2. REVIEW OF LITERATURE
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2.1 Malaria and malaria vectors

Malaria is among most widespread diseases affecting humans in many parts of the world for over 50,000 years. Malaria is caused to human by Plasmodium parasite and considered to incite serious public health damage in tropical and subtropical regions worldwide. World Health Organization and Malaria Atlas Project have suggested that over half of the worlds' human population, mostly living in the developing countries is at risk for malaria transmission (Guerra et al, 2010; Hay et al, 2009; WHO, 2008, 2009, 2010, 2012a). At present 247 million cases are reported from 109 countries annually. Approximately 3.3 billion people are estimated to be at risk and more than 500 million fall severely ill every year while more than 1 million die due to malaria. Malaria is a serious disease in Africa where one in every five (20%) childhood deaths is due to malaria only (Alonso and Tanner, 2013; WHO, 2009, 2010, 2012a).

It is now well known that human malaria is caused by protozoan parasites of the genus Plasmodium and transmitted from man to man by certain Anopheles mosquitoes. The malaria transmission solely relies on the mosquitoes of genus Anopheles which serve as the vector host for malaria parasites. Out of 120 Plasmodium species, 22 species have been reported to transmit malaria in primates, 19 in rodents, bats and other animals and 70 in birds and reptiles. Until recently, only four Plasmodium parasite species namely, P. falciparum, P. vivax, P. malariae and P. ovale were thought to be involved in causing human malaria. P. vivax, P. malariae and P. ovale probably co-evolved in ancient times alongside humans, while P. falciparum is a proportionally more recent human malarial parasite which was passed by monkeys to the humans,
probably between the late Mesolithic and the early Neolithic era (Morelli and Amoroso, 1997; Scotto, 2010).

A few studies have indicated that a primate’s malaria parasite, *P. knowlesi* is emerging as a major malaria parasite throughout the South-East Asian countries. Identification of *P. knowlesi* in Malaysia, Vietnam and Thailand during the early 21st century has drawn the attention of researchers and clinicians to diagnose and treat the malaria patients having *P. knowlesi* infection (Eede *et al.*, 2009; Jongwutiwes *et al.*, 2004; Putaporntip *et al.*, 2009; Singh *et al.*, 2004a). Initially the *P. knowlesi* infections in Malaysia appeared to be an unusually high incidence of *P. malariae* infection, but conclusively it was proved that *P. knowlesi* is a major cause of malaria in Malaysia, particularly on the island of Borneo (Singh *et al.*, 2004a). Younger stages of *P. malariae* and *P. knowlesi* parasites appear very similar under light microscopy, but *P. malariae* multiplies every three days (quartan) and never reaches dangerously high densities in the blood whereas *P. knowlesi* has a daily (quotidian) cycle and can rapidly reach up to potentially lethal densities if remain unchecked (Cox-Singh *et al.*, 2008; Sabbatan *et al.*, 2012). Malaria symptoms usually appear between 10 -15 days after the mosquito bite and include fever, nausea, severe headache and vomiting.

As of now, female *Anopheles* mosquitoes are the definitive host for the human *Plasmodium* parasite, where sexual reproduction of the parasite takes place in the gut. While taking a blood meal needed for egg development, the infectious female mosquito injects the *Plasmodium* parasite in the form of sporozoites into the human body. Unlike human malaria, *Anopheles* mosquitoes are not the primary vectors of avian and reptilian malaria. Studies have revealed that *Aedes, Coquillettidia, Culex* and *Mansonia* mosquitoes are implicated as the primary avian malaria vectors, whereas in addition to
the mosquitoes, biting midges and sand flies are the primary vectors of malaria in reptiles (Cox, 2010; Njabo et al, 2011). The *Anopheles* genus consists of six subgenera of which four subgenera namely, *Kertezia, Lophopodomyia, Nyssorhynchus* and *Stethomyia* are restricted to America and make up only 11% of total *Anopheles* species. Sub genus *Cellia* is found in Africa, Asia, Europe and Australia, whereas subgenus *Anopheles* is cosmopolitan in distribution. Both *Cellia* and *Anopheles* represent 48% and 41% of the total *Anopheles* species respectively (Harbach, 2004; Krzywinski and Besansky, 2003).

