera of mosquitoes were sorted and preserved in petri dishes sealed with masking cello tape these were sent to Dr Nwosu in Enugu for identification.

Fig 1: Eppendurf Tubes Containing Anopheles Mosquitoes for Molecular Analysis

Molecular Analysis of Collected *Anopheline* Mosquitoes

DNA Extraction

DNA extraction was done using modified Collins method (Collins., et al., 1987). Individual, mosquitoes were homogenized in a 1.5 ml Eppendorf tube in 100 µl Bender buffer (0.1 M NaCl2, 0.5 M Tris HCl pH 7.5, 0.05 M EDTA pH 9.1, 0.2 M Sucrose and 0.5% SDS). The homogenate was incubated at 65oC for 30 minutes. 15µl of pre chilled 8M potassium acetate (KAc) was added. This was incubated at 4°C for 30 minutes followed by centrifugation at 14 000 rpm for 5 minutes. The supernatant was transferred into a fresh tube and 250µl absolute ethanol was added, mixed and then incubated at -20°C for 3 hrs. To obtain DNA precipitate. This was followed by centrifugation at 14 000 rpm for 10 minutes and the supernatant discarded to obtain a DNA pellet which was dried by inverting the tube over paper towel. The dried pellet was then re-dissolved in 100µl of sterile double-distilled water, incubated at 4°C for one hour and thereafter kept at -21oC for long storage (to avoid DNA degradation) until required for PCR amplification.

DNA Amplification for *Anopheles Gambiae s.l.*

DNA amplification was done by using thermal cycler machine (S1000TM BIORAD) with initial cycle of denaturation at 94°C for 15 minutes followed by 35 cycles of annealing (94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds) and extension at 72°C for 10 minutes and final hold at 4°C until required for PCR amplification. The master mix used were TEMPase hot start (Ampliqon III, VWRBie Berntsen, Denmark) (dNTP, MgCl2, Taq buffer, and hot start Taq) with the final concentration of 1.Primers used (eurofins mwg/operon-16805692 D4) include a universal primer that shows exact matches with ribosomal DNA for the members of An. gambiae complex (UN= 5'-GTG TGC CCC TTC CTC GAT GT- 3'), The primers which were used to identify both An. merus and An. melas were (ME=5'-TGA CCA ACC CAC TCC CTT GA-3') and specific primers for An. quadriannulatus (QA=5'-CAG ACC AAG ATG GTT AGT AT- 3'), An. gambiae (GA=5'-CTG GTT TGG TCG GCA CGT TT-3') and An. arabiensis (AR=5'-AAG TGT CCT TCT CCA TCC TA-3') according to (Scott et al., 1993).

