Study of malaria transmission dynamics in two different ecosystems in District Deogarh (Odisha)

A thesis submitted to Goa University

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DOCTOR OF PHILOSOPHY

in

ZOOLOGY

By

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STATEMENT

As required under the University ordinance, I hereby state that the present thesis for Ph.D. degree entitled "Study of malaria transmission dynamics in two different ecosystems in District Deogarh (Odisha)" is my original contribution and that the thesis and any part of it has not been previously submitted for the award of any diploma/ degree of any University or Institute. To the best of my knowledge, the present study is the first comprehensive work of its kind from this area.

The literature pertaining to the problem investigated has been duly cited. Facilities availed from other sources are duly acknowledged

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CERTIFICATE

This is to certify that the thesis entitled "Study of malaria transmission dynamics in two different ecosystems in District Deogarh (Odisha)" submitted by Mr. Narayani Prasad Kar for the award of the degree of Doctor of Philosophy in Zoology is based on his original studies carried out by him under our supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma in any University or Institution.

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DEDICATED WITH LOVE TO MY FAMILY AND TEACHERS

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INTRODUCTION

Malaria is a serious life-threatening parasitic disease caused by an endoparasitic protozoan *Plasmodium.* Generally, four species of *Plasmodium* naturally infect human, i.e. *P. falciparum, P. vivax, P. malariae,* and *P. ovale.* Recently a fifth zoonotic species, *Plasmodium knowlesi* is also reported to be naturally infecting humans sporadically (White 2008). Patient may die of falciparum malaria as, occasionally, *P. falciparum* blocks capillaries of the brain, and causes death so is referred to as killer/cerebral malaria. Death may also occur due to the involvement of other organs such as kidneys, spleen, liver and lungs either individually or multi-organ failure. Deaths due to *P. vivax* have also been reported in India in the recent years (Kumar et al. 2007, Kochar et al. 2010). *Plasmodium falciparum* and *P. vivax* are the widespread malaria parasite species worldwide. Latest WHO report of December 2014, approximately estimates 200 million cases, 600,000 deaths per year worldwide and 97 countries and territories with ongoing malaria transmission. Nearly half of the world's population (3.2 billion people) are under endemic malaria risk. *Plasmodium falciparum* and *P. vivax* account for 2.57 billion and 2.5 billion populations respectively (Figure 1) (WHO 2014).

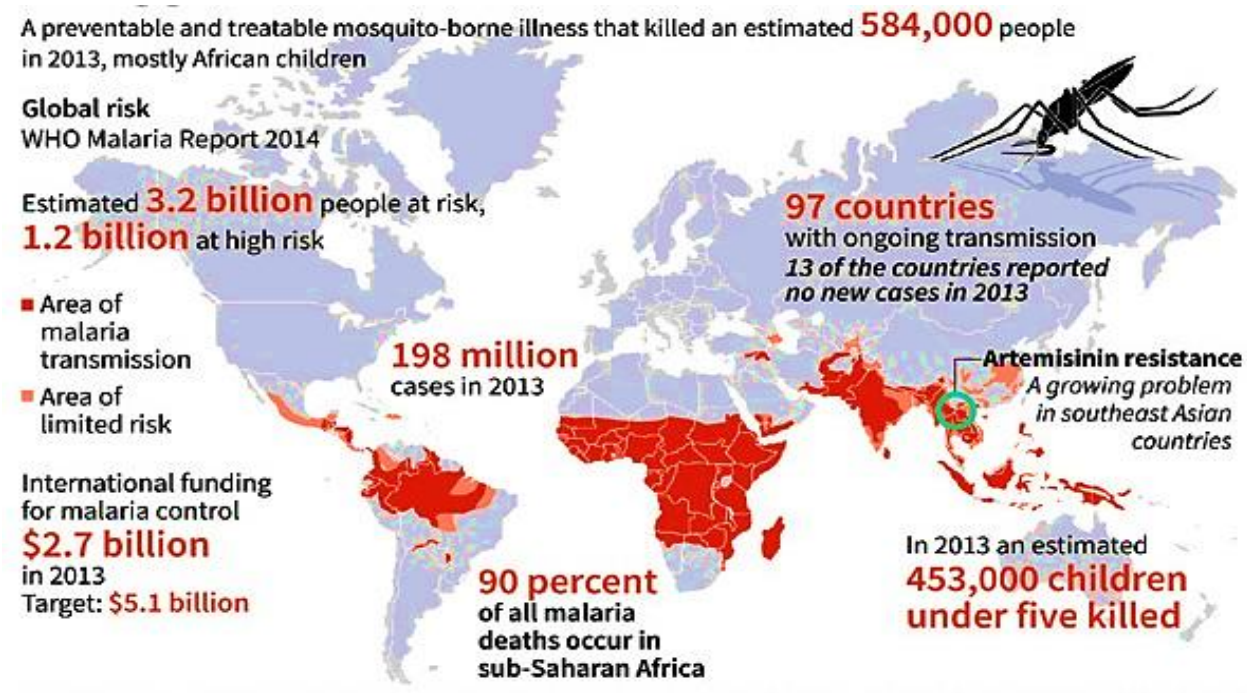

Figure 1: Magnitude of malaria problem world wide

Source: (WHO/Smithsonian institute/malaria.welcome.ac.uk/nih)

1. Milestones in the history of Malaria

Malaria is a very important disease since early times (Vedic periods; 1500-800 B.C.), as ancient script depicts this disease as king of diseases (Sherman 1998) and many historic medical scripts describe the causative environment, it's various aspects and even link malaria with mosquitoes. The first malariologist and father of medicine "Hippocrates" defined various malaria fevers of man and related splenic enlargement with stagnant water in "Hippocratic corpus" about 400 BC. Ancient Indian medical script "Charaka Samhita" classified malaria-fever distinctly dated back to 300 BC.

According to Roman scholar Marcus Terentius Varro (116-27 B.C.), intermittent fever was associated with inhalation of marsh air or miasma carrying invisible "animalcula" (malaria originated from Italian words mala "bad" and aria "air" (Bruce-Chwatt 1980). Around 30 A.D., Celsus described two types of tertian fevers (Sherman 1998). Although the black pigmentation of the brain and spleen was demonstrated in the patient died due to malaria by Italian physician Giovanni Maria Lancisi in 1716 but he failed to link malaria with actual causative agent. Giovanni Rasori (1766-1837) doubted microorganism as a cause of malaria (https://www.ukessays.com/essays/biology/review-of-plasmodiumthe-malaria-parasite-biology-essay.php). The notion of bad air as cause of malaria continued until 1847 when a German physician, Heinrich Meckel, identified protoplasmic spindle-shaped black pigmented granules in the fever patient's blood slides and spleen autopsy. He related the pigments as causative of malaria and coined it as "hemozoin" (Sherman 1998). One year later, in 1848, Schutz witnessed these pigments in the internal tissues of the dead malaria patient. The next year (1849), Virchow confirmed the association of these pigmented bodies with malaria. The investigations continued as Louis Pasteur (1822-1895) and Robert Koch (1843-1895) established involvement of microbes and no evil miasmas causing diseases (Slonczewski and Foster 2009).

Finally, Charles Louis Alphonse Laveran (1845-1922), on October 20, 1880 noticed live microscopic bodies in blood of malaria patient, named the organism as *Oscillaria malariae* and proposed it as the malaria parasite and awarded with the Nobel Prize for physiology, 1907 (Zetterström 2007). In 1884, Marchiafava and Celli found trophozoites in RBC, which they named as *Plasmodium*. Different species of malaria were identified. Laveran identified *P. malariae* in 1880 and Grassi and Feletti identified *P. vivax* in 1890. In 1885 Camillo Golgi (1843-1926), proposed two types of malaria, one with tertian periodicity of

fever (every other day) caused by *P. vivax* and one with quartan periodicity of fever (every third day) caused by *P. malariae*. Subsequently the other human species of Plasmodia have been given the following colloquial names: *P. falciparum* as Malignant tertian (MT), sub-tertian, aestivo-autumnal, Tropical, *P. ovale* as pernicious. He also correlated fever with rupture of schizonts releasing merozoites into the blood stream and the rigorousness with parasite load. From 1891 onwards, after development of the staining method by Dimitri Romanowsky (1861-1921), which stains the *Plasmodium* nucleus with eosin and cytoplasm with methylene blue distinctly, the other *Plasmodium* species causing malaria were easily identified. Welch identified *P. falciparum* in 1897 and Stephens identified *P. ovale* in 1922. In 1896, McCallum, William George and Eugene L. Opie of Johns Hopkins Medical School demonstrated the sexual cycle of malaria parasite. A strong belief about mosquitoes' role in malaria transmission was developed in the late 18th century, (Sherman 1998). Patrick Manson, who recognized mosquitoes as the vector of microfilariae also suspected mosquitoes as malaria vector. In 1896, Amico Bignami claimed the passage of the malaria parasite to mosquito while sucking blood. The major breakthrough in this field took place when Sir Ronald Ross (one of Manson's followers), 'the father of malariology', demonstrated oocysts in the gut of the *Anopheles* mosquito at Secunderabad in India on August 20, 1897 and established mosquito as malaria vector and found sporozoites (1898) in the salivary glands of the mosquito for this epic making discovery he was awarded the Nobel Prize in 1902. Just after Sir Ronald Ross's work, the complete developmental cycles of the *P. vivax* and *P. falciparum* were described by Grassi et al. 1899. By the advent of the 20th century, *Anopheles* mosquito was established as the only vector of human malaria. Malaria parasites were included to the family Plasmodiidae within the order Coccidiida, sub-order Haemosporidiidea. Levine (1978) proposed an alternative system; accordingly, Haemosporidiidea was classified as a sub-order of the Coccidiida. However, Garnham's (1969) classified suborder Haemosporina of class Telospora into family Plasmodiidae (Garnham 1969), Haemosporidiidea (Doflein, 1916) (Corradetti 1938), and Leucocytozoides (Fallis and Bennet, 1961) and is still being maintained.

In 1900, Grassi argued the sporozoite's structure was incapable of penetrating into erythrocyte and hypothesized an intermediary stage during the incubation period after sporozoite inoculation by mosquitoes. In 1903, Fritz Schaudinn claimed to witness sporozoites of *P. vivax* directly penetrating red blood cells. This confused malariologist for decades until 1948, when H. E. Shortt and P. C. C. Garnham revealed the liver stage of the human and primate malaria parasites. In 1912, C. C. Bass and Foster M. Johns were first to culture the

asexual cycle of *P. falciparum* (Kreier 1980). This development led to *in-vitro* validation by Rieckmann in 1971 for development of resistance against chemotherapeutic agents like chloroquine (Rieckmann 1971). The continuous culture of *P. falciparum* was established for the first time by William Trager in 1976 (Trager W. and Jensen J. B 1976). *In-vitro* culture of *P. falciparum* paved path for major breakthrough in malaria research and opened broadways for discovery of new malaria medicines and vaccines. In the late 19th century, malaria vaccine was purposed and the first synthetic Spf66 vaccine against *P. falciparum* was developed by Dr. Manuel Elkin Patarroyo in 1987 (Patarroyo et al. 1987). DNA sequencing arrived in 1990's with a lot of hope. Complete genome sequence of *P. falciparum* and *Anopheles gambiae* were uncovered by year 2002 (Hall et al. 2002, Holt et al. 2002). In 2008, *P. vivax* and *P. knowlesi* genomes were successfully sequenced (Carlton et al. 2008, Pain et al. 2008). Some countries attained zero incidence of locally contracted malaria, for example in recent times; malaria elimination was achieved in United Arab Emirates 2007, Morocco and Turkmenistan 2010 and Armenia in 2011. According to the WHO report of 2015, 55 countries are on the track to reduce malaria up to 75% by 2015. Currently there is no successful vaccine against malaria, but a vaccine against *P. falciparum,* RTS, S/AS01 seems promising and may be launched by the end of 2016 (White et al. 2015).

2. Dynamics of malaria transmission

A female *Anopheles* mosquito transmits malaria from man to man. So for malaria transmission to occur, an infected patient, a vector *Anopheles* and a non-immune receptive person are necessary. A conducive environment is critical for survival, perpetuation and activity of the malaria vector species and also for completion of sexual phase of *Plasmodium* in vector (sporogony) and hence limits the geographical bounds of malaria transmission. Several local biotic and abiotic factors influence malaria transmission and its spatial and temporal distribution.

Life cycle of malaria parasite is complex involving two asexual cycles, one in liver and other in RBCs in human (Secondary host) and sexual and sporogonic development in *Anopheles* vectors (Primary host). The salient features of the life cycle of malaria parasite are depicted in Figure 2.

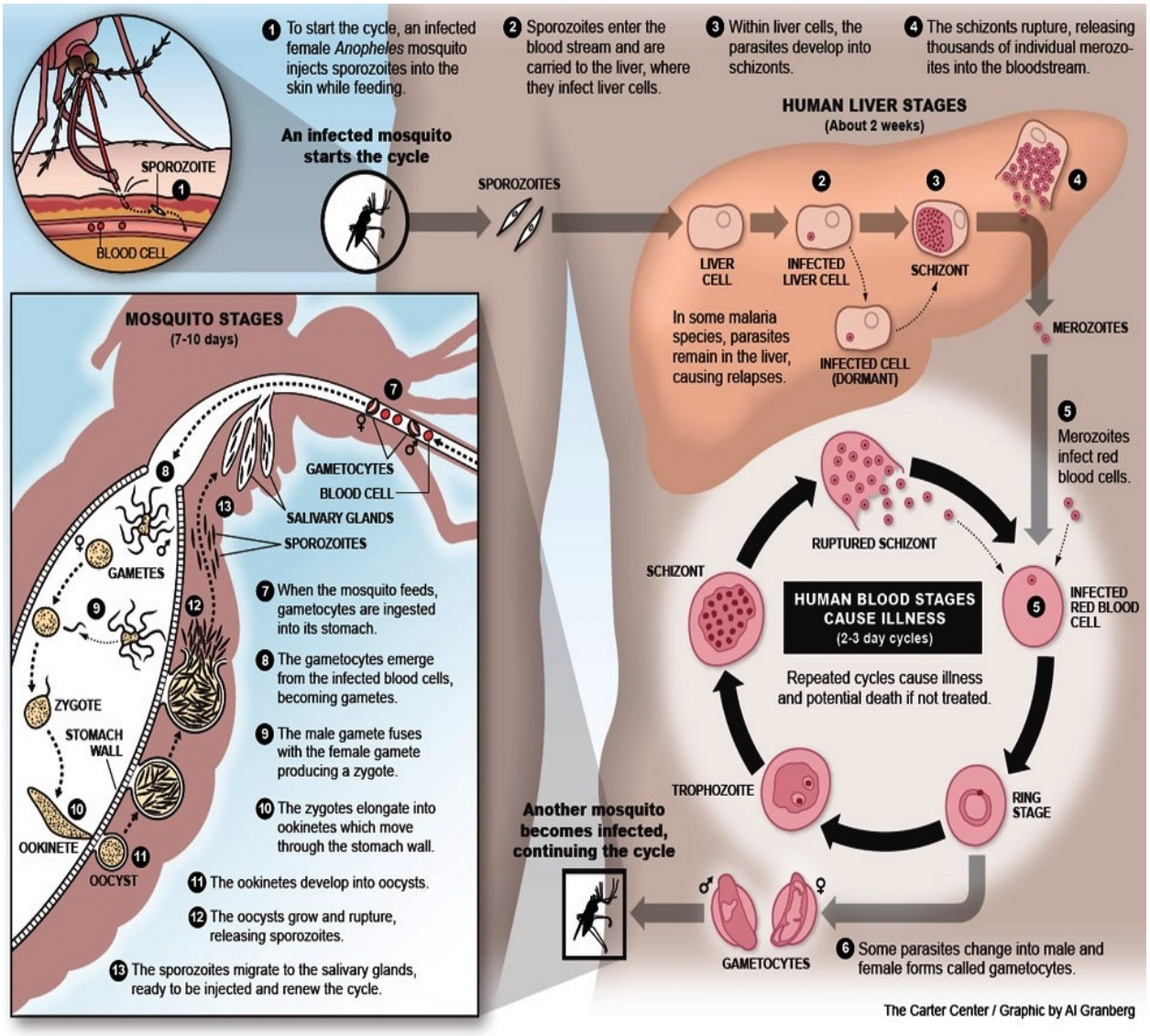

Figure 2: life cycle of malaria parasite

Source: www.cartercenter.org/resources/images/health/malaria/malaria-life-cycle-chart-lg.jpg

Build-up of vector populations depends upon availability of suitable breeding habitats. The longevity of poikilothermic arthropod vector is determined mostly by the environmental conditions like temperature and humidity. The malaria susceptible mosquito must live long enough to support completion of the sporogony of *Plasmodium* in vector and might take between 9-14 days to complete and is essential for the formation of sporozoites and hence successful transmission. The sporogonic development of the malaria parasite in mosquito

slows down in cold conditions or break in extreme temperatures (<14°C). Unlike vector, human host being homoeothermic provides uniform condition for survival of *Plasmodium* irrespective of external environment. Immunity to malaria infection can influence disease severity and may influence intensity of malaria transmission. Human migration may introduce malaria in new geographical area or its perpetuation in an endemic setting. Likewise active or passive vector dispersal may also play role in geographical distribution of malaria.

Due to the age old deleterious effect of malaria on mankind, human genetics has evolved to resist certain strains of malaria, for example, most of the Africans are Duffy antigen negative, which confers resistance to vivax malaria. Similarly sickle cell anaemia and certain other genetic traits confer resistance against malaria. *Plasmodium* grows slower at lower temperature and the duration of maturation is different among different species. *Plasmodium* species with an ability to develop at lower temperature (e.g. *Plasmodium falciparum* in vector at 14°C as opposed to *P. vivax* at 16°C or above) has better survivability and transmissibility. Longevity of the vectors to support a longer incubation period of certain *Plasmodium* species e.g. *Plasmodium malariae* in general or during lower temperatures in particular in case of other species could also play a significant role in malaria transmission. Besides vector longevity, anthropophagy, vector abundances, biting rates, and host preference, refractory status of *Anopheles* towards a *Plasmodium* strain could be other determinants of vector potential and thus malaria transmission.

Preventive and curative services, free of charge, are made available under the National Vector Borne Diseases Control Programme in India. These include antivector services viz., antilarval spraying, source reduction and introduction of larvivorous fishes in urban areas and adulticidal indoor residual spraying (IRS) and/or Long Lasting Insecticide Treated Bed Nets (LLINs). Besides malaria diagnostic and treatment services are made available by fortnightly Active Case Detection (ACD) by Multipurpose Health Workers (MPWs) or Passive Case Detection (PCD) by Village Workers, Accredited Social Health Activists (ASHAs) and in Primary Health Centres, Community Health Centres, Block PHCs and District Hospitals. The treatment is given as per National malaria treatment policy/guidelines depending upon species of malaria parasite. The quality of these services depends on several factors viz., availability of trained manpower, equipment, material, operational feasibility and accessibility. Accessing and availing health facilities and the best utilization of these services depend upon personal preference, quality and reliability of services, and community participation, Information

Education and Communication (IEC) or Behavioural Change Communication (BCC) undertaken by NVBDCP under National Health Mission (NHM).

Malaria transmission is multi-factorial (Figure 3) with high variability in time and space. No one malaria control strategies can work regionally, nationally and globally due to variability and complexities in malaria epidemiology. Local and focal interventions are needed with appropriate situation specific tools. The most pronounced malariogenic conditions can be categorized broadly as ecotypes. From malaria control point of view, India was categorized in five epidemiological paradigms viz. (i) forest/tribal malaria, (ii) rural malaria, (iii) urban malaria, (iv) industrial malaria, and (v) border malaria (Pattanayak et al. 1994, Hoffman et al. 2015). The first two settings encompass most of the geographical area of India. Malaria transmission is most intractable in the forests. Forest malaria is not easily amenable to malaria control due to several technical and operational factors. In this study the major factors that sustain malaria transmission were reviewed. Bed of flooded riverine-plains facilitates large grounds of breeding pools for malaria vectors like *An. culicifacies*. The largely irrigated riverine-plains also support many other vector species. High density of human population near fertile riverine-plains put them at higher risk of malaria. Forests are hotbeds of malaria transmission as they provide ambient conditions such as vegetation cover, temperature, rainfall and humidity that are conducive to distribution and survival of malaria vectors. Forests often lack adequate infrastructure and harbour tribes with distinct genetic traits, socio-cultural beliefs, and practices that greatly influence local malaria transmission dynamics. Various topographical, entomological, parasitological, ecological and socioeconomic factors, which are crucial and shape malaria transmission, are summarized in this thesis. An in-depth understanding and synthesis of the intricate relationship of these parameters in achieving better malaria control in various ecosystems are emphasized. Growing insecticide resistance in malaria vectors and antimalarial resistance in parasites favour transmission of malaria and their management is essential in disease control. Peoples' participation and acceptance of measures of intervention and accessing health facilities are some of the basic necessities of a successful malaria control programme.

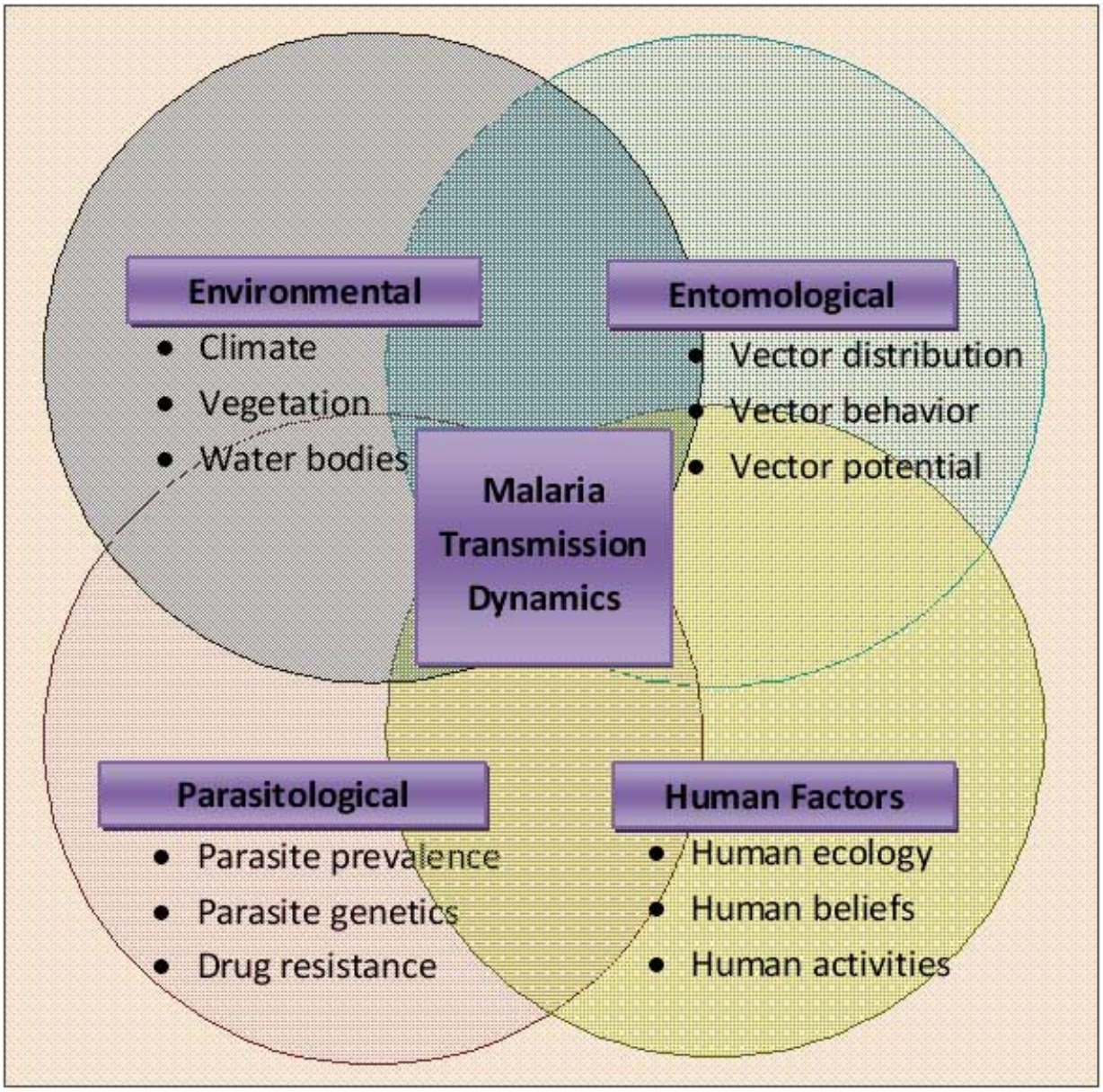

Figure 3: Factors affecting malaria transmission dynamics

Malaria transmission is a dynamic process and involves many interlinked factors, from uncontrollable natural environmental conditions to man-made disturbances to nature. The figure depicts various topographical, entomological, parasitological, human ecological and human behavioural factors, which are crucial and shape malaria transmission. Source: (Kar et al. 2014)

India is endemic to malaria and accounts for about 52% of the total malaria morbidity in Southeast Asia (Pradhan et al. 2016). Interestingly, majority of the malaria morbidity (about 26.9%) and mortality (about 17.6%) is contributed by Odisha state alone, although it comprises about 3% of Indian population (including some aboriginal tribes) (Pradhan et al. 2016). District

Deogarh (Odisha) where the present study has been carried out comprises of typical hilly-forest and riverine-plain ecotypes under Tileibani and Barkot block respectively.

The factors influencing malaria transmission dynamics in different eco-epidemiological settings and the appropriate control measures have been studied in detail under five heads as follows.

1. Analysis of the geographical features and climatic factors that influence malaria transmission in hilly-forest and riverine-plain ecotypes in district Deogarh, Odisha
2. Distribution of malaria vectors, their bionomics and role in transmission in two ecological settings
3. Comparative assessment of the drug resistance pattern in *Plasmodium falciparum* in hilly-forest and riverine-plain ecotypes
4. Assessment of behavioural responses of people in two ecotypes with reference to malaria intervention measures
5. Ascertain the key risk factors of malaria transmission affecting the hilly-forest and riverine-plain ecotypes specifically and the suggested suitable control strategies

Chapter I deals with description of geography, climate, spatio-temporal ecology, and epidemiological profile of the study sites. Open access website based Geographical Information System (Open Web-GIS) of Odisha state government and government of India was accessed. A little explored district with stable malaria from core of the highly malarious zone of India was selected through Web-GIS interface of National Vector Borne Disease Control programme (NVBDCP). One model PHC representing hilly-forest (HF) and other representing riverine-plain (RP) ecotypes from the selected district were taken as study sites. The geography, demography, and availability of communication infrastructure and treatment facility, were evaluated. Climatic variability, i.e. monthly rainfall, temperature, humidity etc. were plotted separately over the two ecotypes to assess seasonal variability. These ecological differences were correlated with epidemiological variability to identify the key factors influencing malaria transmission dynamics.

Chapter II of the thesis includes entomological investigations in HF and RP ecotypes. A detailed study of the distribution pattern and biological attributes of major malaria vectors viz.; *An. fluviatilis* and *An. culicifacies* has been carried out. In addition information on other anophelines including secondary vector *An. annularis* has been generated. Various aspects

studied include sibling species composition of *An. fluviatilis* and *An. culicifacies* complexes, their seasonal prevalence, resting and feeding preferences, response to insecticides used in public health program and role in malaria transmission using standard procedures/ techniques. Based on the information generated, the entomological parameters that influence malaria transmission in two ecotypes have been delineated for planning effective vector control strategies in study areas.

In chapter III information on prevalence of malaria parasite species and genetic variation at the Single Nucleotide Polymorphism (SNP) level in four different genes of *P. falciparum* (viz., *Pfcrt*, *Pfmdr 1*, *Pfdhfr,* and *Pfdhps*) that confer resistance to different antimalarials in two different eco-epidemiological settings, i.e., HF and RP was generated. In addition information on evolution of resistance in *Plasmodium falciparum* isolates collected from two ecotypes against past and present use of antimalarials has been elicited for updating chemotherapeutic strategies in study area.

Chapter IV encompasses study of people's Knowledge, Attitude, and Practices (KAP) against malaria interventions in both the ecotypes. Information on the socio-economic and demographic features of the inhabitants of HF and RP ecotypes and their knowledge regarding malaria, its prevention and control was collected through a questionnaire based survey. In addition data were obtained on the access to health care, adherence to drug regime, behavioural heterogeneity in use of alternative malaria treatments and community's perception and attitude with reference to bednet use and indoor residual spray operation (IRS), that largely influence malaria transmission. The data were analysed using IBM-SPSS software to study variations in the KAP parameters and their association with malaria transmission in HF and RP ecotypes.

Chapter V is the concluding chapter which discusses salient features and risk factors that influences malaria transmission in both the ecotypes. On the basis of information generated on topographic and climatic factors, entomological and parasitological parameters, communities' perception, attitude and practices in relation to intervention measures, situation specific and evidence based measures have been suggested for effective malaria control in two ecotypes. This study has provided in-depth understanding of malaria transmission dynamics in the HF and RP settings, highlighted epidemiological risk factors influencing malaria transmission, and the suggested evidence based situation specific control measures would be useful in curbing malaria transmission in study areas and in other similar ecological settings.

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CHAPTER I

Analyses of the geographical features and climatic factors that influence malaria transmission in hilly-forest and riverine-plain ecotypes in district Deogarh, Odisha

1. Introduction

Study areas were selected in little explored highly malarious Deogarh district of Odisha having Annual Parasite Incidence (API) of malaria as high as > 25 during preceding few years (year 2007-2011) according to National Rural Health Mission (NRHM) Odisha (now known as National Health Mission (NHM)) (http://nrhmorissa.gov.in/mis/SearchDetail.aspx). The Deogarh district (21^{o} 31' N Latitude and 84^{o} 43' E Longitude) is located in the western part of Odisha, India (Figure 1). Deogarh district was carved out from Sambalpur district and came into existence on 1st January 1994 (Wikipedia-Contributors 2014). It is a less developed district predominantly inhabited by both Scheduled Caste (SC) and Scheduled Tribe (ST) people. The people mainly depend on farming. It has abundant forest and river water resources, the obvious lack of sufficient social and developmental infrastructure makes it a backward district with 79% of its population being rural and 'Below Poverty Line' (BPL) of which 29% underprivileged (yearly earnings < Rs.4000). The district consists of only one Municipality, single 'Tehsil' (sub-district) and 3 blocks comprising of 774 villages. District Deogarh has 22% forest out of 2781.66 Sq. km area with a population of 3,09,154, which is at high risk of malaria. Based on the data collected from the district, villages under Tileibani PHC were selected as hilly-forest ecotype (HF), and villages under Bampada PHC were selected as a riverine-plain ecotype (RP). As per the annual PHC report the average API of HF was about 7 times higher than RP during preceding more than a few years.

Figure 1: Map of India highlighting Deogarh district (Odisha state). Sample collection sites in Hilly-Forest ecotype (PHC Tileibani) and Riverine-Plain ecotype (PHC Bampada) are demarcated.

1.1. Malaria burden in District Deogarh, Odisha

Apart from Africa, South-East Asia is the main contributor of malaria. India is endemic to malaria and accounts for about 52% of the total malaria morbidity in Southeast Asia (Pradhan et al. 2016). Interestingly, majority of the malaria morbidity (about 26.9%) and mortality (about 17.6%) is contributed by Odisha state alone, although it comprises about 3% of Indian population (including some aboriginal tribes) (Pradhan et al. 2016). Intense and stable malaria has been reported from tribal areas of Odisha and neighbouring states (http://www.malariasite.com/tag/orissa/) (Nanda et al. 2000, Kumar et al. 2007, Das et al. 2012, Kumar et al. 2012). The state of Odisha consists of two highly malarious clusters; the North-Western (comprising of five districts, *viz.* Deogarh surrounded by Keonjhar, Sundergarh, Anugul and Sambalpur) and the South-Western (comprising of seven districts, *viz*. Koraput, surrounded by Malkangiri, Nawarangpur, Kalahandi, Raygada, Nuapada, and Kandhamal (Mohanty et al. 2009, Sahu et al. 2013, Rao et al. 2015, Pradhan et al. 2016), although other districts too contribute to the total malaria cases (Figure 2).

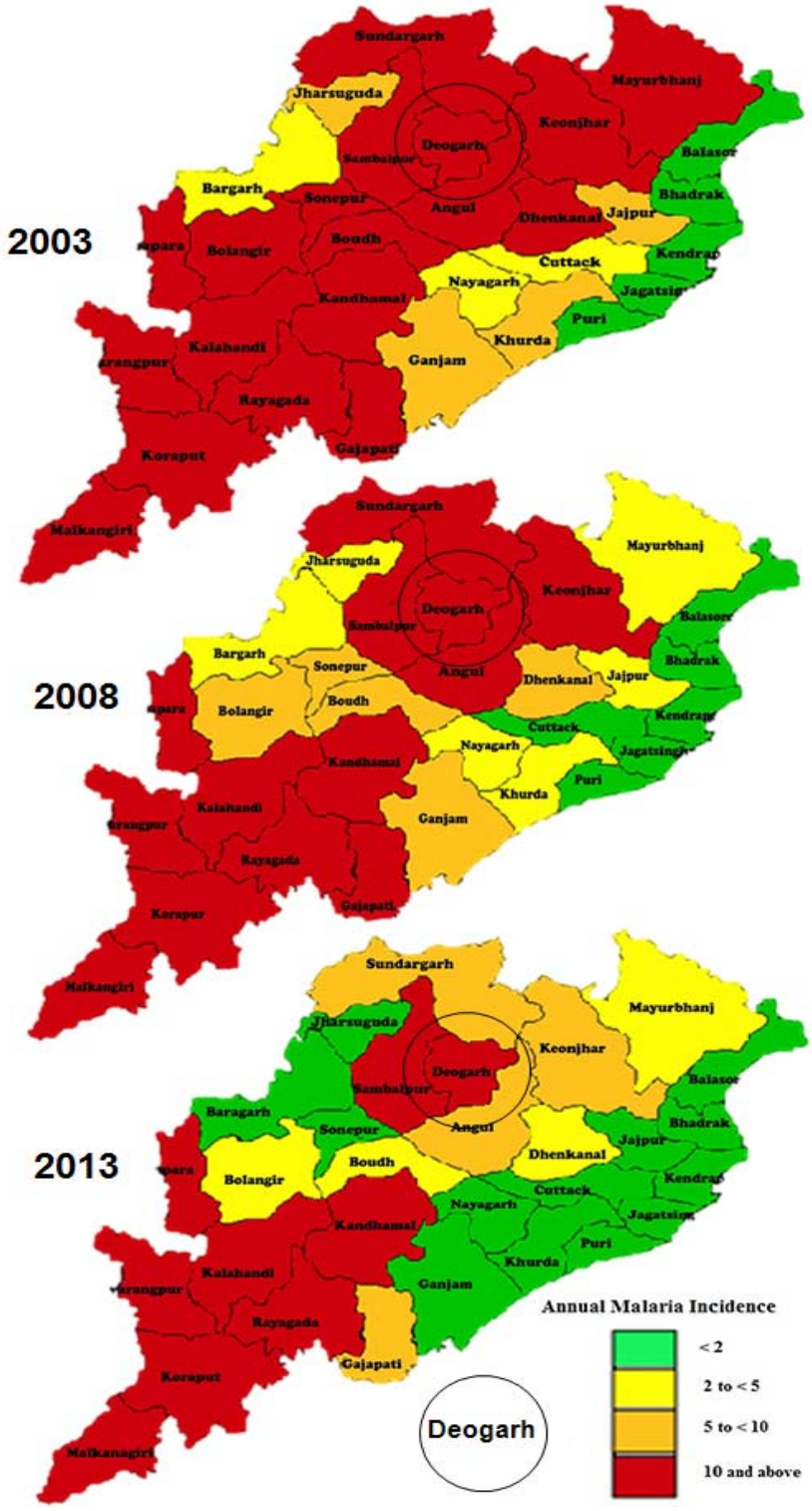

Figure 2: Annual malaria incidence (2003, 2008, and 2013), Odisha.

Source: (Pradhan et al. 2016)

Interestingly, the districts in both the clusters are rich in hills and forests and home for aboriginal tribes (Sahu et al. 2013, Ramar et al. 2014, Pradhan et al. 2016). The Deogarh district is one of the epicentres of high malaria endemicity (Pradhan et al. 2016); comprising of

two distinct ecotypes [Hilly-forest (HF) and Riverine-Plain (RP)], and therefore can serve as a model to understand the influence of micro eco-typical habitats on malaria epidemiological outcome. The tribals (22 % aboriginals of Odisha also called 'Adibasi': aboriginals, 'banabasi': forest dwellers, 'Girijana': Mountain dwellers) cohabited with non-tribal Odias in most of the districts that are endemic to malaria (Nayak 2010). So far, no comprehensive study on the dynamics of malaria transmission has been carried out in this district.

1.2. Health Infrastructure and Malaria Control in District Deogarh

At district level, DHH delivers health care to all the blocks directly and through referral PHCs situated in different blocks. The Chief District Medical Officer (CDMO) and District Malaria Officer organize malaria control interventions of the district as per National Vector Borne Disease Control Programme (NVBDCP) guidelines. Besides, a NVBDCP consultant assists the DMO in malaria control operations. Each PHC has a Medical Officer In-Charge and serves all the villages in its jurisdiction. PHC operates many satellite sub-centres facilitated with one male and one female health worker who work at village level. Accompanying, 'Anganwadi' workers and each of the Accredited Social Health Activists (ASHAs) who are village resident health workers serve 1000 population especially Women and Child Health care and also diagnose and provide treatment of malaria as per NVBDCP guidelines. Indoor residual spraying (IRS) of DDT has been routinely carried out as the main vector control measure. Since 2002, supported by World Bank, Enhanced Malaria Control Program (EMCP) introduced Insecticide Treated Nets (ITNs) in these areas as Deogarh being one of the 100 most malaria endemic districts in India (Patil and Kumar 2011) and later since 2008 ITNs and Long Lasting Insecticidal Nets (LLINs) are distributed through state initiative (My mosquito net) (Pradhan et al. 2016). The CDMO receives mosquito nets and insecticide from the NVBDCP state headquarters in Bhubaneswar, distributes through primary health centres and sub-centres, auxiliary nurse, and midwives (ANMs) and ASHAs. Usually the ANMs during their routine visits to villages announce the availability of insecticide treated mosquito nets at sub-centres and people buy the nets at a subsidized cost of Rs. 25 per net. The ANMs also facilitate the retreatment of the mosquito nets with insecticides (Vijayakumar et al. 2009). District Deogarh is having three PHCs, one each in every block. Bamparada PHC, Tileibani PHC, and Chatabar PHCs correspond to the Barkot, Tileibani, and Reamal blocks respectively. Malaria control intervention is different in these three PHCs in terms of number of rounds of IRS and extent of bednet distribution.

2. Review of literature

2.1. Stratification of malarious region into eco-epidemiological settings

For better understanding of malaria transmission and application of control strategy, malarious areas have been stratified in different eco-epidemiological settings (Sergiev et al. 1994, Rubio-Palis and Zimmerman 1997, Schapira and Boutsika 2012). The stratification is done by selecting a number of variables that may be considered as main determinants of malaria transmission, such as distribution of main vector species and differences in epidemiology (Hackett 1958, Russell 1963, Lal et al. 1999), climatic zones (temperature, humidity, rainfall) (Gill 1938, Boyd 1949, Forattini 1962, Forattini 1973, Trewartha and Horn 1980, Bailey 2009, McGinley 2009) , topography (Forattini 1962), altitude, geological formations and soil characteristics (Srivastava et al. 1999, Githeko et al. 2006, Rosa-Freitas et al. 2007, Manguin 2008, Grillet et al. 2010), and distribution of rural/ urban population (Coene 1993, Okeke and Okeibunor 2010). Forattini classified malaria eco-regions based on vector distribution as (i) littoral, (ii) interior, (iii) Andean, and (iv) bromeliad, and listed primary and secondary vectors in each ecoregions (Forattini 1962). Pattanayak's classification suggested following five epidemiological paradigms viz. (i) forest/tribal malaria, (ii) rural malaria, (iii) urban malaria, (iv) industrial malaria, and (v) border malaria (Pattanayak et al. 1994). Rubio-Palis reviewed previous classifications and classified ecoregions based on vector distribution and important environmental determinants, including type of vegetation, rainfall patterns, mean temperatures, elevation, and geomorphology into 5 regions viz., (i) coastal, (ii) piedmont, (iii) savannah, (iv) interior lowland forest, and (v) high valley (Rubio-Palis and Zimmerman 1997). Omernik defined ecoregions in relation to organism and their environment (Omernik 2004). These variables of classifications are then considered together (Thompson et al. 2004) mapped with iso-lines separating ranges of relevant intensity and overlaid with existing malaria survey maps. With the development of Satellite imagery (Hay et al. 1998, McMahon et al. 2001, Kalluri et al. 2007), and landscape ecology and epidemiology (Kitron 1998, Reisen 2010), spatial classification of malaria ecoregions are now most appreciated for worldwide overall classification (Rogers et al. 2002, Ostfeld et al. 2005, Hay et al. 2006) but these are good if superimposed with maps of regional distribution of vector bionomics, climate, anthropogenic indices, and current malaria situation (Moffett et al. 2007, Rosa-Freitas et al. 2007).