So far approximately 500 species of the genus *Anopheles* have been identified of which 70 have been recognised to be potential vectors of malaria (Harbach, 2004; Hay et al, 2010). The close genetic inspection reveals that majority of *Anopheles* vector species are closely related and morphologically indistinguishable from each other. Most of these species complexes are poorly studied and the vectorial potential is still uncertain (Bashar et al, 2012; Dev and Sharma, 2013; Harbach, 2004; Lardeux et al, 2007). *Anopheles gambiae*, the most extensively studied species of malaria vectors is the highly anthropophilic and most notorious in many parts of the globe.

### 2.2 Malaria and *Anopheles* vectors of North-East India

Malaria continues to cause high rates of morbidity and mortality in India and remains as a major public health concern in many parts of the country. In India, malaria is caused by four species of human malaria parasites, namely *P. falciparum, P. vivax, P. malariae* and *P. ovale*. Of these, *P. falciparum* and *P. vivax* are the most common whereas handful cases of *P. malariae* have also been reported from various parts of India (Mohapatra et al, 2008; Sharma et al, 2006; Sullivan et al, 2012). Few cases of *P. ovale* have also been reported from different parts of the country however its presence
appears to be extremely rare, if not absent (Das et al, 2012; Marathe et al, 2006; Mishra et al, 1999; Prakash et al, 2003; Sahu et al, 2013).


One of the major challenges faced by malaria control programmes is antimalarial drug resistance. There is an increasing trend in the proportion of *P. falciparum* infection in India during the last few decades and the parasite has become resistant to conventional drugs. The spread of malaria to new areas or re-emergence of malaria in areas where the disease has been eradicated can be attributed to antimalarial drug resistance. Human migration, population movement due to development and natural disaster has fueled the spread of drug resistance to new areas. Antimalarial drug resistance has been involved in the occurrence and severity of epidemics in different countries (Bloland, 2001). Out of four species of malaria parasite that naturally infect human, *P. falciparum* and *P. vivax* has developed resistance to antimalarials, however
the geographical distribution of resistance to a single antimalarial drug varies from region to region. Resistance to all classes of antimalarials with the exception of artemisinin has been reported from different regions in recent past decades (Talisuna et al, 2004; White, 2009). Antimalarial resistance to *P. vivax* emerged comparatively later and reported mostly in South-East Asian countries (Achan et al, 2011; Talisuna et al, 2004).

North-Eastern India has recorded very high incidence of antimalarial resistance in last three decades. First case of chloroquine resistance in India was reported in Karbi Anglong district of Assam in 1973 (Chakraborty et al, 1979; Gogoi et al, 1995; Sehgal et al, 1973) and subsequently in different part of North-Eastern India namely, Arunachal Pradesh, Nagaland, Mizoram and Meghalaya during 1979-81 (Mohapatra et al, 2003; Pattanayak et al, 1979). Chloroquine resistance strains of *Plasmodium* multiplied quickly and gradually developed multiple drug resistance (MDR) particularly in the border areas where monitoring is comparatively less (Dev et al, 2003b; Dhiman et al, 2010b; Mohapatra et al, 2003). Increase in malaria cases in the 1970s and subsequent reports of antimalarial treatment failures led to review the entire situation and a new programme called *P. falciparum* Containment Programme (PfCP) was launched in 1978 with the help of Swedish International Development Agency (SIDA). With its main objective to contain the spread of drug resistant *P. falciparum* malaria, the drug resistant foci were diluted in its ten years of operation (Ray et al, 1998). Studies of antimalarial efficacy in the North-Eastern region have shown that chloroquine resistance is widespread. Most of the studies are of localized nature and have involved a limited number of samples. However, in 1997 national level compilation of antimalarial drug resistance included 12863 *P. falciparum* cases and revealed about 24% chloroquine
resistance of varying levels (Shiv et al, 1997). Chloroquine resistance of varying level was reported from different areas of North-East India. Dhiman et al (2010b) reported 35% treatment failure in chloroquine treatment whereas Majumdar et al (2011) reported 67.5% of chloroquine resistance in Tripura. In one of the largest drug sensitivity study conducted in this region, Campbell et al (2006) reported 95.8% treatment failure in chloroquine and sulfadoxine- pyrimethamine (57.1%). However, resistance to mefloquine and mefloquine artesunate combination was not much prevalent. Resistance to sulfadoxine- pyrimethamine has been reported in many forest fringe areas of the North-Eastern region however resistance to artemisinin group drugs has not been reported from this region making it suitable for the treatment of uncomplicated malaria cases. Studies have also reported multi drug resistance in a few areas of this region (Dev et al, 2003b; Dua et al, 2003). Development of chloroquine resistance in P. falciparum, first reported from Assam has emerged as a serious challenge in combating malaria in India. Although resistance to chloroquine has been reported in P. falciparum, it is still effectively used for the treatment of vivax malaria.