RESULT

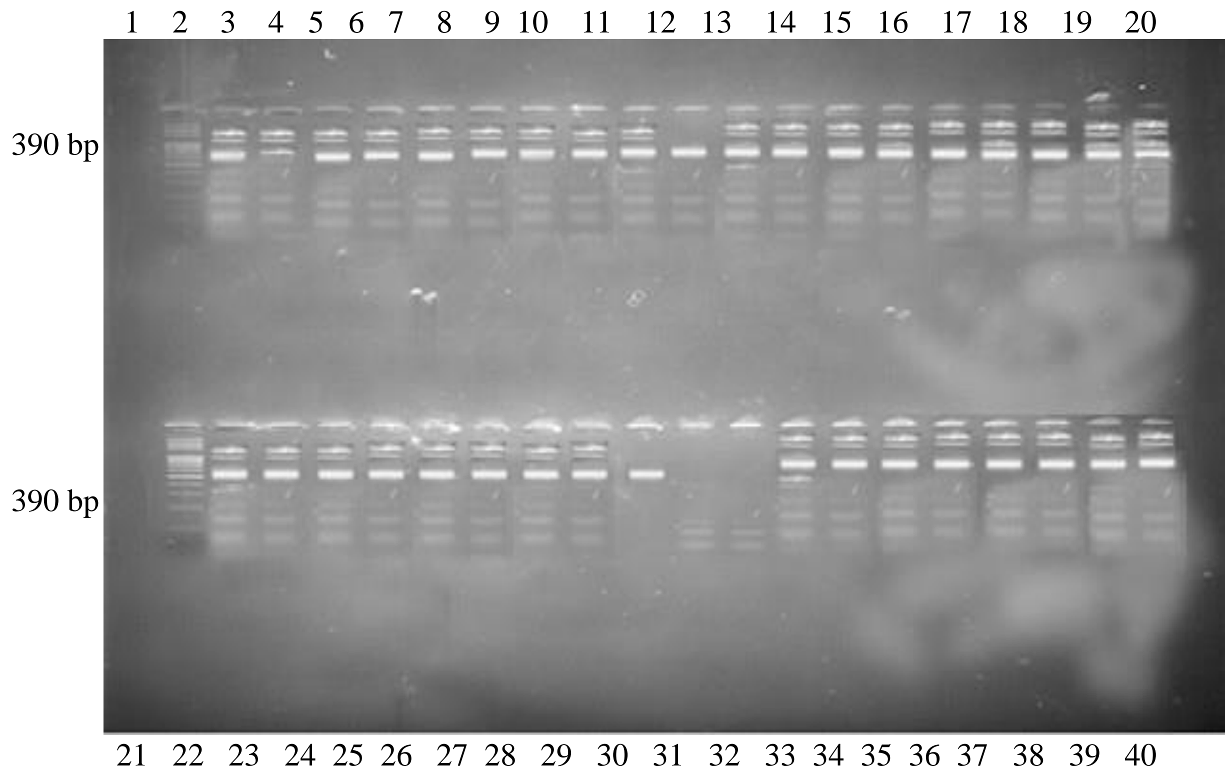

Plate 1: 40 Anopheles Mosquito PCR Result

PCR detection of members of the *An. gambiae* s.l. complex collected Oboburu, Omoku, Obite, Erema, and Ahoada of the Orashi region of Rivers State in the south- south geopolitical zone of Nigeria
Upper part: lane 1-DNA ladder; lane 2-positive control *(A. gambiae s.s.);* lane sample 3-20 positive of *An..gambiae s.s.*
Lower part: lane 21-DNA ladder; lane 22-positive control lane sample 23-30, *(A..gambiae s.s.);* lane 32-33 are negative control lane- samples 32