2.2. Hilly-forest ecotype

The ‘forest ecotype’ is defined by UNESCO as terrain with a tree canopy cover of more than 10% and an area of more than 0.5 hectares, including natural forests and plantations (Guerra et al. 2006) with a minimum tree height of 5 m, including coffee, rubber, cork oak, and fruit tree plantations, wind break and shelter belts with more than 20 m width (Snelder et al. 2008). Forest vegetation is categorized as rain forest, deciduous forest, scrub forest, highland rain forest, and highland alpine forest (Breckle and Walter 2002). The former three are usually distributed in low to mid altitude and the last two are part of the high altitude biome. Hilly forested ecotype is a composite ecosystem comprising both forested and hilly area. Hilly forested region is defined as a land form that extends above forest terrain. The major factors which makes forested hills different than others in relation to malaria transmission dynamics are the slope that forms small rapid streams which facilitates breeding of particular vectors (Rattanarithikul et al. 1995, Prakash et al. 1997), and altitude that influence temperature and humidity facilitating survival and shelter for vectors (Gunasekaran 1994, Gunasekaran et al. 1994, Rubio-Palis and Zimmerman 1997). Uneven land forms, rapids, streams, and dense vegetation which make obstacle in communication, treatment and vector control particularly during rainy season (Lim 1992). In hilly forested area, altitude helps vector survival by shifting up and down with correspondence to increasing and decreasing temperature respectively (Charlwood et al. 2000, O'Loughlin et al. 2008). The forest coverage in hills helps in buffering temperature and prevents temperature fluctuation and also maintains humidity which helps vector survival. Hence temperature, rainfall, humidity and altitude influence each other and simultaneously influence vegetation and vice versa (Los 1998, Maarel 2005, Schultz 2005, Jones and Vaughan 2010).

2.3. Riverine-plain ecotype

Rainwater from highland collectively drains down with rapids; falls to larger streams, when it goes downhill to the plains, hydrodynamics slows down and it descends into rivers. In lowland, riverine-plains are flooded plains formed by sedimentation of particles carried by river (Garcia De Jalon et al. 2014). So the geomorphology of the riverine-plains is sedimentary and soil is loose (Goudie 2004). The loamy river plain tend to form water pools for proficient anopheline breeding supplying river water underneath the clay soils (Castro et al. 2010, De Silva and Marshall 2012). High ground water level was found to be conducive for *Anopheline* breeding in plains (Dongus et al. 2009, De Silva and Marshall 2012). Due to sedimentation in

retention zones of the river sand bar and islands are formed and eventually mainstream run off in a new way forming slackwaters in the riverscape and floodscape. This eventually forms horse shoe shaped natural lakes (Sharma 1973). From headwater to downstream river hydrologic habitat succeeds and the hydro-geomorphic patches facilitate breeding of different malaria vectors (Castro et al. 2010, De Silva and Marshall 2012, Hardy et al. 2015). The riverine-plains are hot bed for agriculture and human settlements grow around agricultural fields. Additionally canals and drains are formed for irrigation, water logging is extensively done in paddy fields and the new manmade ecotype promotes different malaria vectors (Steele et al. 1997). Due to flooding and agriculture grassland ecotype is seen in most of the riverine-plains.

2.4. Impact of ecotype factors on malaria

Most studies of malaria are focused on local factors associated with malaria transmission. These include distance from river, paddy field, forest, impact of deforestation and reforestation, effect of ecotype on microclimate, vector bionomics, *Plasmodium* species survival, and human activities. The underlying factors influencing transmission of malaria is analysed. For example the risk of malaria was shown to be 22 fold higher within 250 m range from riverine-plain in comparison to about a kilometre apart populations in Ethiopia (Peterson et al. 2009). Similar influence of river was also reported from Kenya (Zhou et al. 2007). The biting rate of *An. darlingi* has been estimated to increase 278 times in the hotter non-forested Amazonian riverine-plains (Vittor et al. 2006). Fertile riverine-plains attract more anthropogenic changes like canal systems and irrigation, which prepare fertile beds for anopheline breeding. For example, paddy fields around the Blue and White Nile in Khartoum, Sudan, Dioro, Mali, alongside the Niger River are foci of malaria transmission (Ceesay et al. 2012). Similarly link of agriculture and *Anopheles* habitats was found in Tanzania (Dongus et al. 2009) and India (Schapira and Boutsika 2012). Various Asian vectors are also reported to breed profusely in hot beds of riverine-plains specifically *An. culicifacies* (Barik et al. 2009, Schapira and Boutsika 2012). The riverine-plains are fertile and most human civilization grown crowded in these belts. Human population is higher in the strata below 200 m above sea level and higher population are at risk of malaria in these strata (De Silva and Marshall 2012). Mosquito vectors vary according to locality and their behaviour changes with the micro-climate (Trung et al. 2005); in addition, malaria situation changes with human population, and their social behaviours (Walker et al. 2013, Alias et al. 2014). Forest communities are

generally tribal and cope with poor infrastructure. Certain practices like slash and burn cultivation, overnight stays within forests in order to collect forest produce, hunting, wide open household construction, and cattle ranching, increase vulnerability to malaria. It can be challenging to educate forest communities about malaria control, and without their cooperation it is difficult to control malaria (Ribera and Hausmann-Muela 2011). Now, worldwide malaria communities are aiming at malaria elimination (Tatem et al. 2010, Hamainza et al. 2014), a proposition, which is impractical without prevention of re-introduction / re-emergence from hidden foci / uncontrolled forest malaria (WHO 2008, Tatem et al. 2010). Malaria had declined during the previous eradication era in many regions of the world, some of which subsequently experienced resurgence and suffered from its consequences (Carter and Mendis 2002, Hay et al. 2004, Fuller et al. 2012, Hamainza et al. 2014). The problem of malaria in forests is compounded by hidden reservoirs of malaria infections that are not fully addressed (Charlwood et al. 2000, Trung et al. 2004). The origin and evolution of drug and insecticide resistance are often found associated with the forest and near forest areas (Nosten et al. 1991, Guthmann et al. 2008, WHO 2008, Dondorp et al. 2010). In addition to existing asymptomatic infections, the presence of primate malaria parasites and their zoonotic vectors might pose additional challenges to human health in the forest fringe areas (Deane et al. 1984, Waters et al. 1993, Duarte et al. 2013), where malaria surveillance is generally poor (de Castro Duarte et al. 2008, Duarte et al. 2013). Occasional focal outbreaks (unstable transmission) might occur when malaria transmission extends from the forest shade "*nidus*" to plain, peri-urban and urban areas (Rosenberg 1982), where much higher density of human population and presence of vectors could fuel large epidemics. The major factors, that differentiate hilly forest from riverine-plain ecosystem in relation to malaria transmission dynamics, are the influence of forest on temperature buffering, rainfall (Rubio-Palis and Zimmerman 1997), humidity (Aragao 1960, Minakawa et al. 2002, Koenraadt et al. 2004), tree canopy (Zhou et al. 2007), flora, fauna (Manda et al. 2007), high organic content in breeding pools (Okech et al. 2007), and lack of infrastructure (Lim 1992). Usually it is difficult to develop infrastructure in forests due to their uneven landforms, presence of streams, and dense vegetation. Additionally, poor communication hinders malaria control activities particularly during the rainy season (Lim 1992, Ribera and Hausmann-Muela 2011). Furthermore, forests with hilly land forms are more malariogenic, as their slopes form small rapid streams that facilitate breeding of efficient malaria vectors (Rattanarithikul et al. 1995). Forest influences vector distribution and bionomics, and the distribution of the malaria parasites. Forested areas are primarily inhabited

by tribes (Mandal et al. 2002, Kusel and Adler 2003), whose illiteracy and strong beliefs in age old traditions and practices and a fear of outside world leads to reliance on indigenous treatment for malaria (Pichainarong and Chaveepojnkamjorn 2004).

2.5. Worldwide severity of plain and forest malaria

Sub-Saharan Africa suffers by far the greatest malaria burden worldwide (De Silva and Marshall 2012), malaria in such areas, and plains of mainland of South-Asia and Amazonia are mostly confined to belts of relatively stable agricultural practices around riverine-plains (De Silva and Marshall 2012). The coastal plains of Africa and Eurasia offer favourable condition for malaria. In rest of the plains malaria is now a days rare except socially disturbed areas (Schapira and Boutsika 2012). Forest ecosystems are well known to support transmission of malaria, significantly contributing to the global disease burden. A global assessment reports that "closed forests within areas of malaria risk cover approximately 4.8 million $km^{2"}$ (Guerra et al. 2006). Almost half the malaria risk is estimated to occur among people living in forested areas (1.4 billion) accounting for 11.7, 18.7, 35.1 and 70.1 million population respectively from 1.5 million km^2 in the Amazon region, 1.4 million km^2 in Central Africa, 1.2 million km^2 in the Western Pacific, and 0.7 million km^2 in South–east Asia (Achard et al. 2002, Mayaux et al. 2005). Corresponding forest areas containing these malaria risk zones are 11.16 million to 15.71 million km^2, 6.53 million– 7.80 million km^2, 1.93 million– 5.19 million km^2, 2.70 million–2.72 million km^2(Achard et al. 2002, Mayaux et al. 2005, Guerra et al. 2006). Controlling malaria in these forested regions of the world has been a major challenge (Erhart et al. 2005).

2.6. Influence of topographic parameters in hilly-forest and riverine-plain ecosystems

2.6.1. Bodies of water

Mosquitoes mature in bodies of water (their larval habitat) and disperse according to their flight range. For example in riverine area of Kenya *An. gambiae* and *An. funestus* populations were observed decreasing with increasing distance from the Yala river (Zhou et al. 2007). Even a small change in the distance from bodies of water can influence malaria transmission (Berti et al. 1993, Peterson et al. 2009, Walker et al. 2013). Major South American malaria vector *Anopheles darlingi* larvae preferentially breed in sun exposed river bed than in forested region (Vittor et al. 2009). Similarly regional vector of India *An. culicifacies* also shows preference to breed in sun exposed pools of riverine-plain areas.

Anopheles fluviatilis (Lindsay 2004), *Anopheles maculatus* (Kobayashi et al. 2000, Lindsay 2004) and *An. minimus* (Kobayashi et al. 2000, Lindsay 2004, Obsomer et al. 2007) are prevalent near streams of water in forested areas having cooler climate and tree canopy (Sahu et al. 1990), but *An. dirus* larvae grow well in small, clear and stagnant bodies of water in forested areas of Asia (Lindsay 2004, Obsomer et al. 2007). In Africa, *An. gambiae s.s* larvae grow better in bodies of water under dense forest canopy rather than sparse forest coverage (Tuno et al. 2005). Generally, larvae of forest vectors develop better in bodies of water under tree canopy where the water temperature is buffered and usually 3-3.5 degrees Celsius lower than that of sun-exposed bodies of water (Tuno et al. 2005).

2.6.2. Temperature, rainfall, and humidity:

In East Africa average temperature in the previous month and rainfall in the previous two months have shown a linear-quadratic relationship with *Anopheles gambiae* density (Kristan et al. 2008). Another study in the same region showed that the ratio of rainfall over precipitation/potential evapo-transpiration was the driving force for *An. gambiae* and *An. arabiensis* population increase (Koenraadt et al. 2004). The same vector studied in The Gambia showed rapid population increase towards the end of the dry season and maximally after onset of rains when humidity increases (Jawara et al. 2008). An inverse relation of malaria incidence with rainfall in previous 3 months was recorded in the forested highlands of Kilimanjaro (Drakeley et al. 2005) in contrast to plain areas in the neighboring geographical region (Ijumba et al. 2002, Bodker et al. 2003, Maxwell et al. 2003). This exception may be correlated with flushing of breeding sites, and decrease in temperature with rainfall and altitude in highlands which in turn decrease vector population and prevent parasite development in mosquitoes. Similarly a large number of *An. fluviatilis* larvae were reported in plains after flushed out from hills in India (Sinka et al. 2011). In hilly-forest region, trees in the forests add moisture in the air by transpiration and help in lowering temperature, thus increasing precipitation. The moist environment and breeding sites created by rainfall increase vector population, their longevity and hence increase malaria transmission (Walker et al. 2013).

2.6.3. Altitude

The primary effect of increasing altitude by 165 meters result in 1^{o} C temperature reduction and hence logarithmic linear reduction in vector abundance and, to a lesser extent, a reduction in the proportion of infective mosquitoes (Bodker et al. 2003, Kulkarni et al. 2006). Hence malaria prevalence shows a negative relationship with altitude as decrease of 19% and 21% malaria /100-m altitude increase observed, respectively, in Kilimanjaro and Tanga (Drakeley et al. 2005).

2.6.4. Vegetation:

Vegetation near human habitation increases the population of forest malaria vectors and thus increases malaria transmission (Pavlovsky 1966, Mouchet and Carnevale 1997, O'Loughlin et al. 2008). Villages with more broadleaf forests, and wetland vegetation in Belize and in forested villages of Bangladesh have higher malaria rates (Hakre et al. 2004, Haque et al. 2011) due to effective density of forest vectors (Haque et al. 2011). Forest vectors usually prefer tree canopy coverage (Afrane et al. 2006, Zhou et al. 2007) and are known to take shelter in tree holes (Gunasekaran et al. 1989, Yadav et al. 1997). Forest flora and sugar availability have also been shown to be crucial determinants of vectorial capacity. The availability of plant sugar increased egg numbers (Gary Jr and Foster 2004, Manda et al. 2007) and survival potential of *An. gambiae* beyond ages at which they are old enough to transmit malaria (Okech et al. 2003). In addition, leaves falling into larval habitats assure sustainable micro-climatic conditions and food for larvae, which favour vectors like *An. dirus* in South East Asia (Obsomer et al. 2007).

2.7. Ecological impact of deforested land on malaria epidemiology

Reduction of dense tree shade increases exposure of vector breeding sites and resting places to sunlight, hence altering vector habitats. Studies have shown preference for forest shade by, *An. dirus* (Obsomer et al. 2007, Yasuoka and Levins 2007), *An. fluviatilis* (Sharma et al. 1991, Yasuoka and Levins 2007), *An. minimus* (Service 1991, Yasuoka and Levins 2007), and *An. funestus* (Service 1989, Service 1991), *An. darlingi* (Yasuoka and Levins 2007) and contrastingly, preference for sunlight is shown by some of the species of *An. gambiae* (Service 1989, Yasuoka and Levins 2007), and *An. maculatus* (Gilles and Warrell 1999, Kobayashi et al. 2000, Lindsay 2004). Changing density of anophelines due to deforestation has been reported worldwide, and its relation to niche width and sunlight preference were reviewed in

meta-analysis / tabulation (Yasuoka and Levins 2007), and it was found that changes in anophelines density and malaria incidence varied by type of development, agriculture, and locality (Yasuoka and Levins 2007). It was predicted that deforestation in central Africa and tropical America might increase malaria (Guerra et al. 2006, Vittor et al. 2009), whereas in Asia deforestation would result in reduction in malaria (Walsh et al. 1993). As predicted in the Sahara region, malaria incidence increased due to deforestation as a consequence of increased vector density of *An. gambiae* and *An. arabiensis* (Yasuoka and Levins 2007), and increase in *An. funestus* and *An. gambiae* population in Sub-Saharan Africa (Yasuoka and Levins 2007). Similarly deforestation increased the population of the South American vectors *An. darlingi* and *An. aquasalis* (Yasuoka and Levins 2007), accompanied by increased malaria in Guyana and Amazonia (Yasuoka and Levins 2007). The predicted reduction in malaria in deforested regions of Asia may be due to a decrease in forest-loving (halo phobic) vectors like *An. dirus* in Thailand and *An. fluviatilis* in India (Sharma et al. 1991, Taylor 1997). However, malaria transmission was accelerated by *An. minimus* due to deforestation in Thailand and India (Das et al. 2004), as well as *An. culicifacies* in Nepal and Sri Lanka (Yasuoka and Levins 2007), and *An. philippinensis, An. annularis,* and *An. varuna* in India (Das et al. 2004). A risk of increased malaria in response to deforestation exists if vectors like *An. darlingi* are present in a deforested habitat (Elliott 1972). Thus, deforestation affects malaria transmission depending upon the vector diversity of a particular region.

2.8. Ecological impact of reforested land on malaria epidemiology

Man-made forests, including significantly large plantation areas or reforestation also cause habitat change and influence malaria vector abundance leading to changes in malaria transmission scenarios. For example malaria increased due to a coffee plantation in Thailand (Singhasivanon et al. 1999), palm plantations in Cameroon (Tanga et al. 2011), Papua New Guinea (Pluess et al. 2009) and Malaysia (Chang et al. 1997); rubber plantation in Cameroon (Bigoga et al. 2012), Thailand (Singhasivanon et al. 1999) and orchard plantations in Thai-Myanmar and other South-East Asia regions (Singhasivanon et al. 1999, Wangroongsarb et al. 2012, Basurko et al. 2013). Commercial plantations and reforestation, which increase human insurgence, increases man-vector encounter and malaria transmission in those areas (Walsh et al. 1993, Singhasivanon et al. 1999, Wangroongsarb et al. 2012, Basurko et al. 2013).

3. Material and methods

3.1. Selection and characterization of study sites by Geographical Information System

Geographical Information System (GIS) was used to select study sites. Indian National Geographical and Malaria Information System of National Vector Borne Disease Control Programme **(**NVBDCP) under National Rural Health Mission (NRHM) of Directorate of Health Services in coordination with the Department of Health and Family Welfare was used to select study state then district from core of most malarious areas having high API (http://nvbdcp.gov.in/maps.htm). Districtwide spatial malaria transmission was observed through a government open access web GIS portal of Odisha State Malaria Information System (http://nrhmorissa.gov.in/mis/SearchDetail.aspx). Google Earth was used initially to visualise satellite picture, explore altitude, latitude, longitude and a rough outline of the study area (https://www.google.com/earth/explore/products/plugin.html). Geomorphology of study sites was defined by National Remote Sensing Centre web GIS service "Bhuvan" of ISRO's geoportal (http://bhuvan.nrsc.gov.in/bhuvan_links.php#). Maps of the topography and demography of district Deogarh were obtained from web GIS for Odisha composed by National Informatics Centre (NIC) (http://gis.ori.nic.in/). Customized thematic maps were prepared in the block level web GIS service "Odisha Sampad" provided by Orissa Space Applications Center (ORSAC), Bhubaneswar (http://www.odishasampad.in/app/).

The study utilised available government web GIS systems for selection, categorization and mapping malaria related themes. Competent government authorities manage display subsystems i.e. data input, storage, retrieval, manipulation, and display output. Only thematic mapping analysis was done in this study. Remote sensing detection of vector habitat and selection of high risk areas were done on the basis of data available on environmental parameters. Following modules were used for thematic mapping of study sites by dynamic web GIS which provides continues updated information from authentic sources.

1. Overlay analysis of different thematic data was done by multi-criteria modelling.
2. Buffer zone analysis was done with logical radius for health services availability.
3. Network analysis of healthcare catchment was done.
4. Quantification of various categories (demography, topography and meteorological parameters etc.) was done by statistical analysis.
5. Queries of condition and trends were mapped.
6. Non-sampled areas were mapped for malaria situation by extrapolation.

Block and village boundaries were demarcated, and slope, forest, plain, river, drain, water body, and human settlements were defined for both the study blocks. Land geomorphology and land use pattern were mapped. Village wise API of malaria was plotted for Tileibani and Bamparada PHCs for the year 2010 (preceding year of initiation of the study). Road infrastructure was demarcated. Villages within one kilometre distance from road were identified. Health care units, Anganwadi centre, and ASHA were located in both the ecotypes. Villages within one kilometre and 3 Km from PHC were identified. Villages having at least one doctor or any health care unit or with telephone were identified. Demographic data like block wise population distribution, male-female, ST and SC distribution were mapped.

All these maps were superimposed and compared in parallel to presume two sets of study villages representing hilly-forest and riverine-plain settings. Following set of parameters were considered for selecting ideal study villages of hilly-forest block named "Tileibani" (PHC Tileibani).

1. Villages with hilly geomorphology and slope more than 15°
2. Villages within 1km of forest and 2 km of streams
3. Villages having API of malaria more than 50 in the year 2011
4. Settlements within 30 minutes walking distance from road

Following set of parameters were considered for selecting ideal study villages of riverine-plain block named "Barkot" (PHC Bamparada).

1. Villages with hilly geomorphology and slope less than 5°
2. Villages 10 km away from forest and within 2 km of river
3. Villages having API of malaria more than 5 in the year 2010
4. Settlements within 30 minutes walking distance from road

3.2. The survey

Surveys were carried out during March, September and November 2011, and July 2012 in both the ecotypes. The block wise or PHC wise monthly malaria incidence data (NVBDCP consultant reports) were collected from Chief District Medical Officer (CDMO) through proper channel during the field visits. Other malaria intervention data like distribution of Long Lasting Insecticidal Nets (LLINs), Indoor Residual Spray IRS, and antimalarial treatment reports were also collected. The PHC wise monthly malaria incidence report was plotted in a

graph. Malaria situation in Tileibani and Bamparada PHCs of district Deogarh for the years 2006-2013 (before and after the study) were plotted.

3.3. Meteorological data analysis

Data of daily temperature range, humidity, cloud cover, wet days, and precipitation from year 2007 to 2012 were collected from Sericulture Research Centre, Deogarh and Krishi Bigyan Kendra, Deogarh for hilly-forest Tileibani Block and riverine-plain Barkot block. Monthly average of the collected climatic variables were calculated and recorded. Additionally historic and updated daily rainfall data were obtained from Odisha rainfall monitoring system (http://as.ori.nic.in/rainfall/PubRainChart.asp) for double check with our collected data. Highest, lowest, mean, and difference of temperatures were plotted separately in a graph. The six-year monthly climatic data from both the ecotypes were collected (http://www.imdorissa.gov.in/), and crosschecked with the data collected from Sericulture Research Centre. The meteorological data were tabulated and representative mean values were plotted in graphs. Arithmetic mean was calculated and used for all the average calculations.

Geomorphology, land use pattern and spatial meteorological data were evaluated to project suitability of malaria vectors habitat. Availability of health care and communication infrastructure, demographic characteristics of both ecotypes were evaluated. Based on evaluation of all the parameters (malaria vector abundance, demographic vulnerability, and healthcare availability) the study sites were selected.

4. Results

4.1. Selection and characterization of study sites by Geographical Information System

Malaria is endemic in most of the places of India except in high altitudes like Himalaya and some patches in India. The Eastern and North Easter parts of India were found to be more malarious having high annual malaria incidences. These are Odisha, Chhattisgarh, West Bengal, Jharkhand, Assam, and Arunachal Pradesh. Out of 42 highly malarious (API>10) districts in India 13 are from Odisha. Districts bordering Odisha, Chhattisgarh, Jharkhand form largest highly malarious zone of India (Figure 3).

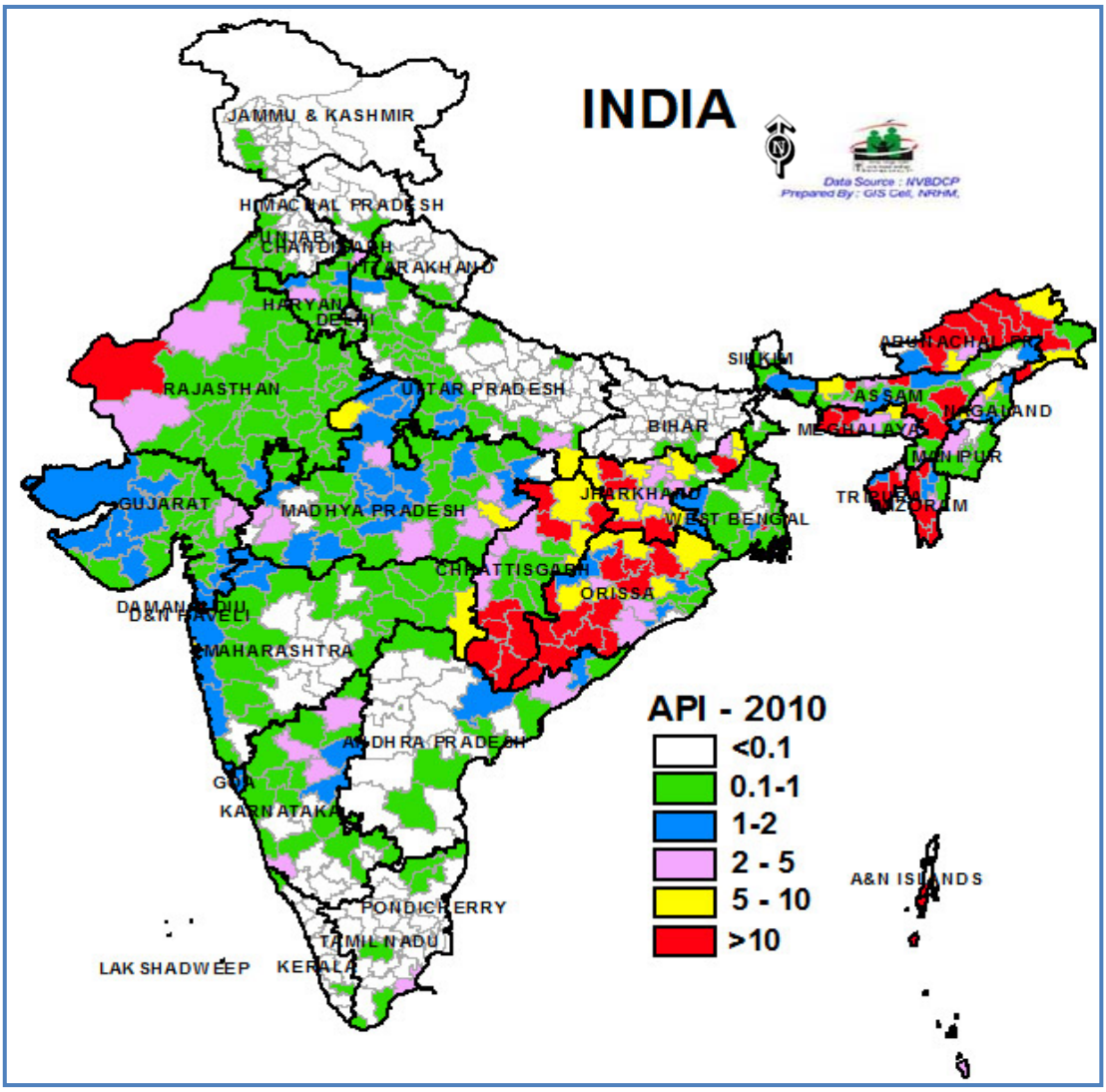

Figure 3: Map of India showing Annual Parasite Incidence of malaria in different states (base API 2010)

There are two clusters of malarious districts in Odisha i.e. Southern cluster and North-Western cluster. District Deogarh was reported to have consistently high malaria API, whereas

neighbouring districts showed reducing trend in malaria API from year 2010-2011 (Figure 2-4). District Deogarh is situated in the centre of North-Western malarious cluster. Deogarh district was selected as it is least explored and lies in the core of the North-Western malarious clusters.

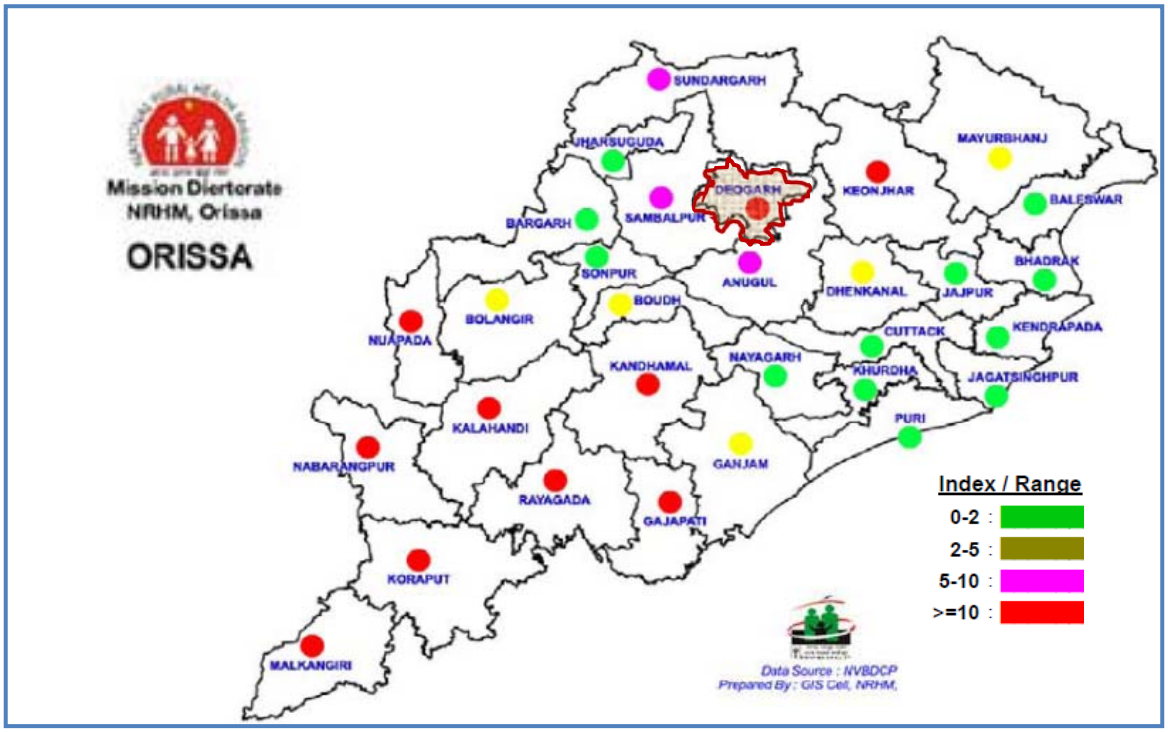

Figure 4: Malaria share of district Deogarh, Odisha (Annual Parasite Index-2011)

Table 1: Distribution of malaria cases among three blocks of district Deogarh (2011)

Name of the PHC	Species wise positivity			Sex wise positivity		Total	API
	BSC/BSE	*Pv*	*Pf*	Male	Female		
Bamparada	13016	72	1068	612	528	1140	10.9
Tileibani	26910	223	5425	2925	2733	5648	74.4
Chatabar	27266	97	2436	1339	1194	2533	23.8
DHH, DGH	9918	157	556	435	278	713	31.7
Total	77110	549	9485	5311	4733	10034	32.4

(Epidemiological profile of District Deogarh for year 2011 by NVBDCP)

The overall Annual Parasite Incidence (API) of malaria in district Deogarh was 32.4 in the year 2011. API of Bamparada PHC was 10.9, whereas it was 74.4 in Tileibani PHC. The API of the third block was reported 23.8 and API at District Headquarter Hospital was 31.7 (Table 1).

4.2. Geo-referencing of study district

District Deogarh is located between cordinates 21° 44 09’ N, 84° 23 46’ E in it’s west, 21° 30 54’ 85° 13 40’ in it’s east, 21° 45 45’, 84°39 32’ in it’s north and 21°07 52’ and 84° 48 05’ in it’s south. Figure 5 shows satelite imagery of forest cover and submerged areas due to Rengali dam construction.

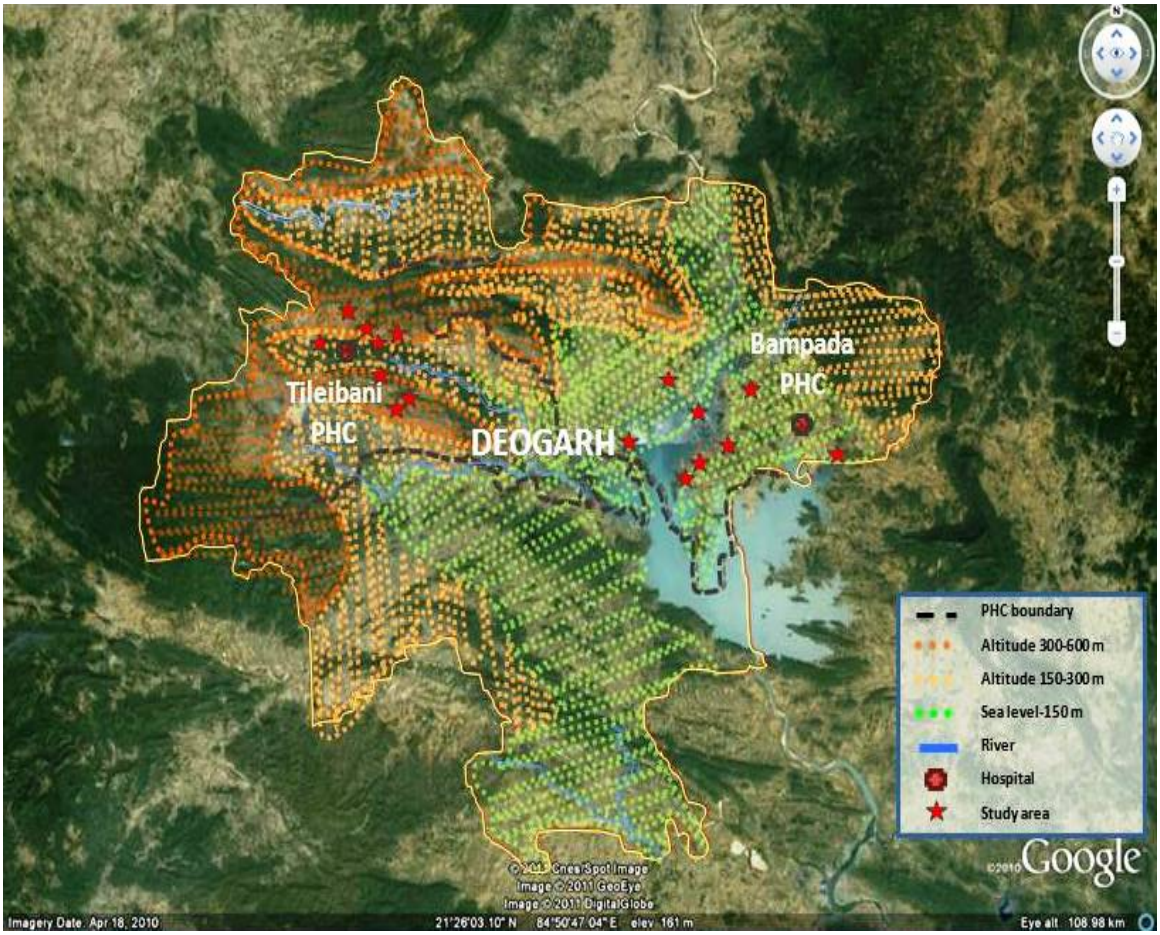

Figure 5: Satellite imagery of hilly-forest and riverine-plain areas in district Deogarh

4.3. Difining Urban and rural boundaries of district Deogarh

District Deogarh is majorly rural as only Deogarh town is under urban township category. Deogarh town is located in Tileibani block and the district administrative headquarter. Deogarh town is in the foothill valley area surrounded by hilly-forest belts (Figure 6).

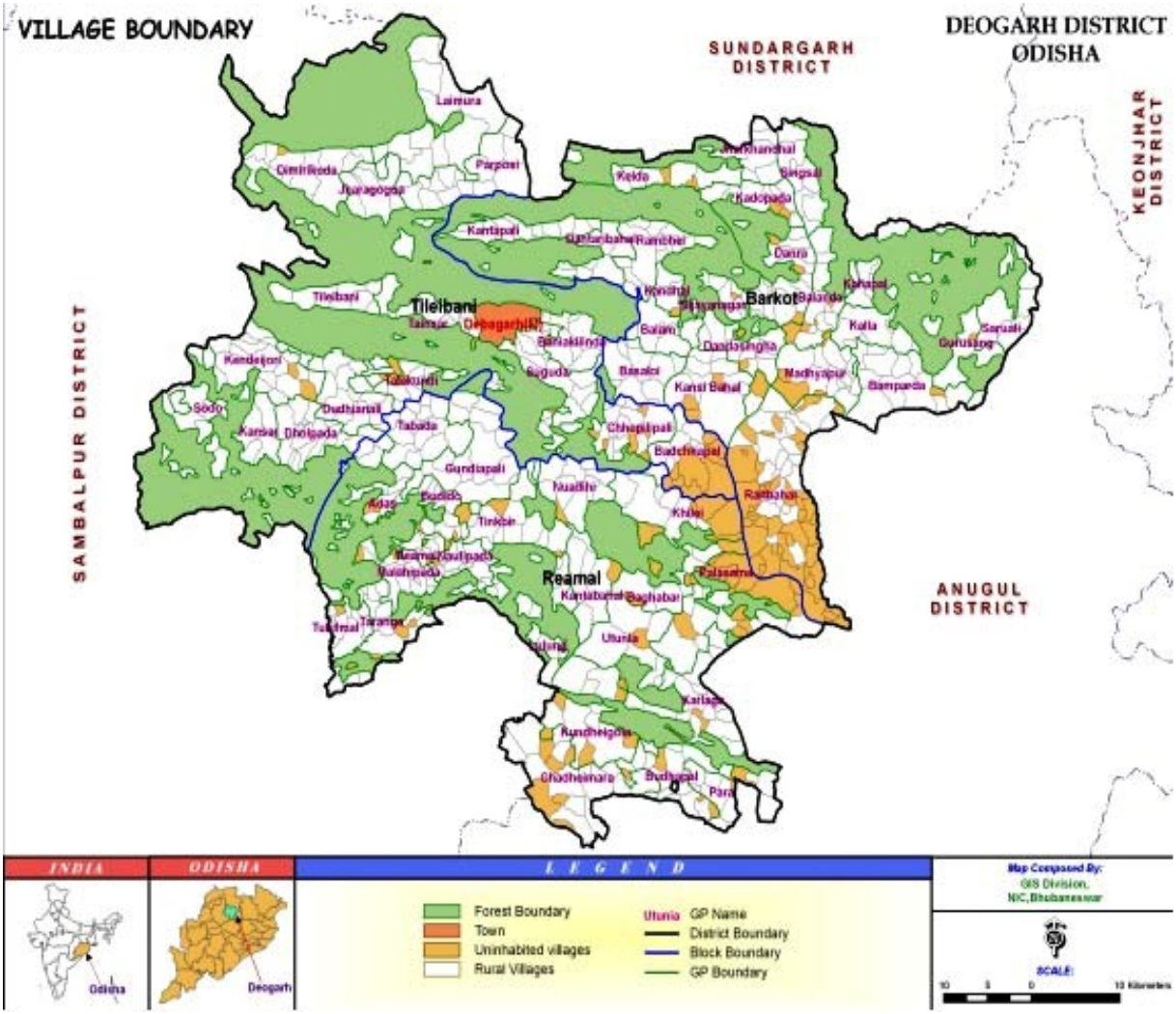

Figure 6: Urban and rural boundaries of district Deogarh

There were number of villages uninhabited majorly due to subsequent flooding after construction of a dam at Rengali (Figure 6). In the consequence of Rengali project government, displaced large numbers of peoples from flood affected riverine-plains and resettled them in the hilly-forest areas. There were number of resettled population in the hilly-forest settings. There are 242 and 278 villages in Tileibani and Barkot block respectively. Tileibani's block headquarter is titled "Tileibani" and Barkot block headquarter is titled "Balanda". Tileibani was constituted by 16 gram panchayats and Barkot was constituted by 25 gram panchayats. Tileibani block is bordered by district Deogarh's other two blocks boundary i.e. Reamal and Barkot and also borders other districts border i.e. Sambalpur and Sundergarh (Figure 6). Barkot blocks is bordered by district Deogar's other two blocks boundary i.e. Reamal and Tileibani and also borders other districts border i.e. Anugul and Sundargarh (Figure 6).