Malaria transmission has been a daunting epidemiological challenge in the North-Eastern states of India as its distribution is heterogeneous and intensity is governed by many biological, climatic and physiological risk factors (Dev et al, 1996a, b, 2004; Dhiman et al, 2009, 2012b; Pardal et al, 2009). Difficult terrain, hilly forest, inadequate health infrastructure and lack of awareness aggravated the malaria situation in the region (Dev et al, 2003a; Dhiman et al, 2010a, 2011). Assam is largest state (population wise) among eight states in the North-East India and shares 71% population and maximum number of malaria positive cases annually. Arunachal Pradesh, geographically largest state contributes 2.6% population of the North-East and 15% of
total malaria positive cases in the region. The hills and foothill areas of Arunachal Pradesh – Assam border are highly endemic for malaria with annual parasitic index (API) of > 15 (Das et al, 2002, 2004, 2007).

Meghalaya, Manipur, Nagaland, Mizoram and Tripura are hot spots for *P. falciparum* and many cases are reported annually. A recent study in Meghalaya has revealed that malaria transmission was perennial and persistent with seasonal peak during May-July corresponding to the months of high rainfall. West Garo hill district is co-endemic for *P. falciparum* and *P. vivax*, but *P. falciparum* was the predominant infection (> 82%). *An. minimus* was the predominant vector species that was incriminated by detection of sporozoites in salivary glands (infection rate - 2.3%) and was ascertained to be fully susceptible to DDT (Dev et al, 2010). Tripura, surrounded by Bangladesh on three sides and one side by Assam and Mizoram, consists of 3 districts. South Tripura district is the most endemic contributing 62% and 58.6% of malaria positives and *P. falciparum* cases of the state respectively. In an endemic state of North-Eastern India, Dhiman et al (2010b) have found the slide positivity rate (SPR) of 25.2% with *P. falciparum* as the predominant malaria parasite (slide falciparum rate-22.3%). The incidence rates of *falciparum* malaria varied significantly among the age groups (*p < 0.001*) and 2-4 year olds were most affected. Major malaria vectors recorded in light trap collections were *An. dirus*, *An. minimus* and *An. philippinensis/nivipes*.

The North-Eastern region of India is strategically important and large numbers of troops are deployed along highly malaria endemic borders with neighbouring Bhutan, Myanmar, Bangladesh and China where the movement into infested area is a regular phenomenon which exposes them to the risk of acquiring infections. The activities of
the armed forces create thousands of breeding places for the vector mosquitoes and thus greatly increase the transmission. Even in recent years, extensive movement of non-immune soldiers, night vigils and other activities like cine-viewing, inadequate use of mosquito nets and other protection methods, failure to take chemoprophylaxis or even in chemoprophylaxis and its adverse effects have contributed to the rising cases of malaria in war time as well as in peace (Dhiman et al, 2010a).

Dev (1996a) reported a notable increase in the number of fever cases along with corresponding increase in the number of malaria positives cases from May to September. This increase was largely due to *P. falciparum* infection and coincided with the wet season (monsoon). For the remaining year transmission continued but there was a marked decrease in the number of cases. Over the study period the SPR ranged from 22.9- 36.5% with the majority being *P. falciparum* infections (74%). Focal outbreaks of malaria are common in the North-Eastern region accounting for 8-12% of all reported malaria cases in India. *P. falciparum* remains the major malaria parasite causing >60% of malaria infections (Baruah et al, 2004; Das et al, 2002; Dev et al, 2010; Dhiman et al, 2010a, b, 2012a, b; Rabha et al, 2012; Nath et al, 2013; Yadav et al, 2012a, b).