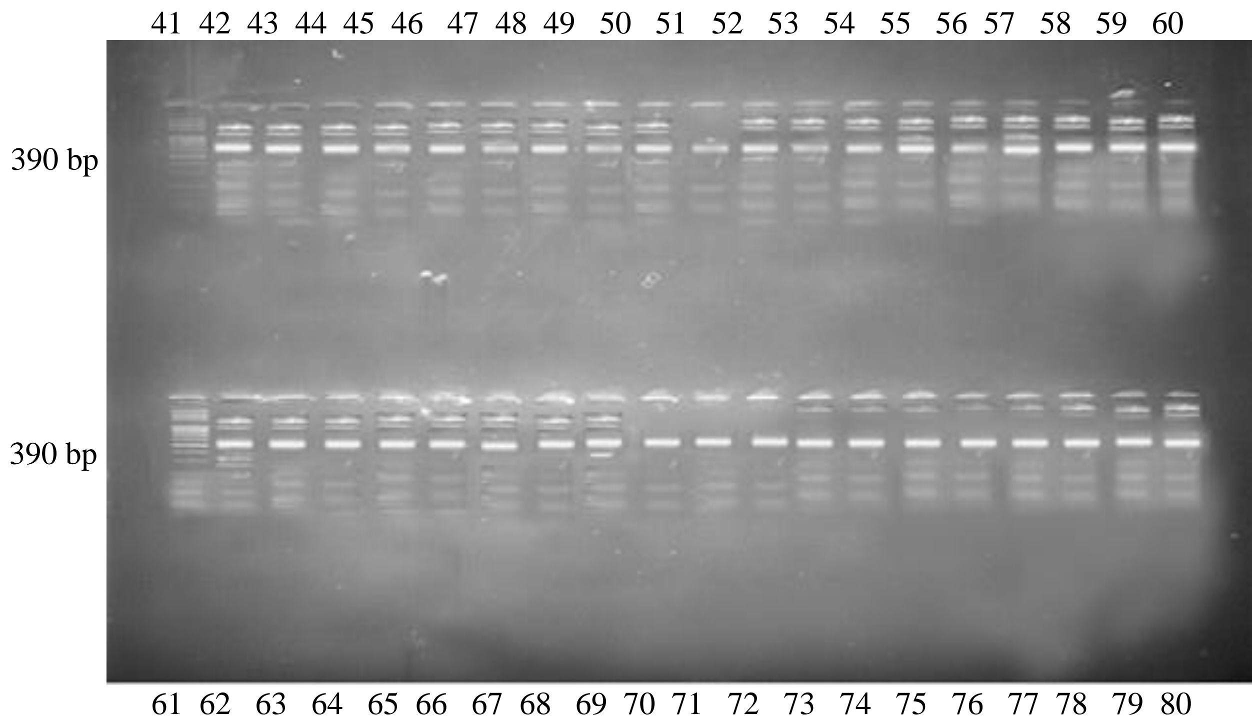

Plate 2: 40 Anopheles Mosquito PCR Result

Upper part: lane 41-DNA ladder; lane 42-positive control lane *Anopheles gambiae s.s* - 390 base pair sample 43-60 positive *(A. gambiae s.s.);*
Lower part: lane 61-DNA ladder; lane 62-positive control lane *Anopheles gambiae s.s* - 390 base pair sample 63- 80npositive *(A.gambiae s.s)*

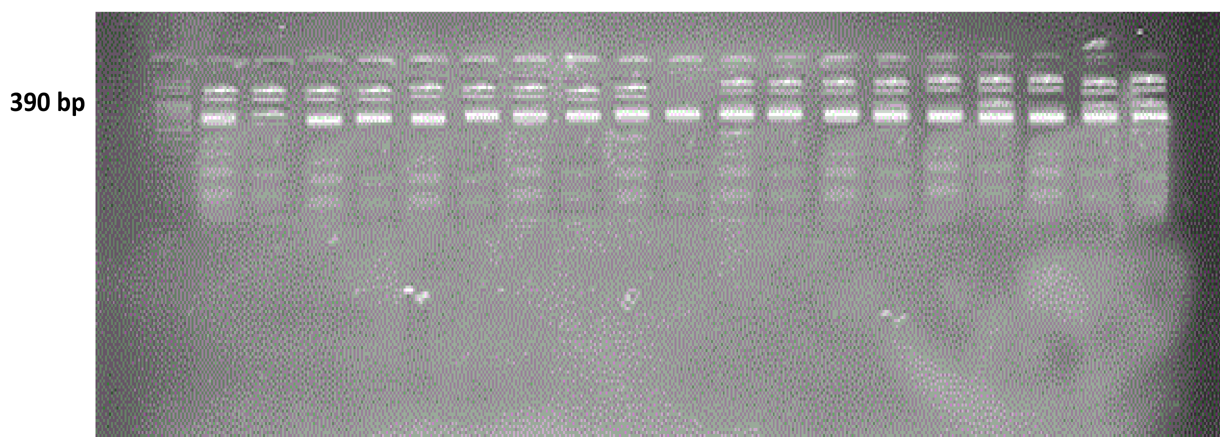

Plate 3: 20 Anopheles Mosquito PCR Result

lane 81-DNA ladder; lane 82-positive control lane 3-95 -100 bp. (*A.gambiae s.s*)