4.4. Geomorphology of the hilly, forested, and riverine-plain ecotype villages

The geomorphology of Tileibani block is majorly of structural origin (Lithologically these consist of more metamorphic rocks that retain less water to support vegetation), highly dissected hills and valleys with marginal denudational origin (low relief hills mostly covered with vegetation) and pediment-pedi-plain complex (concave surfaces developed at Junction of hills with the plains which supports agriculture) (Figure 7). Barkot block is largely denudational origin and pediment-pedi-plain complex with marginal structural origin, moderate to highly dissected (undulated) hills and valleys (Figure 7). Most of the fluvial origin (flood plains) are submerged due to Dam construction at Rengali and categorized as anthropogenic terrain (Figure 7). The soil morphology of Tileibani block is gravelly loamy to fine-loamy to fine to fine-cracking soil which supports moderate forest and streams. The soil morphology of Barkot block is principally fine soil to coarse-loamy soil to fine-loamy to fine cracking soil to gravelly loamy soil which supports cultivation, and that collects underground water to form bodies of water supporting anopheline breeding (Figure 7).

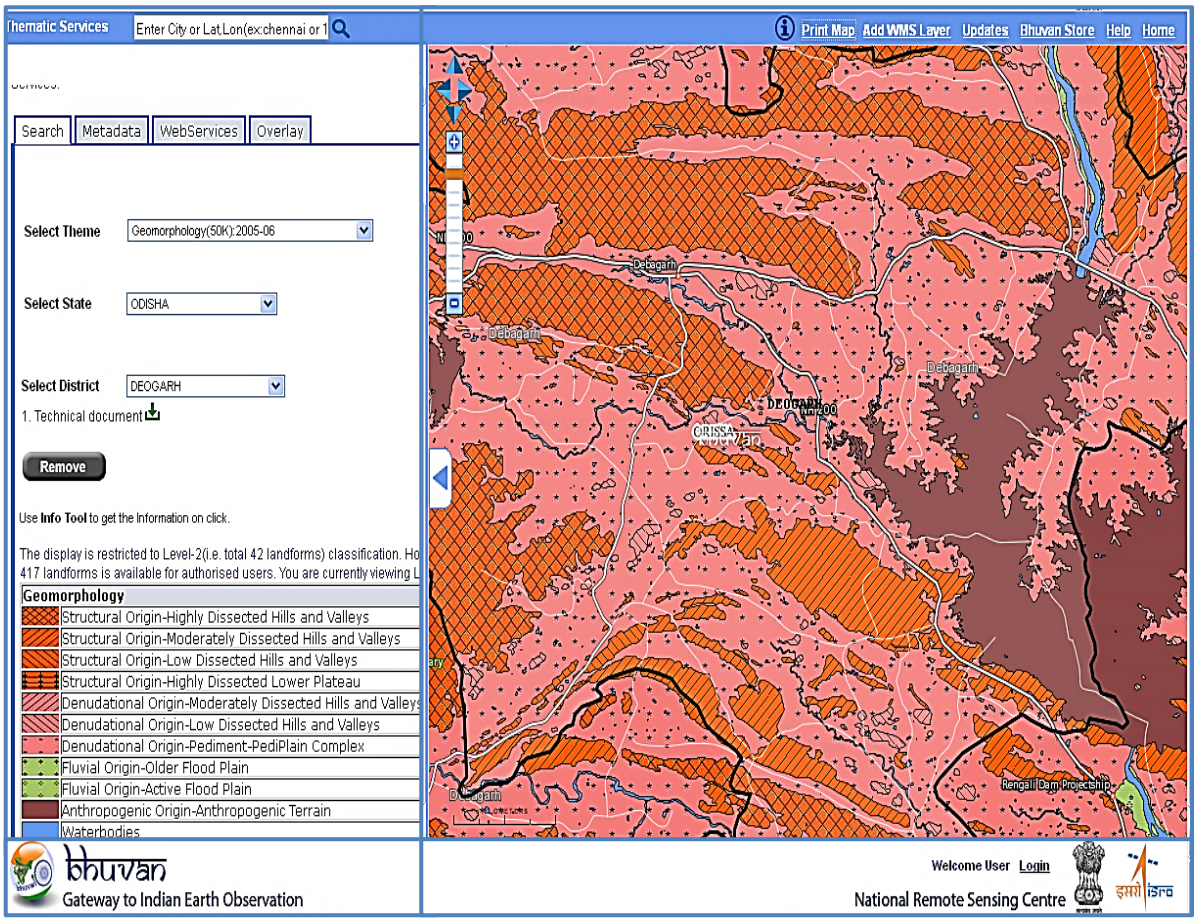

Figure 7: Geomorphology of the villages of Tileibani and Barkot

4.5. Defining vegetation and land use pattern of Tileibani and Barkot block

The superimposed maps of vegetation and land use pattern of both the blocks show distinct pattern. Tileibani block is full of hills but in contrast Barkot block is rather with plains (Figure 8 and 9). The hill ranges have elevation ranging from 610 meters to 762 meters from the mean Sea Levels. Total forest cover 612 Sq. km. area (22%) of district Deogarh is of deciduous type. Thick forests occupy Tileibani block and there is open forest outlined in forest fringe areas. Barkot block is mostly non-forest area and comprises of riverine-plains (Figure 9). Despite dominance of forest cover, large-scale resettlements, and land allotment for agricultural purpose forms a good percentage in hilly-forest area (Figure 8). Due to the Rengali dam construction much of the riverine-plains are submerged in water, remaining riverine-plains are agricultural lands (Figures 8 and 9).

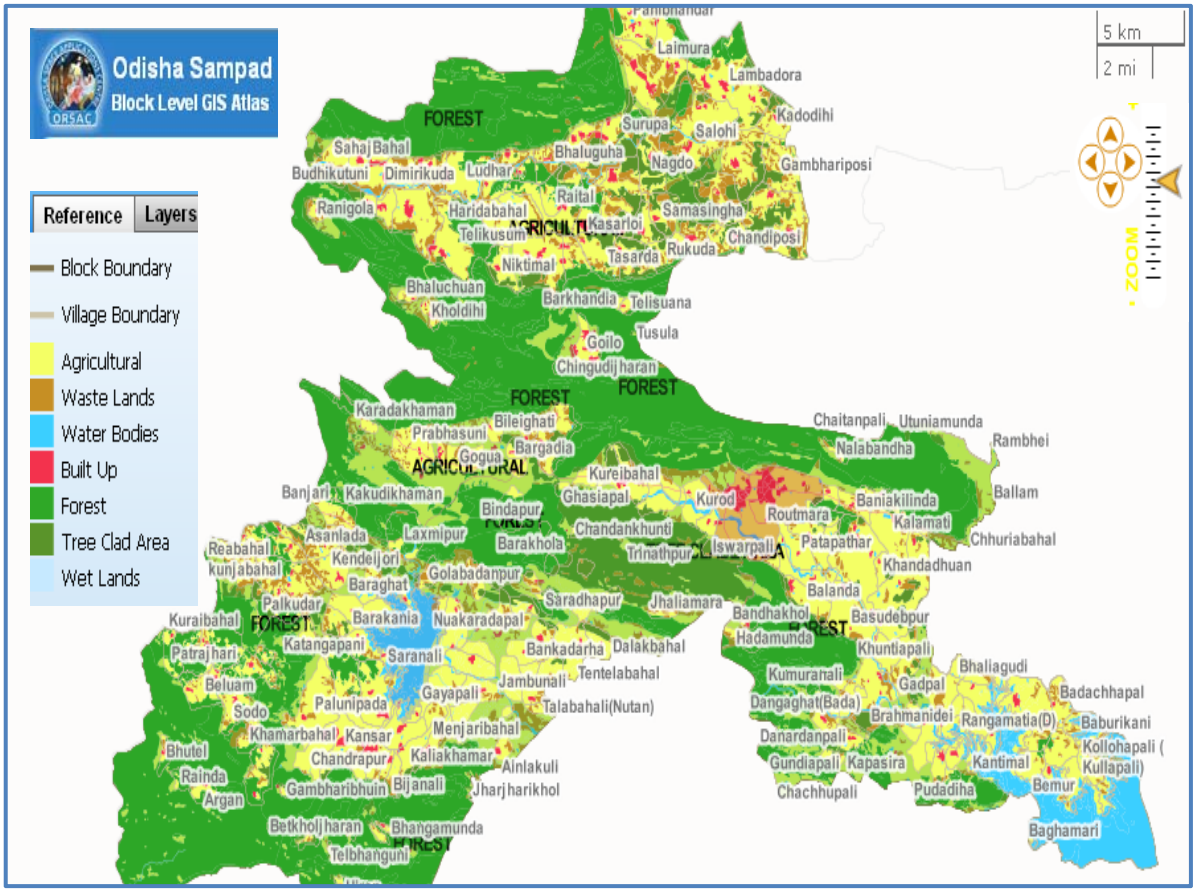

Figure 8: Defining vegetation and land use pattern of Tileibani block (Tileibani PHC)

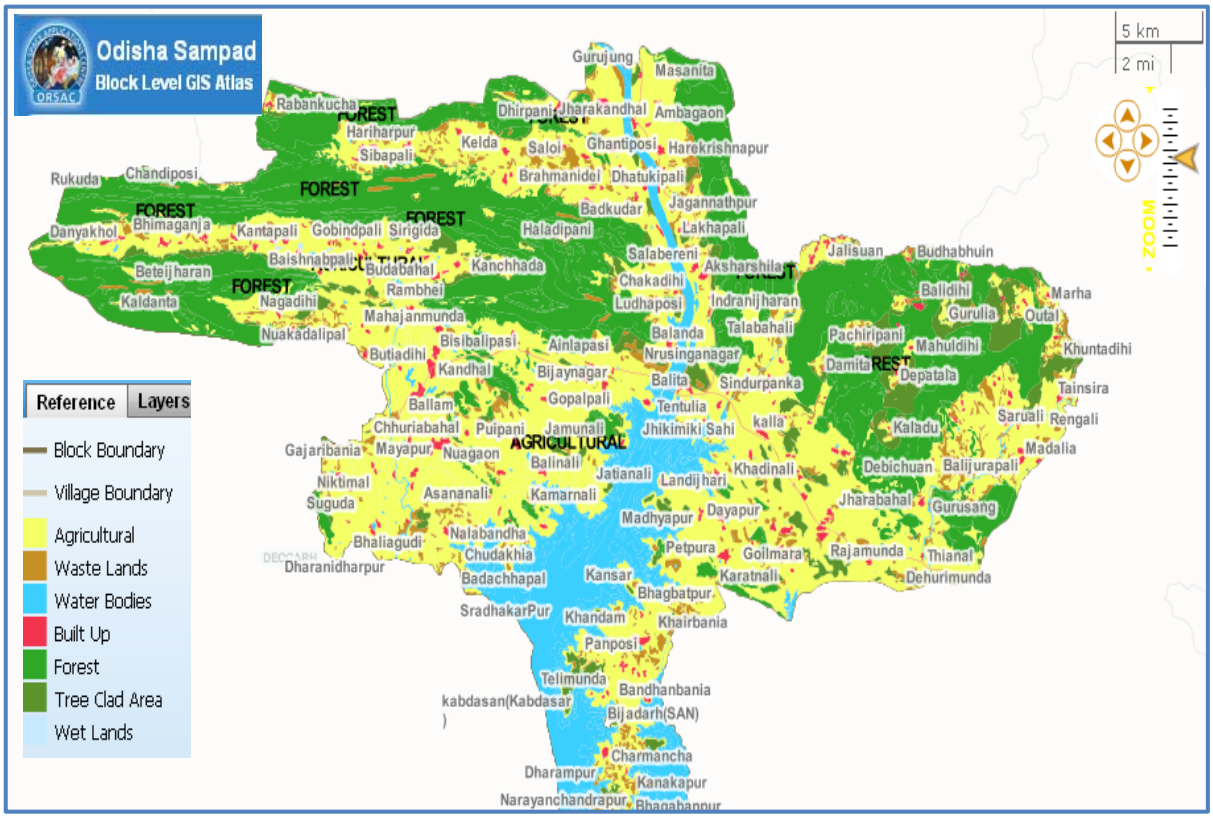

Figure 9: Defining vegetation and land use pattern of Barkot block (Bamparada PHC)

4.6. Defining water bodies in Deogarh district

The map shows downstream bigger river, drains and canal irrigated riverine-plain ecotype in Barkot block whereas upstream ecotype is shown in Tileibani block (Figure 10). Rainwater of Tileibani block is drained by numerous seasonal and perennial streams collectively into the three main drains called; “Gahira”, “Jaraikala” and “Lambodara”. Gahira drain is having a small water reservoir called “Gahira reservoir”. Very limited canal system is constructed from the Gahira reservoir. Brahmini River is crossing through Barkot block and due to dam construction at Rengali called Rengali Dam, large portion of Barkot is found submerged in water. Most of the block’s water drains to four drains called “Kaunsi”, “Kumbaidak”, “Barjor”, and “Madhuali” which open in mainstream of Brahmini River. The riverine-plain areas of Barkot are irrigated with canal system called “Bamra canal” from Rengali reservoir (Figure 10).

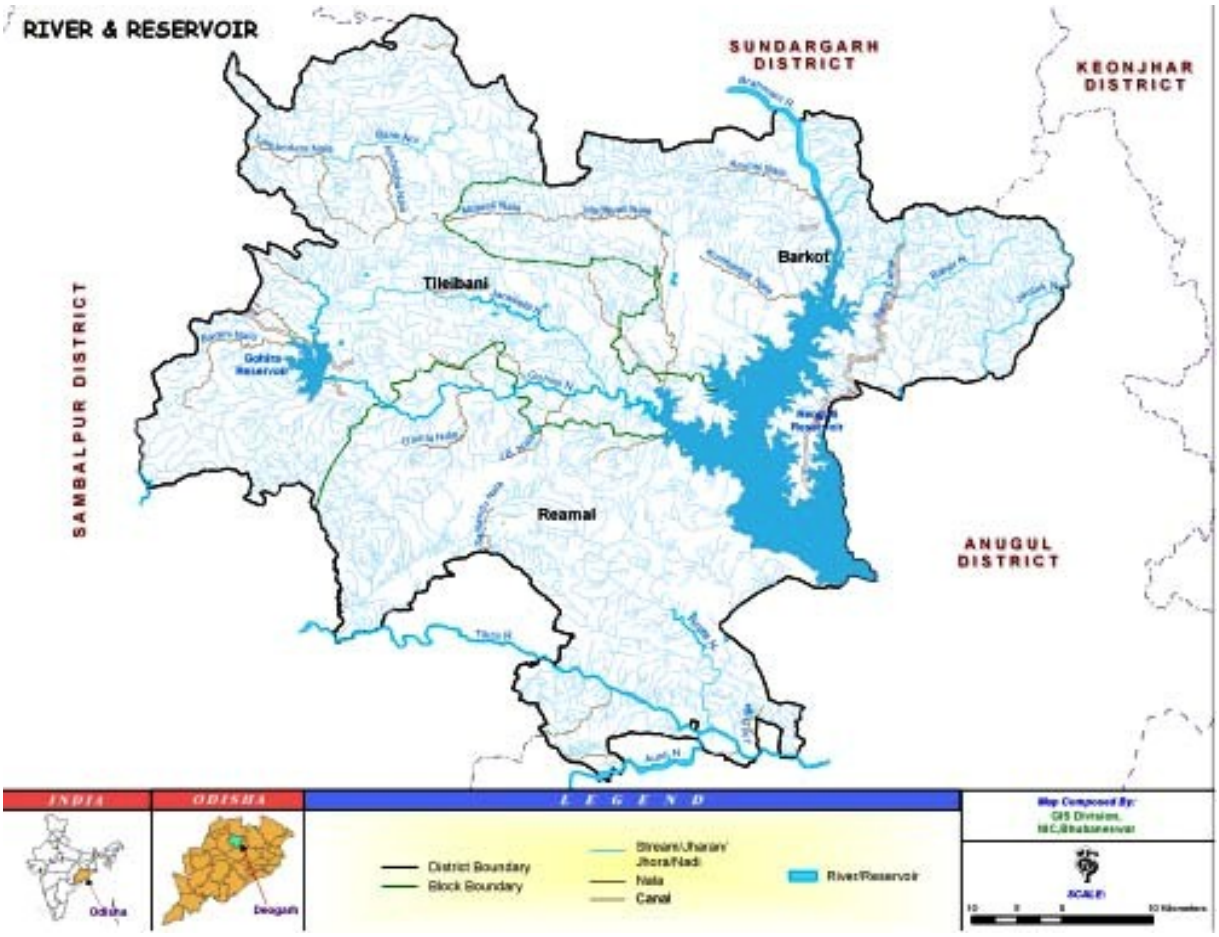

Figure 10: Defining water bodies in Deogarh district

(Locally, small rapids are called as "Jharan", streams are called "Jhara", Drains are called "Nala", wider drains are called "Nai", and bigger rivers are called "Nadi")

4.7. Defining water bodies and slopes with reference to study sites in Tileibani and Barkot blocks

Streams and backwater river ecotype were extrapolated correspondingly in Tileibani and Barkot blocks. The vector breeding sites were distinct in both the blocks. Human settlements in both the blocks and their proximity to water bodies have been shown in Figures 11 and 12. The block wise map of water bodies superimposed over map of terrain slopes showed high-slope streams in Tileibani block whereas in Barkot block, low-slope, and wider river and canals were found (Figures 10-12). By analysing the maps of human settlements near major and minor water bodies in respect to the slope of the terrain (Figures 11 and 12), the study sites in distinct ecotypes were selected.

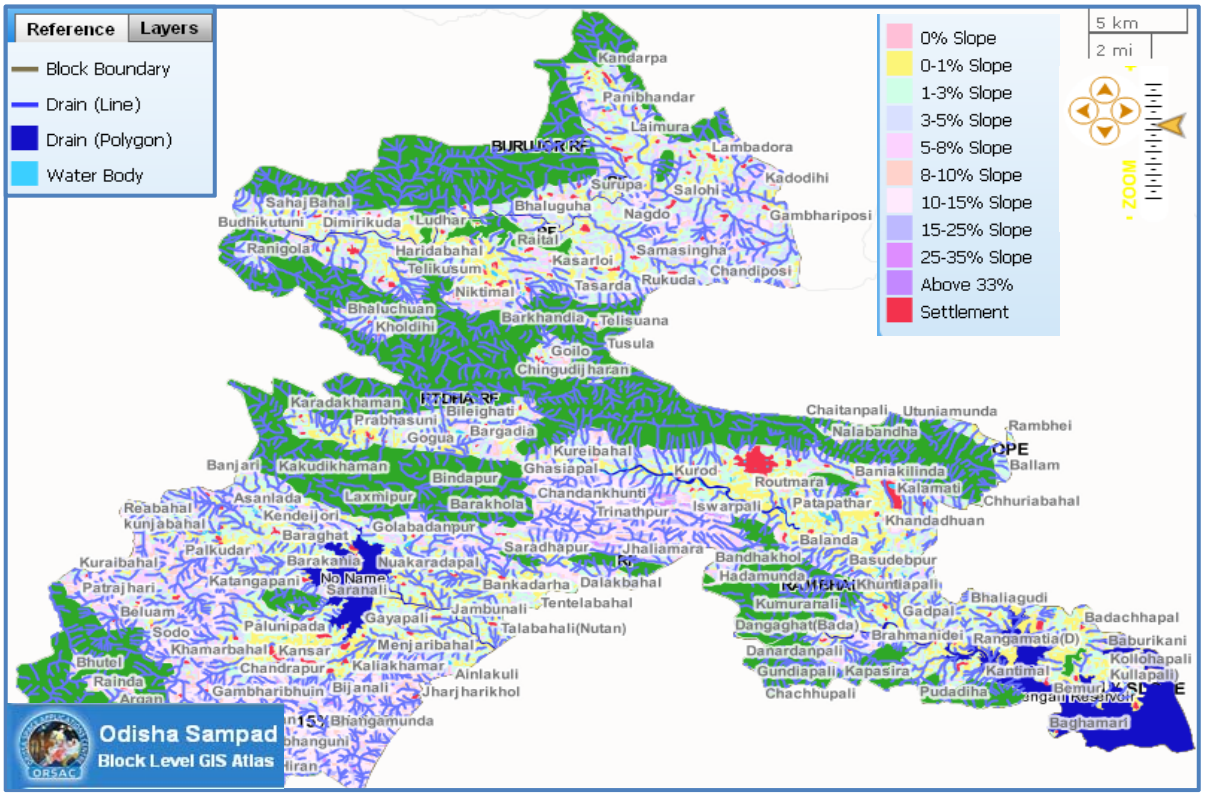

Figure 11: Defining streams and water bodies with slopes of Tileibani block study sites

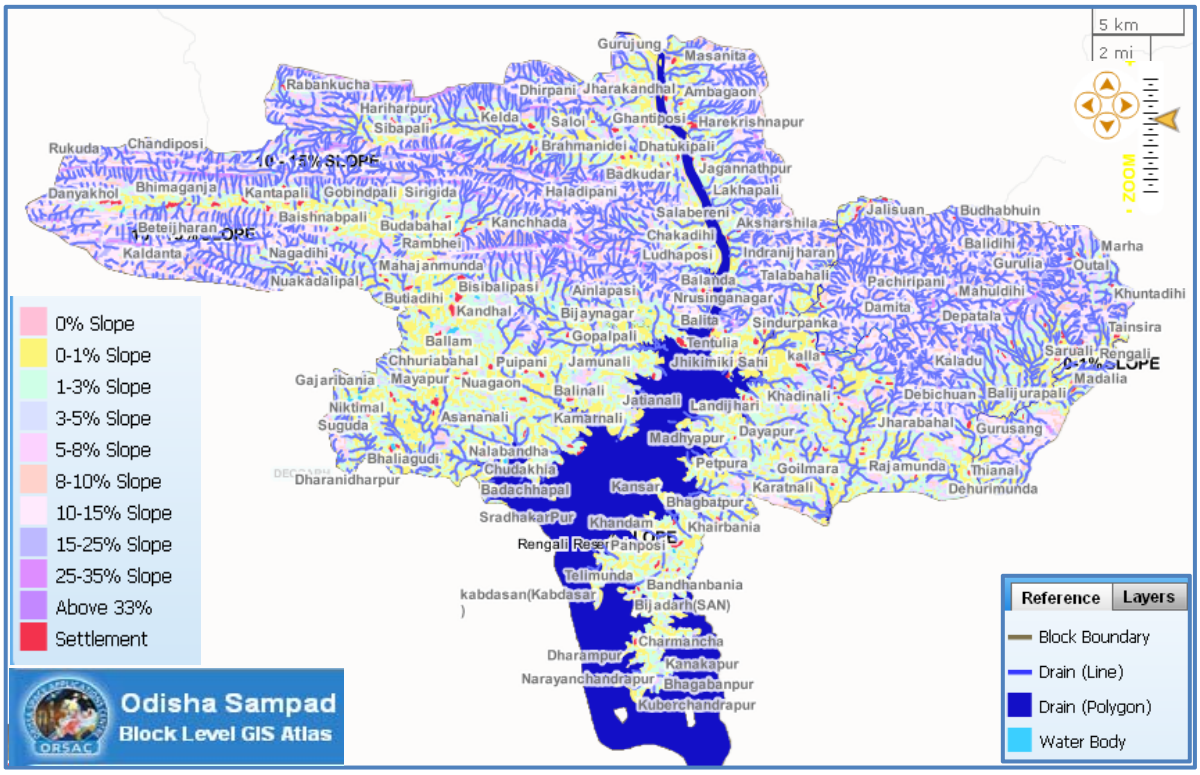

Figure 12: Defining streams and water bodies with slopes of Barkot block study sites

4.8. Annual Parasite Incidence (API) of malaria in villages of Tileibani and Barkot block in the year 2011

Mapping the Annual Parasite Incidence (API) in villages of Tileibani and Barkot blocks depicted clustering of malarious areas in both the blocks. Most of the malaria was found clustered in villages located in Pravasuni Reserve Forest under Tileibani block (Figure 13). In Barkot block a malarious cluster was found around block-headquarter named "Ballanda" and the second cluster of high malaria-reporting villages, was found in villages located in Bamparada PHC (Figure 14). By superimposing the topographical and malaria incidence maps the study sites in hilly-forest and riverine-plain ecotypes were selected. The hilly-forest study villages under Tileibani PHC are Kailash, Jareikela, Jhaliamara, Raipur, sukapadan, Kaunsikhol, Prabhasuni, Jialibhanga, and Bileighati. The riverine-plain study villages under Bamparada PHC are Singhasala, Thianala, Kulusura, Jhikimiki, Dandasingha, Saida, Puruna Barkot, and Godabhanga. The number of villages, their total population, and the number of households in these villages are given in Table 2.

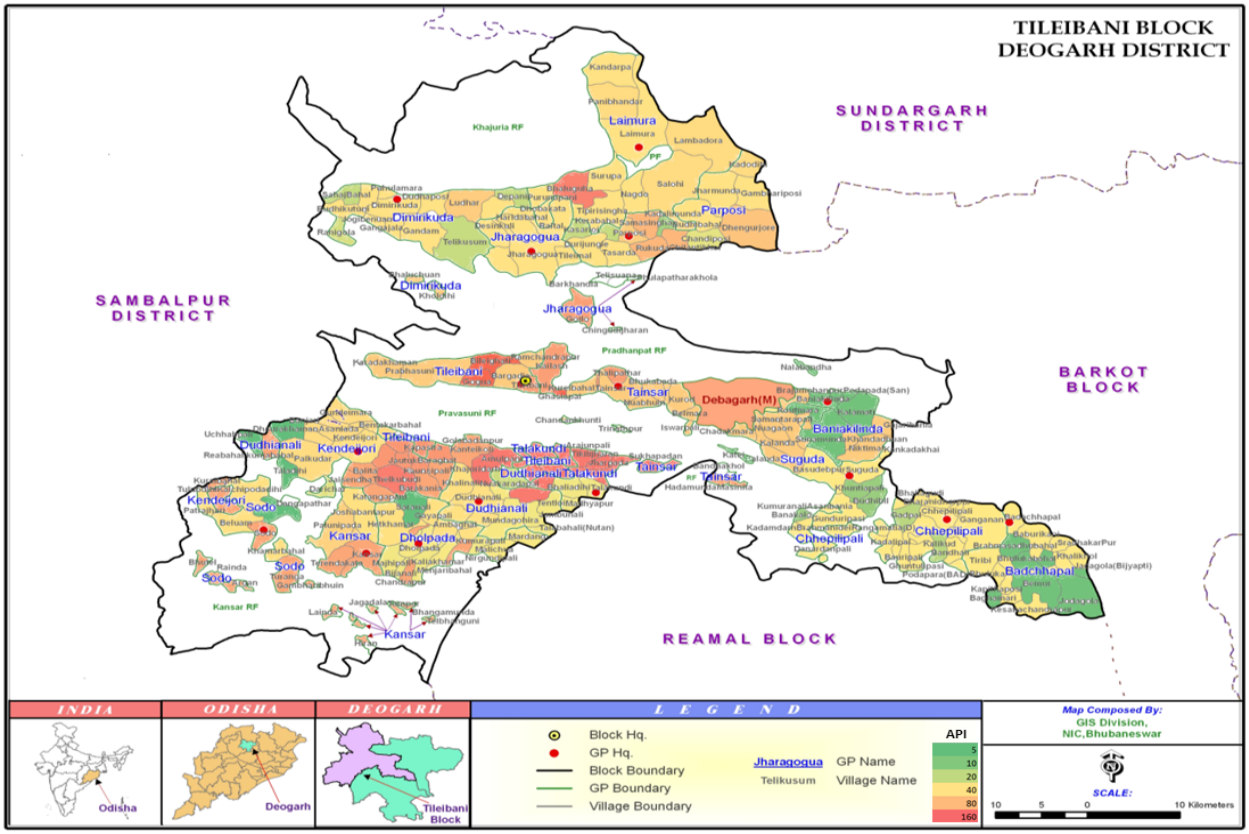

Figure 13: Annual Parasite Incidence (API) of malaria in villages of Tileibani block

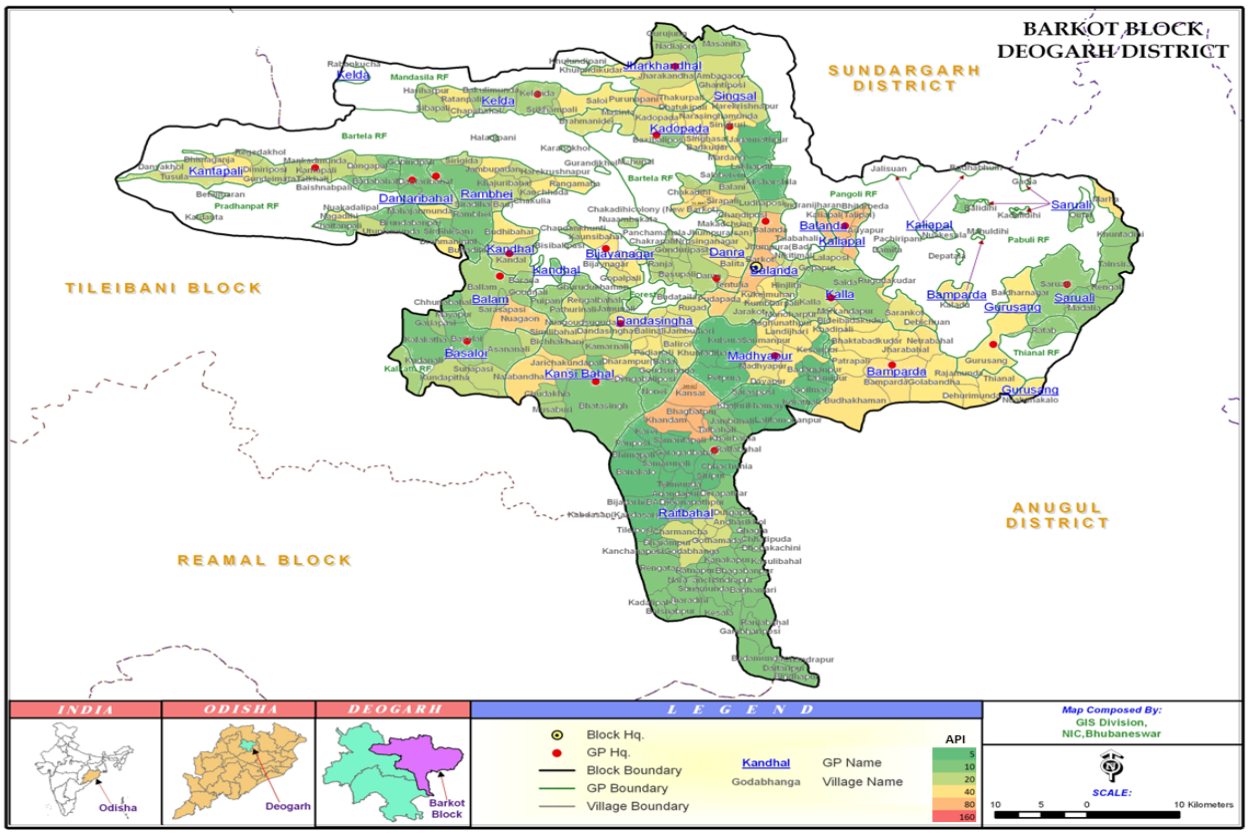

Figure 14: Annual Parasite Incidence (API) of malaria in villages of Barkot block

Table 2: Population calculation of selected study villages

Village attributes	Hilly-forest	Riverine-plain	Total
Number of selected study villages	9	8	17
Number of people in the villages	3289 (34.6%)	6226 (65.4%)	9515
Total number of households in the villages	645 (32.3%)	1350 (67.7%)	1995

4.9. Availability of infrastructure and health care facilities in the study sites

4.9.1. Delineating road network of Tileibani and Barkot blocks

Maps of road network of Tileibani (Figure 15) and Barkot (Figure 16) blocks showed that the National Highway number six (NH-6) is crossing both the blocks. District Deogarh's Headquarter Hospital named "Deogarh DHH" and Tileibani PHC under Tileibani block are connected with NH-6 whereas Bamparada PHC under Barkot Block connected with NH 23 which ultimately gets connected to NH 6. There were other road networks in both the blocks connecting different places of these blocks. State Highway 10A passes through Barkot block only. This network of roads connects many of the villages in district Deogarh. Map of district Deogarh with villages located within 1 Km distance from road is shown in Figure 17. The map shows that there are number of villages in both the blocks which do not have a connecting road

within a distance of one kilometre. However, there is lesser number of villages under Tileibani PHC which are located within one kilometre from the road as compared to those under Bamparada PHC.

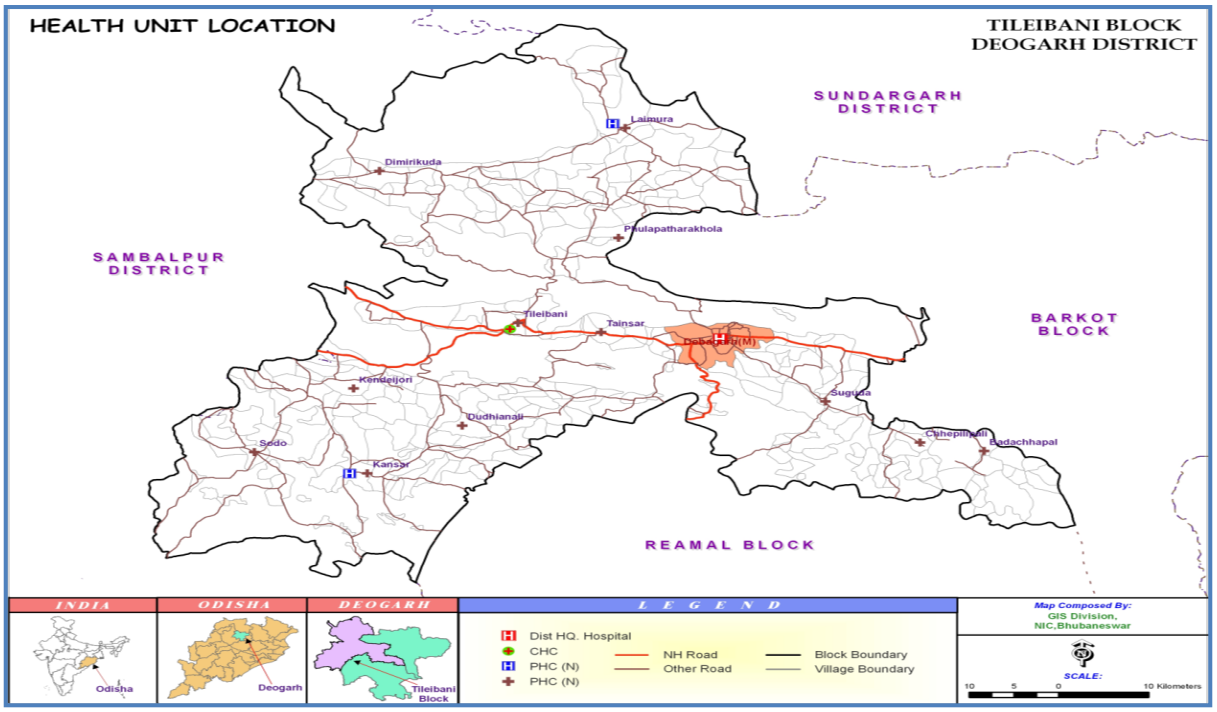

Figure 15: Locations of health unit and connectivity with roads in Tileibani block

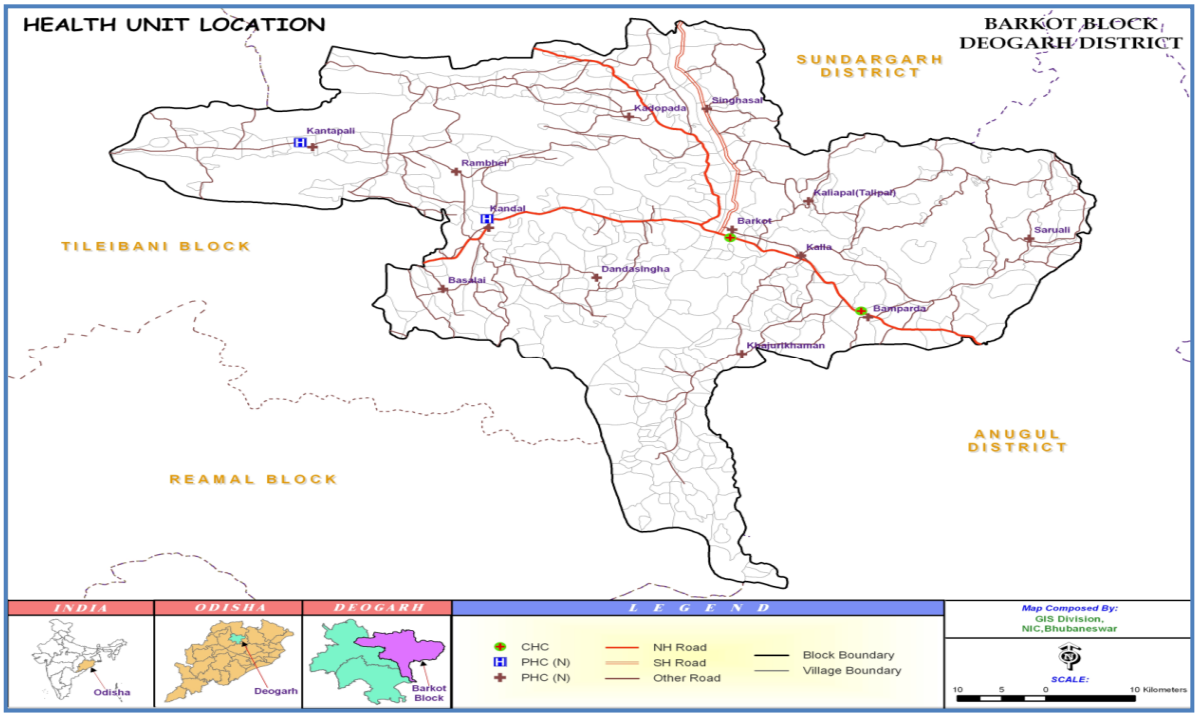

Figure 16: Locations of health unit and connectivity with roads in Barkot block

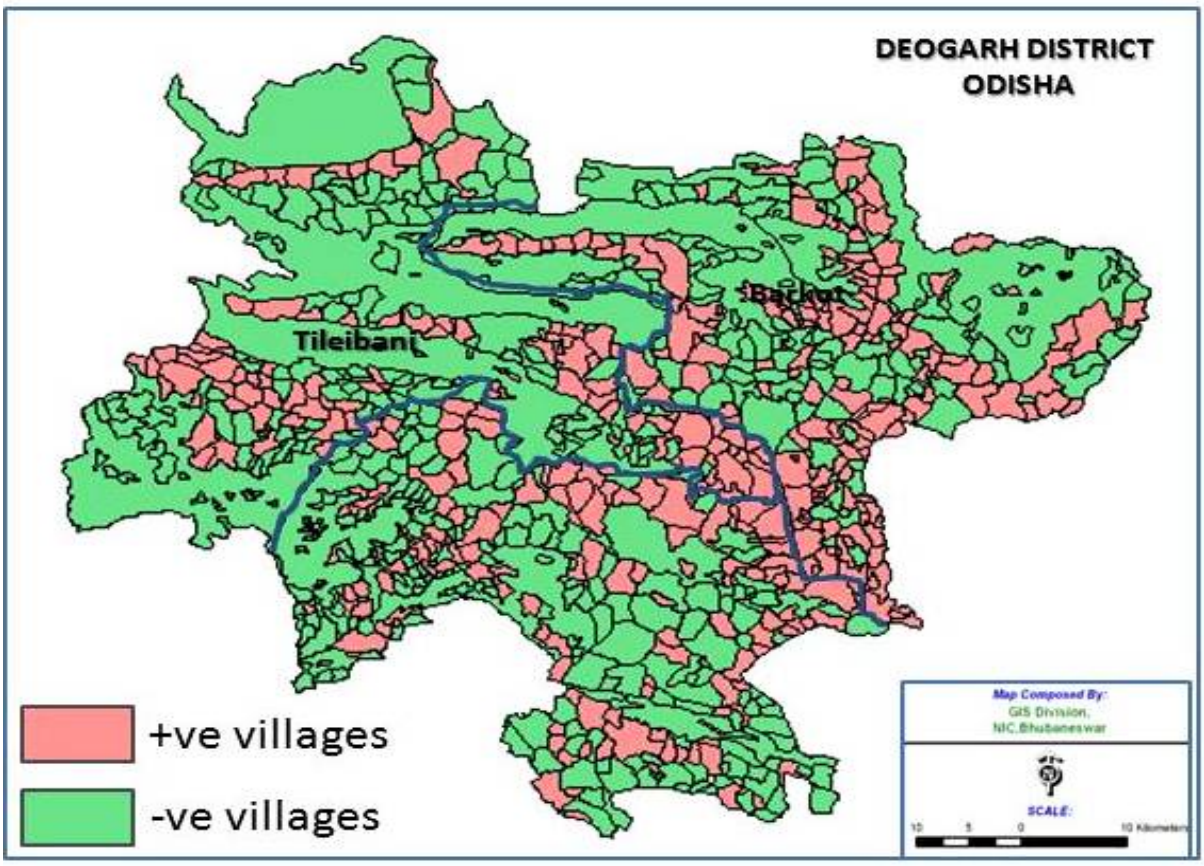

Figure 17: Villages within one kilometre distance from road in district Deogarh.