Climate change and deforestation significantly influence the malaria pattern. A recent study (Nath et al, 2012) analysed the malaria epidemiological data from 1994 to 2005 in a district of Assam and showed that the entire study area is endemic with perennial malaria infection and each village contributing considerably. The overall SPR ranged from 5.1% in 1997 to 44.4% in 2005. There was a significant increase in the SPR during the study years and maximum SPR (28.2%) was recorded in 2005 (F = 2.5; p > 0.01). In the initial years SPR was found to be lower than 20% in many villages, however the increasing pattern of SPR was observed over the subsequent years. A
significant linear trend was found among the SPR recorded during the study years (slope = 0.47; \( r^2 = 0.12; \ p < 0.002 \)). The comparison of SPR among years 2000, 2003 and 2005 showed that the increase observed in 2005 was statistically significant (\( p = 0.03; \ \chi^2 = 5.06 \)) along with a significant linear trend (slope = 6.751; \( r^2 = 0.27; \ p = 0.01 \)). On the other hand the forest covered area of study area was found to decrease from 2000 to 2005. The forest covered area in 2000, 2003 and 2005 was 23.6%, 18.6% and 15.4% of the total area respectively. The decrease in forest cover in 2005 was found statistically significant (\( p < 0.0001; \ \chi^2 = 34.5 \)). The correlation obtained between the SPR and forest cover during 2000, 2003 and 2005 was not quite significant (\( r^2 = 0.94; \ p = 0.09 \)); however, the percentage of deforestation was associated with increase in malaria incidence in the study area. The study has suggested that there might be a change in the spread of malaria transmission due to change in various climatic parameters and malaria may decrease in African countries while significant increase could be observed in other parts (Ermert et al, 2011; Gething et al, 2010).

Malaria episodes have been reported from the armed forces all around the world as the troops were not able to take precautions during operation and duty hours (Kitchener et al, 2003; Tuck et al, 2003). In malaria hyper-endemic areas such as North-Eastern region of India, the military forces have always been at the risk of malaria infection probably due to duty demand and sometimes movement of susceptible soldiers from the non-endemic zone (Dhiman et al, 2010a). Malaria statistics in forces changes every year in the adjacent units and even in the same unit which may be associated with the number of fresh soldiers moved into the unit or a new unit raised/ moved in from other regions. The troops are on chemoprophylaxis still incidence of malaria are recorded due to potential burden of parasite in highly malarious ecosystem. In such
situation the demand for extra investment in case detection, warning or forecasting system, emergency responses by government or non government organizations may not be the most appropriate and cost-effective method for control. However, investments in sustainable approaches to vector control (spraying households with residual insecticide, i.e., IRS), promoting individual protection (ITBN and repellents, i.e., PPM) and effective case management are more useful. Health education among soldiers at grassroots level is of paramount importance for effective translation and implementation of the designed technology (Nicholas, 2006).

Various socio-economical, ethnic and behavioural aspects have significantly contributed to the malaria burden in North-Eastern states. Little work has been done on the role of social factors in the resurgence of malaria because the focus of public health and malariology in particular has been narrowly fixed on the parasite and the mosquito vector. The bigger picture has been neglected, namely the increased rates of malaria morbidity although directly influenced by changes in the parasite and vector are more directly caused by human behaviour. Those behaviours are related to individual culturally coded patterns and larger scale sociological phenomena including the political-economic level (Dhiman, 2009; Sharma et al, 2007). The socio-economic conditions, myths and beliefs and awareness have also been listed as determinant factors for the spread of malaria in a region.

The transmission of disease is facilitated by six vectors, namely An. minimus, An. fluviatilis, An. philippinensis, An. culicifacies, An. annularis and An. dirus. However, few other vectors have also been found involved in malaria transmission but their status is still uncertain. Hot and humid climate prevailing in the region is ideal for the survival, proliferation and multiplication of malaria vectors (Dev et al, 2003a, b,
Various ecological changes, particularly deforestation, urbanization, agricultural developments, migration of population and new settlements have been influencing the vector distribution and composition to a greater extent (Dev et al, 1996b; Nath et al, 2012). These factors have influenced the breeding ecology and in turn species composition of mosquitoes as whole which have resulted in appearance, disappearance or introduction of previously unknown and new species (Jambulingam et al, 2005). Malhotra (1998) reported 45 species of *Anopheles* mosquitoes in the North-Eastern region of India. Of these, *An. dirus* (*An. balabacensis*), *An. fluviatilis*, *An. minimus* and *An. philippinensis* (*An. nivipes*) were regarded as important malaria vectors. Besides, natural infections were found occasionally in *An. aconitus*, *An. annularis*, *An. culicifacies*, *An. jeyporiensis* and *An. maculatus* (Rao, 1984). These *Anopheles* species are known to transmit the disease singly or in association resulting in varying degree of malaria intensity in the region. A study conducted in Dimapur (Nagaland) 20 anopheline species were recorded including six potential vector species namely, *An. annularis*, *An. culicifacies*, *An. dirus*, *An. fluviatilis*, *An. minimus* and *An. philippinensis*. *An. annularis*, *An. culicifacies*, and *An. philippinensis* were observed to have their peak density during the month of July, August or September coinciding with maximum rainfall in the area. *An. minimus* although maintained relatively high density throughout the year, manifested two peaks in June and October. *An. fluviatilis* and *An. dirus* though found in relatively low density their seasonal prevalence was suggestive of a post-monsoon and monsoon species respectively (Baruah et al, 2004).