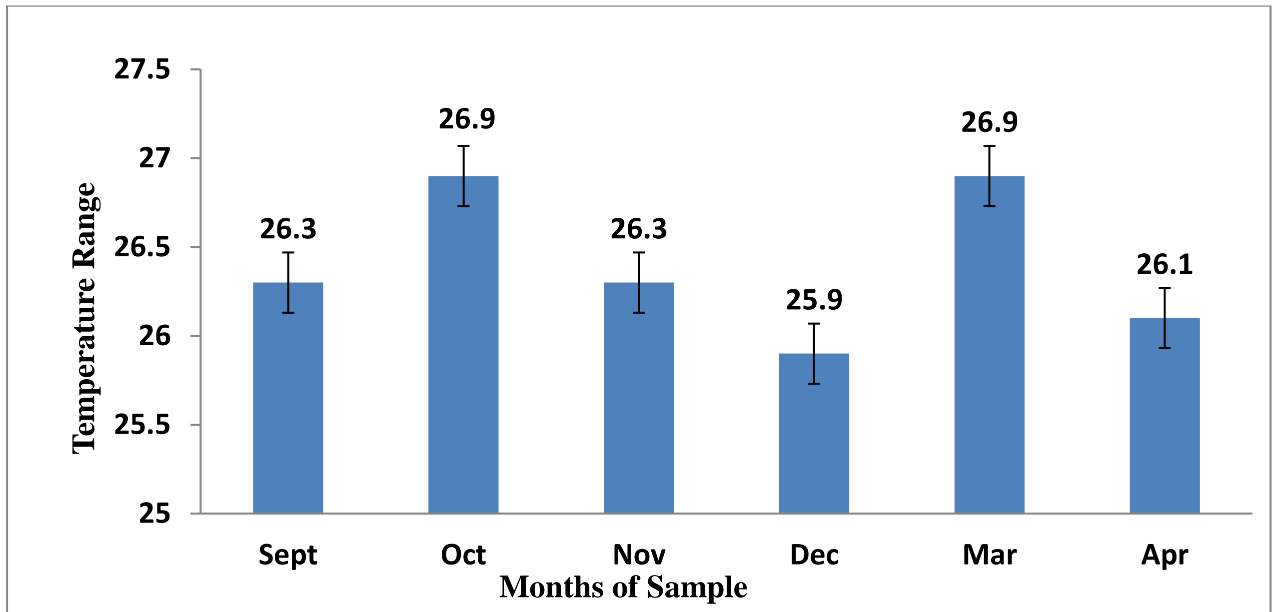

Fig: 2. Mean Temperature Across the Months of Sampling

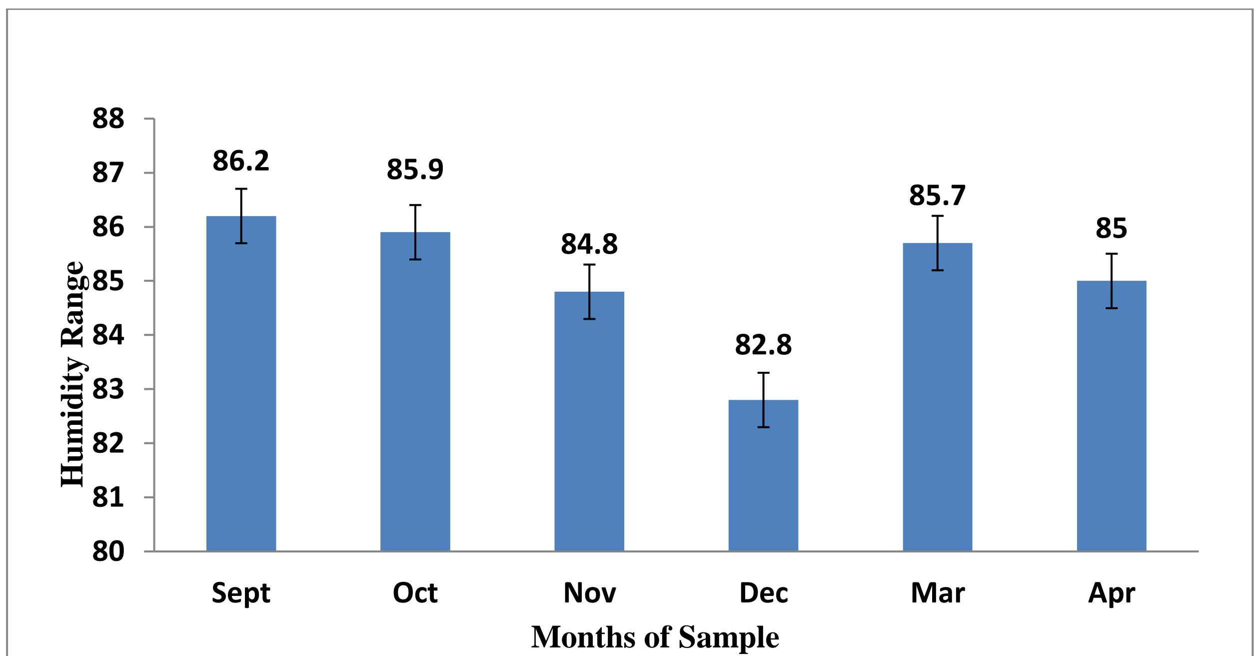

Fig 3: Mean Humidity Across Months of Sampling

RESULT

Molecular results revealed that out of 100 Anopheles that were subjected to molecular analysis (100%) were confirmed to be Anopheles. *gambiea s.s.* (Plate: 1-3). This is as a result of a favorable temperature and humidity of the study area which varied slightly across the months from 25.9° – 26.9° and 82.8 – 86.2 respectively. Mosquitoes are capable of breeding in the environment and getting a blood meal from the occupants of the houses which is used for egg production which result to continuation of life cycle as a result of good weather conditions (Fig 2 and 3).

DISCUSSION

The mosquitoes in the Orashi region of Rivers State were relatively high and varied from location to location across the various months of sampling this was due to favorable environmental adaptations and the availability of suitable habitat such as, grass land, water log, drainages, swamps, and broad leaves and a suitable temperature and humidity which support the growth of various species of the mosquito. In this study, one amongst the species of *Anopheles* was *Anopheles gambiea s.s.*

A Similar report was published by (Mfonobong and iyang 2014) that two anophelines species were collected using the pyrethrum spray catch (PSC) sampling methods revealed that, a Polymerase Chain Reaction (PCR) based test on the *An. gambiae* complex identified (96.0%) as *An. gambiae sensu stricto*. The study also revealed that the resting behaviour of *An. gambiae* complex species in this area is endophilic and exophagic/exophilic