4.9.2. Village level health care facilities existing in Tileibani and Barkot blocks

Figures 18 and 19 show that there are 164 and 107 ASHA in Tileibani and Barkot blocks respectively. There are 64 and 124 villages without ASHA in Tileibani (Figure 18) and Barkot (Figure 19) blocks respectively and there were 135 and 99 Anganwadi Centres in Tileibani and Barkot blocks in that order. The data revealed that there are more number of ASHA and Anganwadi Centres in Tileibani block in comparison to Barkot. Mapping of the healthcare facilities revealed that there are only 25 and 55 villages within two kilometre range from PHCs Tileibani and Bamparada respectively (Figure not shown) and only 50 and 65 villages were located within 5 kilometres distance from the PHCs (Figure 20). Only one village was having registered private doctor in Tileibani PHC area and seven villages were having registered private doctors under Bamparada PHC area (Figure not shown). A total of 201 and 196 villages were having at least one health care facility i.e. PHC, ASHA, Anganwadi Centre, or registered private doctors in Tileibani and Barkot blocks respectively. Overall the availability and accessibility of health care facilities were better in villages under Barkot block as compared to Tileibani block. (Figures 15-20).

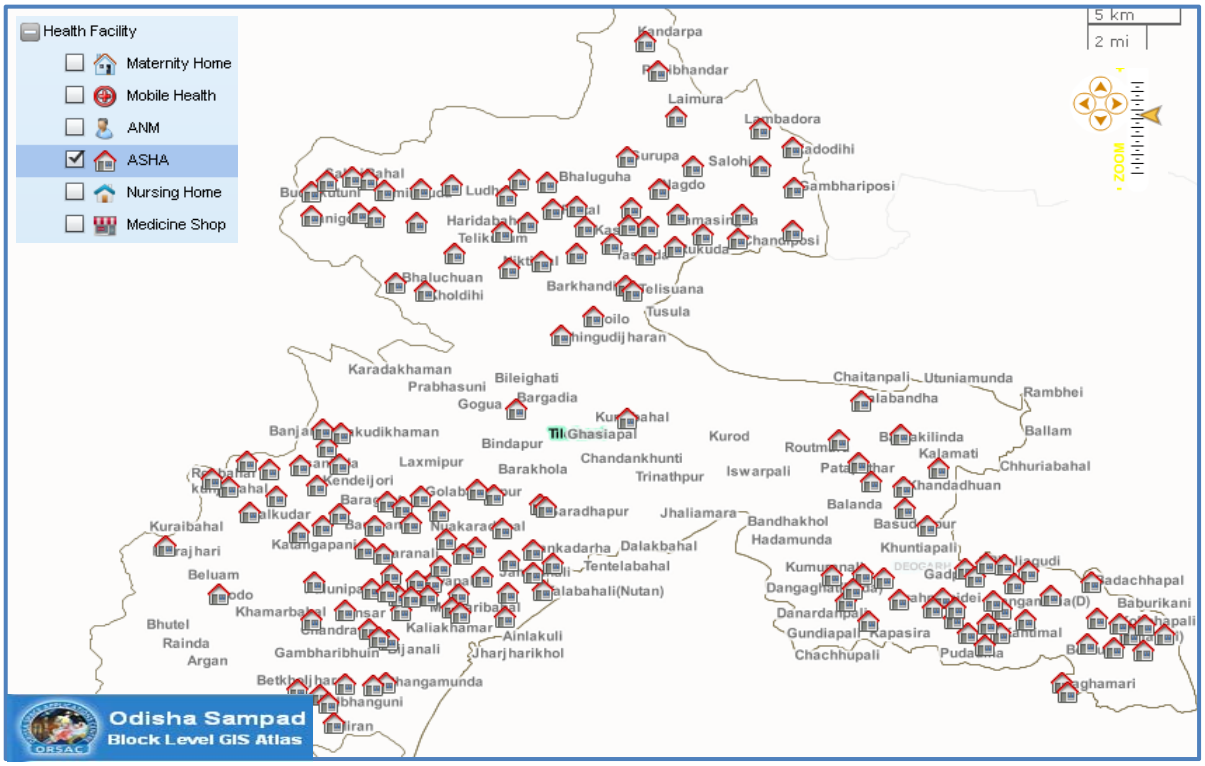

Figure 18: Availability of ASHA in Tileibani block

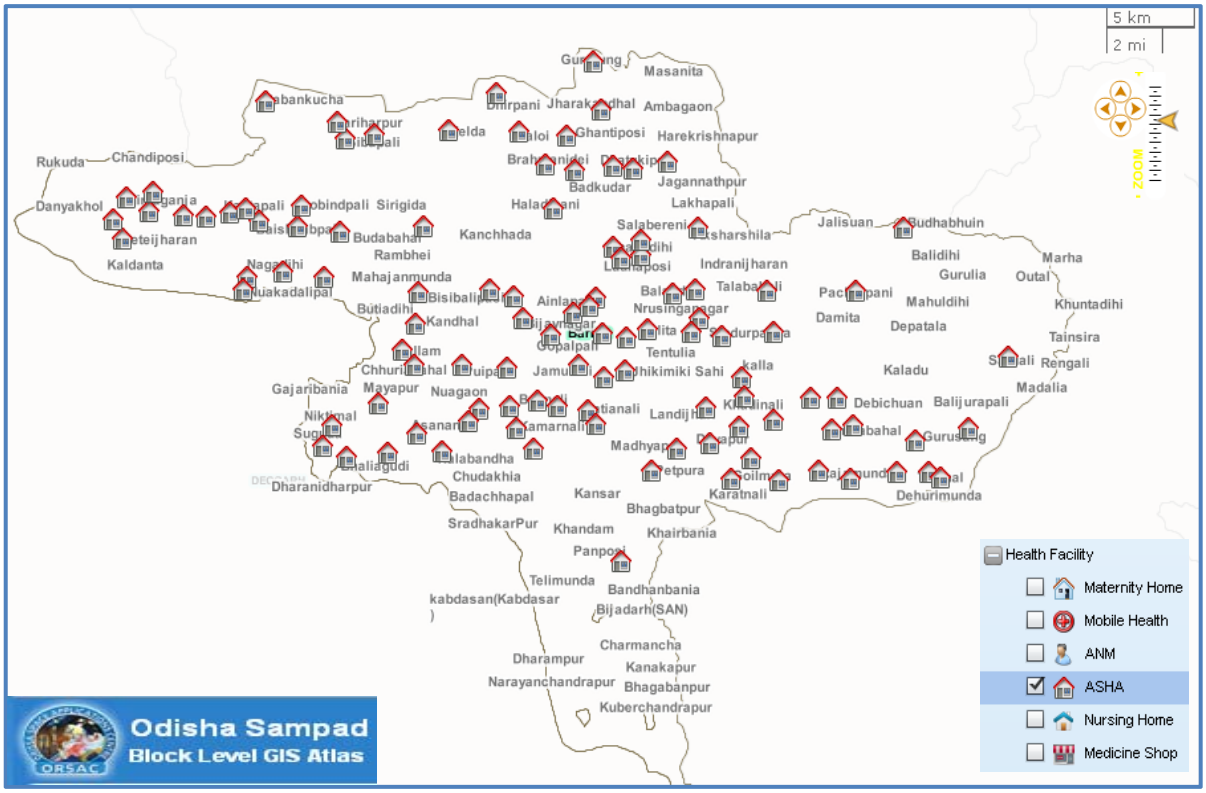

Figure 19: Availability of ASHA in Barkot block

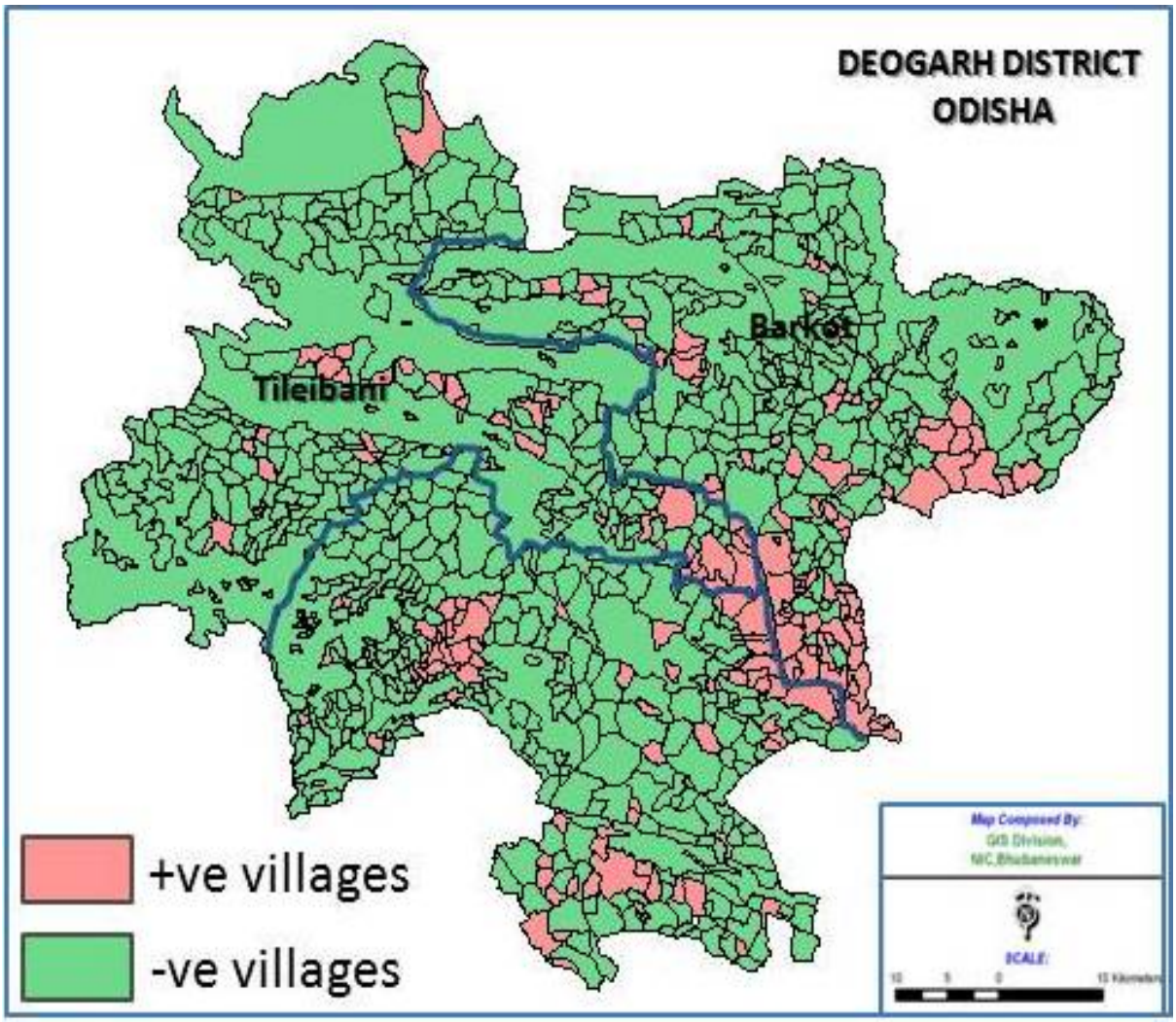

Figure 20: Villages within five kilometres of PHC, in blocks of Deogarh district

4.10. Demographic characteristics of study sites

Figures 21-26 depict population density, male-female ratio and caste distribution in Tileibani and Barkot blocks of district Deogarh. Overall villages under Tileibani block were less densely populated then in Barkot block (Figure 21). The male-female ratio was almost balanced in both the blocks (Figure 21). Tileibani and Barkot blocks were found majorly inhabited by Scheduled Tribe (ST) and Scheduled Caste (SC) population. Mapping of ST and SC population distribution had shown the ST dominance in majority of the villages under Tileibani block whereas villages under Barkot block revealed SC dominance (Figure 22). The detailed block wise village level ST/SC population density was mapped in Figures 23-26.

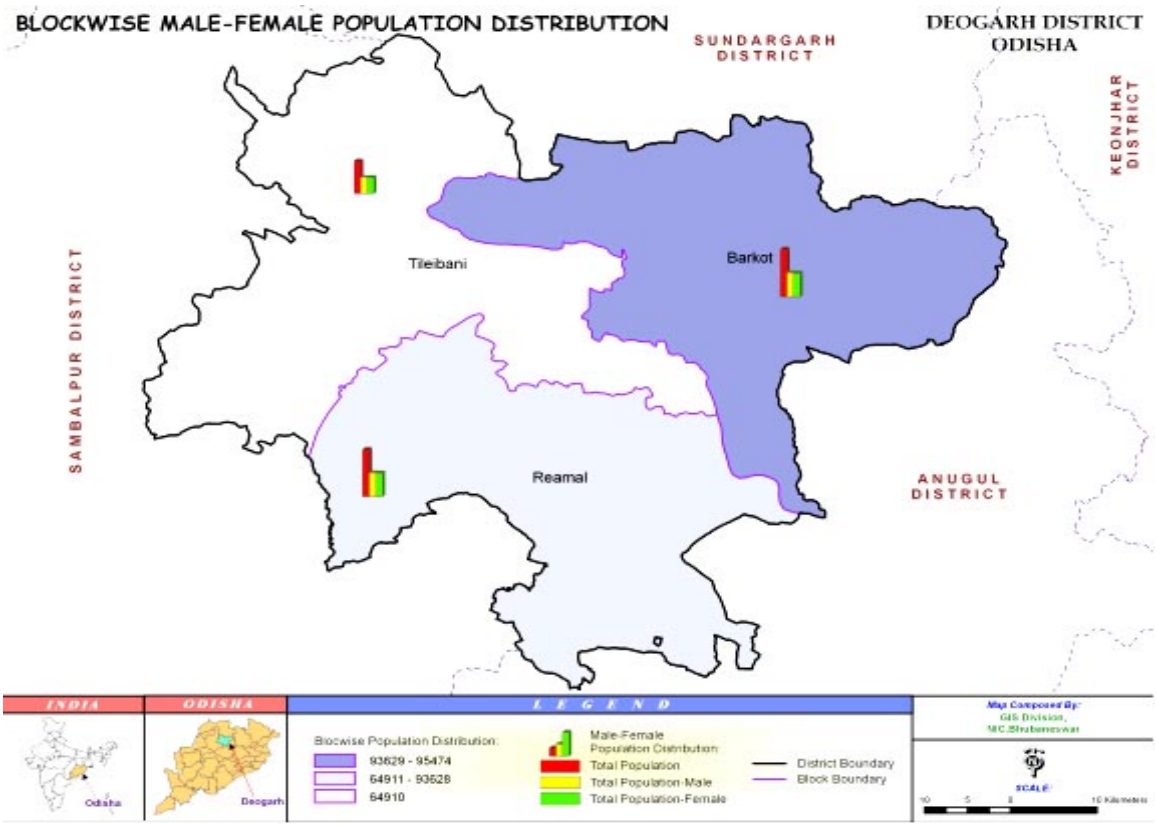

Figure 21: Block wise population and male-female distribution in Deogarh district

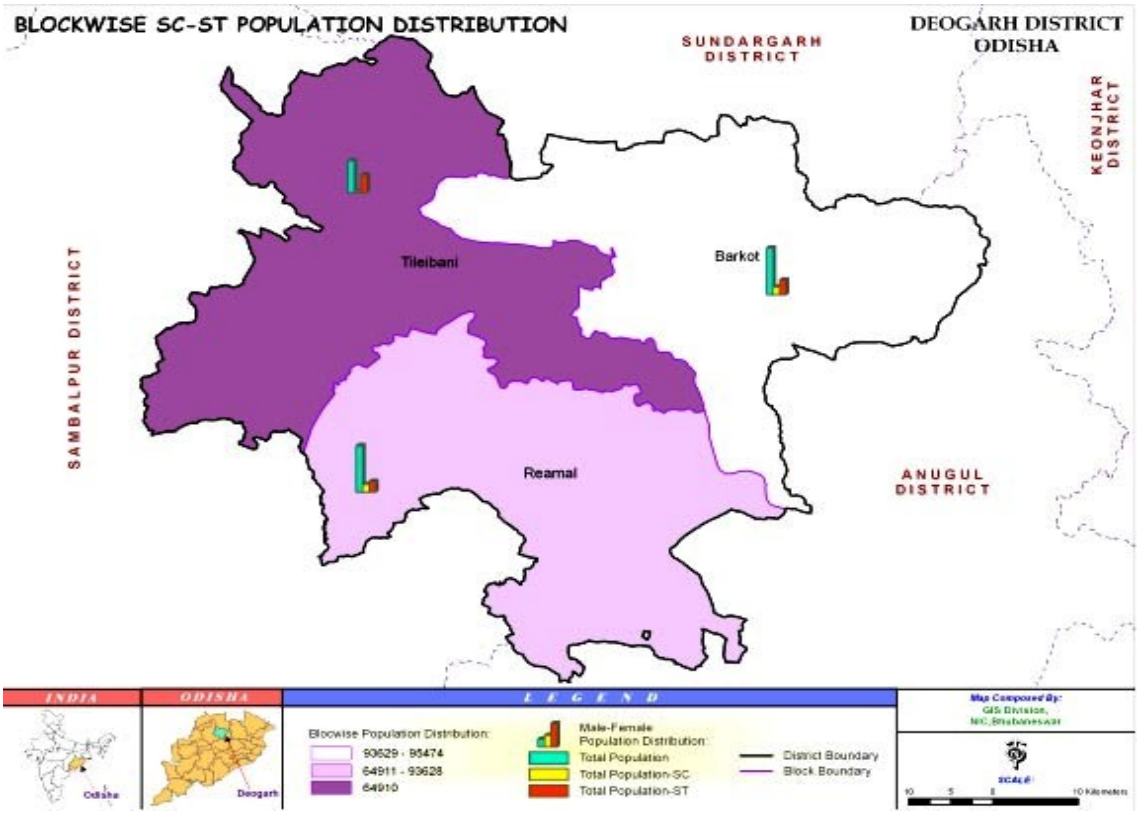

Figure 22: Block wise Scheduled Caste (SC) and Scheduled Tribe (ST) population distribution in Deogarh district

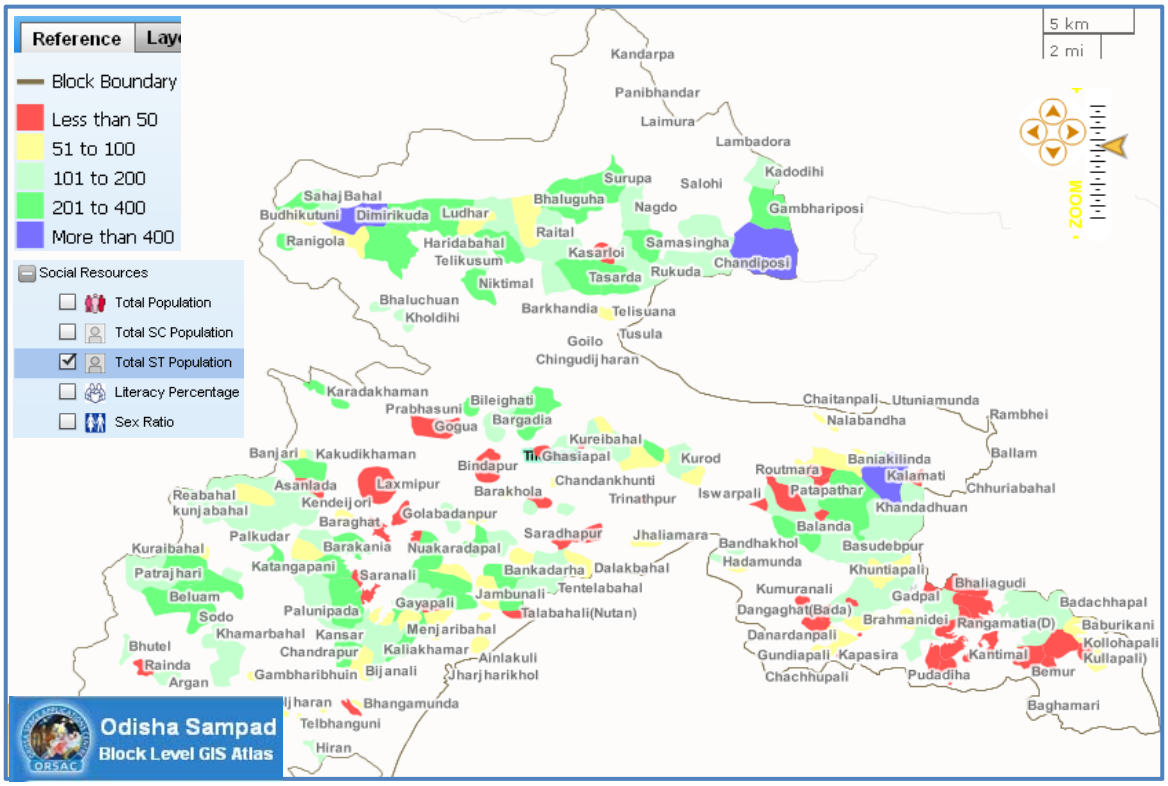

Figure 23: Scheduled Tribe (ST) population distribution in Tileibani block (Tileibani PHC)

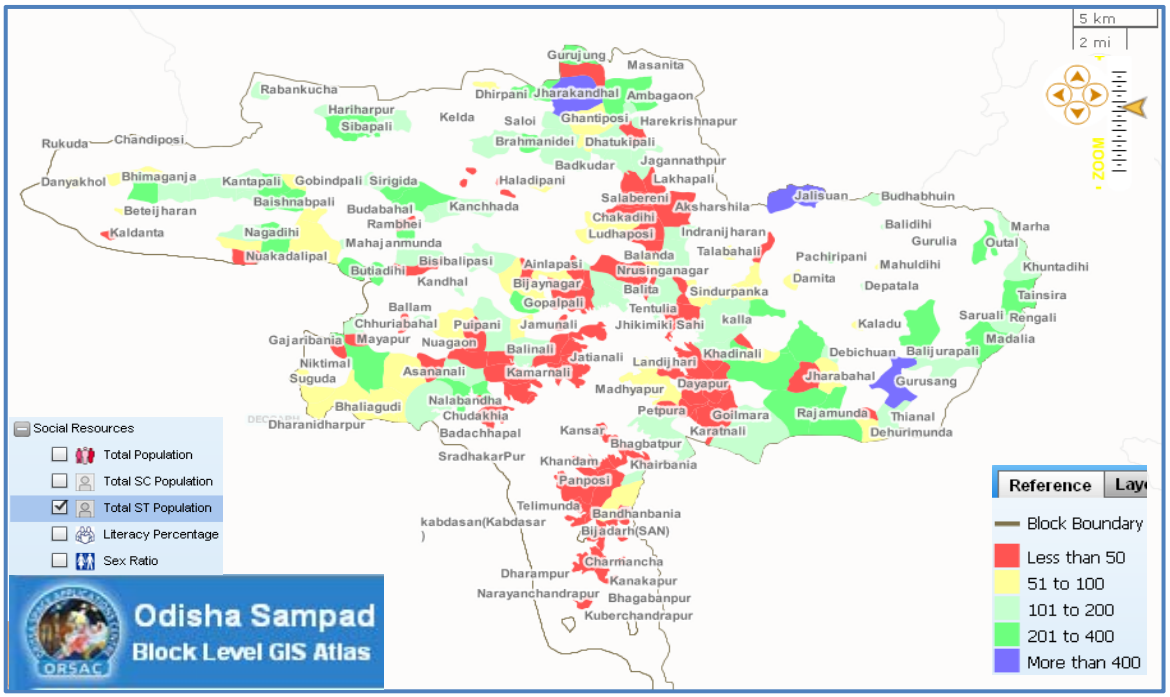

Figure 24: Scheduled Tribe (ST) population distribution in Barkot block (Bamparada PHC)

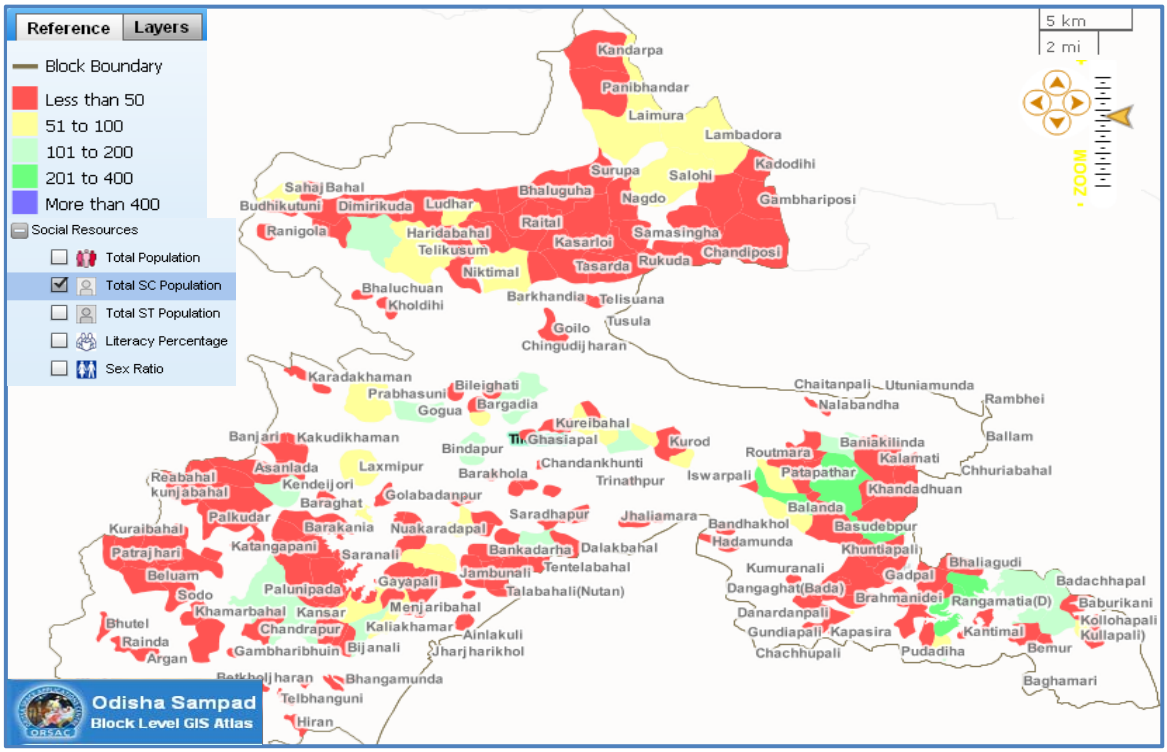

Figure 25: Scheduled Caste (SC) population distribution in Tileibani block (Tileibani PHC)

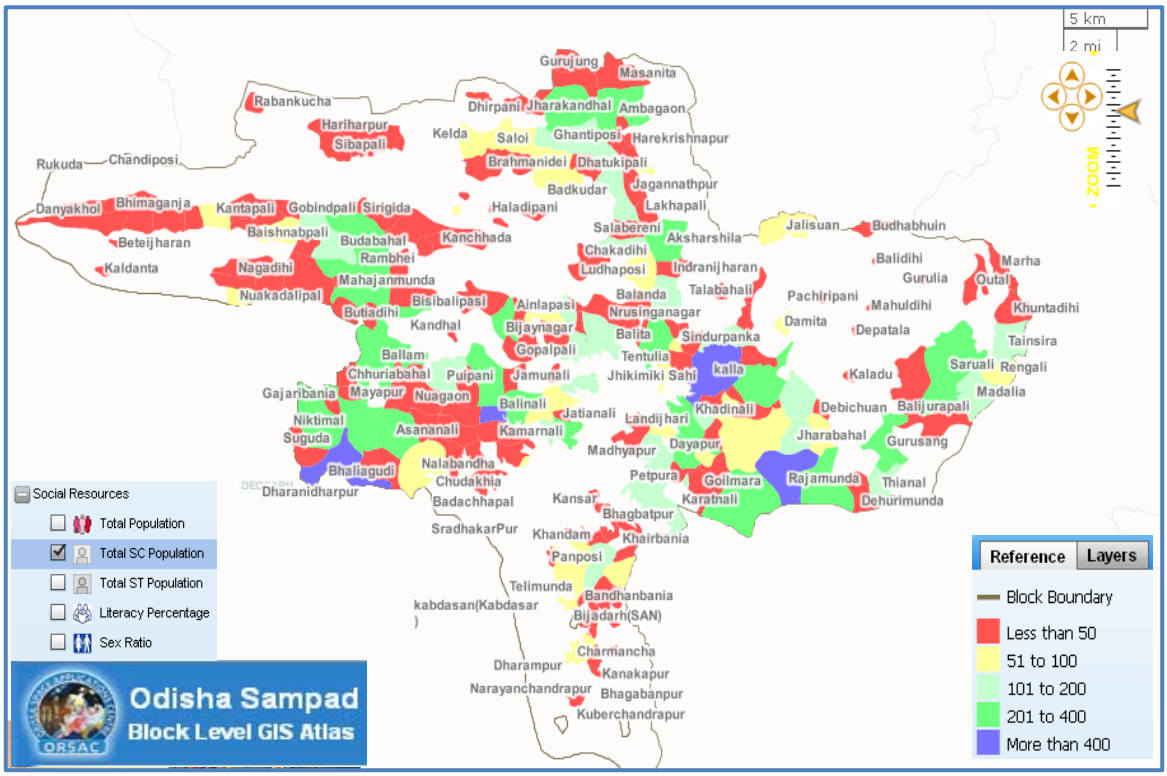

Figure 26: Scheduled Caste (SC) population distribution in Barkot block (Bamparada PHC)

4.11. Malaria transmission pattern

Month wise malaria incidence pattern of district Deogarh as a whole shows highest incidence in November-December. However, block wise monthly malaria incidence pattern was different. Barkot block which includes study villages under Bamparada PHC, the monthly malaria incidence was found to be highest during monsoon (July-August). Whereas in Tileibani block which includes study villages under Tileibani PHC, the pattern of monthly malaria incidence was similar to that of Deogarh district as a whole with peak in November-December (Figure 27). The Tileibani block was found to be contributing more malaria cases reported from the district.

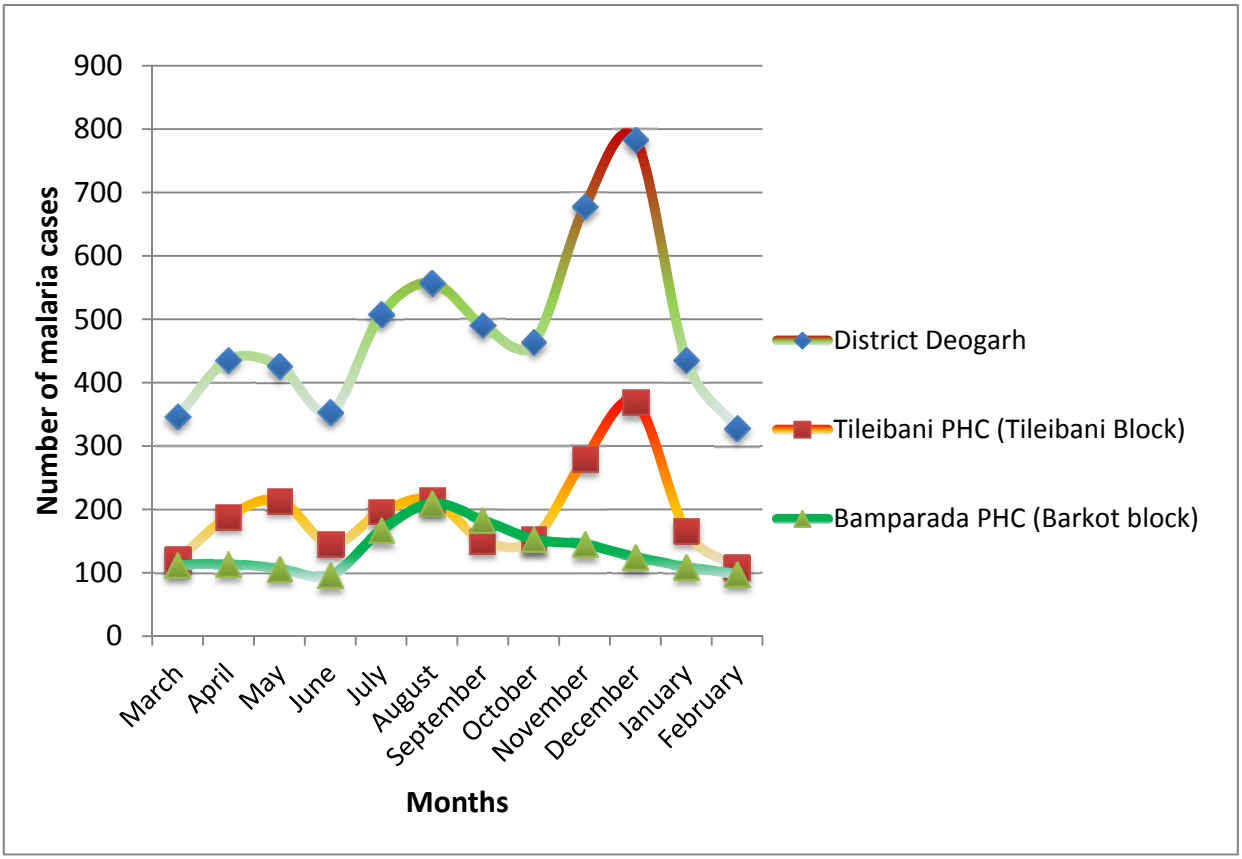

Figure 27: Month wise malaria incidence pattern of Tileibani PHC, Bamparada PHC and entire district Deogarh, for the years 2006-12 (Pooled data)

4.12. Meteorological reports

4.12.1. Temperature

In Barkot block the monthly minimum temperature ranges from 12.9°C in winter (January) to 25.8°C in summer (May) whereas in Tileibani block the monthly minimum temperature ranges from 13.3°C in winter (January) to 26.7°C in summer (May) (Tables 4 and 5). Barkot block's monthly maximum temperature ranges from 26.4°C in winter (January) to 39.2°C in summer (May). Tileibani block's monthly maximum temperature ranges from 25.5°C in winter (January) to 36.6°C in summer (May) (Table 6). The monthly mean of Barkot block's minimum and maximum temperature varies from 19.7°C in winter (January) to 32.5°C in summer (May) (Table 7). The monthly mean of Tileibani block's minimum and maximum temperature varies from 19.4°C in winter (January) to 31.7°C in summer (May) (Table 8). The range of Barkot block's circadian temperature varies from 6.5°C in August to 14.3°C in April (Table 9). The range of Tileibani block's circadian temperature varies from 4°C in August to 12.3°C in February (Table 10). January was found to be the coldest month in both Barkot and Tileibani blocks as the average monthly minimum temperature recorded is as low as 12.9°C (Table 4) and 13.3°C (Table 5) respectively. May was found to be the hottest month in Barkot and Tileibani blocks as having average monthly maximum temperature recorded was as high as 39.2°C and 36.6°C respectively (Tables 6 and 7). Month wise minimum, maximum, circadian mean and circadian range of temperatures from year 2007-2012, along with average of all the years' monthly data were tabulated in Tables 3-10 and representative average data was presented in graph (Figure 28).