The study carried out in Boko area of Assam (Nandi et al, 1993) identified 19 anopheline species collected from cattle baits whereas only 15 species were collected
from human dwelling. *An. philippinensis* was the predominant species followed by *An. minimus*. *An. dirus* and *An. fluviatilis* were found in low density but sporozoite infection was detected in them along with *An. minimus*. The high malaria incidence predominantly with *P. falciparum* was closely related to sporozoite infection in the vectors and their densities. The human landing collection revealed that the biting cycle of *An. minimus* was at peak in the first half of the night. There was a gradual build up in species composition with the onset of monsoon and maximum density was recorded in July suggestive of a monsoon species like *An. dirus, An. annularis, An. jeyporiensis, An. maculatus, An. culicifacies* and *An. philippinensis* were observed to be predominant. *An. fluviatilis* though known to be anthropophilic in this area could not be captured on human bait. The study carried out by Dhiman *et al* (2010c) in Tripura recorded 17 anopheline species. *An. minimus* was found in high density whereas *An. dirus* was sparsely distributed. *An. philippinensis/nivipes* was widely distributed in the region (94.9%) forming 10.8% of the indoor resting collections. *An. fluviatilis, An. annularis* and *An. culicifacies* were prevalent in low numbers. *An. vagus* was widely distributed (91.0%) and formed 6.5% of the indoor resting collections.

2.3 Insecticide resistance problem

The malaria incidence largely depends upon various elements related to anopheline vectors, such as prevalence of potential *Anopheles* vector, availability of the human host, anthropophilic behaviour, conducive ecology for vector growth and proliferation and suitable environmental factors supporting vector longevity (Dev *et al*, 2004, 2006a; Dhiman *et al*, 2012a; Rabha *et al*, 2012). Vector control relies primarily on two interventions, namely long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Despite marked increase in the use of both during the past 10 years,
malaria cases are still comparatively high. The active ingredients of all products for IRS come from only four classes of insecticides: pyrethroids, organochlorines (dichlorodiphenyltrichloroethane, DDT), organophosphates and carbamates. All currently recommended LLINs are treated with pyrethroids from the point of both safety and effectiveness. The pyrethroids are the best insecticides ever developed for public health use and account for the majority of IRS coverage worldwide (WHO, 2010, 2012a). The reliance of modern malaria control on pyrethroids and the increasing resistance of malaria vectors to these products put current global efforts at risk.

The use of insecticides for vector control will continue to play a major role in the control programs. However, extensive and indiscriminate use of insecticides for the control of vector-borne diseases has resulted in the development of resistance in many malaria vectors (WHO, 1992, 1998a, b, 2012b). The development and spread of resistance among malaria vector mosquitoes against commonly used insecticides is the major factor of prime concern. This is reflected by the repeated failure of an insecticide to achieve the expected level of control when used according to the recommendations for that particular vector insect (Brogdon and McAllister, 1998; Brown, 1986; Clark and Shamaan 1984; Davies et al, 2007; Martinez-Torres et al, 1998). Insecticide resistance is mediated by behavioural, metabolic or physiological factors and usually result from one or more of three different mechanisms namely, reduction in insecticide penetration, an increased metabolism of insecticide by metabolic enzymes and insecticide target site modification (Wilkinson, 1976). Furthermore, the insecticide resistance management becomes complicated if cross resistance or multiple insecticide resistance develops within a species (Farnham and Sawicki, 1976; Hemingway et al, 1989; Hemingway and
Multiple insecticide resistance occurs when insects develop resistance to several compounds and thereby limiting the choice of insecticides that can be used. The selection of suitable insecticide require resistance management strategies involving comprehensive information of malaria vector species composition in the area of interest, susceptibility to the insecticides proposed to be used for their control and understanding of the underlying resistance mechanisms. There have been instances when resistance has been recorded with long-term use of pesticide in both agriculture and health (Masendu et al., 2005; Soderlund and Bloomquist, 1989; Usherwood et al., 2005). Insecticide resistance mechanism has a biochemical basis which involves the modification of target-site to which the insecticide no longer binds and detoxification enzyme based resistance, which occurs when the activities of esterases, oxidases or glutathione S-transferase (GST) are modified to prevent the insecticide from reaching its site of action (Prapanthadara et al., 1996; Brogdon and McAllister, 1998). An additional mechanism based on thermal stress response has also been proposed (Patil et al., 1996) but its significance has not been assessed.

