1. INTRODUCTION
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1.1 Malaria vectors and epidemiology

Mosquitoes are important group of blood feeding insect pests which cause physical, psychological and financial loss to the mankind in particular and society at large. Mosquitoes not only inflict irritating and painful bites but also spread highly debilitating diseases such as malaria, dengue, chikungunya, filariasis and many types of encephalitis in many parts of the world. However among all the diseases, malaria is considered as the worst enemy of a malarious country because it irreversibly affects the public health and overall development (Sarkar and Vijay Veer, 2010). It was not until 1880 when the scientists found that malaria was not a foul air related disease but a parasitic disease transmitted by the *Anopheles* mosquito. Not long after that they found out that the infected mosquitoes infect the host with a parasite called *Plasmodium* which is transmitted from person-to-person through the bite of the female mosquito while taking blood for egg development (Ross, 1901). Malaria imposes great burden on public health and socio-economics. Along with six other diseases, namely diarrhoea, AIDS, tuberculosis, measles, hepatitis-B and pneumonia, it accounts for 85% of global infectious diseases burden (Murray and Lopez, 1996, 1997). World Health Organization (WHO) has estimated that about 40% of the human population mostly living in developing countries is at risk of malaria (Kumar *et al*, 2007, 2012; WHO, 2008-2010, 2012a).

There are more than 500 *Anopheles* species of which 70 have been shown to be vectors of human malaria in different countries. However 52 species have been identified as dominant vector of human malaria globally and distributed widely from the tropics to temperate regions (Hay *et al*, 2010). In India, about 59 anopheline species
have been well described of which natural *Plasmodium* infection was found in 14 species.

Malaria is a serious disease in Africa where it accounts for one in every five childhood deaths (20%); thereby killing an African child every 30 seconds is due to malaria only. Malaria has not only been found associated with poverty but also a cause of poverty and an important obstacle to overall economic development (Oaks *et al*, 1991; Roshanraven *et al*, 2003; Scopel *et al*, 2004; Sharma, 2003; Teklehaimanot and Mejia, 2008; WHO, 2010). Malaria is caused by a microscopic parasite known as *Plasmodium* transmitted from human to human by the bite of infected female *Anopheles* mosquito species. As of now five species of human malaria parasites namely, *Plasmodium falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi* have been reported from various parts (Eede *et al*, 2009; Sahu *et al*, 2013; Singh *et al*, 2004a). The fifth species, *P. knowlesi* is distributed mainly across the South-East Asia and is often misdiagnosed as *P. Malariae*. Studies have evidenced that *P. knowlesi* has jumped out the host species barrier from monkey to man and now spreading rapidly in humans in Malaysia and its infection has been found to be potentially more serious and even life-threatening (Cox-Singh *et al*, 2008; Singh *et al*, 2004a).

Malaria clinical symptoms in the early stages are sometimes similar to those of many other bacteria, viruses or parasites infection that can begin with flu like manifestations including fever with chills, headache, nausea, profuse sweating and sometime vomiting. However in some cases the symptoms may include dry cough, back pain and enlarged spleen which can lead to the impaired function of brain and spinal cord and cause seizures and loss of consciousness. Only four species have been reported from India, of these, *P. falciparum* and *P. vivax* are the most common, whereas handful
cases of *P. malariae* and *P. ovale* has been reported from the different parts of the country (Das *et al*, 2012; Marathe *et al*, 2006). Nine species of anopheline mosquitoes are responsible for malaria transmission in India of which six are of primary importance whereas remaining three have role at regional level (Bhattacharyya *et al*, 2010; Dev *et al*, 2003a, b). Development of chloroquine resistance in *P. falciparum*, first reported from the North-Eastern state of Assam has emerged as a serious challenge in combating malaria in India (Sehgal *et al*, 1973). Systematic monitoring of drug resistance is undertaken by the National Vector Borne Disease Control Programme (NVBDCP) and the latest studies indicate that chloroquine resistance in *P. falciparum* is widespread in the country (Awasthi *et al*, 2011; Mittra *et al*, 2006; NVBDCP, 2010; Sharma, 2012; Vathsala *et al*, 2004).