Table 3: Year 2007-12, month-wise minimum temperature of Barkot (in °C)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	12.4	14.7	20.2	21.2	25.3	26.7	24.4	23.8	23.4	21.3	18.5	15.4
2008	13.2	16.9	18.7	23.2	26.2	27.4	24.5	24.8	24.2	22.9	18.4	13.1
2009	12.0	17.2	19.9	24.9	25.1	25.1	23.8	23.9	23.5	21.8	16.5	14.3
2010	14.4	16.5	19.4	24.1	25.6	24.6	24.5	24.3	23.5	22.0	17.4	12.5
2011	12.6	16.3	20.0	23.2	26.0	24.3	23.7	24.0	24.3	22.4	19.0	13.6
2012	13.2	16.5	20.3	23.3	26.6	25.5	24.7	23.5	23.3	21.5	16.6	14.0
2007-12	12.9	16.3	19.7	23.3	25.8	25.6	24.3	24.0	23.7	22.0	17.7	13.8

Table 4: Year 2007-12, month-wise minimum temperature of Tileibani (in °C)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	12.6	14.8	20.6	22.0	26.2	27.4	25.0	24.3	24.0	21.8	19.0	15.8
2008	13.5	17.0	19.1	24.1	27.2	28.0	25.2	25.5	24.8	23.3	18.6	13.3
2009	12.2	17.4	20.5	25.9	25.8	25.6	24.5	24.6	23.9	22.2	16.8	14.5
2010	14.8	16.7	20.0	24.9	26.5	25.3	24.9	24.9	24.3	22.2	17.9	12.8
2011	12.8	16.5	20.8	24.1	27.0	24.9	24.4	24.7	24.7	22.8	19.8	13.9
2012	13.7	16.8	21.1	24.0	27.6	26.2	25.5	24.0	24.0	21.8	17.4	14.4
2007-12	13.3	16.5	20.3	24.2	26.7	26.2	24.9	24.7	24.3	22.3	18.3	14.1

Table 5: Year 2007-12, month-wise maximum temperature of Barkot (in °C)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	25.4	28.5	33.4	35.5	38.8	37.2	31.1	30.2	31.1	31.2	30.0	27.3
2008	27.0	29.2	32.4	37.0	39.2	37.5	31.3	30.9	31.4	31.9	29.0	26.3
2009	25.2	30.4	34.8	39.6	38.7	35.3	30.9	30.0	30.2	31.5	28.6	26.1
2010	27.4	28.2	33.0	38.6	38.7	34.5	31.0	31.0	30.9	31.8	29.7	26.5
2011	26.4	29.9	34.2	37.2	39.3	33.5	30.1	30.7	32.0	31.4	29.7	27.2
2012	27.0	30.4	34.9	37.9	40.3	35.7	31.9	30.2	31.1	31.3	28.9	27.8
2007-12	26.4	29.5	33.8	37.6	39.2	35.6	31.0	30.5	31.1	31.5	29.3	26.9

Table 6: Year 2007-12, month-wise maximum temperature of Tileibani (in °C)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	24.8	28.2	32.2	33.4	36.3	35.3	29.3	28.7	29.3	29.7	28.8	26.1
2008	26.1	28.9	31.1	34.5	36.4	36.0	29.4	29.0	29.8	30.9	28.3	25.7
2009	24.6	29.9	33.2	36.8	36.6	34.1	28.8	27.8	28.9	30.3	27.7	25.7
2010	26.1	27.6	31.4	36.5	36.3	32.6	29.7	29.2	28.8	31.1	28.1	25.8
2011	25.7	29.2	31.8	34.8	36.5	31.9	28.2	28.6	30.8	30.5	27.5	26.2
2012	25.7	29.6	32.8	36.0	37.6	33.6	29.8	28.7	29.3	30.7	26.8	26.6
2007-12	25.5	28.9	32.1	35.3	36.6	33.9	29.2	28.7	29.5	30.5	27.9	26.0

Table 7: Year 2007-12, month-wise means temperature of Barkot (in °C)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	18.9	21.6	26.8	28.4	32.1	32.0	27.8	27.0	27.2	26.2	24.3	21.4
2008	20.1	23.1	25.5	30.1	32.7	32.5	27.9	27.8	27.8	27.4	23.7	19.7
2009	18.6	23.8	27.4	32.2	31.9	30.2	27.4	26.9	26.8	26.7	22.6	20.2
2010	20.9	22.3	26.2	31.4	32.2	29.6	27.7	27.6	27.2	26.9	23.5	19.5
2011	19.5	23.1	27.1	30.2	32.7	28.9	26.9	27.4	28.1	26.9	24.3	20.4
2012	20.1	23.5	27.6	30.6	33.5	30.6	28.3	26.8	27.2	26.4	22.8	20.9
2007-12	19.7	22.9	26.8	30.5	32.5	30.6	27.7	27.3	27.4	26.8	23.5	20.3

Table 8: Year 2007-12, month-wise means temperature of Tileibani (in °C)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	18.7	21.5	26.4	27.7	31.3	31.4	27.2	26.5	26.6	25.7	23.9	21.0
2008	19.8	22.9	25.1	29.3	31.8	32.0	27.3	27.3	27.3	27.1	23.5	19.5
2009	18.4	23.6	26.9	31.4	31.2	29.8	26.7	26.2	26.4	26.2	22.2	20.1
2010	20.5	22.1	25.7	30.7	31.4	29.0	27.3	27.0	26.5	26.6	23.0	19.3
2011	19.3	22.9	26.3	29.4	31.8	28.4	26.3	26.7	27.7	26.6	23.7	20.1
2012	19.7	23.2	26.9	30.0	32.6	29.9	27.6	26.4	26.6	26.2	22.1	20.5
2007-12	19.4	22.7	26.2	29.7	31.7	30.1	27.1	26.7	26.9	26.4	23.1	20.1

Table 9: Year 2007-12, month-wise circadian temperature range of Barkot (in °C)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	13.0	13.9	13.2	14.3	13.5	10.5	6.8	6.5	7.7	9.9	11.5	12.0
2008	13.8	12.3	13.7	13.8	12.9	10.2	6.7	6.0	7.2	9.0	10.6	13.3
2009	13.2	13.3	14.9	14.6	13.7	10.2	7.2	6.1	6.8	9.8	12.1	11.7
2010	13.0	11.8	13.6	14.5	13.1	9.9	6.5	6.7	7.4	9.8	12.3	13.9
2011	13.8	13.7	14.3	14.0	13.2	9.2	6.4	6.8	7.8	9.0	10.6	13.5
2012	13.8	13.9	14.6	14.5	13.7	10.2	7.2	6.8	7.8	9.8	12.3	13.8
2007-12	13.5	13.1	14.1	14.3	13.4	10.0	6.8	6.5	7.4	9.6	11.6	13.1

Table 10: Year 2007-12, month-wise circadian temperature range of Tileibani (in °C)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	12.2	13.4	11.6	11.4	10.1	7.9	4.3	4.4	5.3	7.9	9.8	10.3
2008	12.6	11.8	12.0	10.4	9.2	8.0	4.2	3.5	5.1	7.7	9.7	12.4
2009	12.4	12.5	12.8	10.9	10.8	8.5	4.3	3.2	5.0	8.1	10.9	11.2
2010	11.3	10.9	11.4	11.6	9.8	7.3	4.8	4.3	4.5	8.9	10.2	13.1
2011	13.0	12.7	11.0	10.7	9.5	7.1	3.8	3.9	6.1	7.7	7.8	12.3
2012	12.1	12.8	11.7	12.0	10.0	7.3	4.2	4.7	5.3	8.9	9.4	12.1
2007-12	12.2	12.3	11.8	11.2	9.9	7.7	4.3	4.0	5.2	8.2	9.6	11.9

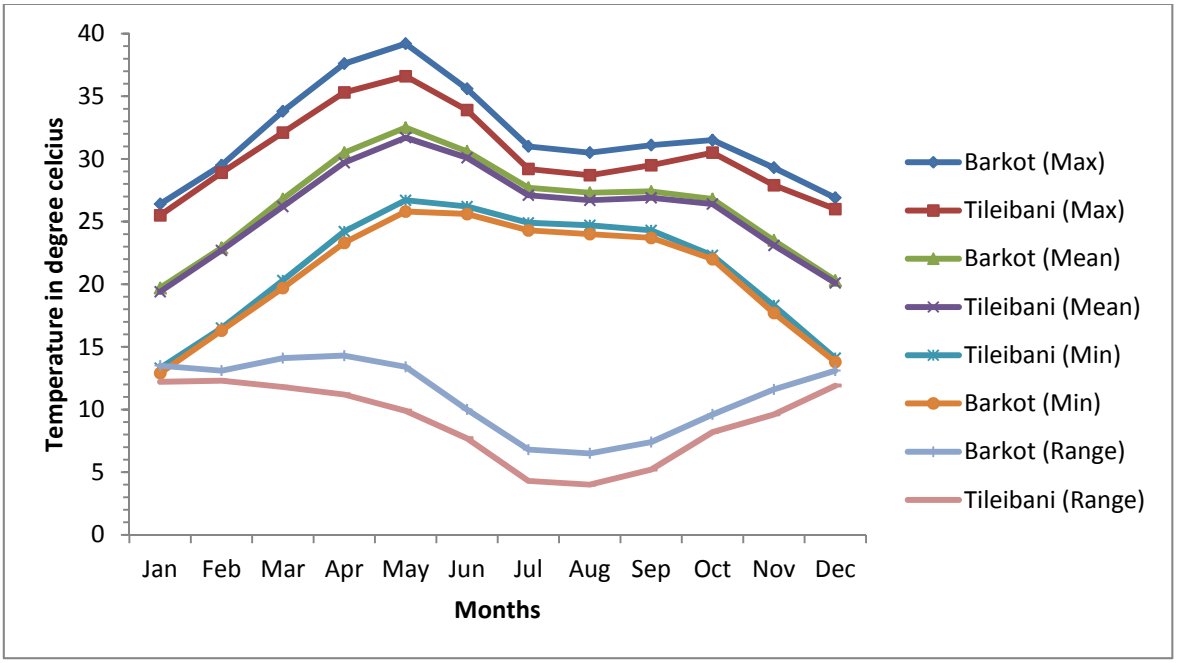

Figure 28: Minimum, maximum, mean, and range of month wise average circadian temperature of Tileibani and Barkot blocks for the years 2007-12 (Pooled data)

4.12.2. Vapour pressure (Humidity)

The recorded vapour pressure in Barkot block was found to be as low as 16 Pa in the month of December (winter) and as high as 31.7 Pa in the July (monsoon) (Table 11). The recorded vapour pressure in Tileibani block was found to be as low as 16.2 Pa in the December and as high as 32.5 Pa in the July (Table 12). The year wise monthly vapour pressure of Barkot and Tileibani blocks for the years 2007-2012 is given in Tables 11 and 12 separately and the comparative graph of both the blocks is shown in Figure 29.

Table 11: Years 2007-12, month-wise vapour pressure (in Pa) in Barkot block

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	16.3	17.2	20.7	21.1	27.3	32.5	31.4	31.3	30.8	26.0	21.2	17.0
2008	16.5	18.5	20.1	23.1	27.5	33.6	31.7	31.3	30.8	27.8	22.0	15.2
2009	15.6	18.8	20.6	24.7	27.3	30.7	31.2	31.3	31.1	25.9	19.8	16.5
2010	17.3	18.9	20.6	24.1	27.3	29.6	31.2	31.3	30.7	26.8	19.8	15.6
2011	16.2	18.2	20.1	22.9	27.3	29.9	32.0	31.3	31.0	26.7	22.0	15.6
2012	16.3	18.4	20.1	23.0	28.2	30.7	32.8	31.3	30.8	26.1	19.8	16.0
2007-12	16.4	18.3	20.4	23.1	27.5	31.2	31.7	31.3	30.9	26.6	20.8	16.0

Table 12: Years 2007-12, month-wise vapour pressure (in Pa) in Tileibani block

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	16.9	17.6	21.9	23.2	29.8	34.3	33.2	32.9	32.6	27.5	22.5	18.2
2008	17.4	18.9	21.4	25.5	30.3	35.2	33.5	33.1	32.4	28.8	22.6	15.9
2009	16.2	19.4	22.2	27.4	29.4	32.0	33.4	33.4	32.4	27.2	20.8	16.9
2010	18.5	19.6	22.2	26.2	29.7	31.5	32.5	33.1	32.8	27.5	21.4	16.2
2011	16.9	18.9	22.5	25.4	30.0	31.5	33.9	33.4	32.3	27.7	24.1	16.6
2012	17.5	19.2	22.3	24.8	30.8	32.8	35.0	32.8	32.6	26.8	21.9	17.3
2007-12	17.2	18.9	22.1	25.4	30.0	32.9	33.6	33.1	32.5	27.6	22.2	16.8

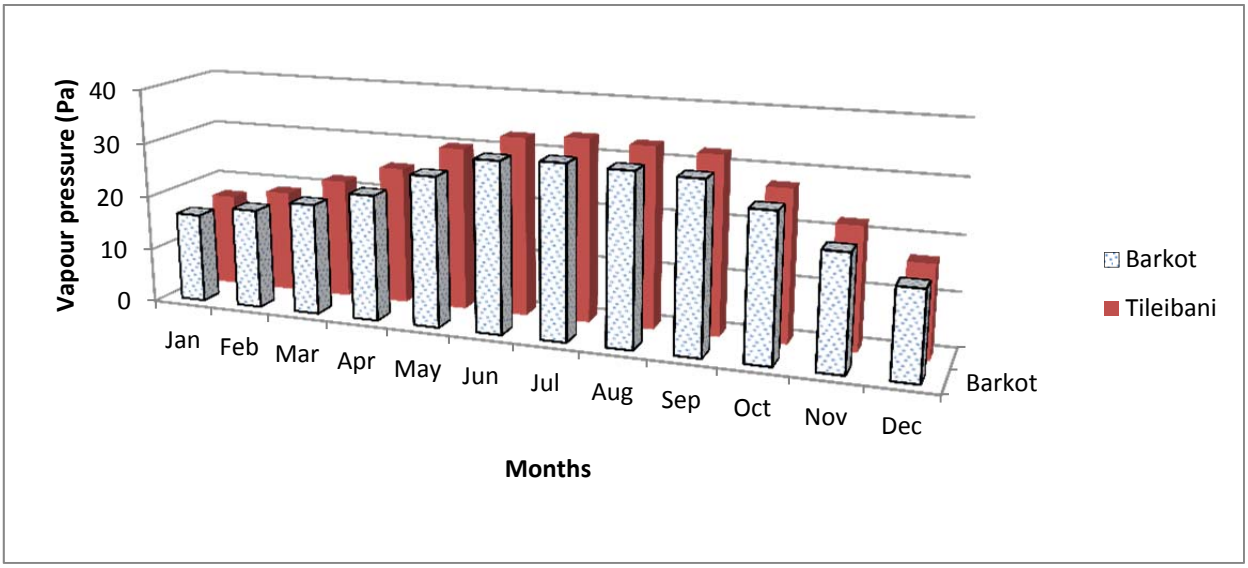

Figure 29: Average monthly vapour pressure of Barkot and Tileibani blocks for the years 2007-12 (Pooled data)

4.12.3. Precipitation (rainfall)

Precipitation in Barkot block is less during December as average rainfall recorded was only 2.5 mm and it was maximum during August with an average of 385.6 mm of rainfall (Table 13). Similarly, precipitation in Tileibani block is less during December as average rainfall recorded was only 3.4 mm and maximum during August with an average of 387.6 mm rainfall (Table 14). The year wise monthly rainfall of Barkot and Tileibani blocks for the years 2007-2012 is given in Table 13 and 14 respectively and comparative graph of both the blocks is represented in Figure 30.

Table 13: Years 2007-12, month-wise precipitation (in mm) of Barkot block

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	16.9	7.5	65.0	33.2	30.2	147.3	349.4	348.7	212.2	20.8	33.3	14.9
2008	8.8	63.4	11.5	17.8	50.4	175.7	268.2	206.6	233.3	161.1	50.6	.0
2009	2.4	3.2	.3	.2	82.7	199.5	249.9	608.8	135.5	48.4	7.7	.0
2010	1.3	87.2	.3	26.2	39.6	329.3	225.0	299.4	127.7	24.3	4.7	.3
2011	.4	2.3	43.4	19.4	45.8	329.3	308.3	403.0	116.6	71.7	24.0	.0
2012	36.0	10.6	8.7	25.4	52.5	166.7	275.3	447.1	168.1	42.2	16.5	.0
2007-12	11.0	29.0	21.5	20.4	50.2	224.6	279.4	385.6	165.6	61.4	22.8	2.5

Table 14: Years 2007-12, monthly precipitation (in mm) of Tileibani block

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	17.6	7.8	66.3	35.4	32.9	149.3	351.4	350.4	214.1	22.4	34.7	16.2
2008	9.7	63.8	12.8	20.4	53.4	177.4	270.2	208.6	235.0	162.1	51.3	.7
2009	3.1	3.8	1.9	3.1	85.0	200.9	252.2	611.1	136.9	49.8	8.7	.4
2010	2.7	87.9	2.0	28.5	42.2	331.3	226.3	301.4	129.9	25.0	6.4	.9
2011	1.0	3.1	45.9	22.0	48.7	331.0	310.3	405.3	117.9	72.7	26.3	1.0
2012	37.3	11.4	11.0	27.4	55.3	169.0	277.6	448.8	170.0	42.9	18.8	1.4
2007-12	11.9	29.6	23.3	22.8	52.9	226.5	281.3	387.6	167.3	62.5	24.4	3.4

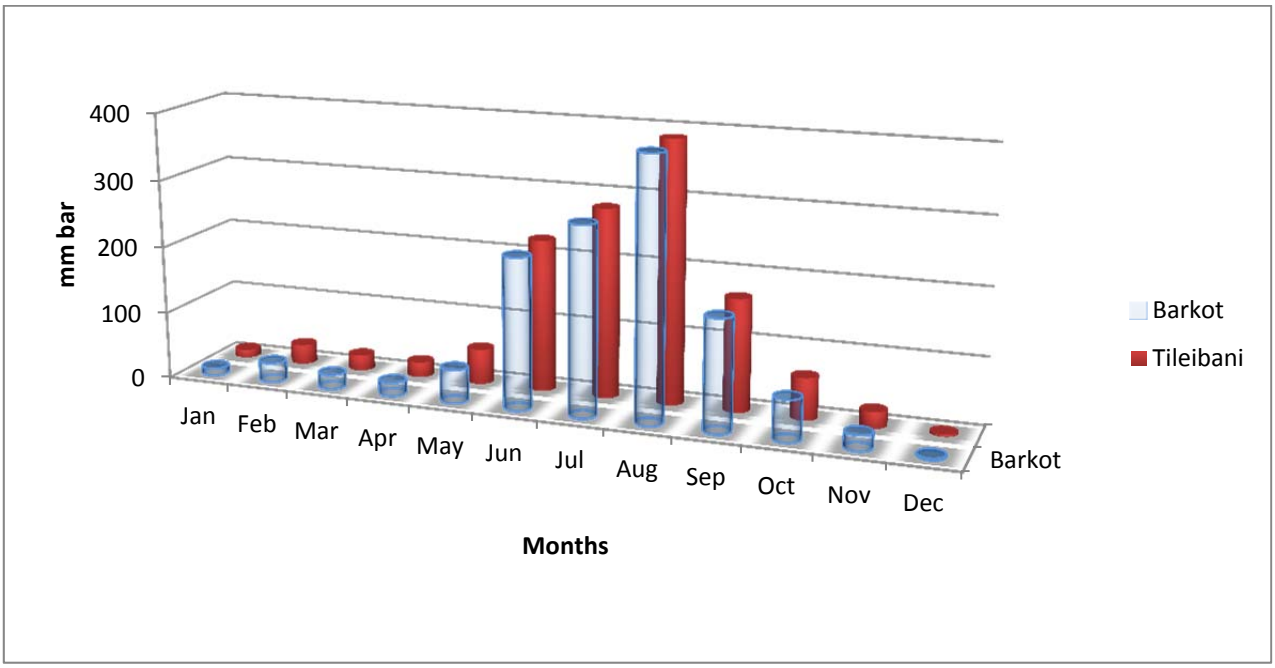

Figure 30: Average monthly precipitation of Barkot and Tileibani blocks for the years 2007-12 (Pooled data)

4.12.4. Wet days (number of rainy days)

In Barkot block, the minimum number of wet days (1 day) was recorded during January and maximum numbers of wet days (15 days) were observed during August. Similarly there were minimum numbers of wet days (1.2 day) were observed during January and maximum numbers of wet days (15.6 days) were observed during August in Tileibani block (Table 16). The year wise monthly wet days of Barkot and Tileibani blocks for the year 2007-2012 are given in Tables 15 and 16 respectively and comparative graph of both the blocks is represented in Figure 30.

Table 15: Years 2007-12, month-wise wet days of Barkot block

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	1.4	1.0	2.8	2.3	3.1	6.7	15.1	15.0	10.2	2.4	2.0	1.0
2008	1.3	3.0	1.1	1.8	4.2	8.0	13.7	11.5	10.8	6.7	2.2	.0
2009	.0	1.0	.3	.2	4.5	8.7	13.1	18.7	8.5	3.6	1.0	.0
2010	1.0	3.1	.3	2.0	3.0	10.7	12.2	12.9	7.9	2.6	1.0	.3
2011	.0	1.0	2.4	1.9	3.6	10.6	13.8	15.3	7.4	4.5	1.5	.0
2012	2.1	1.0	1.0	2.3	3.3	7.9	12.7	16.4	8.5	3.8	1.3	.0
2007-12	1.0	1.7	1.3	1.7	3.6	8.7	13.4	15.0	8.9	3.9	1.5	.2

Table 16: Years 2007-12, month-wise wet days of Tileibani block

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2007	1.7	1.1	3.2	3.0	4.0	7.3	15.7	15.6	10.9	3.0	2.4	1.4
2008	1.6	3.2	1.5	2.7	5.2	8.5	14.4	12.1	11.4	7.0	2.5	.0
2009	.0	1.2	.8	1.2	5.3	9.1	13.8	19.4	9.0	4.1	1.4	.0
2010	1.4	3.4	.8	2.7	3.8	11.4	12.7	13.6	8.7	2.9	1.6	.5
2011	.0	1.3	3.2	2.8	4.6	11.1	14.5	16.1	7.8	4.8	2.3	.0
2012	2.6	1.3	1.8	3.0	4.2	8.6	13.5	17.0	9.1	4.0	2.0	.0
2007-12	1.2	1.9	1.9	2.6	4.5	9.4	14.1	15.6	9.5	4.3	2.0	.3

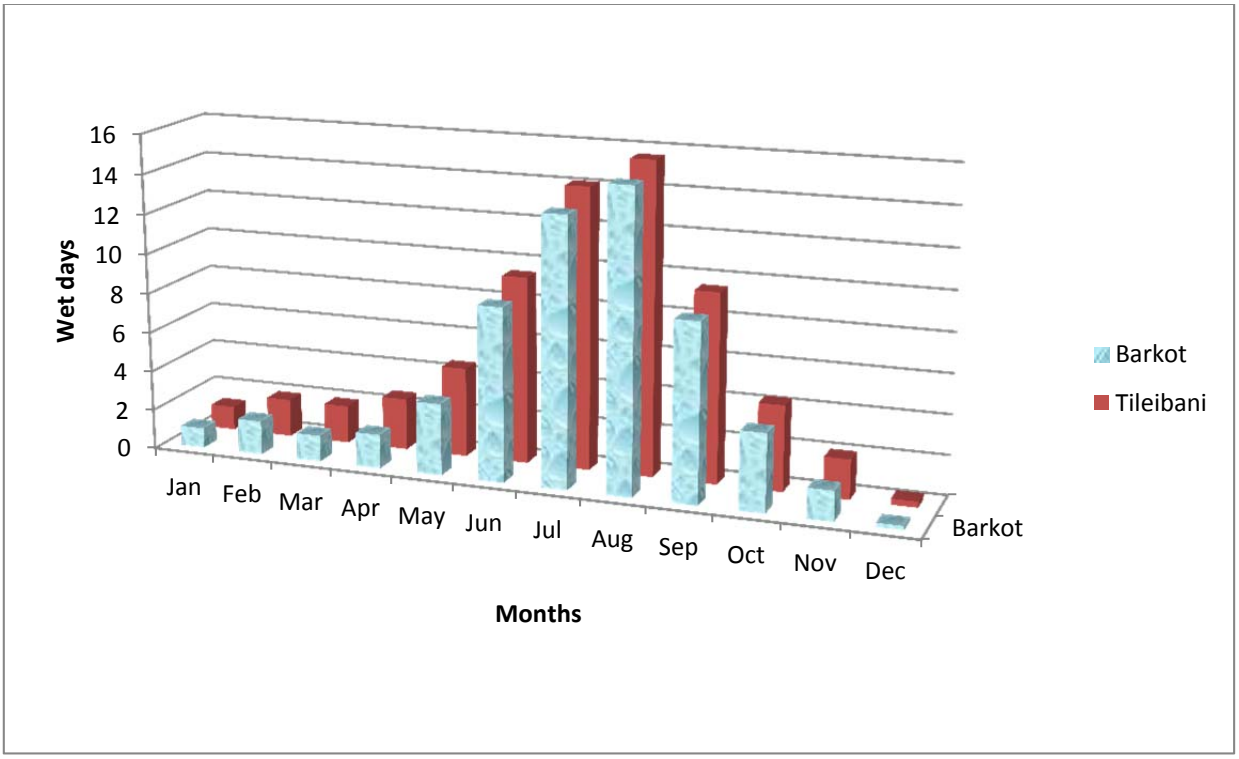

Figure 32: Average monthly wet days of Barkot and Tileibani block for the years 2007-12 (Pooled data)

5. Discussion

In district Deogarh, Odisha, the malaria incidence was manifold in Tileibani PHC as compared to that in Bamparada PHC. These two PHC areas typically represent most important hilly-forest and riverine-plain malaria ecotypes of rural malaria respectively. Representative villages were selected from each eco-epidemiological setting based on high and low slopes, presence and absence of forest, closeness to streams and river, and high and low malaria incidence respectively for HF and RP ecotypes. Both the ecotypes were inhabited majorly by socio-economically backward classes and the degree of prevalence of backward classes was higher in HF as it was predominated by Scheduled Tribe (ST) followed by Scheduled Caste (SC) and Other Backward Classes (OBC) whereas in RP, there was dominance of SC followed by ST and OBC. This breakup of communities is necessary to understand the practices and health seeking choice they make when suffering from malaria (Sabin et al. 2010, Singh et al. 2013, Sharma et al. 2015). Though, the healthcare services in HF were more in number and proportion in comparison to RP, the healthcare centres were distantly located and difficult to reach in HF as compared to RP due to poor roads and public transport facilities (Figures 15-17 and 20). The houses in HF ecotype were in close proximity to water bodies e.g. streams which were covered with thick vegetation providing suitable breeding sites for malaria vectors whereas in RP ecotype, the major vector breeding sites were riverbeds, water pools farther away from households (Figures 11 and 12). Regarding environmental parameters, the rainfall pattern, the number of rainy days in a month, the vapour pressure, were found to be similar in both HF and RP ecotypes but in HF the high humidity and lesser temperature extremities were observed (Figure 28) which provides conducive conditions for survival and proliferation of highly efficient sylvatic vectors like *An. fluviatilis* and *An. minimus* during all seasons (Kobayashi et al. 2000, Lindsay 2004, Obsomer et al. 2007).

The observation and interpretation of the results in this chapter reflect distinct demographic, topographic, and climatic differences in the hilly-forest and riverine-plain ecotypes. In addition, differences in the number and location of healthcare units were also found to be different in HF and RP ecotypes. The above-mentioned factors are important in shaping malaria transmission dynamics in a given ecotype (Koenraadt et al. 2004, Jawara et al. 2008, Kristan et al. 2008) (Drakeley et al. 2005). These factors not only influence the distribution and survival of the malaria vectors but also have a direct bearing on health seeking behaviour and treatment practices of the community (Bodker et al. 2003, Staedke et al. 2003, Kulkarni et al. 2006). The relevance of these parameters have been dealt and discussed in detail in subsequent chapters.

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CHAPTER II

Analyses of the vectors' prevalence, their biological attributes and transmission potential in hilly-forest and riverine-plain ecotypes

1. Introduction

Asia is second in global malaria stake holding after Africa (Brown and Rogerson 2016). Highest numbers of people are at risk of malaria in Central Asia and South-East Asia as unlike Africa, population density is much more in this region and people are susceptible to vivax malaria. Unlike Amazonia Central- South-East Asia comprises many potential vectors of malaria and homes 19 out of 41 dominant vector species of world (Sinka et al. 2012). In Asia, India contribute 75% of malaria and there are ten malaria vectors of which six are considered primary and four play secondary role in malaria transmission (Dash et al. 2008). In India, vector control by DDT was successful to curb malaria in the late 1960s (Gunasekaran et al. 2005). The success of curbing malaria was mainly due to vast reduction in *An. culicifacies* population. However, malaria control in forested and remote areas was not successfully achieved. Effect of DDT era in India was evidential in curbing *An. minimus* up to a great extent but the control of exophagic vectors like, *An. dirus/An. baimaii* with insecticides in the forests was impractical. Malaria reappeared as vector populations re-established with added challenges, like insecticide resistance, ecological succession of vectors due to deforestation and climate change.

Targeting malaria vectors with appropriate vector control tools is key to the success of malaria control. Correct identification of vectors is important prior to expense resources because misidentification might lead to waste of resources (Coluzzi 1988). For example in Vietnam, *An. varuna* that is zoophagic and inefficient vector was misidentified as *An. minimus* and drained vector control resources in vain (Sinka et al. 2011). Knowledge of bionomics of specific culprit vector species of malaria and taking advantage of their specific behaviour is a novel idea. For example; blocking streams by micro dams in hilly forested areas of Odisha successfully reduced *An. fluviatilis* breeding (Sharma et al. 2008, Sahu et al. 2014a), flushing irrigation channel in India reduced *An. culicifacies* breeding (Barik et al. 2009).

More than 80% of the India's malaria is credited to rural vectors, *An. culicifacies* (65%) and *An. fluviatilis* (15%) (Subbarao et al. 1988, Sharma and Dev 2015). More than 65% of India's malaria remained confined to remote/forest fringe/tribal belts of Eastern/ Central/ North-Eastern states (Kumar et al. 2007, Narain 2008). The most important ecological setting of malaria is forest ecotype which is often remote and inhabited by tribals. The second most important eco-epidemiological setting of rural parts of India is riverine-plain ecotype. Two malaria vectors majorly responsible for malaria in these two eco-epidemiological settings are *An. culicifacies* and *An. fluviatilis*. *Anopheles culicifacies* has wide distribution in India, prevalent throughout the plains and also in the hills (Sharma and Dev 2015). *Anopheles fluviatilis* is an important vector in hilly forested and foothill region (Dev and Sharma 2013). This vector has been recorded in plains but is not reported to play major role in malaria transmission in plains (Sinka et al. 2011). In India, both *An. culicifacies* and *An. fluviatilis* have been established as species complexes consisting of cryptic/ sibling species (WHO 2008, Sinka et al. 2011). The variation in vector forms is accompanied by differences in vectorial capacity, biting habits and differential resistance to insecticides, thus influencing vector control strategies (Massebo et al. 2013, Ngom et al. 2013) in response to malaria transmission (Subbarao and Sharma 1997, Sinka et al. 2010a). *Anopheles culicifacies* is a complex of five sibling species provisionally designated as species A, B, C, D, and E. These vary in their biological features and malaria transmission potential (Subbarao 1988, Subbarao et al. 1988, Subbarao et al. 1992, Kar et al. 1999, Dash et al. 2007, WHO 2008). Similarly the Fluviatilis Complex consists of 4 sibling species (S, T, U and V), which exhibit distinct variations in their biology and vectorial potential (Subbarao et al. 1994, Sharma et al. 1995, Nanda et al. 1996, Shukla et al. 1998, Nanda et al. 2000, Nanda et al. 2013).

An. stephensi is distributed throughout India and is primarily responsible for urban malaria. Despite its wide occurrence, it contributes only 12% of total malaria cases in India (Sharma 1999). Moreover, the members of *An. minimus* and *An. dirus* complexes play important role in malaria transmission in North-Eastern parts of India and *An. sundaicus* is the only malaria vector in Andaman and Car-Nicobar islands. There are sporadic reports of incrimination of certain *Anopheles* as malaria vectors like *An. annularis* in Eastern state (Odisha), *An. varuna*, *An. jeyporiensis, An. nivipes/ An. philippinensis* and *An. maculatus* in East and North-East states and *An. subpictus* in semi-urban localities in North-India. These vectors appear to play secondary and focal role in malaria transmission in the area of their dominance (Kumar et al. 2012).

1.1. Review of literature

1.1.1. Regional abundances of malaria vectors and their ecotypes

Malaria transmission is unique to the ecoregions of world according to the presence of vector species, and the influence of inhabitant's behaviour and environment (Rubio-Palis and Zimmerman 1997, Rosa-Freitas et al. 2007, Grillet et al. 2010). Different vector species are responsible for malaria transmission in distinct geographical regions in the world (Knight and Stone 1977, Foley et al. 2008). Particular vector responsible for malaria in mesoclimatic situation is considered as local vector and may be widely responsible for malaria situation in entire ecoregions and considered as regional vector (Rubio-Palis and Zimmerman 1997). Central Asia and South East Asian region is rich in potential vector diversity (Sinka et al. 2010b). *Anopheles culicifacies* is the major vector in rural riverine-plain areas of Middle and South East Asia and *An. stephensi* is the major malaria vector in urban ecotype (Sharma 1999, Manguin et al. 2008, Barik et al. 2009, Sharma and Dev 2015). *Anopheles dirus*, *An. maculatus, An. fluviatilis,* and *An. minimus* are the major vectors of forest ecotype of South East Asia. The coast of South East Asia surrounded by Pacific and Bay of Bengal is also malaria endemic but *An. sundaicus* is the predominant vector (Rahman et al. 1997, Tin et al. 2001, Trung et al. 2005, Manguin et al. 2008).

1.1.2. Distribution of malaria vectors in India.

India is a country with diverse ecotypes and home to 58 anopheline species (Nagpal and Sharma 1995), of which only six are designated as the primary malaria vectors namely, *An. culicifacies, An. stephensi, An. fluviatilis, An. dirus, An. minimus,* and *An. sundaicus*. Primary vectors of malaria in India, and their sphere of influence is mapped in Figure 1. Beside, some are considered secondary vectors namely, *An. philippinensis-nivipes*, *An. varuna*, *An. annularis* and *An. jeyporiensis* (Shanna 1998, Dash et al. 2008, Dua and Acharya 2012).

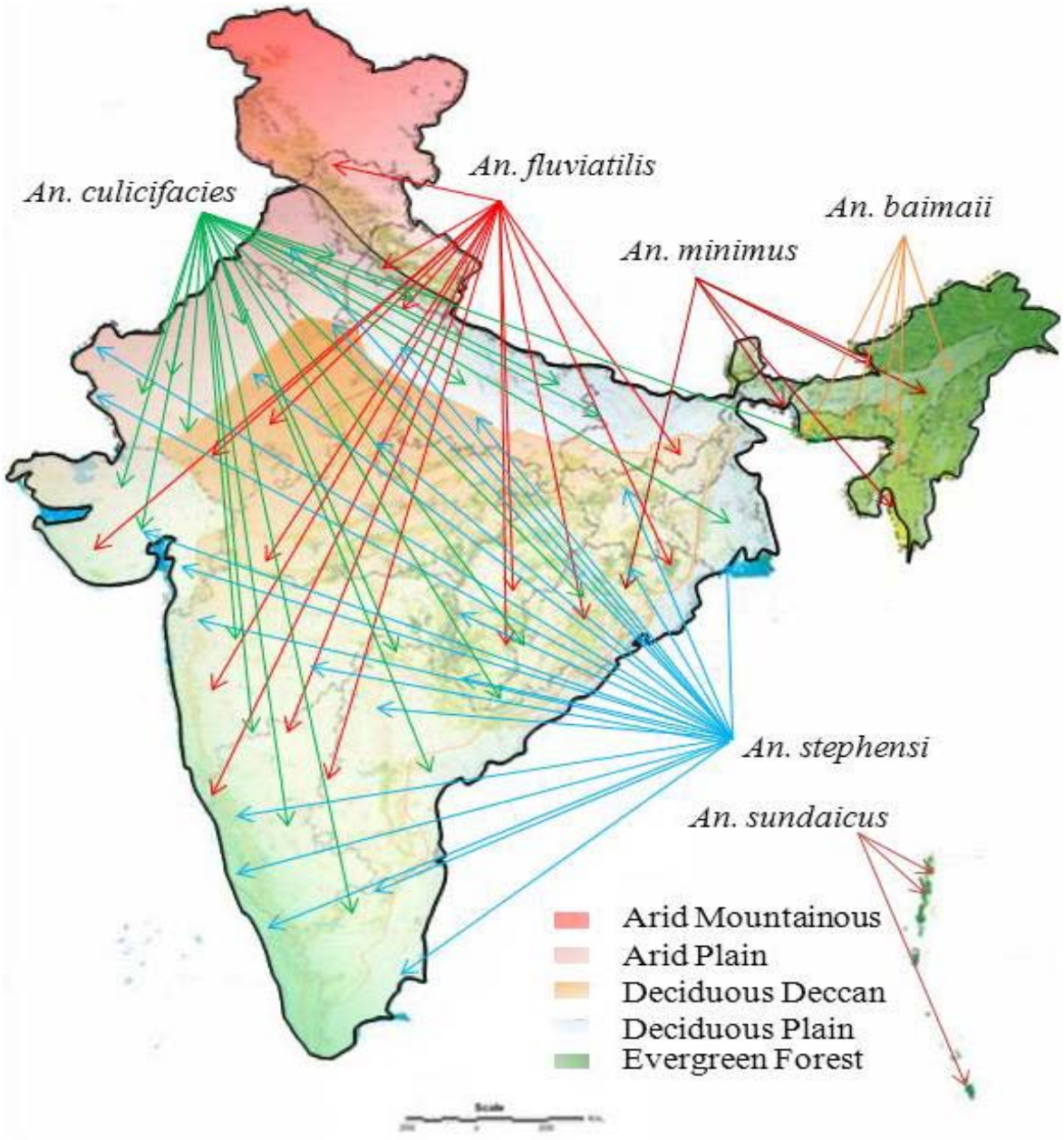


Figure 1: Distribution of primary vectors of malaria in India

1.1.3. Species complexes of malaria vectors in India

Of the six primary malaria vectors of India, five have been established as species complexes (Sarala K. Subbarao 1998, WHO 2008, Sinka et al. 2011). The *An. culicifacies* complex comprises of five members designated as species A, B (Green and Miles 1980), C (Subbarao et al. 1983), D (Vasantha et al. 1991), and E (Kar et al. 1999) and all are found in India. All the four members of the *An. fluviatilis* complex i.e. S, T, U (Subbarao et al. 1994) and V (Nanda et al. 2013) are also found in India.

Only two sibling species of *An. dirus* (*An. baimaii* formerly sp. D, *An. elegans* formerly sp. E) out of total 7 (A-F, and *An. takasagoensis*) (Sallum et al. 2005, Sinka et al. 2011)) are found in India. Out of three members (A, C and E) of the *An. minimus* complex only *An. minimus sensu stricto* (formerly species A) is prevalent in India (Harbach 2004). Apart from three members (species A, B and C) of the *An. sundaicus* complex, only a "new cytotype D" has been reported from India (Sukowati and Baimai 1996, Sukowati et al. 1999, Nanda et al. 2004, Alam et al. 2006). Apart from primary vectors, the secondary vector *An. annularis* is also a species complex consisting of two sibling species viz. A and B and both are found in India (Atrie et al. 1999). The Philippinensis-Nivipes Complex (comprising of *An. philippinensis* and *An. nivipes*) is also found in India (Ramachandra Rao 1984, Nagpal and Sharma 1987). The Subpictus Complex comprises of four sibling species, A, B, C and D and all are reported from India (Suguna et al. 1994, WHO 2008).

The sixth primary vector *An. stephensi* comprises of three ecological races based on egg-dimension and number of ridges present on the egg float and all the three forms have been reported from India i.e. '*var. mysoriensis*' Sweet & Rao (Sweet and Rao 1937, Rao et al. 1938), '*type form*' (typical) and the intermediate form (Subbarao et al. 1987, WHO 2008).

1.1.4. Distribution and bionomics of malaria vectors in Odisha state

The state of Odisha is highly malarious and *Anopheles culicifacies* and *An. fluviatilis* are the most important vectors of malaria in Odisha (Gunasekaran et al. 2014b, Sharma and Dev 2015). The distribution and relative proportion of *Anopheles culicifacies* and *An. fluviatilis* varies in different parts of the state (Swain et al. 2010) but *An. culicifacies* is the predominant species in Odisha (Tripathy et al. 2010, Sharma and Dev 2015). *Anopheles fluviatilis* is considered to be the more important vector (prime vector) in hilly forested ecotype (Nanda et al. 2000, Sharma et al. 2006, Sahu et al. 2014b, Sharma and Dev 2015). Only species S and T of the Fluviatilis Complex have been reported from Odisha.

Anopheles fluviatilis S has been reported to be predominant vector species in forested areas of Odisha (Sahu et al. 2009, Tripathy et al. 2010). *Anopheles fluviatilis* S and T are highly selective and prefer to breed in slow moving streams in hilly-forest and foothill forest fringe (Sahu et al. 1990, Nanda et al. 2000, Sharma et al. 2006). Although *An. fluviatilis* was found in plains, it was not reported to be a malaria vector in plains of Koraput district (Sahu et al. 1990, Gunasekaran 1994). *Anopheles fluviatilis* densities build up during July and August

and peak in winter (November), and then very few survive by April (Gunasekaran 1994, Tripathy et al. 2010). In dense hilly forested regions of Northern Odisha where *An. fluviatilis* sibling species S prevails, *An. fluviatilis* exhibited marked endophily, anthropophagy and endophagy (Nanda et al. 1996, Nanda et al. 2000, Gunasekaran et al. 2005, Sharma et al. 2005), but in less forested region of southern part of Odisha where *An. fluviatilis* sibling species T is significantly more than northern part, *An. fluviatilis* exhibited marked exophagy and zoophagy (Das et al. 1990, Collins et al. 1991, Parida et al. 2006). The peak biting time of *An. fluviatilis* was observed at early quarter of night in more dense forest region of Malkangiri whereas in less dense forest region of Jeypore the peak biting time was midnight (Gunasekaran et al. 1994). The four sibling species of the Fluviatilis Complex have been reported to vary in their biological characteristics and vectorial potential (Sharma et al. 1995, Nanda et al. 1996, Shukla et al. 1998, S.K. Subbarao 1998, Nanda et al. 2000). Species S has been primarily found resting indoors in human dwellings, highly anthropophagic with high sporozoite rate of malaria vectors in hilly forested areas of Odisha (Nanda et al. 1996, S.K. Subbarao 1998, Nanda et al. 2000, Sharma et al. 2006). The anthropophagic index of species S was found to be ranging from 60-98 according to different localities of Odisha (Parida et al. 2006, Mohanty et al. 2007, Tripathy et al. 2010). Species T is primarily zoophagic and found resting in cattle sheds. Though previously considered as poor vector of malaria (Sharma et al. 1995, Nanda et al. 1996, Shukla et al. 1998), recently this species has been incriminated in parts of central India (Singh et al. 2013, Singh et al. 2015).

Indoor Residual Spray (IRS) with DDT is still being used in vector control program by NVBDCP in Odisha, besides Long Lasting Insecticidal Nets (LLINs) have been distributed and in some regions synthetic pyrethroids are being used in IRS (Dash et al. 2012, Dash and Sahu 2014, Sahu et al. 2014b). *An. fluviatilis* was found susceptible to DDT, malathion and deltamethrin in most of the southern districts (Sharma et al. 2004, Dash and Sahu 2014, Sahu et al. 2014b) but it had started developing resistance in northern district Mayurbhanj, where 95 and 87.5% corrected mortality (CM) was observed against DDT and malathion respectively (Sharma et al. 2004). The delayed knock-down effect of deltamethrin in *An. fluviatilis* in many districts indicated emerging resistance in Odisha (Sharma et al. 2004).