Pyrethroids primarily act by binding and blocking the voltage-gated sodium channel of the nerve membranes. Knock-down resistance (kdr) or insensitivity of this target site is now unequivocally attributed to structural modifications in a sodium channel protein. The same amino acid substitution in a sodium channel protein confers a "basal" kdr phenotype in a range of insect species including mosquito. This phenotype may subsequently be enhanced by further mutations that also recur between species.
resistance takes a variety of forms. Therefore, local strategies must be tailored to the type of resistance present. Resistance mechanism, metabolic resistance and target-site resistance includes multiple forms which are of varying importance for different classes of insecticide. A further complication is ‘cross-resistance’ between insecticides with same mode of action for killing mosquitoes. For example, vectors that are resistant to pyrethroids and have \textit{kdr} target-site resistance will probably also be resistant to DDT. Cross-resistance restricts the choice of alternative insecticide available for resistance management. The study carried out by Bhatt \textit{et al} (2012) to assess susceptibility status of \textit{An. culicifacies} to DDT, malathion and deltamethrin in Chhattisgarh revealed that with DDT the mortality in \textit{An. culicifacies} varied from 3.2 to 33.7%, whereas against malathion mortality varied from 39.4 to 73.5%. On the other hand mortality against deltamethrin varied from 68 to 98.7%. The study concluded that the species has developed resistance to DDT and malathion in all the districts, whereas deltamethrin resistance was observed in many places.

\textit{An. culicifacies} was found resistant to DDT in all study places and to malathion in some districts of Madhya Pradesh. The species registered resistance against deltamethrin in the majority of the study areas. The observed mortality in different study areas varied from 0 to 28.3% for DDT, 57.1 to 100% for malathion and 64.3 to 100% for deltamethrin. These results indicated that the species was highly resistant to DDT while moderately resistant to malathion and deltamethrin (Mishra \textit{et al}, 2012). Another study by Tikar \textit{et al} (2011) determined the susceptibility of malaria vectors \textit{An. stephensi} and \textit{An. subpictus} collected from various locations of India against DDT, malathion, deltamethrin and larvicide bioassay of fenthion, temephos, chlorpyryphos and malathion using diagnostic doses. Both the species were found to
exhibit variable resistance to DDT and malathion from majority of location. Adults of both the species were susceptible to deltamethrin. Whereas the larvae of both the species showed some evidence of resistance to chlorpyriphos and fenthion and were found susceptible to temephos and malathion. The study conducted in Tripura (Dhiman et al, 2010b) revealed that the mortality rates with 4% DDT against An. minimus and An. philippinensis/nivipes were below 98 percent, whereas 100% with 0.05% deltamethrin for both the species. The study indicated that since the potential vectors were found to developed resistance to DDT, the intervention using DDT in vector control may not produce desired results. Previous studies have indicated that malaria vectors An. culicifacies and An. stephensi have been found to be resistant to DDT in India (Dash et al, 2008) and the emergence of resistance to synthetic pyrethroids in An. culicifacies has also been recorded (Singh et al, 2002). Many studies have reported the development of physiological resistance to DDT and deltamethrin insecticides in Culex quinquefasciatus mosquitoes which are vectors of bancroftian filariasis in India (Sarkar et al, 2009a, b, c) however, the data on Anopheles susceptibility to the commonly used insecticides is scanty.