1.2 Malaria in North-East India

The North-East region of India spreads over an area of 2,62,179 square kms and comprised of eight states, namely Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim. It is stretched between 89.46 degree to 97.30 degree East longitude and 21.57 degree to 29.30 degree North latitude. North-East India has 4,500 km long international border with five foreign countries namely Bangladesh, Bhutan, Burma (Myanmar), China and Nepal. The whole of the North-East region is connected with the rest of the country by a tenuous 22 kilometers land corridor through Siliguri in the Eastern state of West Bengal - a link that has come to be referred to as 'Chicken's Neck'. In the North-Eastern states, malaria transmission has been a daunting epidemiological challenge as its distribution is heterogeneous and intensity is governed by many biological, physiological, climatic, socio-economical, behavioural and geopolitical risk factors (Bergquist, 2001; Bhattacharya *et al*, 2006; Dev *et al*, 2003a, b,
2006a, b; Dhiman, 2009; Nath et al, 2012). Focal outbreaks of malaria are common in this region which accounts for 8-12% of all the reported malaria cases in India. *P. falciparum* is a major malaria parasite in this region causing >60% of malaria infections (Das et al, 2004; Dev et al, 2004, 2006a, 2010; Dev and Sharma, 2013; Dhiman et al, 2010 a, b, 2011; Rabha et al, 2012). Six major vectors, namely *Anopheles minimus*, *An. fluviatilis*, *An. philippinensis*, *An. culicifacies*, *An. annularis* and *An. dirus* facilitate malaria transmission round the year.

The North-East region of India has rich biodiversity and experience high rainfall which provides hot and humid climate suitable for mosquito breeding and malaria transmission annually. The region has perennial malaria transmission under the uninterrupted influence of monsoon species (*An. dirus*), winter species (*An. fluviatilis*) and perennial species (such as *An. minimus*). The incidence of malaria largely depends upon various elements such as parasitic load in the population in a given time, prevalence of potential *Anopheles* vector, availability of the human host, conducive ecology for vector growth and proliferation, adequate host vector interaction, health status of the host and suitable environmental factors supporting vector longevity. Further, socio-economic conditions, myths and beliefs and awareness have also been listed as determinant factors for the spread of malaria in a region (Dev, 1996a, b; Dev et al, 2003a, b, 2004, 2006a, b; Dhiman, 2009; Rabha et al, 2012).

### 1.3 Anopheles vectors and human blood meal identification

Xenomonitoring of malaria in vector mosquitoes and preference of host to obtain a blood meal are important epidemiological aspects of malaria transmission (WHO, 2002; Moreno et al, 2004). These are influenced by several factors including parasitic load, host availability, nutritional requirements, intrinsic host preferences of
the species and vector density. Comprehensive information about the blood meal preference of *Anopheles* vector mosquitoes is helpful in providing information on anthropophilic behaviour (Bashar *et al.*, 2012; Chaves *et al.*, 2010; Dhiman *et al.*, 2012a; Swami *et al.*, 2012). Malaria transmission in the many regions of India is uninterruptedly supported by major vectors, *An. minimus*, *An. fluviatilis* and *An. dirus*. However, *An. annularis*, *An. culicifacies* and *An. philippinensis/nivipes* have also been incriminated as vectors in some areas, and are thought to have a lesser role in overall malaria transmission (Bhattacharyya *et al.*, 2010; Chandra, 2008; Mahapatra *et al.*, 2006; Prakash *et al.*, 2004). However, the host preference and incrimination data of certain malaria vectors which appear in large density in malaria endemic areas is still scanty.

### 1.4 Malaria vectors and their control using insecticides

Vector control using insecticide has been a method of choice since long and insecticides in various forms at personal and community levels such as indoor residual spray, fogging and bed net treatment are commonly used in malaria endemic countries (WHO, 2010, 2012a, b). Wide scale use of insecticides have lead to spectacular decrease in the malaria incidences in many regions and now these interventions have become cornerstone of malaria control programmes in many countries. But, extensive and indiscriminate use of recommended and non-recommended insecticides in vector control has resulted in development of resistance in many mosquito vectors (Brogdon and McAllister, 1998; Clark and Shamaan 1984; Davies *et al.*, 2007; Dhiman *et al.*, 2013). There are currently 12 insecticides from four classes of insecticides (organochlorines, carbamates, organophosphates and pyrethroids) recommended by WHO for the use in indoor residual spray, however only one class (pyrethroid) is approved for use in insecticide treated bed nets (ITNs) and long lasting insecticidal nets.
LLINs). These insecticide classes are widely used in agriculture, hence exposing mosquitoes to a sub-lethal dose when the insecticide percolates their larval habitat. Development and spread of resistance among vector mosquitoes against commonly used insecticides which is reflected by repeated failure of an insecticide to achieve the expected level of control when used for that particular vector insect is an important factor in vector control. Insecticide resistance is mediated by behavioural and physiological factors and usually results from one or more of three different mechanisms (Hemingway et al, 1989; Reddy et al, 2011; Russell et al, 2013; Tikar et al, 2011). Two major resistance mechanisms namely, molecular genotype and phenotype resistance have been reported in the mosquito vector population. The molecular genotype resistance occurs due to gene mutation while phenotype resistance is the development of an ability of mosquitoes to tolerate insecticides at a dose which is lethal to normal population of same species. Vector control programmes are intended to kill the mosquitoes therefore phenotypic resistance is most commonly discussed and determined in the public health. Phenotypic resistance is measured by using WHO approved bioassay susceptibility test which determine the mortality of vector mosquitoes at standard insecticide dose and assesses the susceptibility status (WHO, 2010, 2012a). Insecticide resistance situation is further complicated, when cross resistance or multiple insecticide resistance develops within a species and thereby limiting the choice of insecticides (Ranson et al, 2000; Sarkar et al, 2009a, b, c; Usherwood et al, 2005). Since there are a limited number of insecticides for malaria vector control, the insecticide resistance may have negative impact on vector control interventions. Suitable insecticide selection in vector control requires comprehensive information of malaria vector species composition and susceptibility to the insecticides.
proposed to be used for their control. Despite the marked increase in indoor residual spray (IRS) and insecticide treated bed nets (ITBNs), malaria cases are still high which necessitate the insecticide susceptibility test of malaria vector populations. Information on the susceptibility status of vector populations would be useful in preserving the sensitivity of vector mosquitoes to insecticides.