Anopheles culicifacies has been considered as the principle malaria vector in riverine-plain ecotype and deforested habitat (Nagpal and Sharma 1986, Nanda et al. 2000, Kumari et al. 2009, Sharma and Dev 2015). In a recent study *An. culicifacies* sibling species E was also

found along with other sibling species in Odisha and the sequence of abundance from high to low was B, C, E, D and A (Tripathy et al. 2010, Das et al. 2013). In a study carried out in different districts of Odisha reports that *An. culicifacies* A is better adapted to dry climate, direct and indirect sunlit hotter and sedimented clear fresh water bodies including wells for breeding. *Anopheles culicifacies* D prefers fresh direct or indirect sunlit rainwater collected pools. *Anopheles culicifacies* C breeds mainly in hilly forested perennial slow moving or stagnant water bodies with vegetation (Sahu et al. 1990, Tripathy et al. 2010, Das et al. 2013). *An. culicifacies* B is better adapted to downstream riverine backwater and flooded turbid water open pools in the plains for breeding. *Anopheles culicifacies* E prefers to breed in sun lit hot riverine backwater bodies, pools and adapted for breeding in rice fields and man-made aquifers and in brackish water to some extent (Das et al. 2013). In northern Odisha, *An. culicifacies* densities increase in March, ceases in April and peak in July (Chand et al. 1993). In other parts of Odisha *An. culicifacies* density increases through July and August and is maintained up to November then it declines by April (Das et al. 1990, Tripathy et al. 2010). In Odisha, the peak biting time of *An. culicifacies* was observed in early quarter of night in more dense forest region of Malkangiri whereas in less dense forest region of Jeypore the maximum biting time was during midnight (Gunasekaran et al. 1994). The five sibling species of Culicifacies Complex have been reported to vary in their biological characteristics and malaria transmission potential (Subbarao et al. 1988, Subbarao et al. 1992, Kar et al. 1999, Dash et al. 2007). In Odisha the *An. culicifacies* E was reported to be the highly efficient vector with highest anthropophilic index, resting mainly in human dwellings and was incriminated for both *Plasmodium falciparum* and *Plasmodium vivax* sporozoites thus proving it to be the most efficient vector among the members of Culicifacies Complex (Das et al. 2013). *Anopheles culicifacies* A, B, C and D are largely zoophilic, and rest in human dwellings and cattle sheds. The reported anthropophagic indices were, 5 for species A and 0.5-2 for species B, C and D (Parida et al. 2006, Tripathy et al. 2010, Das et al. 2013), beside prevailing species E the sporozoite rate is maximum in D followed by A and C (Das et al. 2013).

The reports revealed that in Odisha *An. culicifacies* is resistant to DDT in almost all the studied districts, and has developed resistance to malathion and deltamethrin in many of the malarious districts. Since first report of DDT resistance in *An. culicifacies* in Odisha (Das 1966), there has been gradual spread of resistance against DDT (Sahu et al. 1990, Chand and Yadav 1991) and for both DDT and HCH (Sahu and Patra 1995) and now resistance is being reported in both *An. culicifacies* and *An. fluviatilis* against DDT, malathion and synthetic

pyrethroids such as deltamethrin and from many parts of Odisha (Sharma et al. 2004, Gunasekaran et al. 2014b, Raghavendra et al. 2014, Sahu et al. 2014b, Singh et al. 2014a). According to recent report from 10 southern malarious districts of Odisha corrected mortality (CM) for *An. culicifacies* against DDT ranged from 10-17%, malathion (63-87%) and deltamethrin (82-100%) (Sahu et al. 2014b).

Anopheles minimus was recently reported in Keonjhar district of Odisha after 45 years of its disappearance since DDT era (Jambulingam et al. 2005), and incriminated as a malaria vector (Sahu et al. 2008). It was found to be *An. minimus* A (now known as *An. minimus sensu stricto*) (Sahu et al. 2009). This species appeared to share same ecological niches and behaviour of *An. fluviatilis* S (majorly resting in human dwellings and highly anthropophagic) (Sahu et al. 2009). Insecticide resistance status against DDT was shown to be under verification required category (96.2%) as per new guideline of WHO (Dash et al. 2012). Reports are now claiming this species to be one of the prime vectors of malaria and warranting consideration of this species in vector control intervention in this ecoregion (Gunasekaran et al. 2014a).

Anopheles annularis is a secondary malaria vector comprising of two sibling species (A and B) (Atrie et al. 1999, Alam et al. 2007). Only species A is reported from Odisha and incriminated (Sinka et al. 2011) while species B is a non-vector (WHO 2008).

Anopheles subpictus comprises of 4 sibling species of which species B is largely anthropophagic (Sinka et al. 2011, Singh et al. 2014b). *Anopheles subpictus* was also incriminated in Malkangiri district of Odisha (Sahu 1998). However, the sporozoite rate in this species was found to be less than that in *An. culicifacies*, but it was found to be more anthropophagic than *An. culicifacies* in district Angul (Kumari et al. 2009). *An. subpictus* has been reported abundantly in highly malarious Keonjhar district (Sahu et al. 2009).

Apart from above mentioned anopheline species, *An. aconitus, An. varuna, An. philippinensis,* and *An. pallidus* are widespread in Odisha (Swain et al. 2010).

2. Methodology

2.1. Study area, duration, mosquito collection and processing

2.1.1. Study area and study period

Highly endemic hilly-forest villages under Tileibani PHC (Tileibani block) and moderately endemic riverine-plain villages under Bamparada PHC (Barkot block) were selected in district Deogarh, Odisha. The geography and environment of the selected areas have been elaborated in Chapter 1. Surveys were carried out during March 2011 (the pre monsoon period), September 2011 (the post monsoon period), November 2011 (the winter period), and July 2012 (the monsoon period) from both the ecotypes.

2.1.2. Description of study sites

Mosquito collections were done from 9 and 8 selected villages in HF and RP ecotype respectively. Most of the houses in study villages were having mud-plastered-walls and tiled roofs in both the ecotypes (Figures 2 and 3) and only a few houses were having stem-wall without plaster and wide openings (Figure 4). Mean man: cattle ratio was 1:1 in HF and 2: 1 in PR. There were lesser number of separate cattle sheds and the cattle are usually kept under a single roof near human dwelling separated by incomplete or no wall (Figures 2 and 3). These were categorized as “mixed dwellings (MD)”. Hence, households with no cattle were considered as “human dwellings” (HD) (Figure 4). In few household cattle were kept separately under independent roof and were considered as the cattle shed (CS). There were more number of mixed dwellings in both the ecotypes. Villages were situated near streams or river that act as source of anopheline breeding in HF and RP as described in Chapter I. In HF most of the households were found within the range of 20 m to 1 km distance from perennial streams (Figure 5) and in RP most of the households were found within the range of 100m to 2 km distance from the river (Figures 7). Apart from streams and river bed pools which serve as major breeding sites for malaria vectors, the paddy-fields/ cultivation lands were also observed breeding anophelines within a range of 20 m to 2 km from households in HF ecotype whereas paddy-fields/ cultivation lands and irrigation canals were found breeding anophelines within a range of 100 m to 2 km in RP ecotype (Figures 6 and 8).

Both HF and RP population were socio-economically backward though the magnitude of remoteness and backward ness was relatively higher in HF than RP. HF was sparsely populated with predominance of backward tribes ST followed by SC, OBC and few general caste people. RP was relatively more populous than HF and had predominance of SC followed by ST, OBC and few general caste people. Detailed demography, malaria parasite prevalence, and socioeconomic status of inhabitants in selected study sites have been described in Chapter I, III and IV respectively.

Figure 2: Household having mud plastered walls and tiled roof with adjacent cattle shed in HF ecotype

Figure 3: Typical household with mud-plastered-wall with tiled roof and a nearby cattle shed in RP ecotype

Figure 4: Typical non-plastered house with thatched roof in HF

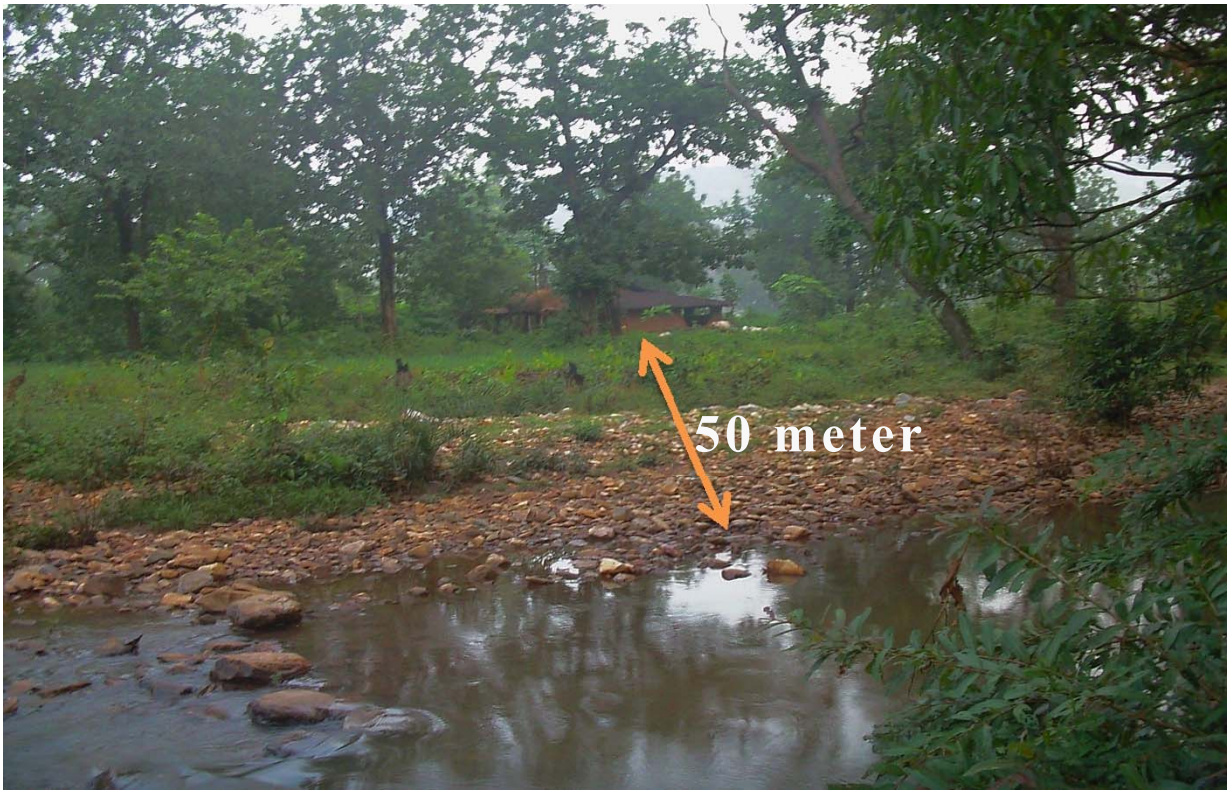


Figure 5: Stream near HF households breeding *An. fluviatilis*

Figure 6: Paddy fields breeding anophelines near HF households

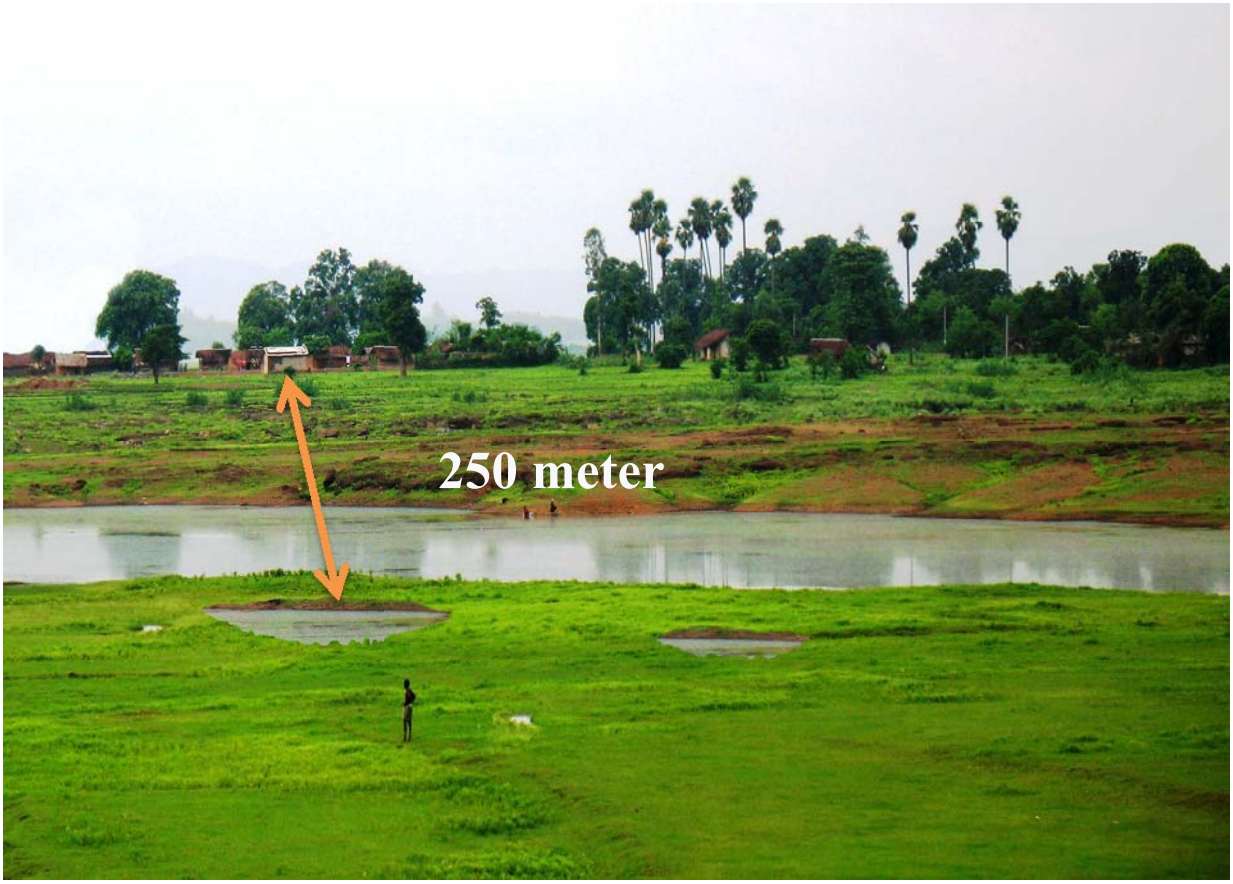


Figure 7: River and riverbed pools near RP households, the preferred breeding sites for *An. culicifacies*

Figure 8: Canal and paddy fields near RP households breeding anophelines

2.1.3. Collection of relevant data from district health department

During the field visits the village wise malaria intervention data pertaining to distribution of long lasting insecticide nets (LLINs), and Indoor Residual Spray (IRS) were collected from Chief District Medical Officer (CDMO) (NVBDCP consultant reports) through proper channel. The schedule of 1st round of DDT spray was reported to be during June-July in HF and RP and second round of DDT spray was restricted to HF and during November-December. Malaria transmission was perennial in both HF and RP and the intensity was much higher (hyper-endemic) in former ecotype. The malaria transmission peaks after monsoon in HF whereas in RP it peaks during monsoon. The detailed ecotype wise malaria transmission pattern has been presented in Chapter I.

2.1.4. Mosquito collection

2.1.4.1. Indoor resting mosquito collection by hand catch method

A team of three trained insect collectors collected indoor resting anophelines from 10-12 randomly selected households per village between 5.00AM to 8.00 AM following standard procedure (Figure 9) (WHO 1975). Collections were made from HD/ MD/ CS and mosquitoes collected from different types of dwellings were kept in separate cages.

Figure 9: Indoor resting mosquito collection by hand catch method

2.1.4.2. Outdoor mosquito collection by CDC light trap

CDC light trap collections were also attempted outdoors from dusk to dawn. CDC light traps were placed around 50 m away from human habitation. Light traps were emptied in the morning and the anophelines were separated out.

2.1.4.3. Morphological identification of anophelines

The anophelines collected were identified morphologically to species following standard keys (Christopher 1933, Nagpal and Sharma 1995) and man-hour densities (MHD) anopheline species collected by hand-catch method were estimated using the standard formula:

MHD = number of mosquitoes collected ÷ total collection time in minutes × 60.

2.1.5. Processing of morphologically identified vector species for identification of their sibling species, blood meal source identification and detection of *Plasmodium* sporozoites

Blood fed vectors captured alive during hand catch collection were transferred into labelled muslin cloth cages and kept under ambient conditions. The mosquitoes were provided cotton swabs soaked with 10% glucose solution and used in insecticide susceptibility test and sibling species identification. For cytotaxonomic identification of sibling species, the morphologically identified vectors were allowed to reach half-gravid stage. From the individual half-gravid female (in late III Christophers stage), ovaries were extracted and preserved in separate vials containing modified Carnoy's fixative (1:3 acetic acid: methanol).

For identification of blood meal source blood from the mid-gut of half gravid female was smeared on Whatman No.1 filter paper (Whatman International Inc., Maidstone; England). For detection of sporozoites (CS antigen) in the salivary glands, heads and thoraxes were separated, dried, and preserved in individual microfuge tubes with silica gel beads to avoid moisture. Rest of the body parts including legs were held in reserve as DNA source in individual 1.5 ml Eppendorf tubes containing isopropanol. All parts of a specimen were coded identically for correlating the results. Gravid and unfed *An. culicifacies* were discarded as cytotaxonomy with these stages is not possible and PCR based taxonomy was not done for Culicifacies Complex in this study.

For *An. fluviatilis,* both cytotaxonomy and molecular taxonomy were done. The half gravid *Anopheles fluviatilis* females were used for cytological identification. In PCR method of identification, head and thorax of gravid and unfed *An. fluviatilis* were dissected for incrimination studies and preserved as per standard protocol and rest of the body parts were preserved in individual 1.5 ml Eppendorf tubes containing isopropanol for molecular taxonomical identification of sibling species using allele specific PCR assay.

For other vectors, cytotaxonomy was used for identification of *An. annularis* sibling species and PCR assay for identification of *An. minimus* sibling species.

2.2. Sibling species identification of malaria vectors by cytological method

Preserved ovaries of vectors were processed in 50% propionic acid and stained with 2% lacto-aceto orcein according to the method of Green and Hunt (Green and Hunt 1980), for making polytene chromosome preparations. The chromosome complement of individual mosquitoes was examined under a Zeiss Axioplan universal microscope for species-specific diagnostic inversions in the polytene chromosomes for the identification of the members of Culicifacies Complex (Sarala K. Subbarao 1998), Fluviatilis Complex (Figure 11) (Subbarao et al. 1994) and Annularis Complex (Atrie et al. 1999) as shown in Figures 10-12.

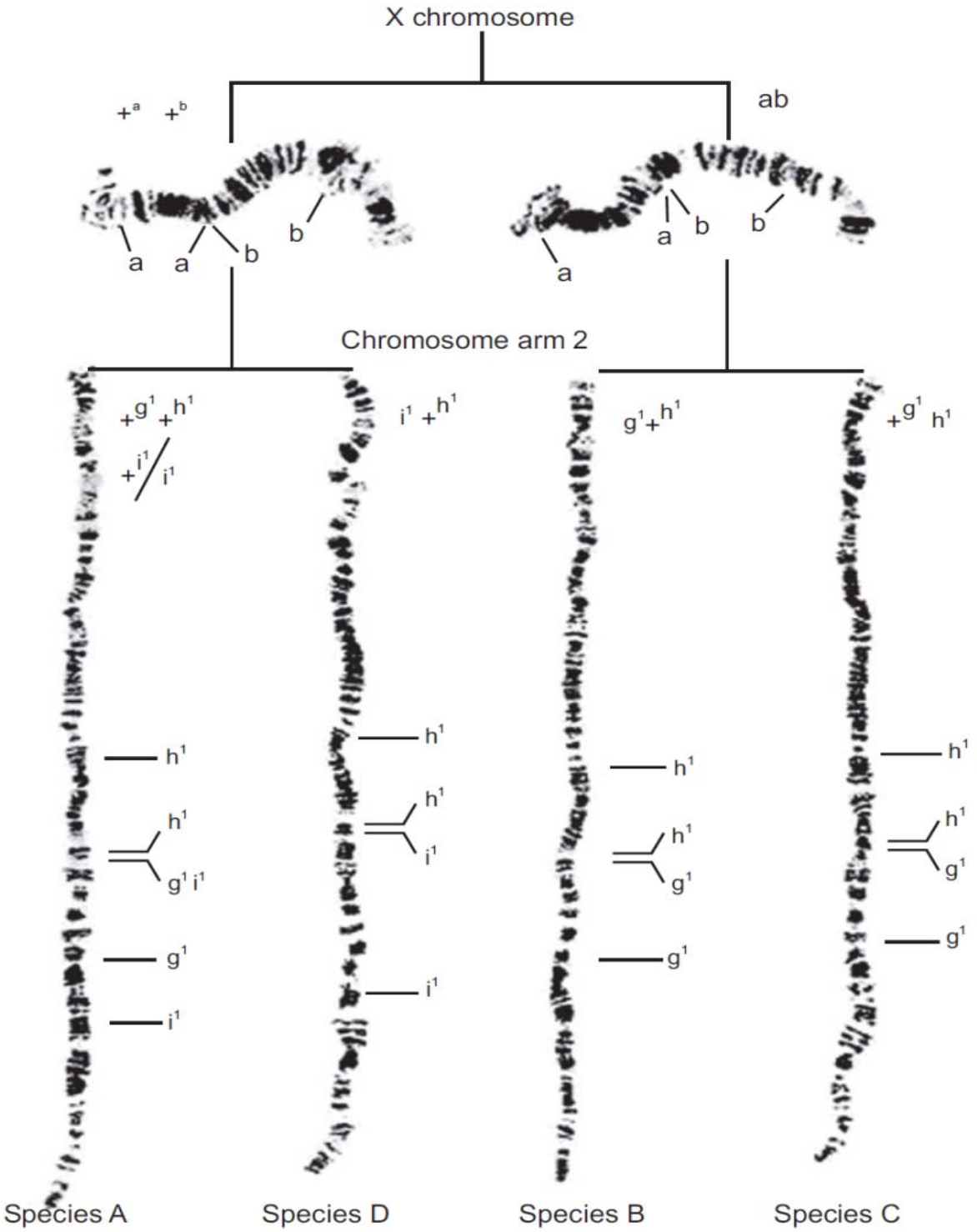


Figure 10: Schematic representation of polytene chromosomes of *Anopheles culicifacies* sibling species

source: (Sarala K. Subbarao 1998)

Figure 11: Photomap of the polytene chromosomes of *An. fluviatilis* ovarian nurse cells

Break points of paracentric inversions q1 and r1 on chromosome arm 2 are shown
Source: (Subbarao et al. 1994, WHO 2008)

Figure 12: Photomap of polytene chromosomes of *An. annularis* species A

The break points of inversions are marked with the letter designations on the right side of chromosome arms. Arrows indicate the centromeric ends of the chromosome arms
Source: (Atrie et al. 1999)

Table 1: Identification key for *An. culicifacies*, *An. fluviatilis* and *An. annularis* polytene chromosomes

Species	Sibling species	Karyotype
***Anopheles culicifacies* Complex**	**A**	**X+a+b; 2+g1+h1; +i1/i1**
	B	**Xab; 2g1+h1**
	C	**Xab; 2+g1h1**
	D	**X+a+b; 2i1+h1**
	E	**Xab; 2g1+h1**
***Anopheles fluviatilis* Complex**	**S**	**2+q1+r1+s1;3+S**
	T	**2q1+r1+s1;3+S**
	U	**2+q1r1+s1;3+S**
	V	**2+q1+r1 s1;3S**
***Anopheles annularis* Complex**	**A**	**$2+j^1$**
	B	**$2j^1$**

Source: (Atrie et al. 1999, WHO 2008, Nanda et al. 2013)

2.3. Sibling species identification of Fluviatilis complex by multiplex PCR method

Body parts of *An. fluviatilis* and *An. minimus* stored individually were processed for isolation of genomic DNA in 1.5 ml tubes following protocol of Black & Du Teau 1997 and modified as per (Coen et al. 1982, Cornel et al. 1996), finally suspended in 200µl $T_{10}E_1$ (10 mM Tris- HCl, 1mM EDTA, pH 8.0) and stored at -20°C. The DNA samples were subjected to

allele specific PCR assay using species specific primers developed from variable D3 domain of 28S rDNA for differentiation of *An. fluviatilis* sibling species (Singh et al. 2004) (Figure 13) and *An. minimus* sibling species (Phuc et al. 2003).

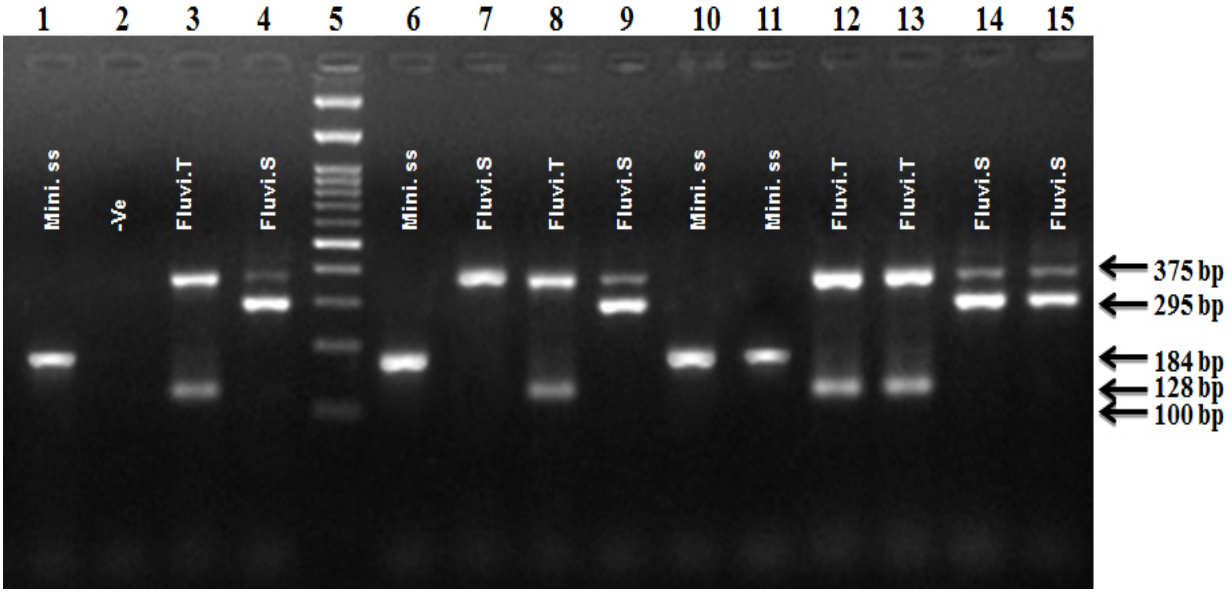


Figure 13: Agarose gel picture showing sibling species specific identification bands for the members of Fluviatilis and Minimus Complexes using multiplex PCR methods

Illustration of Polymerase Chain Reaction (PCR) assay for differentiation of members of the *Anopheles fluviatilis* and *Anopheles minimus* complexes. The PCR products were subjected to electrophoresis on a 2% agarose gel containing ethidium bromide and visualized under ultraviolet illumination. Lane 1: positive control for *Anopheles minimus* sensu stricto, lane 2: negative control without DNA, lane 3 and lane 4: positive control for species T and species S of Fluviatilis Complex, lane 5: 100-basepair (bp) ladder; lanes 6, 10 and 11: positive sample of *Anopheles minimus* sensu stricto; lane 7: unidentified (may be species U); lane 8, 12 and 13: positive sample of species T of Fluviatilis Complex; lane 9, 14 and 15: species S of Fluviatilis Complex.

2.4. Blood meal source identification of vectors using Counter Current Immuno-Electrophoresis (CCIE)

Mid-gut blood smears of *An. fluviatilis, An. culicifacies* and *An. annularis* specimens that were identified to sibling species were subjected to blood meal source identification using human and bovine anti-sera with counter current immunoelectrophoresis (Bray et al. 1984), the gold standard robust method using Barbitone buffer (Culliford 1964). Blood elutes were loaded in duplicate sets against anti-human and anti-bovine antisera (antibody) horizontally in wells cut in 0.8% mid electro endosmosis-type (Mid-EEO) Agarose. Pair of positive controls of known human and bovine blood samples were included in every plate. The loaded plate was

placed between anode and cathode orienting cationic charged blood elutes and anionic antisera on analogous sides in a submarine gel electrophoretic tank. The buffer was not filled to submerge the gel but poured up to 5/6th of both anode and cathode chamber keeping both the chambers separate. The circuit was completed with two thick filter papers soaked with the buffer keeping the gel in between so that the electric current was passed through the sandwich bridge from cathode to anode chamber. The current was passed at constant 200 volts for 20 minutes.

The gels were washed 2 times with approximately 330 ml normal saline in every 10 minutes after electrophoresis and retained in the same normal saline for another 10 minutes. After electrophoresis the precipitin bands were viewed with naked eyes and scored against an ordinary light source. The human blood index (HBI) was calculated for each of the sibling species following formula: HBI = total positive for human blood ÷ total identified

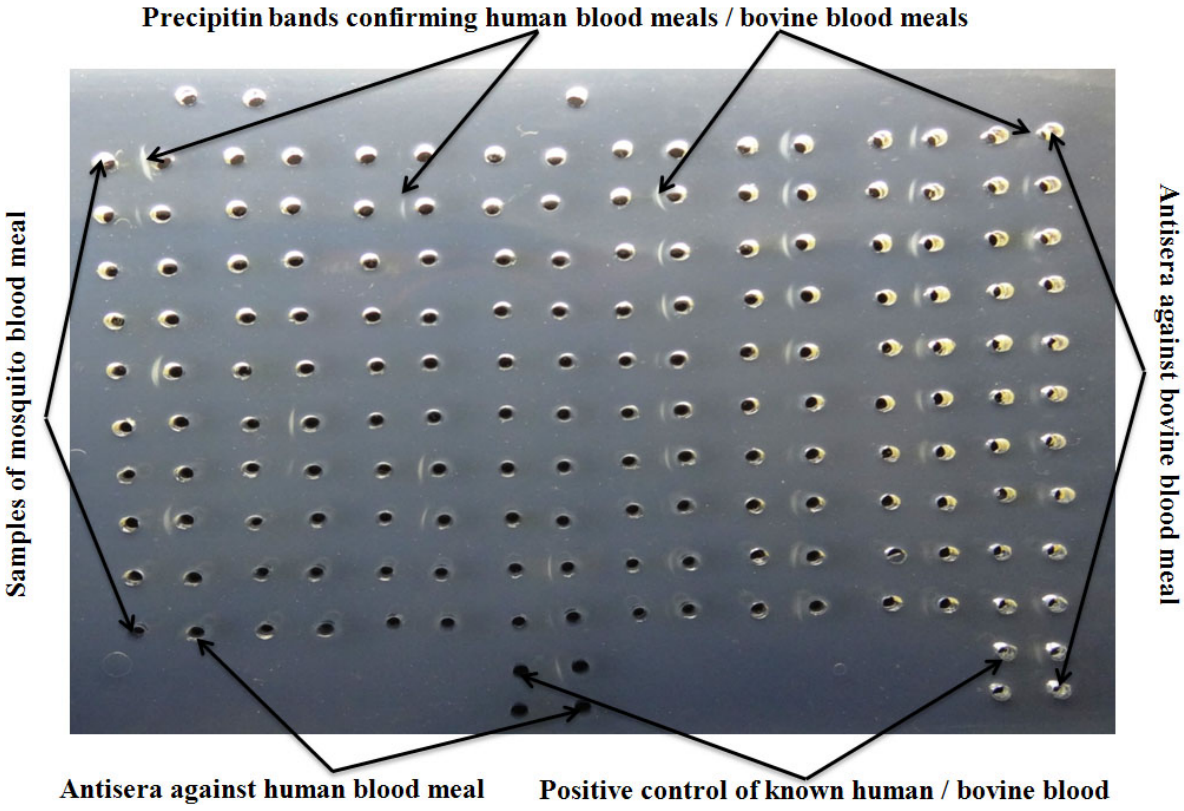


Figure 14: Illustration of precipitin bands visualization in Counter Current Immuno-Electrophoresis (CCIE)

2.5. Detection of Circumsporozoite antigen of *Plasmodium* species in vectors by Enzyme-linked Immunosorbent Assay (ELISA)

Head and thorax were dissected out from *An. fluviatilis* and *An. culicifacies* samples that were identified to sibling species by cytotaxonomy/ PCR assay. Homogenates of head and thorax of individual mosquitoes were screened for the presence of circumsporozoite antigen in the salivary glands. Presence of *Plasmodium* species was detected by set of monoclonal antibodies detecting circumsporozoite proteins of *P. falciparum*, *P. vivax* 210, and *P. vivax* 247 distinctly in ELISA (Wirtz et al. 1985, Wirtz et al. 1989).

Figure 15: One of the photographs of a micro-titre plate illustrating vector incrimination test by Enzyme-linked Immunosorbent Assay (ELISA) using *Pv* and *Pf* monoclonal antibodies against CS antigens.

A1: Positive control of *Plasmodium vivax* 210 antigen, B1-H1: negative controls, A2-H12 test samples, E11: *An. culicifacies* field collected sample positive for *Pv* 210 CS antigen.

2.6. Insecticide susceptibility tests of field collected adult malaria vectors

Susceptibility tests were performed on the wild caught blood-fed females using WHO adult mosquito resistance monitoring test kits following the enclosed instruction sheet, criteria document, insecticide impregnated papers and other test materials (WHO 1998). The field collected mosquitoes were provided with 10 % sugar water soaked in cotton pads and brought to the camp laboratory in the cubic ft. mosquito cages (Das 1966) wrapped with a wet towel.

The temperature and relative humidity in the camp laboratory were maintained at 25 ± 2 °C and 70-85 per cent, respectively.

Morphologically identified wild caught blood-fed female vector mosquitoes from individual ecotype were exposed for one hour to discriminating dosages of insecticides-DDT (4%), malathion (5%) and deltamethrin (0.05%) in 1-3 replicates, with parallel controls (Figure 16). Mortality was recorded after 24-hour recovery period in holding tube. Observed mortality was calculated as total number of dead mosquitos' ÷ total sample size × 100. The tests with >20% mortality in control were discarded. If control mortality was >5 % but <20 % then by using Abbott's formula corrected per cent mortality was calculated (Abbott 1987). According to the new WHO criteria (WHO 2013), mosquito species with a corrected mortality of >98% is considered as 'susceptible', <90% 'resistant' and Corrected Mortality between 90-97% falls under 'verification required' category.

Figure 16 : Insecticide susceptibility tests of field collected adult malaria vectors

3. Results

3.1. Seasonal abundances of malaria vectors and other anophelines in the HF and RP ecotypes

The relative proportion of indoor resting anopheline species collected from both the ecotypes i.e. hilly-forest (HF) and riverine-plain (RP) are given in Table 2. Most of the anophelines were found resting indoors and a very few anophelines were collected in CDC light traps. A total of 1347 anophelines comprising 12 species were collected from nine HF villages during the study period. Two known malaria vectors viz. *Anopheles culicifacies* and *An. fluviatilis* were mainly found during the surveys. *Anopheles culicifacies* was the predominant species accounting for 34% of total anophelines collected followed by *An. fluviatilis* 29%, *An. annularis* 12%, *An. subpictus* 8%, *An. vagus* 7.5%, and other anopheline species were found in very low number, including *An. minimus* (0.45%). From eight RP villages 1416 anophelines collected belonged to six species. *Anopheles culicifacies* was predominant 72% followed by *An. subpictus* 13%, *An. annularis* 11%, *An. vagus* 5%, *An. maculatus* 0.28% and *An. aconitus* 0.14%. *Anopheles fluviatilis* was not found in study villages of RP ecotype (Table 2). *Anopheles culicifacies* was predominant species in both HF and RP ecotypes, its average per-man hour density (MHD) was low (2 and 7) during winter (November) and high (10 and14) during monsoon (July) in HF and RP respectively. The prevalence of *An. fluviatilis* was confined to HF. The average MHD of *An. fluviatilis* was very low (0.1) during monsoon (July) and high (8.9) during pre-monsoon (March) (Table 3 and Figure 17). *Anopheles minimus* prevalence was also restricted to HF it was collected in very low numbers during pre and post monsoon surveys with and average MHD of 0.15 (Table 3). Relative proportion of *An. culicifacies* collected from cattle shed (CS) was maximum in RP followed by human dwelling (HD) and mixed dwelling of man and cattle (MD). In HF villages *An. culicifacies* was mainly collected from MD. *Anopheles fluviatilis* was mostly found in MD followed by HD (Table 4 and Table 5 a and b). Apart from major vectors, *An. annularis,* which has been reported to play a role in malaria transmission in Odisha, was found resting mainly in MD and CS in both the ecotypes with average MHD ranging from 1-4. Other anopheline species collected from study villages of both the ecotypes and their MHDs are given in Table 3.