2.4 Malaria diagnosis for intervention

In the North-Eastern region malaria control relies on early active and passive case detection and prompt treatment through malaria camps and village level health workers. Since malaria associated with fever and other symptoms is the most common diagnosis and treatment in the rural settings the incorrect diagnosis may have severe public health implications (Dhiman et al, 2011; Jain et al, 2014). A considerable proportion of patients who are treated and medicated for malaria actually do not have malaria while those who have malaria do not get malaria treatment due to various
reasons. Further, the missed out malaria cases may act as epicentres for disease spreading at local level (Nath et al, 2013; Yadav et al, 2012a, b). Correct diagnosis followed by appropriate treatment of all malaria cases could be useful in reducing malaria burden in endemic areas. Until recently, microscopic examination of blood smears has been a standard method for malaria diagnosis (Ansah et al, 2010; Johnston et al, 2006; Mohapatra et al, 2008). Microscopy however, requires well trained technical staff and is also labour intensive and time consuming (Maltha et al, 2010). Non-microscopic immunochromatographic rapid diagnostic tests (RDT’s) are simple, sensitive and specific with achievement of good results in various endemic regions (Msellem et al, 2009; Murray and Bennett, 2009). Since, introduction during the late nineties RDT’s have undergone many changes and at present three-band RDT’s for detecting both P. falciparum specific antigen and Plasmodium specific antigen of the four species (PAN-specific) have been developed. Although the performance of the RDT in diagnosis of malaria has been validated throughout the malaria endemic regions, however it was unable to identify the mixed infections due to the common PAN specific antibody capture band as compared to the polymerase chain reaction (PCR) assay (Ansah et al, 2010; Iqbal et al, 1999; Moody, 2002). The RDT’s have been used effectively in detecting the filaria in various parts of the world (Weil et al, 1997; Weil and Ramzy, 2007); however, the RDT’s have not been found suitable to diagnose malaria where the parasite count was as low as <50 parasite/μl (Iqbal et al, 1999; Johnston et al, 2006). The pLDH based OptiMAL-IT has some limitations, but as of today it has been proved to be one of the best RDT available for on spot detection of malaria parasites (Iqbal et al, 1999; Johnston et al, 2006; Msellem et al, 2009; Zakeri et al, 2002).
Recently, PCR assay has been proved to be a sensitive method to diagnose the malaria parasite at species level, the mixed infections and also to detect parasite at very low level. PCR cannot be considered a rapid technique as compared to the conventional methods for the initial diagnosis of malaria parasite. However, its value lies in its sensitivity with the ability to detect all five human malaria parasites and also in less than 1 microliter of blood (Snounou et al, 1993). Nested and multiplex PCR methods could be useful in obtaining valuable information during attempts to identify parasites to the species level. Malaria parasite in many cases of severe infection appears with two or sometime three species in the same patient. In such cases, one species could be misidentified as other species, leading to complications in the diagnosis and treatment as well (Cox-Singh et al, 2008; Singh et al, 2004a). Single and multiplex PCR assays have been developed for the detection of malaria parasite by targeting its DNA from the whole blood of the patients. These PCR based assays have been used for the initial diagnosis following the response to treatment (Iqbal et al, 1999; Johnston et al, 2006; Snounou et al, 1993). The 18S rRNA and circumsporozoite genes have been used as targets for the differentiation of different species of Plasmodium parasite. Methods using nested PCR and reverse transcription PCR enable quick and easy identification of all the Plasmodium species. PCR assay based techniques are capable of detecting the malaria parasites in patients with low parasitemia levels and identify them upto the species level. Several workers have investigated that parasitic DNA persists in the blood after antimalarial treatment (Kain et al, 1994). However, it has been concluded that if the PCR yielded positive results for some days even after treatment the therapeutic failure might be predicted possibly due to the antimalarial drug resistance. P. malariae is still uncommon in India and only few systematic studies have reported it in the
country. In North-Eastern region only two studies have been able to report the presence of *P. malariae* malaria, but only one study could evidence the persistence transmission of *P. malariae* in the region (Dev, 2000; Mohapatra et al, 2008; Sharma, 2009; Sitalakshmi et al, 2005).

2.5 Xenomonitoring and host preference of malaria vector

A mosquito must take at least two blood meals in order to facilitate uptake of the pathogens from an infected human and eventual transmission to a susceptible human. Hence, the human-vector contact is an important component in disease transmission and used to evaluate the risk of vector-borne disease. In addition to the prevalence of *Anopheles* mosquitoes, its host preference and parasite transmission rate provide insight into the malaria epidemiology in an area of interest (Lardeux et al, 2007). *Anopheles* mosquito species which prefer for human blood are considered as important vectors of malaria, whereas those with a preference to blood meal of multiple hosts may increase the rate of arboviruses transmission (Snow, 1983; Anderson and Brust, 1995).