A study carried out by Kilama (1986) showed that Analysis of the membership in the *An. gambiae* complex by molecular techniques revealed the presence of *An. arabiensis* as the only sibling species in the study area. In an earlier study conducted in a village of Buiko located near the current study site, Mnzava and documented the presence of *An. gambiae* s.s., *An. merus* and *An. arabiensis* were comfirmed. Studies have suggested that the scale up of insecticide treated nets may be one of the causes of change the composition of the *An. gambiae* complex (Russell *et al.,* 2011; Mutuku *et al*., 2011; Derua *et al*., 2012).

Implication

1. This study is going to form the baseline information for subsequent researcher who will work on malaria vectors in the study area.
2. The study has been able to show the *Anophiline* species of mosquitoes in the area (the exact malaria vector species in the area)

CONCLUSION

In this study *Anopheles gambiea s.s* has been confirmed to be the potential malaria vector in Orashi region on Rivers State, So preventive measures must be taken in order to reduce the factors such as breeding site of this vector in the locality, a workshop program should be prepared to reduce the epidemic of the parasite which been transmitted by the vector.

Future Research

1. Further studies should be carried out on a larger scale to tackle various diseases caused by the species of mosquitoes found in the area.
2. Also, Non-Governmental Bodies should support research plan to provide full scale information (knowledge) on the vector endemic cases around this area.

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