Table 2: Relative proportion of indoor resting anopheline species collected from study villages in HF and RP ecotypes*

Anophelines	Total collected (N)		% proportion	
	HF	**RP**	**HF**	**RP**
An. culicifacies	453	1011	33.63	71.40
An. fluviatilis	391	0	29.03	0.00
An. annularis	160	155	11.88	10.95
An. subpictus	110	175	8.17	12.36
An. vagus	102	69	7.57	4.87
An. aconitus	65	2	4.83	0.14
An. jeyporiensis	20	0	1.48	0.00
An. pallidus	20	0	1.48	0.00
An. maculatus	11	4	0.82	0.28
An. varuna	7	0	0.51	0
An. minimus	6	0	0.45	0.00
An. splendidus	2	0	0.15	0.00
Total	**1347**	**1416**	**100**	**100**

*Pooled data of study villages

Table 3: Average per man hour density (MHD) of anophelines in HF and RP ecotypes in different seasons*

Anophelines	**March (Pre-Monsoon)**		**July (Monsoon)**		**September (Post-Monsoon)**		**November (Winter)**	
	HF	**RP**	**HF**	**RP**	**HF**	**RP**	**HF**	**RP**
An. Fluviatilis	8.8	0	0.1	0	3.8	0	3.5	0
An. Culicifacies	4.1	11	9.9	14.1	2.8	10.4	2.1	6.7
An. Annularis	1.5	1.5	1	1	3.5	3	2	2.25
An. Subpictus	1	2.5	2.5	3.5	1.25	1.75	0.75	1
An. Vagus	0.5	0.2	2.5	2	1.1	0.75	1	0.5
An. aconitus	0.75	0	0.5	0	1	0.1	1	0
An. jeyporiensis	0.25	0	0	0	0.25	0	0.5	0
An. maculatus	0	0.1	0	0	0.55	0.1	0	0
An. pallidus	0	0	0.5	0	0	0	0.5	0
An. minimus	0.15	0	0	0	0.15	0	0	0
An. splendidus	0	0	0	0	0	0	0.1	0
An. varuna	0	0	0	0	0.1	0	0.25	0
Total	**17.05**	**15.3**	**17**	**20.6**	**14.5**	**16.1**	**11.7**	**10.45**

*Pooled data of study villages

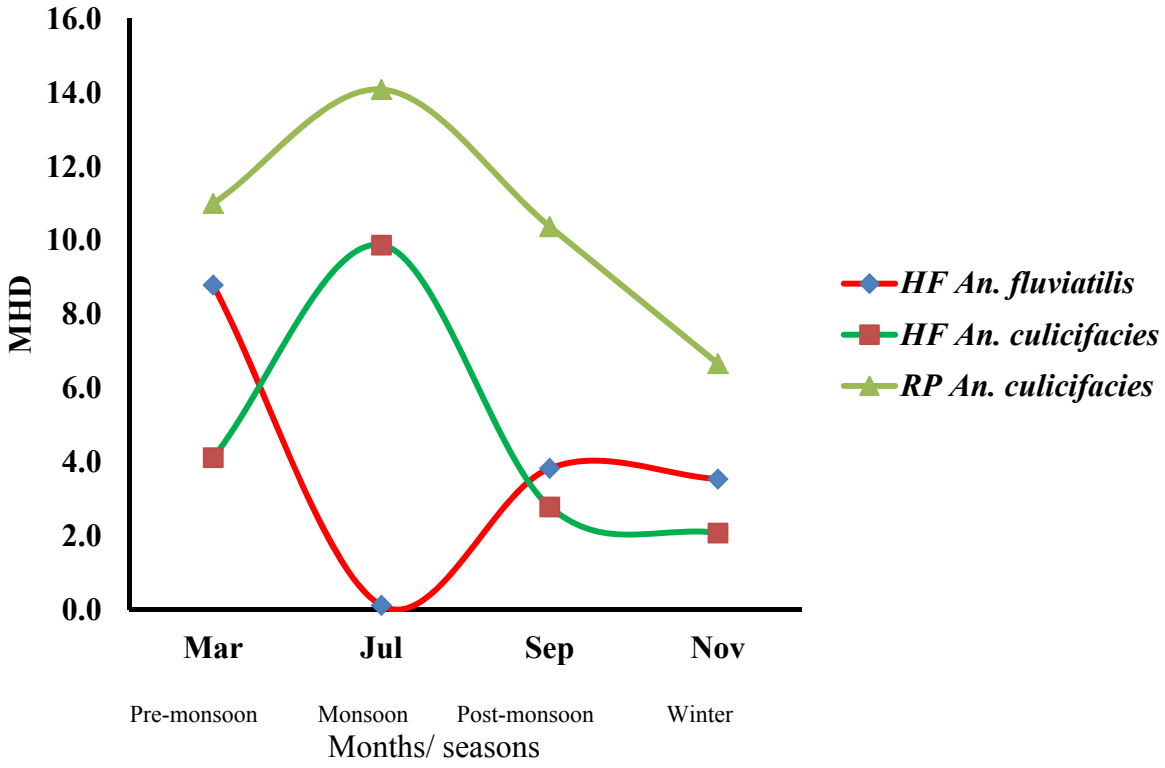


Figure 17: Average per man hour density of *An. fluviatilis* and *An. culicifacies* in HF and RP ecotypes in different seasons

Table 4: Dwelling-wise total number and the average per man hour density (MHD) of primary vectors in HF and RP ecotypes in different seasons*

Season	Vector species	Dwelling	Total number		MHD	
			HF	RP	HF	RP
Pre-Monsoon (March)	*An. fluviatilis sl.*	HD	34	0	1.4	0.0
		MD	177	0	7.4	0.0
	An. culicifacies sl.	HD	0	76	0.0	3.2
		MD	99	48	4.1	2.0
		CS	0	140	0.0	5.8
	An. minimus sl.	MD	3	0	0.1	0.0
Monsoon (July)	*An. fluviatilis sl.*	HD	1	0	0.0	0.0
		MD	2	0	0.1	0.0
	An. culicifacies sl.	HD	12	26	0.5	1.1
		MD	225	306	9.4	12.8
		CS	0	6	0.0	0.3
Post-Monsoon (September)	*An. fluviatilis sl.*	HD	58	0	2.4	0.0
		MD	30	0	1.3	0.0
		CS	4	0	0.2	0.0

	An. culicifacies sl.	HD	20	14	0.8	0.6
		MD	6	207	0.3	8.6
		CS	41	28	1.7	1.2
	An. minimus sl.	HD	3	0	0.1	0.0
Winter	*An. fluviatilis sl.*	HD	15	0	0.6	0.0
(November)		MD	70	0	2.9	0.0
	An. culicifacies sl.	HD	0	100	0.0	4.2
		MD	50	60	2.1	2.5

*Pooled data of study villages

3.2. Resting behavior of malaria vectors

The relative proportion of primary malaria vectors collected in different types of dwellings HD, MD and CS and during different seasons is shown in Table 5a and b. In HF ecotype, of the total 453 *An. culicifacies* collected, 7%, 84% and 9% were collected from HD, MD and CS respectively and of the 391 *An. fluviatilis* collected 28%, 71% and 1% were found resting in HD, MD and CS respectively during different seasons. In RP ecotype, of the total 1011 *An. culicifacies* collected, 21%, 61% and 17% were collected from HD, MD and CS respectively (Figure 18).

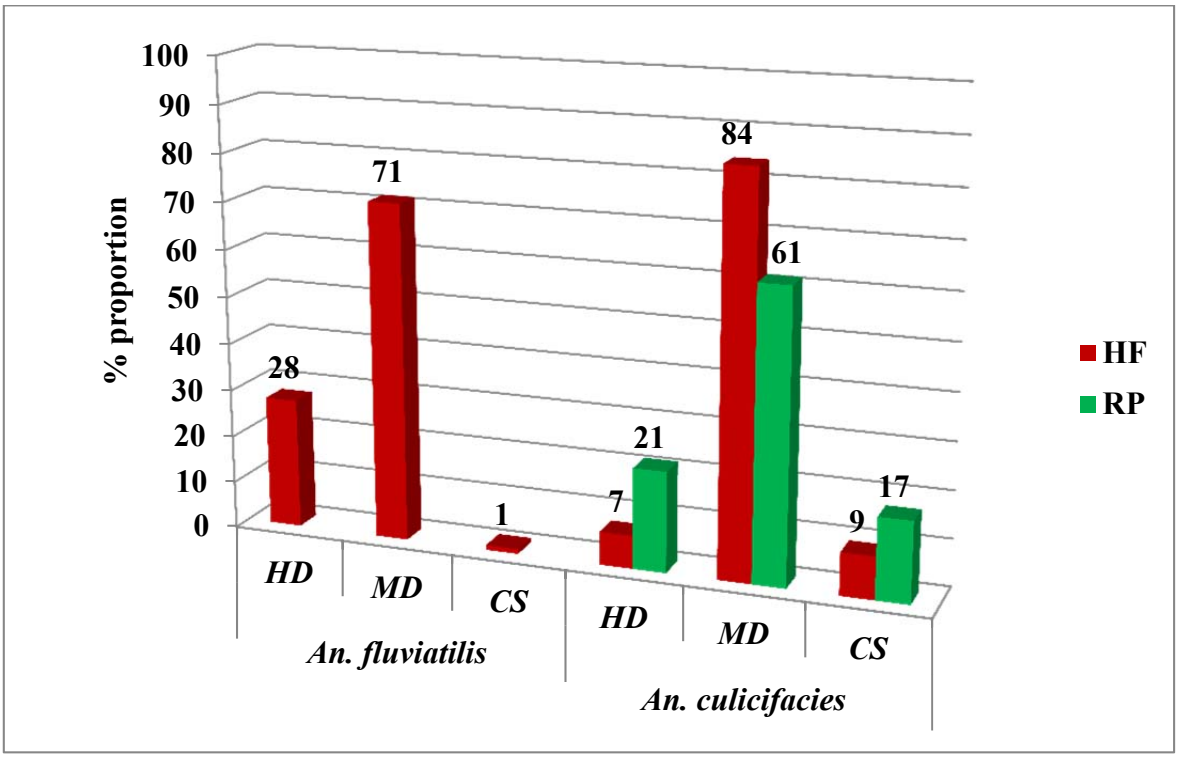
Table 5: Relative proportion of *An. fluviatilis* and *An. culicifacies* collected from different types of dwellings in HF and RP ecotypes in different seasons

a) Hilly-forest ecotype

Seasons	***An. fluviatilis***				***An. culicifacies***			
	Total	**% proportion**			**Total**	**% proportion**		
	(N)	**HD**	**MD**	**CS**	**(N)**	**HD**	**MD**	**CS**
March (Pre monsoon)	211	16	84	0	99	0	100	0
July (Monsoon)	3	33	67	0	237	5	95	0
September (Post monsoon)	92	63	33	4	67	30	9	61
November (Winter)	85	18	82	0	50	0	100	0
Total	**391**	28	71	1	**453**	7	84	9

b) Riverine-plain ecotype

Seasons	*An. fluviatilis*	*An. culicifacies*			
	Not found	Total (N)	% proportion		
			HD	MD	CS
March (Pre monsoon)		264	29	18	53
July (Monsoon)		338	8	91	2
September (Post monsoon)		249	6	83	11
November (Winter)		160	63	38	0
Total		**1011**	21	61	17



HD: Human dwelling, MD: Mixed dwelling of human and cattle, CS: Cattle shed

Figure 18: Relative proportion of primary malaria vectors collected from different types of dwellings in HF and RP ecotypes (pooled data of study villages for different seasons)

3.3. Distribution of the members of Culicifacies and Fluviatilis complexes in HF and RP ecotypes

The sibling species composition of the Fluviatilis and Culicifacies Complexes in the study areas is presented in Table 6. *Anopheles fluviatilis* species S and T were prevalent in HF and species U and V were not found. Only species B and C of *An. culicifacies* were prevalent in HF and RP as revealed by cytotaxonomy. Of the total number of *An. fluviatilis* collected from different dwellings and identified to sibling species, the relative proportion of species S and T was 43.7%: 56.3% respectively. In case of *An. culicifacies* the relative proportion of species B and C was 71.9%: 28.1% in HF and 78.9%: 21.1% in RP (Table 6). In each ecotype, only one inversion heterozygote between species B and C was observed. The relative proportions of the members of Culicifacies and Fluviatilis Complexes during different seasons are given in Table 6 and graphically depicted in Figure 19. The cytological examination of *Anopheles annularis* collected from HF and RP ecotypes (N=126) revealed prevalence of only species A of the Annularis Complex.

Table 6: Relative proportion of *An. fluviatilis* and *An. culicifacies* sibling species in HF and RP ecotypes during different seasons

Seasons	Hilly-forest				Riverine-plain	
	An. fluviatilis		*An. culicifacies*		*An. culicifacies*	
	Sibling species	**N (% proportion)**	**Sibling species**	**N (% proportion)**	**Sibling species**	**N (% proportion)**
March (Pre-Monsoon)	S	72 (48.0)	B	33 (57.9)	B	17 (65.4)
	T	78 (52.0)	C	24 (42.1)	C	9 (34.6)
July (Monsoon)	S	1 (33.3)	B	41 (80.4)	B	53 (86.9)
	T	2 (66.7)	C	10 (19.6)	C	8 (13.1)
September (Post-Monsoon)	S	41 (61.2)	B	38 (90.5)	B	49 (89.1)
	T	26 (38.8)	C	4 (9.5)	C	6 (10.9)
November (Winter)	S	11 (16.7)	B	16 (57.1)	B	34 (65.4)
	T	55 (83.3)	C	12 (42.9)	C	18 (34.6)
Total	**S**	**125 (43.7)**	**B**	**128 (71.9)**	**B**	**153 (78.9)**
	T	**161 (56.3)**	**C**	**50 (28.1)**	**C**	**41 (21.1)**

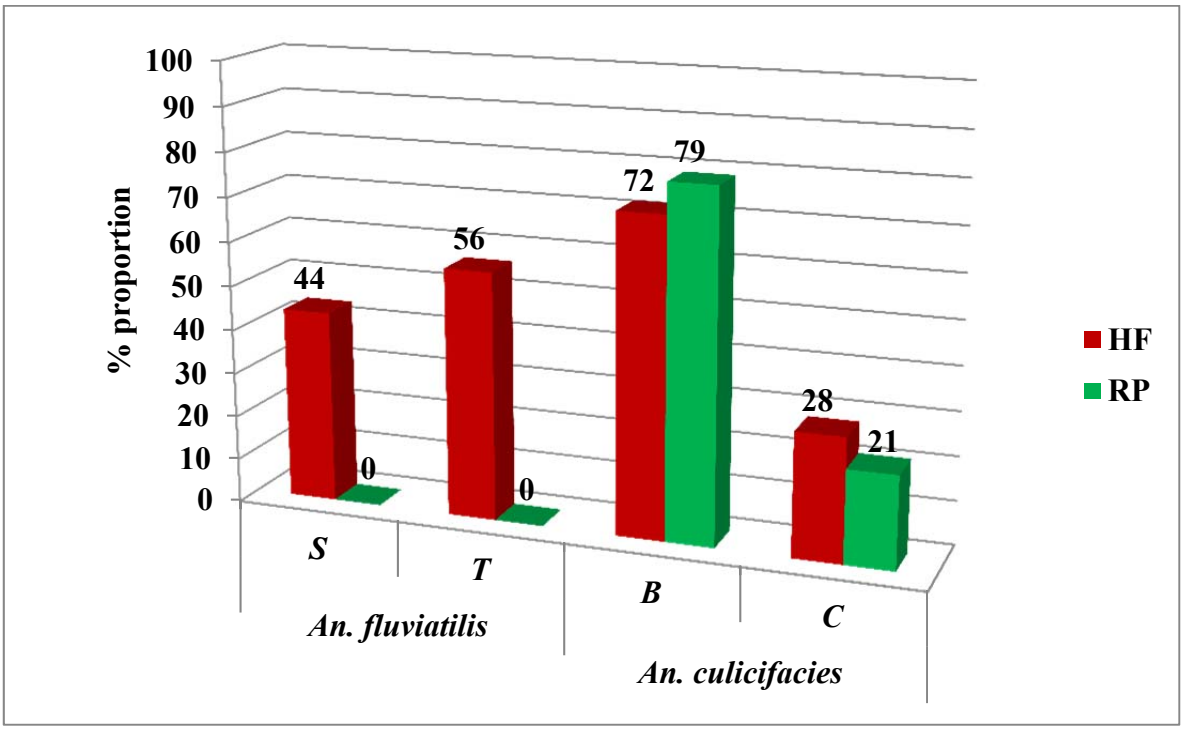


Figure 19: Relative proportion of *An. fluviatilis* and *An. culicifacies* sibling species in HF and RP ecotypes (pooled data for different seasons)

3.4. Resting behavior of the *An. fluviatilis* and *An. culicifacies* sibling species in different months/ seasons in HF and RP ecotypes

In HF ecotype, of the total 125 *An. fluviatilis* species S identified, 47.2%, 52.8% and 0% were collected from HD, MD and CS respectively and of the 161 *An. fluviatilis* species T identified 16.8%, 80.7% and 2.5% were found resting in HD, MD and CS respectively. Of the total 128 *An. culicifacies* species B identified in HF ecotype, 20.3%, 66.4% and 13.3% were collected from HD, MD and CS respectively, and of the total 50 *An. culicifacies* species C identified, 2%, 94% and 4% were collected from HD, MD and CS respectively. In RP ecotype, of the total 153 *An. culicifacies* species B identified, 23.5%, 58.2% and 18.3% were collected from HD, MD and CS respectively, and of the total 41 *An. culicifacies* species C identified, 43.9%, 48.8% and 7.3% were collected from HD, MD and CS respectively. The relative proportions of the members of Culicifacies and Fluviatilis Complexes collected from different dwellings during different seasons are given in Table 7 (a) and (b) and graphically depicted in Figure 20.

Table 7: Relative proportion of *An. fluviatilis* and *An. culicifacies* sibling species collected from different types of dwellings in HF and RP ecotypes in different seasons.

a) Hilly-forest

	An. fluviatilis sibling species				*An. culicifacies* sibling species			
	Numbers	% proportion			Numbers	% proportion		
		HD	MD	CS		HD	MD	CS
March (Pre-monsoon)	S (72)	30.6	69.4	0	B (33)	0	100.0	0
	T (78)	5.1	94.9	0	C (24)	0	100.0	0
July (Monsoon)	S (1)	.0	100.0	0	B (41)	24.4	75.6	0
	T (2)	50.0	50.0	0	C (10)	0	100.0	0
September (Post-Monsoon)	S (41)	78.0	22.0	0	B (38)	42.1	13.2	44.7
	T (26)	42.3	42.3	15.4	C (4)	25.0	25.0	50.0
November (Winter)	S (11)	45.5	54.5	0	B (16)	0	100.0	0
	T (55)	20.0	80.0	0	C (12)	0	100.0	0
Total	**S (125)**	**47.2**	**52.8**	**0**	**B (128)**	**20.3**	**66.4**	**13.3**
	T (161)	**16.8**	**80.7**	**2.5**	**C (50)**	**2.0**	**94.0**	**4.0**

b) Riverine-plain

Season	*An. fluviatilis*	*An. culicifacies*			
	Not found	Numbers	% proportion		
			HD	MD	CS
March (Pre-monsoon)		B (17)	23.5	76.5	.0
		C (9)	66.7	33.3	.0
July (Monsoon)		B (53)	26.4	62.3	11.3
		C (8)	.0	100.0	.0
September (Post-Monsoon)		B (49)	20.4	34.7	44.9
		C (6)	.0	50.0	50.0
November (Winter)		B (34)	23.5	76.5	.0
		C (18)	66.7	33.3	.0
Total		**B (153)**	**23.5**	**58.2**	**18.3**
		C (41)	**43.9**	**48.8**	**7.3**

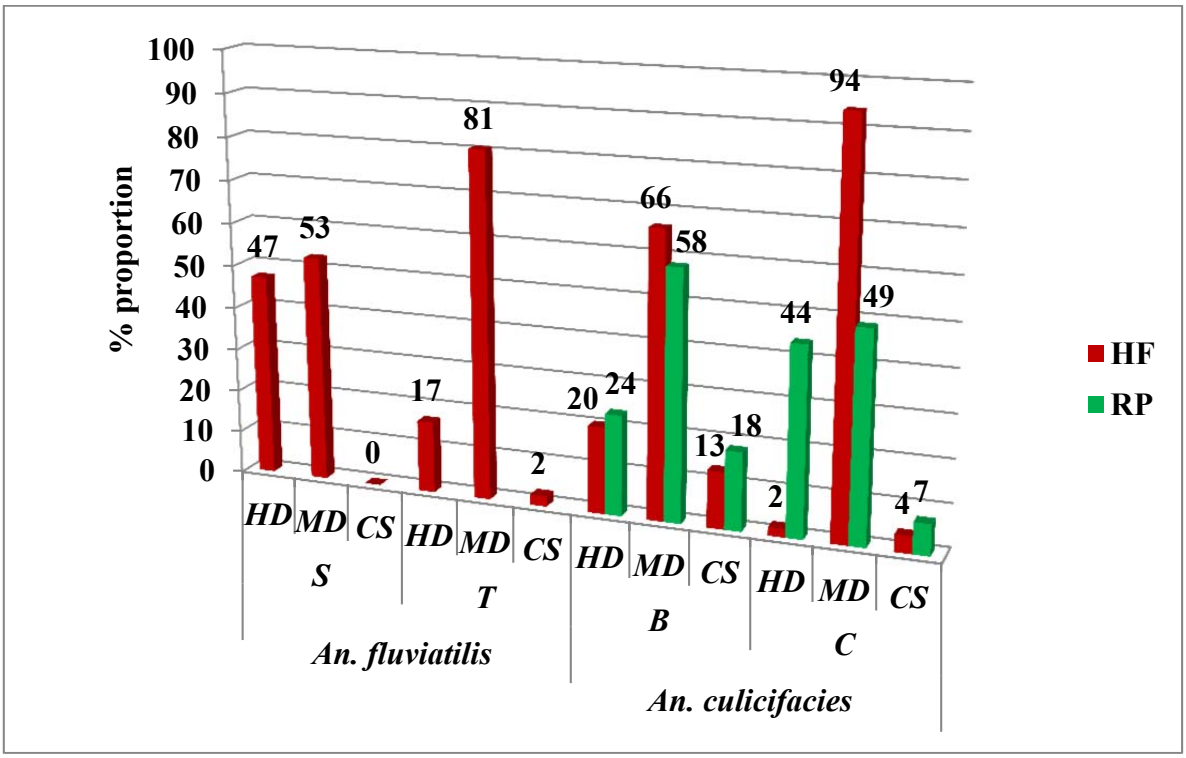


Figure 20: Relative proportion of sibling species of *An. fluviatilis* and *An. culicifacies* collected from different types of dwellings in HF and RP ecotypes.

3.5. Host preference of *Anopheles fluviatilis* and *An. culicifacies* sibling species

The results of the blood meal source identification of sibling species of *An. fluviatilis* and *An. culicifacies* are shown in Table 8. In HF, species S of the Fluviatilis Complex was found to be predominantly anthropophagic with human blood index (HBI) of 0.73. In contrast, species T was found to be primarily zoophagic as 95% of the specimens were found to have fed on bovine blood, feeding occasionally on humans (HBI=0.05). The relative proportion of feeding on humans was low in both species B and C of *An. culicifacies* in both the ecotypes and the HBI was 0.1 and 0.03 for species B and 0.12 and 0.15 for species C in HF and RP in that order (Figure 21). Host feeding pattern of the members of Culicifacies and Fluviatilis Complexes collected during different seasons is shown in Table 8 and graphically depicted in Figure 21. *Anopheles annularis* species A was exclusively zoophagic in both the ecotypes as none of the *An. annularis* tested (N=126) was found to have fed on human blood.

Table 8: Host preference of sibling species of *An. fluviatilis* and *An. culicifacies* collected in HF and RP ecotypes during different seasons

Season	Sibling species	Total tested	Human blood (H+)	Bovine blood (B+)	Mix blood (H+B+)	Non-reactive to H and B	Human blood index
			Hilly-forest				
An. fluviatilis							
March	S	72	50	21	0	1	0.69
(Pre-monsoon)	T	78	2	76	0	0	0.03
July	S	1	1	0	0	0	1.00
(monsoon)	T	2	0	2	0	0	0.00
Sep	S	41	33	8	0	0	0.80
(Post-monsoon)	T	26	3	23	0	0	0.12
Nov	S	11	4	3	3	1	0.64
(winter)	T	55	3	45	0	7	0.05
Total	**S**	**125**	**88**	**32**	**3**	**2**	**0.73**
	T	**161**	**8**	**146**	**0**	**7**	**0.05**
An. culicifacies							
March	B	33	0	33	0	0	0.00
(Pre-monsoon)	C	24	1	23	0	0	0.04
July	B	41	5	31	5	0	0.24
(monsoon)	C	10	0	9	1	0	0.10
Sep	B	38	0	36	2	0	0.05
(Post-monsoon)	C	4	0	3	1	0	0.25
Nov	B	16	1	15	0	0	0.06
(winter)	C	12	3	9	0	0	0.25
Tot	**B**	**128**	**6**	**115**	**7**	**0**	**0.10**
	C	**50**	**4**	**44**	**2**	**0**	**0.12**
			Riverine-plain				
An. culicifacies							
March	B	17	1	16	0	0	0.06
(Pre-monsoon)	C	9	0	9	0	0	0.00
July	B	53	0	52	0	1	0.00
(monsoon)	C	8	1	7	0	0	0.13
Sep	B	49	1	46	0	2	0.02
(Post-monsoon)	C	6	1	3	2	0	0.50
Nov	B	34	2	32	0	0	0.06
(winter)	C	18	2	16	0	0	0.11
Tot	**B**	**153**	**4**	**146**	**0**	**3**	**0.03**
	C	**41**	**4**	**35**	**2**	**0**	**0.15**

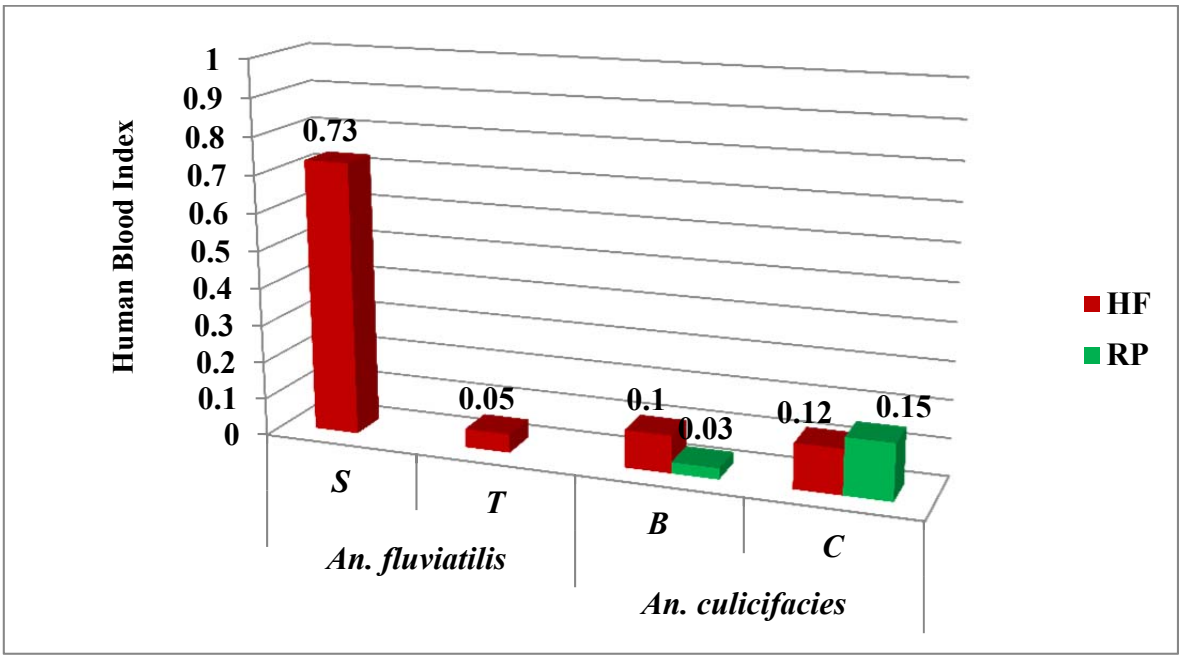


Figure 21: Human Blood Index of *An. fluviatilis* and *An. culicifacies* sibling species in HF and RP ecotypes (Pooled data for different seasons)

3.6. Malaria vector incrimination reports in both the ecotypes during study months

The results of detection of the circumsporozoite antigens of *P. falciparum* and *P. vivax* in sibling species S and T of *An. fluviatilis* and sibling species B and C of *An. culicifacies* by ELISA method in two ecotypes during different seasons are presented in Table 9. Out of 125 samples of *An. fluviatilis* S tested against *P. falciparum* and *P. vivax* monoclonal antibodies, 4 were found harboring CS antigen of *P. falciparum* in March, September and November months with an overall sporozoite rate of 3.2%. None of the *An. fluviatilis* T (N=161) was found positive for circumsporozoite antigen of any of the *Plasmodium* species. In HF ecotype one out of 50 *An. culicifacies* species C was found positive for *P. falciparum* CS antigen in July whereas none of the *An. culicifacies* species B was found to be positive for circumsporozoite antigen of any of the *Plasmodium* species. In RP ecotype one out 41 *An. culicifacies* species C was found positive for *P. falciparum* CS antigen in July and one out of 153 *An. culicifacies* B was found positive for *P. vivax* CS antigen in September. The sporozoite rate of *An. culicifacies* C was found to be 2.0 and 2.44 and in case of species B it was 0.0 and 0.65 in HF and RP ecotypes respectively. The relative rates of malaria parasite infectivity in primary malaria vectors during different seasons in both the ecotypes are presented in Table 9. In case of *An. annularis* none of the cytologically identified specimens (N=126) from HF and RP ecotypes was found positive for CS antigens of malaria parasites.

Table 9: Detection of circumsporozoite (CS) antigens* of *Plasmodium* species in the members of Fluviatilis and Culicifacies complexes by ELISA† in HF and RP ecotypes

Seasons	Species	Sibling species	Total tested	Total positive	*Pf*	*Pv*-210	*Pv*-247	Sporozoite rate
			Hilly-forest					
March	*An.*	S	72	**1**	**1**	0	0	1.39
(Pre-monsoon)	*fluviatilis*	T	78	0	0	0	0	0.00
July		S	1	0	0	0	0	0.00
(Monsoon)		T	2	0	0	0	0	0.00
September		S	41	**2**	**2**	0	0	4.88
(Post-Monsoon)		T	26	0	0	0	0	0.00
November		S	11	**1**	**1**	0	0	9.09
(Winter)		T	55	0	0	0	0	0.00
Total		**S**	**125**	**4**	**4**	**0**	**0**	**3.20**
		T	**161**	**0**	**0**	**0**	**0**	**0.00**
March	*An.*	B	33	0	0	0	0	0.00
(Pre-monsoon)	*culicifacies*	C	24	0	0	0	0	0.00
July		B	41	0	0	0	0	0.00
(Monsoon)		C	10	**1**	**1**	0	0	10.00
September		B	38	0	0	0	0	0.00
(Post-Monsoon)		C	4	0	0	0	0	0.00
November		B	16	0	0	0	0	0.00
(Winter)		C	12	0	0	0	0	0.00
Total		**B**	**128**	**0**	**0**	**0**	**0**	**0.00**
		C	**50**	**1**	**1**	**0**	**0**	**2.00**
			Riverine-plain					
March	*An.*	B	17	0	0	0	0	0.00
(Pre-monsoon)	*culicifacies*	C	9	0	0	0	0	0.00
July		B	53	0	0	0	0	0.00
(Monsoon)		C	8	**1**	**1**	0	0	12.50
September		B	49	**1**	0	**1**	0	2.04
(Post-Monsoon)		C	6	0	0	0	0	0.00
November		B	34	0	0	0	0	0.00
(Winter)		C	18	0	0	0	0	0.00
Total		**B**	**153**	**1**	**0**	**1**	**0**	**0.65**
		C	**41**	**1**	**1**	**0**	**0**	**2.44**

*Circumsporozoite proteins of *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*-210 and 247). † Enzyme-Linked Immunosorbent Assay.

OD values (at 450 nm) for field samples (*An. fluviatilis* and *An. culicifacies*) positive for CS antigen of *Pf* ranged from 0.876-1.247. OD values for *Pf* CS antigen (+ve control) ranged from 0.62-1.803 and OD values for lab reared uninfected mosquitoes (–ve control) ranged from 0.021-0.056. OD values for single field sample positive for CS antigens of *Pv*210 was 0.456. OD values for *Pv*210 CS antigen (+ve control) ranged from 0.259-0.553 and OD values for –ve control ranged from 0.019-0.048. OD values for *Pv*247 CS antigen (+ve control) ranged from 0.904-0.1.598 and OD values for –ve control ranged from 0.015-0.094. None of the field samples was found positive for *Pv*247 CS antigen.

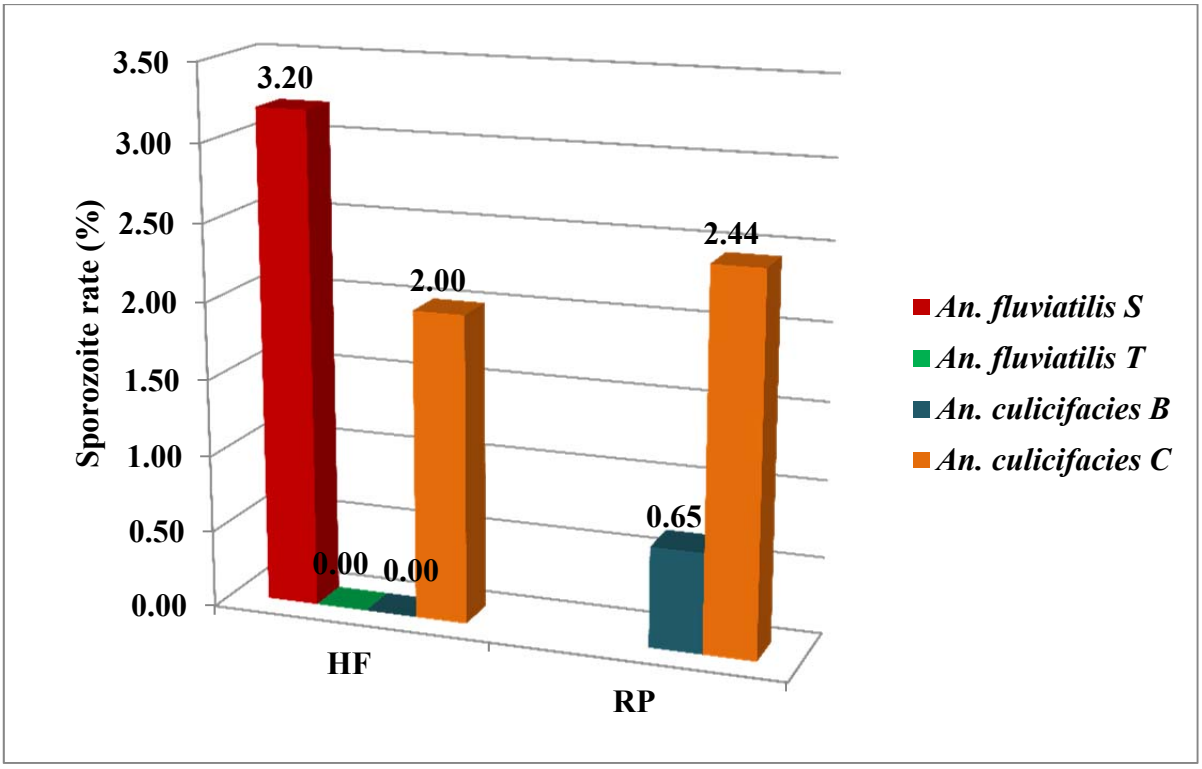


Figure 22: Sporozoite rate in the members of Fluviatilis and Culicifacies Complexes for human *Plasmodia* in HF and RP ecotypes

3.7. Susceptibility status of *An. fluviatilis s.l.* and *An. culicifacies s.l.* against different insecticides in HF and RP ecotypes

Susceptibility status of *An. fluviatilis* (prevalent only in HF ecotype) against diagnostic dose of 4% DDT was found under verification required (VR) category according to new WHO criteria (2013) but this species was found susceptible to 5% malathion and 0.05% deltamethrin. As per new WHO criteria, *An. culicifacies s.l.* was found resistant to all the insecticides used in public health. In *An. culicifacies* the corrected mortality was 48.3% and 40.0% against 4% DDT; 76.7% and 85.6% against 5% malathion and 80.0% and 81.9% against 0.05% deltamethrin in HF and RP ecotypes respectively. The detailed results of tests are presented in Table 10 (a) and (b). However, as per the old criteria (WHO 1998) *An. culicifacies* falls under verification required category against 0.05 deltamethrin in both the ecotypes. According to new WHO criteria *An. culicifacies* also resistant to deltamethrin (Table 10).

Table 10 a: Susceptibility status of *An. fluviatilis s.l.* and *An. culicifacies s.l.* against different insecticides in HF ecotype

Vector species	Insecticide	Control			Test			Corrected Mortality (%)	Status
		Exposed (N)	Dead (N)	Mortality (%)	Exposed (N)	Dead (N)	Mortality (%)		
An. fluviatilis	DDT 4%	30	2	6.7	60	54	90	89.3	VR
	Malathion 5%	20	0	0.0	40	40	100.0	100.0	S
	Deltamethrin 0.05%	20	0	0.0	40	40	100.0	100.0	S
An. culicifacies	DDT 4%	30	0	0.0	60	29	48.3	48.3	R
	Malathion 5%	30	0	0.0	60	46	76.7	76.7	R
	Deltamethrin 0.05%	30	1	3.3	60	48	80.0	80.0	R

S- Susceptible, R-Resistant, VR- Verification required.

Table 10 b: Susceptibility status of *An. culicifacies s.l.* against different insecticides in RP ecotype

Vector species	Insecticides	Control			Test			Corrected Mortality	Status
		Exposed (N)	Dead (N)	Mortality (%)	Exposed (N)	Dead (N)	Mortality (%)		
An. culicifacies	DDT 4%	115	3	2.6	200	80	40	40.0	R
	Malathion 5%	80	0	0.0	125	107	85.6	85.6	R
	Deltamethrin 0.05%	80	1	1.3	160	131	81.9	81.9	R

S- Susceptible, R-Resistant, VR- Verification required.

4. Discussion:

So far, Deogarh district in northern Odisha has not been explored for entomological aspects influencing malaria transmission. In the present study analyses of the results of entomological investigations in Deogarh district revealed distinct differences in the vector species prevalence in hilly-forest (HF) and riverine-plain (RP) ecotypes. Among primary vectors of malaria, *An. culicifacies*, *An. fluviatilis* and *An. minimus* were prevalent in HF ecotype whereas in RP, only *An. culicifacies* was found (Table 2). This is because of the preference of *An. fluviatilis* and *An. minimus* to breed in slow moving streams and stream channels around the HF villages (Ramachandra Rao 1984, Nagpal and Sharma 1995). *Anopheles culicifacies* and *An. fluviatilis* have been reported to be responsible for majority of malaria problem in India, specifically in the eastern plateau covering Odisha. The former is considered as a major vector in both RP and HF ecotypes of rural settings and the latter is designated as principal vector in HF areas (Nanda et al. 2000, Sharma et al. 2006, Sahu et al. 2014b, Sharma and Dev 2015). In the present study, the density of *Anopheles culicifacies* peaked during monsoon months, and declined in winter in both the ecotypes whereas in HF, an increase in *An. fluviatilis* population was observed in post monsoon and winter months, and it peaked in March revealing seasonal fluctuation in the populations of primary malaria vectors in two ecotypes (Figure 17). Both these vector species were found resting indoors (Table 5 a and b). The seasonal trend of vector abundances and resting behaviour of primary malaria vectors in Deogarh district were in agreement with other reports from neighbouring districts (Chand et al. 1993, Nanda et al. 2000, Sharma et al. 2006). In RP, the increase in malaria incidence in monsoon season can be attributed to peak density of *An. culicifacies* during that period whereas in HF high malaria incidence during post monsoon and winter months can be correlated with build-up of *An. fluviatilis* population. Though *An. minimus* was found in very low numbers in HF ecotype it is suspected to play an important role in malaria transmission. In recent reports it is claimed that *An. minimus* is the prime vector of malaria in Northern Odisha, developing resistance against insecticides, and sharing ecological niches of *An. fluviatilis* (Gunasekaran et al. 2014a).

Analyses of sibling species composition of major malaria vectors, their feeding preference, and malaria transmission potential revealed prevalence of *An. fluviatilis* sibling specie S and T in study villages of hilly-forest area. Both the sibling species were sympatric and found in almost equal proportion (Figure 19). Species S was found to be predominantly anthropophagic whereas species T was primarily zoophagic (Table 8 and Figure 21).

Sporozoite positive specimen belonging to species S were found in the months of September, November and March indicating that this species is highly efficient malaria vector and responsible for extended malaria transmission season up to onset of summer in district Deogarh (Table 9). Though species T was not detected positive for malaria sporozoite in the present study it might also be contributing to malaria transmission in HF as this species has been shown to support the sporogonic development of *Plasmodium* species in laboratory experiments (Adak et al. 2005) and there are recent reports where this species has been incriminated in Central India (Singh et al. 2013, Singh et al. 2015).

Regarding Culicifacies Complex, the cytotaxonomic studies revealed that species B and C were prevalent in study villages of both HF and RP ecotypes. Recent studies report prevalence of all five species of Culicifacies Complex in southern districts of Odisha (Tripathy et al. 2010, Das et al. 2013). In these studies, multiplex PCR assays have been used for differentiating the members of Culicifacies Complex (Goswami et al. 2006). This method has been reported to be ambiguous and not accurate in differentiating all the members of Culicifacies Complex and at best the members of Culicifacies Complex can be divided in two subgroups A/D and B/C/E (Raghavendra et al. 2009, Manonmani et al. 2013). Therefore, cytotaxonomic method appears to be more accurate and feasible. The finding of this study is in agreement with previous reports of prevalence of species B and C in the districts of Northern Odisha (Nanda et al. 2000, Sharma et al. 2006, Tripathy et al. 2010). Though *An. culicifacies* species B and E are homo-sequential in polytene chromosome complement and cannot be differentiated cytotaxonomically (Kar et al. 1999), the possible existence of species E in district Deogarh is remote as this species has been reported to be the highly efficient vector with highest anthropophilic index among the members of Culicifacies Complex (Das et al. 2013). In present study, *An. culicifacies* species B and C were found sympatric and species B was predominant in study areas of HF and RP ecotypes. Both species B and C were found to be primarily zoophagic in both the ecotypes (Figure 21). *An. culicifacies* species C which is an established vector of malaria (WHO 2008) was incriminated in both HF and RP ecotypes during monsoon season. In addition species B which is considered as poor vector of malaria (WHO 2008) was also incriminated in RP ecotype in post monsoon season (Table 9). Since *An. culicifacies* was the only primary vector found in RP ecotype, the prevalent sibling species of Culicifacies Complex are mainly responsible for malaria transmission in this ecotype. *Anopheles culicifacies* was sympatric with *An. fluviatilis* in HF ecotype and species C was incriminated during monsoon period when *An. culicifacies* density was found to be high (Table

3 and Table 9). These findings suggests that *An. culicifacies* is significantly contributing to malaria transmission in HF ecotype as well. Similar observations were made in other districts of northern Odisha (Sharma et al. 2006, Tripathy et al. 2010). Apart from primary malaria vectors, *An. annularis* which is reported to play secondary and focal role in malaria transmission (Kumar et al. 2012), was found prevalent in both the ecotypes (Table 2). Cytotaxonomic study revealed that the *An. annularis* population comprised of only species A. *Anopheles annularis* A was found to be exclusively zoophagic and none of the specimens tested was found positive for *Plasmodium* sporozoites. Therefore, *An. annularis* might be playing a marginal role in malaria transmission in study areas of district Deogarh.