The choice of blood meal is influenced by several factors including host availability, nutritional requirements, intrinsic host preference of the species and vector density. Studies have suggested that mosquitoes foraging preference changes in organisms with niche shifts during their life cycle. Therefore, several factors influencing the mosquito blood intake behaviour are still unknown underlining the need for further understanding of blood feeding patterns that are of importance in studying and managing human diseases. The proper and sufficient knowledge of blood meal preference in different *Anopheles* vector mosquitoes particularly from the human host and from different locations provides information on anthropophilic behaviour and is
useful to demonstrate the changing patterns of host preference and disease management (Bashar et al, 2012; Chavas et al, 2010; Mohanty et al, 2007; Swami et al, 2012).

Malaria transmission in the region is uninterruptedly supported by *An. minimus*, *An. fluviatilis* and *An. dirus*. In addition, *An. annularis*, *An. culicifacies* and *An. philippinensis/nivipes* have also been incriminated as vectors of secondary importance (Bhattacharyya et al, 2010; Chandra, 2008; Dhiman et al, 2012a; Mahapatra et al, 2006; Mohanty et al, 2007; Prakash et al, 2004). Some studies have suggested that the anopheline vectors have seasonal tendencies toward obtaining the blood meal from a host (Basseri et al, 2010). *An. fluviatilis* feeds on human blood during the entire year but the feeding behaviour of *An. stephensi* and *An. culicifacies* varied according to the seasons. The study also indicated that the abundance of the female mosquito positive for human blood was 4.25% per human shelter as compared to 17.5% per animal shelter indicating that the vectors had a tendency to rest in animal shelters after feeding on human. Bashar et al (2012) in their study in Bangladesh has found that out of 21 collected *Anopheles* species, 17 were tested positive for human blood meal. The majority of positives were collected from the human house indoors. *An. baimai* was highly anthropophilic followed by *An. minimus*, *An. annularis* and *An. pallidus*. The study indicated that *An. baimai* and *An. minimus* preferred for human blood. Whereas, *An. annularis*, *An. maculatus* and *An. pallidus* exhibited some opportunistic blood-feeding behaviour and preferred to feed on accessible hosts either human or animal. A study in Rajasthan, India (Swami et al, 2012) revealed that *An. subpictus* had a preference towards cattle blood compared to human while *An. culicifacies* and *An. stephensi* preferred human blood. *An. annularis* gut content revealed the presence of
bovine blood only indicating that *An. annularis* apparently seems to be zoophagic specifically in the study area.

In Bangladesh, it has been found that *An. baimai, An. minimus, An. annularis, An. jamesi, An. maculatus* and *An. pallidus* are more or less anthropophilic, whereas the other anopheline species are zoophilic. Ten species namely, *An. annularis, An. baimai, An. barbirostris, An. jeyporiensis, An. karwari, An. kochi, An. minimus, An. peditaeniatus, An. philippinensis* and *An. vagus* were found to have *Plasmodium* parasite. *An. vagus* and *An. philippinensis* are dominant species in Bangladesh present almost throughout the year with high density in March and comparatively lesser in September, but *An. baimai* is found during monsoon season only. Rainfall and temperature were found to be the most significant variables influencing *An. baimai, An. vagus* and *An. subpictus* density and their abundance was positively related to malaria cases (Bashar, 2012). Analysis of blood meal preference and vectorial status in central India (Nanda et al, 2012) revealed that *An. fluviatilis* is highly anthropophagic and rested in human dwellings. Incrimination studies have shown the high sporozoite rate in *An. fluviatilis* confirming its vectorial efficiency. *An. culicifacies* was found to be exclusively zoophagic.

Molecular xenomonitoring in mosquitoes for malaria has been a sensitive and accurate method for assessing the vector/pathogen distribution by detecting DNA in different strains. It has been found a useful tool for evaluating filariasis elimination programs (Farid et al, 2007; Ramzy et al, 1997, 2006; Williams et al, 2002). Various methods have been deployed to detect the filarial DNA by dissection and by PCR assays in different mosquito strains which concluded that PCR is much more sensitive than dissection for detecting filarial larvae, especially their remnants in mosquitoes.
(Chanteau et al, 1994; Farid et al, 2007; Fischer et al, 2007; Hoti et al, 2008; Sakthidevi et al, 2010). The host preference and incrimination data of Anopheles vectors are scanty and only a few systematic studies have been carried out earlier. Various methods, such as salivary gland dissection, enzyme linked immunosorbent assay (ELISA) and gel diffusion methods were used to detect the blood meal in mosquito however, the PCR based methods are more reliable and sensitive. In addition to recognise the blood meal DNA ingested by mosquito, PCR based methods are specific to detect the Plasmodium species present within the vector mosquito.