1.5 Malaria parasite detection

Malaria control strategy in India relies on early case detection so that prompt treatment can take place (Das et al, 2002; Dev et al, 2003b, 2006b, 2010; Dhiman et al, 2010b; Dutta et al, 2004; Sharma, 2003, 2009; Sharma et al, 2006). Malaria associated with fever and other symptoms is the most common diagnosis and treatment in the rural areas. However in many endemic settings the asymptomatic patients serve as reservoir for the malaria parasite and hence mediate uninterrupted transmission throughout the year. Therefore, a considerable proportion of patients who are treated for malaria actually do not have malaria while the others who actually have malaria do not get malaria treatment. The missed out malaria cases may act as epicentres for disease spreading at local level (Nath et al, 2013; Yadav et al, 2012a, b). Accurate diagnosis of malaria cases could be useful in lessening such conjunctures and ultimately reducing the malaria burden to a greater extent. Microscopy requires well trained technical staff, and is labour intensive and time consuming, but considered a standard method for malaria diagnosis (Ansah et al, 2010; Maltha et al, 2010). Immunochromatographic rapid diagnostic tests (RDT) are very useful in the field conditions for on the spot detection of malaria parasite (Murray and Bennett, 2009). These are simple and sensitive and good results have been achieved in various endemic regions. The pLDH based RDT, although having some limitations, but as of today has been proved to be one of the best tool
available for on the spot detection of malaria parasite (Iqbal et al, 1999; Zakeri et al, 2002). Polymerase chain reaction (PCR) assay which involves the detection of parasitic nucleic acid fragment in the blood has been proved to be a sensitive tool to detect the malaria parasite at species level, in mixed infection and even when the parasite count is very low (Johnston et al, 2006). *P. falciparum* and *P. vivax* are common whereas *P. malariae* is still uncommon in India and only few systematic studies have reported it across the country (Dev, 2000; Mohapatra et al, 2008; Sharma, 2009; Sitalakshmi et al, 2005).

1.6 **Aim of present investigation**

The present research has been undertaken to estimate the malaria epidemiology among some vulnerable population groups in Indo-Bangladesh border in South Tripura and Dhalai districts in Tripura and seven malaria sensitive tea estates in Assam. Known malaria vectors were identified along Indo-Bangladesh International border areas in Tripura, Indo-Bhutan International border area in Tamulpur, Assam- Meghalaya border area in Khasi hills and four military stations namely Tezpur, Dinjan, Sibsagar and Missamari in Assam to understand the composition of prevalent malaria vectors in the region. Human host preference and molecular xenomonitoring of malaria parasite in field collected *Anopheles* mosquitoes was carried out in Tezpur cantonment area and Khasi hills area of Assam to identify the potential vectors and their anthropophilic behaviour among commonly prevailing known malaria vectors. Comparisons of microscopy with PCR and pLDH based rapid diagnostic kit were made using the blood samples collected from malaria endemic Missamari and Hozai areas of Assam to assess the sensitivity of all these methods in malaria diagnosis. Known malaria vectors which prevailed in large number were tested using WHO insecticide susceptibility bioassay in
Indo-Bangladesh International border area in South Tripura district of Tripura, Indo-
Bhutan International border area in Tamulpur, Chandubi area of Assam-Meghalaya
border and three military cantonments in Tezpur, Dinjan and Sibsagar of Assam. Additionally knock-down resistance and behavioural resistance has also been
determined in Chandubi and Rani areas of Assam.

Although many studies on malaria epidemiology have been conducted in Assam
but not much information is available on malaria incidences and vector prevalence in
border areas and among some marginalised vulnerable population groups living in the
region. Prevalence and abundance of known anophelines may be useful in updating the
malaria vectors database whereas identification of potential malaria vectors, their
human host preference and possible role in malaria transmission would be useful in
targeting vectors species. DDT and deltamethrin susceptibility of certain malaria vectors
which are recorded abundantly and may have a role in malaria transmission would be
useful to provide an informed selection of insecticides in light of available malaria
vectors and their susceptibility status. The outcome could be useful for implementation
in an effective malaria control programme in the region.