Study of the insecticide susceptibility status of primary malaria vectors against different insecticides used in public health was carried out in HF and RP ecotypes. The observations revealed that *An. fluviatilis* which was confined to HF was fully susceptible to diagnostic doses of malathion and deltamethrin whereas this species has developed tolerance / resistance to DDT (Table 10 a). Similar observations were made in district Mayurbhanj (Sharma et al. 2004) and resistance against *An. fluviatilis* has been reported from Maharashtra and the adjacent state Jharkhand (Singh et al. 2014a). In this study *An. culicifacies* was found highly resistant to DDT and malathion and the percent corrected mortality against deltamethrin ranged between 80%-82% in two ecotypes. These findings are in accordance with the reports from other districts of Odisha state (Raghavendra et al. 2014, Sahu et al. 2014b). Information obtained from district health department revealed that presently Indoor Residual Spray (IRS) with DDT is being carried out, two rounds in HF and one round in RP ecotype. Keeping in view the susceptibility status of primary malaria vectors and their prevalence period it would be appropriate to replace DDT with synthetic pyrethroids in IRS operations and minimum of two rounds of IRS are required in both ecotypes with good coverage of human and mixed dwellings. Though *Anopheles culicifacies* has shown resistance/ tolerance to deltamethrin, timely spray of two rounds of an appropriate synthetic pyrethroid with good quality and coverage would have a desired epidemiological impact. An additional round of IRS after winter in HF ecotype would be useful in reducing extended transmission by highly efficient *An. fluviatilis* S which is prevalent in good numbers in spring season. In tribal areas where *An. fluviatilis* S is prevalent and maintains extended transmission and there is predominance of *P. falciparum,* it can be categorized as high risk areas. In such areas, a selective additional round with suitable insecticide can be advocated as a short term intervention measures (NMEP 1995, NMEP 1996, NVBDCP 2009).

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CHAPTER III

Comparative assessment of the prevalence of mutations in the *Plasmodium falciparum* drug-resistant genes in hilly-forest and riverine-plain ecotypes

1. Introduction

Considering malaria as a local and focal disease, epidemiological understanding of different microhabitat ecotypes of malaria can help in devising novel control measures. One of the major hurdles in malaria control lies on the evolution and dispersal of the drug-resistant malaria parasite of *Plasmodium falciparum* (*pf*). Most of the malaria in India is contributed by rural areas which majorly comprise of Hilly-Forest (HF) and Riverine-Plain (RP) ecotypes. Patterns of genetic variations in genes conferring drug-resistance in micro eco-epidemiological settings in malaria are not known. Malaria is intense in HF due to many a factors and such ecological setting having high transmission is associated with the rapid evolution of antimalarial resistance. The rural settings of RP are also endemic or epidemic for malaria. Comparing the two distinct eco-epidemiological settings separated by short geographical distances can reveal the differences in the status and level of drug resistance between these two most important malaria ecotypes.

Chemotherapy is the primary means of treating malaria infections. A problem confronting antimalarial treatment is the ability of the *Plasmodium* to mutate and to survive despite intake of a drug in doses equal to or higher than those recommended but within tolerance of the human (https://en.wikipedia.org/wiki/Antimalarial_medication). Effective chemotherapy mainly depends on the exploitation of the metabolic differences between the pathogen and the host (http://www.tulane.edu/~wiser/protozoology/notes/drugs.html). Summary of the activity of the most widely used antimalarials throughout the life cycle of *Plasmodium* is schematically represented in Figure 1 and the mechanism of action of main antimalarials is depicted in Figure 2. The severity of drug resistance increases with accumulation of multiple resistance conferring mutations (SNPs) against multiple antimalarials hence, mounting multidrug resistant *Pf* population. The history of antimalarial chemotherapy and resistance is presented in Figure 3. The acquired drug resistance in *Pf* spreads very fast to broad geographical areas due to migration of *Pf* patients across countries and continents (Figure 4). The build-up and falling-off of multidrug resistant *Pf* population are influenced by

use of antimalarials and eco-epidemiological settings. So, drug resistance status should be monitored regularly in order to formulate appropriate antimalarial drug policies.

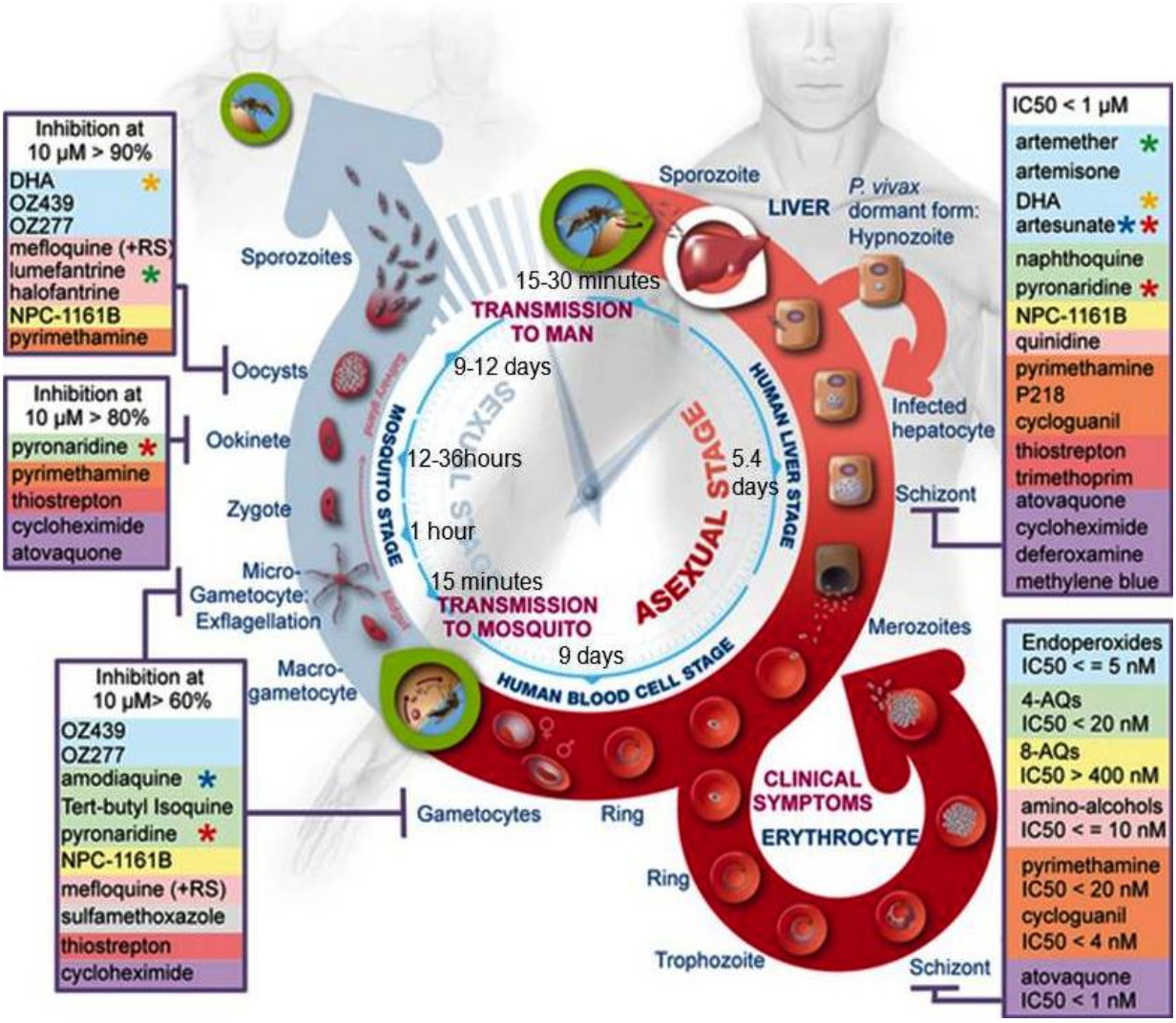


Figure 1: Action of major antimalarials on the lifecycle stages of *Plasmodium*

Different stages in the life cycle phase of *Plasmodium* are targeted for breaking the malaria transmission cycle viz. preventing liver stages, treating erythrocytic stages, and blocking gametocytes. Different pathways and cellular compartments of *Plasmodium* are targeted i.e. Antifolates targets folate biosynthesis in cytosol, Quinolines targets haemozoin formation in lysosomes, Atovaquone targets cyt c reduction in mitochondrion, Antibiotics targets protein translation in Apicoplast, and Artimisinin targets ATPase 6 calcium pump in endoplasmic reticulum (ER). Source: (Delves et al. 2012)

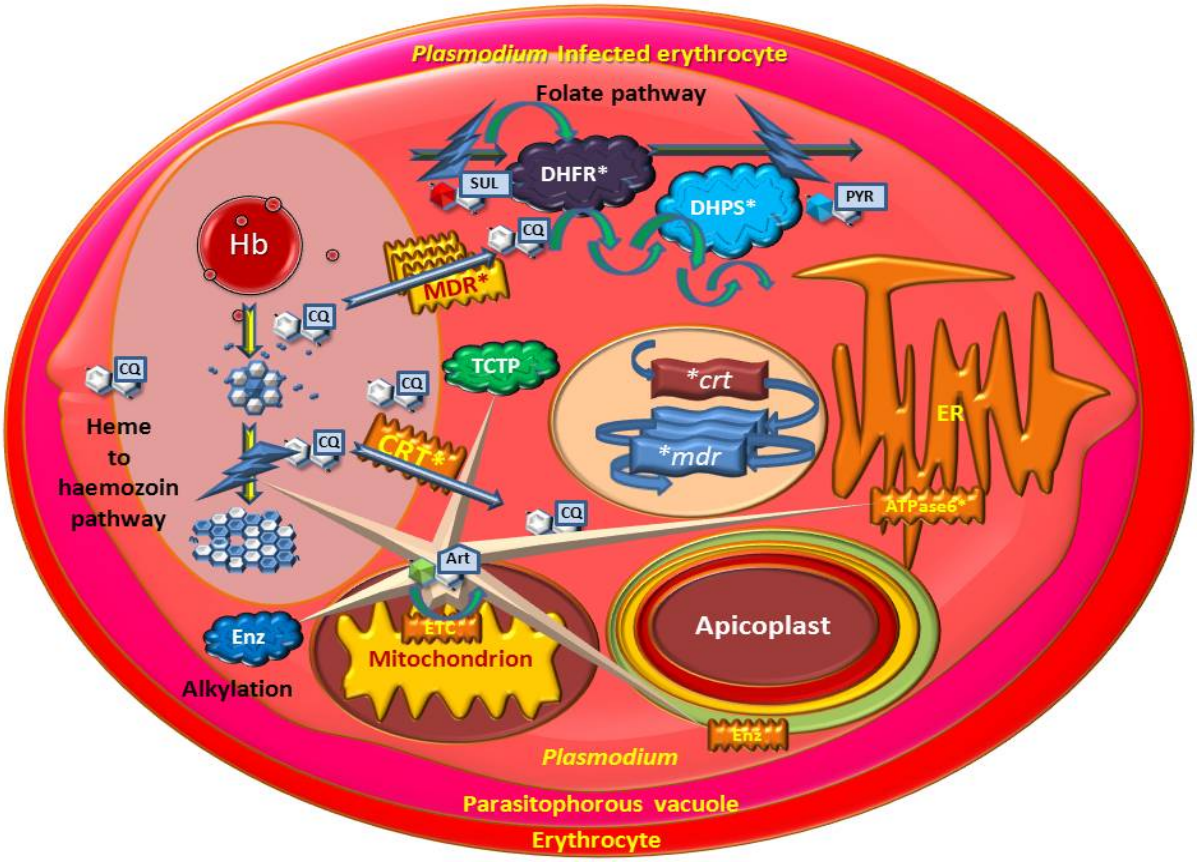
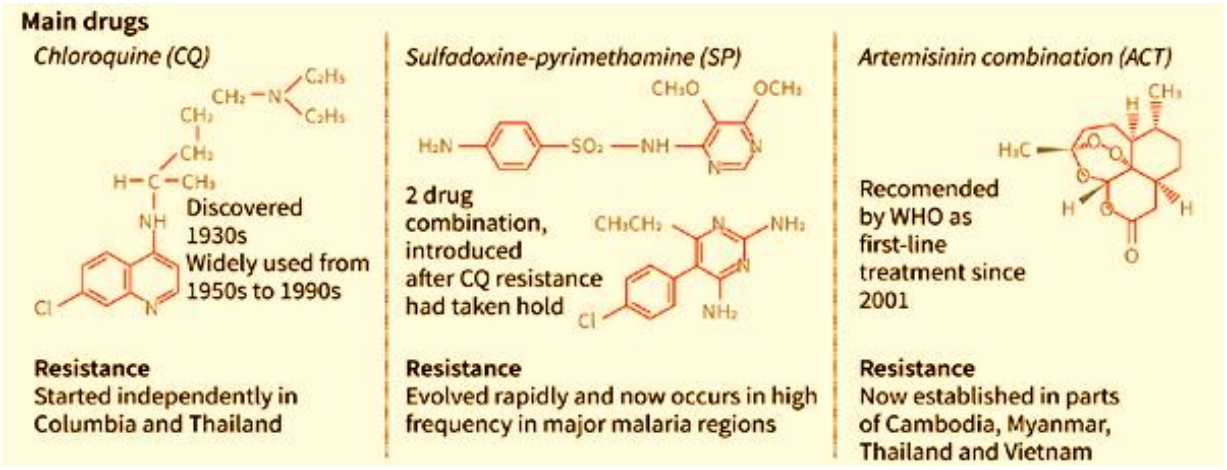


Figure 2: Mechanism of action of major antimalarials

The concept for this schematic presentation is derived from the sources: (Ridley 2002, Ding et al. 2011, Ding et al. 2012, Shahinas et al. 2013). Digestion of haemoglobin (Hb) free Heme (ferriprotoporphyrin IX or hematin or FP) is toxic to the parasite (lyse membranes, inhibition of several enzymes activity). Parasite detoxifies free Heme by sequestration into haemozoin (malarial pigment); Chloroquine (CQ) binds with hematin and blocks the development of the hemozoin crystals, blocking clearing of hematin and killing the parasite. The resistant *P. falciparum* parasite expels CQ, 40 to 50-folds more rapidly than the susceptible parasite (Krogstad et al, 1988). As *Pf* has higher rate of replication the parasite requires greater amount of nucleotides as precursors for DNA synthesis and thus is particularly sensitive to antifolates. The two primary targets of antifolates [Sulfadoxine (SUL) and Pyramethamine (PYR)] are the enzymes dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) which *de novo* synthesize folates. The malaria parasite synthesizes folates *de novo* whereas the human host must obtain preformed folates and cannot synthesize folate. The inability of the parasite to utilize exogenous folates makes folate biosynthesis a good target of Sulfadoxine and Pyramethamine (SP) drug. Mutations in the genes *Pfcrt* and *Pfmdr 1* were identified as genetic markers associated with the inheritance of CQ resistance and mutations in the genes *Pfdhfr* and *Pfdhfr* were identified as genetic markers associated with the inheritance of SP resistance in *Pf*. Putative targets of Artemisinin (Art) viz. alkylation of enzymes like translationally controlled tumor protein (TCTP), inhibiting haemozoin formation and endoplasmic reticulum (ER) protein ATPase 6, interfere with mitochondrial functions etc. are indicated (Ridley 2002, Ding et al. 2011, Ding et al. 2012, Shahinas et al. 2013).



Source: (WHO/Smithsonian institute/malaria.welcome.ac.uk/nih)

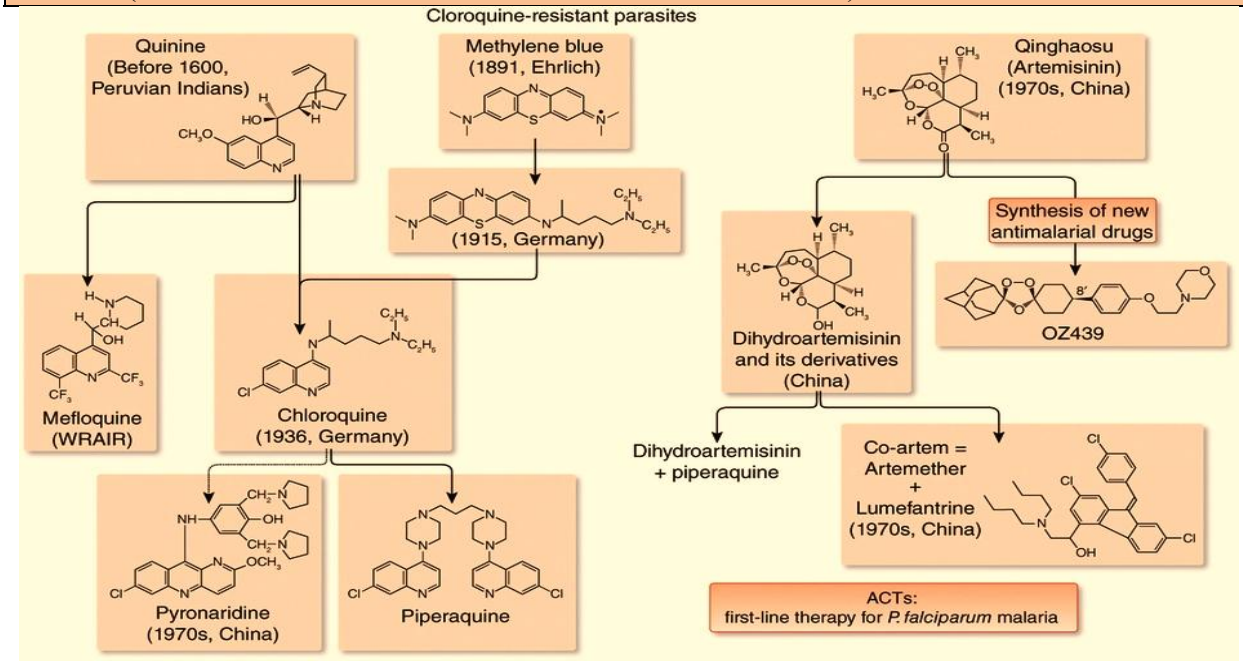


Figure 3: Trend of developing resistance against major antimalarials

At present, broadly three types of antimalarials are being used viz. (i) 4-aminoquinolines and amino alcohols, (ii) sulphonamides and sulphones, and (iii) sesquiterpenes. Quinine had been known by Peruvian Indian before 16th century, introduced as antimalarial in 1632 and the resistance was noticed for the first time in 1910. CQ was introduced in 1945 and first resistance was noticed in 1957, Proguanil was introduced in 1948 and resistance was noticed the next year, SP was introduced in 1967 and resistance was noticed in the same year, Mefloquine was introduced in 1977 and resistance was noticed after five years, Atovaquone was introduced in 1996 and resistance was noticed in the same year. Source: (Miller et al. 2013)

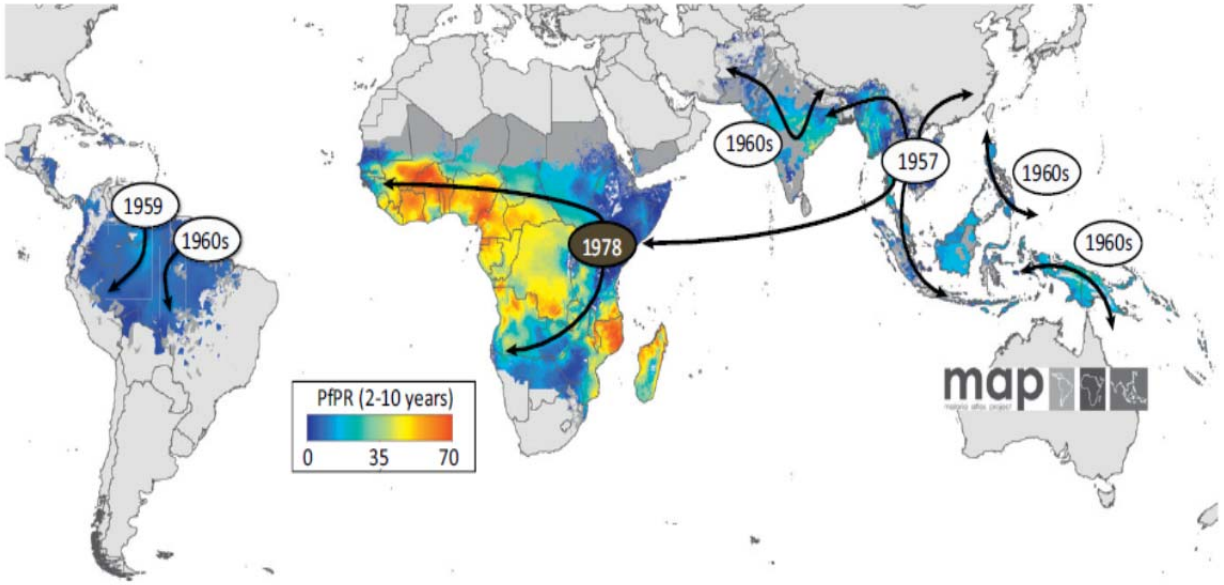


Figure 4: Evolution and spread of chloroquine (CQ) resistant *P. falciparum*

Foci of CQ resistant *P. falciparum* were detected in Colombia and at the Cambodia-Thailand border during the late 1950s. Resistant strains from these foci spread steadily in the 1960s and 1970s through South America, Southeast Asia, and India. Africa was spared until the late 1970s, when resistance was detected in Kenya and Tanzania; the sweep of resistant *Pf* across that continent followed within a decade. Source: (Ecker et al. 2012)

Therefore a study has been carried out on genetic variation at the Single Nucleotide Polymorphism (SNP) level in four different genes of *Pf* (*Pfcrt*, *Pfmdr1*, *Pfdhfr,* and *Pfdhps*) that confer resistance to different antimalarials in two different eco-epidemiological settings, *i.e.* Hilly-Forest (HF) and Riverine-Plain (RP), in a high malaria endemic district of Odisha state, India.

1.1. Review of literature

Epidemiological outcome of malaria infection differs in different ecotypes of the globe (Okwa et al. 2009, A. Das et al. 2012, Kar et al. 2014, Kaewwaen and Bhumiratana 2015, V. P. Sharma et al. 2015), and therefore, malaria is considered as a local and focal disease (Rath 2004, Dash et al. 2008, Conn et al. 2015). Several studies involving different ecological and climatic settings have provided evidence that malaria epidemiology can be significantly variable across small eco-climatic scales (Jambulingam et al. 1991, Kaga and Ohta 2012, Schapira and Boutsika 2012). For example, malaria epidemiological outcomes including distributional prevalence of mosquito vectors and malaria transmission were correlated with different ecotypes in Nigeria (Okwa et al. 2009), Kenya (Ingasia et al. 2015), Brazil (Rosa-

Freitas et al. 2007), Southeast Asia (Seng et al. 1999) and India (Jambulingam et al. 1991, Shukla et al. 2007, Ramar et al. 2014, Singh et al. 2015). Moreover, malaria outcome was found to be significantly higher in forest ecotype in comparison to no-forest ecotype (Kar et al. 2014) as observed in Belize (Hakre et al. 2004), Bangladesh (Haque et al. 2011), Nepal (Reisen et al. 1993), and India (Sharma et al. 2006, Shukla et al. 2008, Nath and Mwchahary 2012). In India, studies conducted in the Sundargarh districts of Odisha state (high malaria endemic) showed that villages in forest and plain areas (separated by short geographical distances) have distinct malaria transmission pattern (Sharma et al. 2006), which could have been a consequence of prevalence of different species and vectorial behavior of a particular species of the mosquito vectors (Singh et al. 1996, Nanda et al. 2000, Manguin et al. 2008, Das 2015).

Considering the evolution and spread of malaria parasites resistant to different antimalarials [*viz*. Chloroquine (CQ), Sulfadoxine and Pyrimethamine (SP) *etc.*] that highly influence malaria epidemiological outcome and pose strong impediment to malaria control programs (Hastings 2003, Das and Dash 2007, V. Singh et al. 2009, Mallick et al. 2013b), whether different local micro eco-climatic factors have influenced genetic changes at the genes conferring resistance to different antimalarials, needs to be evaluated (Sorosjinda-Nunthawarasilp and Bhumiratana 2014).

Needless to mention, such information will be of enormous benefit to the local malaria control program (Dash et al. 2008). This is because, in high malaria transmission areas resistance against CQ and SP in the malaria parasite *Plasmodium falciparum* spreads fast, whereas in low transmission areas, drug pressure plays a much crucial role (Talisuna et al. 2002, Hastings and Watkins 2005, Mallick et al. 2013a, Mallick et al. 2013b, Malisa et al. 2016). It has further been proposed that the predominance of tribal groups along with unrestricted use of inappropriate antimalarials, population movements, resettlements, and presence of sylvatic mosquito vectors promote rapid evolution of antimalarial resistance and therefore high malaria transmission settings encompassing this type of ecotype were proposed to be centre of origin of drug resistance (Malakooti et al. 1998, Chareonviriyaphap et al. 2000, Keiser et al. 2005, N. Singh et al. 2009, Kar et al. 2014). Malaria morbidity and asymptomatic malaria are often under reported among these groups (Erhart et al. 2005, Lokki et al. 2011). Along with submicroscopic circulation of malaria parasites in the blood, some immunity develops against malaria in such groups (Trape et al. 1985, Jha et al. 2012). Illiteracy, language

and pronunciation differences, and social discrimination make them reluctant to seek health care facilities (Sheik-Mohamed and Velema 1999, Kar et al. 2014).

Tracking the patterns of mutations, estimating genetic diversities at the Single Nucleotide Polymorphism (SNP) level and asserting linkage among the SNPs in populations are the most efficient ways to understand the evolution of that particular gene (Sutar et al. 2013, Carlton et al. 2015, Pelleau et al. 2015, Malisa et al. 2016). Several studies following these methodologies in genes conferring resistance to antimalarials in *Pf* have indicated evolutionary potential of these genes both at the global scale and also in India (Das and Dash 2007, Awasthi et al. 2011, Brown et al. 2015, Kumar et al. 2015, Li et al. 2015, Rouhani et al. 2015).

It has been known that chloroquine (CQ) was a very useful drug in the global fight against malaria in the 20th century, relieving the first drug quinine (QN). Later CQ failure due to spread of resistance limited its use (Valderramos et al. 2010). Sulfadoxine and Pyrimethamine (SP) recipe was effective against *Plasmodium falciparum* (*Pf*) malaria, but not as effective against *Plasmodium vivax* malaria compared to CQ and unlike CQ it was an operational tool for a very limited time due to resistance issues (Kinzer et al. 2010) (Figure 3). However, it is currently being used as a partner drug with the very potent Artemisinin (AS) derivatives, which is at present a very efficient combination to treat *Pf* cases. The partnership of AS+SP (ASP) was projected to complement each other in blocking the folate pathway and safeguard each other as AS is fast-acting while SP is slow-acting with a longer half-life. Mutations in the *Pf dihydrofolate reductase* (*Pfdhfr)* and *Pf dihydropteroate synthase* (*Pfdhps)* are responsible for the failure of these partner drugs (Peterson et al. 1988, Brooks et al. 1994). It was anticipated that change in drug policy and withdrawal of CQ against *Pf* treatment will revert back the *Pfcrt* and *Pfmdr 1* mutations and support of Artemisinin will discontinue evolution of *Pfdhfr* and *Pfdhps* resistance (Hastings and Donnelly 2005). Despite high resistance established against CQ in most endemic areas, CQ was being used chiefly as it was affordable (Valderramos et al. 2010). This reduces the chances of reverting the CQ resistant gene pool and hope of reintroducing CQ for treating *Pf* malaria.

Mutations in the gene encoding a *Pf* CQ resistance transporter (*Pfcrt*) and resulting change in single amino acid (AA) locus 76 from K to T (K76T) were proven a strong marker for CQ resistance (Fidock et al. 2000, Valderramos et al. 2010). Similarly, mutations in the *Pf* multi-drug resistance gene (*Pfmdr1*) conferring single AA change at point 86 from N to Y

(N86Y) and/or multi-copy number of *Pfmdr1* was further reported to be linked with K76T and CQ resistance conferring synergistic increase in resistance when combined (Foote et al. 1990, Price et al. 1999, Babiker et al. 2001, Mwai et al. 2009, Chauhan et al. 2014). The linkage of N86Y with K76T and mechanism is still unclear and under debate (Ghanchi et al. 2011). The most convincing mechanism is diagrammatically represented in Figure 2. Furthermore, mutations (S436A and A437G) in the *Pf* dihydrofolate reductase enzyme coding gene singly pose mild resistance to SP drugs, and when linked with mutations A581G and /or K540E and /or A613S/T, confer high resistance (Peterson et al. 1988, McCollum et al. 2012, Rouhani et al. 2015). Moreover, polymorphism in the *Pfdhps* gene encoding S108N is the core mutation, but this confers comparatively lower resistance when present singly (Brooks et al. 1994, Reeder et al. 1996, Triglia et al. 1997, McCollum et al. 2012). Of particular importance is the correlation on the number of different mutations a parasite possesses to the ability to resist an antimalarial. For example, double mutant of the *Pfcrt*-$S_{72}V_{73}M_{74}N_{75}T_{76}$ *and* triple mutant $C_{72}V_{73}I_{74}E_{75}T_{76}$ haplotypes were more prevalent along with wild type $C_{72}V_{73}M_{74}N_{75}K_{76}$ and reflected more successful resistant haplotypes (Nagesha et al. 2003, Ghanchi et al. 2011). The former mutant was abundant in South-East Asia and Africa, whereas the latter was prevalent in South America, Papua New Guinea and Philippines (Ghanchi et al. 2011). Global occurrences of AA mutations in *Pfcrt* are reviewed and schematic view of *Pfcrt* mutation sites is represented in Figure 5 (a and b). Spread of CQ resistant *Pfcrt* haplotypes in India is represented in Figure 6.

(a) Major reported haplotypes of PfCRT - an extraordinarily polymorphic protein

Region of origin	Isolates (examples)	CQ response	PfCRT position and encoded amino acid																
			72	74	75	76	97	144	148	160	194	220	271	326	333	334	350	356	371
Wild-type haplotype																			
All regions	HB3, 3D7, D10	S	C	M	N	K	H	A	L	L	I	A	Q	N	T	S	C	I	R
Mutant haplotypes																			
Africa	106/1 (revertant?)	S	C	I	E	K	H	A	L	L	I	S	E	S	T	S	C	I	I
SE Asia, Africa, W Pacific	Dd2, 102/1, PH4	R	C	I	E	T	H	A	L	L	I	S	E	S	T	S	C	T	I
SE Asia, Africa	FCB, PAR	R	C	I	E	T	H	A	L	L	I	S	E	S	T	S	C	I	I
SE Asia, Africa	Cam742, GB4	R	C	I	E	T	H	A	L	L	I	S	E	N	T	S	C	I	I
SE Asia	Cam783	R	C	I	E	T	H	A	L	L	I	S	E	N	T	S	C	T	I
SE Asia	Cam738	R	C	I	D	T	H	A	I	L	T	S	E	N	S	S	C	I	R
SE Asia	Cam734	R	C	I	D	T	H	F	I	L	T	S	E	N	S	S	C	I	R
SE Asia	TM93-C1088	R	C	I	E	T	L	A	-	L	-	S	E	S	-	-	-	T	I
China	e	UN	C	I	E	T	H	A	-	L	-	S	E	-	-	-	-	-	R
China	d	UN	C	I	E	T	H	Y	-	L	-	A	E	-	-	-	-	-	R
China	b	UN	C	I	D	T	H	Y	-	L	-	A	E	-	-	-	-	-	R
China	c	UN	C	I	D	T	H	Y	-	L	-	A	E	-	-	-	-	-	I
W Pacific	PH1	R	C	M	N	T	H	T	L	Y	I	A	Q	D	T	S	C	I	R
W Pacific	PH2	UN	S	M	N	T	H	T	-	Y	-	A	Q	D	-	-	C	I	R
W Pacific	PNG4	R	S	M	N	T	H	-	-	-	-	A	Q	D	-	-	-	L	R
W Pacific	2300	UN	C	I	K	T	H	A	L	L	I	S	E	S	T	S	C	I	I
S America, W Pacific, Africa	7G8, PNG1905	R	S	M	N	T	H	A	L	L	I	S	Q	D	T	S	C	L	R
S America	H209	R (low)	S	M	N	T	H	A	L	L	I	S	Q	D	T	S	R	L	R
S America	Ecu1110	R	C	M	N	T	H	-	-	-	-	S	Q	D	-	-	-	L	R
S America	TU741	R	C	M	N	T	H	A	L	L	I	S	Q	D	T	N	C	L	R
S America	Jav	R	C	M	E	T	Q	A	L	L	I	S	Q	N	T	S	C	I	T
S America	TA4641	R	C	M	E	T	Q	A	L	L	I	S	E	N	T	S	-	I	-
S America	TA4640	R	C	M	E	T	Q	A	L	L	I	S	Q	N	S	S	-	I	I

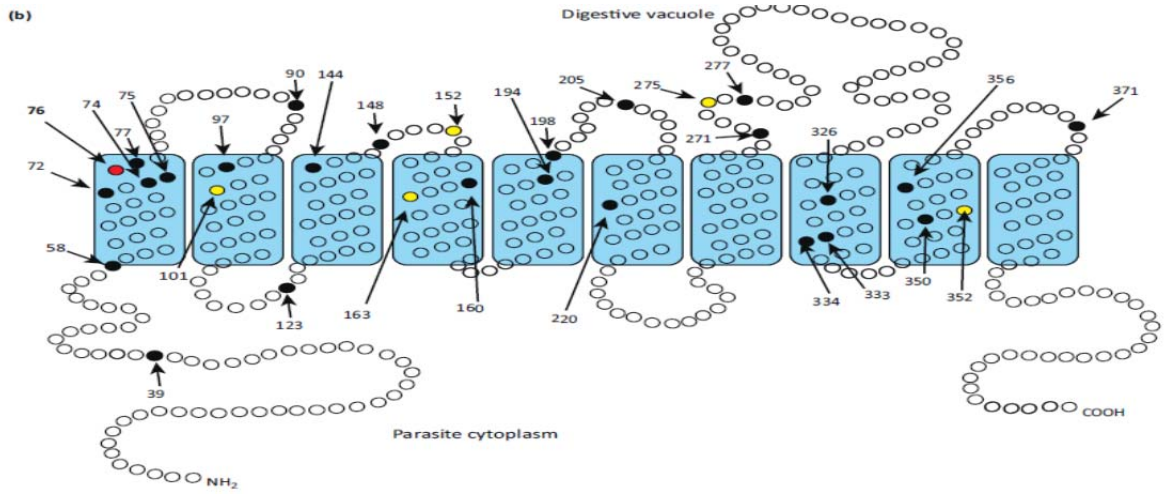


Figure 5: Globally reported haplotypes of *Pfcrt*

(a) The diagrammatic representation of the same mutant amino acids (b) Aligned view of amino acid mutations in worldwide reported *Pfcrt* associated with CQ resistance. Grey shading indicates residues that differ from the wild-type allele. Residues that were not reported are indicated by -. S=CQ-sensitive; R=CQ-resistant; UN=unknown Source: (Ecker et al. 2012)

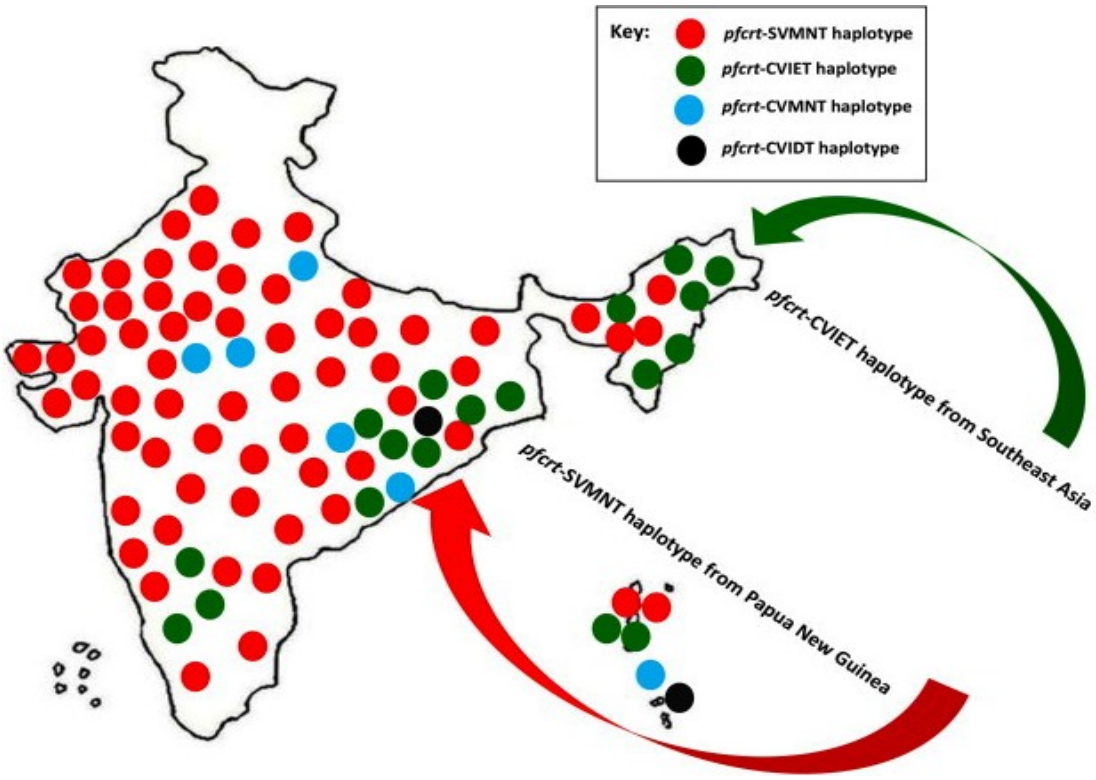


Figure 6: Spread of CQ resistant *Pfcrt* haplotypes in India

Four types of CQ resistant *Pfcrt* haplotypes were observed in India and all four were reported from Odisha viz. SVMNT, CVIET, CVMNT and CVIDT. CVIET is thought to have Southeast Asian origin whereas SVMNT is thought to have originated in Papua New Guinea. Source: (Das 2015)

Moreover, accumulation of multiple mutations in *Pfdhfr* gene resulting in double mutants (C59R/S108N and N51I/S108N) shows moderate levels of resistance, the triple mutants N51I/C59R/S108N show a significant level, and the quadruple mutant parasite (N51I/C59R/ S108N/I164L) is considered to be completely resistant to pyrimethamine (Sirawaraporn et al. 1997, McCollum et al. 2012). Very similarly, co-accumulation of resistance-conferring mutations in both the *Pfdhfr* and *Pfdhps* genes synergistically diminishes the success of SP (Ahmed et al. 2004, Rouhani et al. 2015).

Collectively sextuple (six) and added mutations harboring haplotypes comprising at least triple mutations in respective genes strongly predict resistance to SP but quintuple mutations are shown to be significantly linked to clinical failures (Pathak et al. 2014). The quintuple haplotype *Pfdhfr* $I_{51}R_{59}N_{108}$ and *Pfdhps*$G_{437}E_{540}$ were significantly linked to clinical failures of SP in Northern Borneo (Abdullah et al. 2013) and other parts of Africa. ASP treatment failures were reported in patients with *Pfdhfr* $I_{51}R_{59}N_{108}$ and *Pfdhps* G_{437} or $A_{436}E_{540}$ in West Bengal (Pathak et al. 2014).The same haplotype has been reported from Odisha (Pathak et al. 2014). The triple *Pfdhfr* haplotype $R_{59}N_{108}L_{164}$ had also been reported from Assam, furthermore, quadruple *Pfdhfr* haplotype $I_{51}R_{59}N_{108}$ L164 and *Pfdhps* with triple or quadruple resistance-conferring SNP haplotypes were reported from Andaman and Nicobar islands (Pathak et al. 2014). SP replaced CQ, in 1982 by National Drug Policy of India within drug resistant areas and subsequently, ASP combination was introduced and replaced "SP" monotherapy as the second line drug for use in CQ resistant areas in 2004–2005. In 2007, ASP became the first line of treatment. The failure rate of ASP (9.5%) has been reported in West Bengal which is approaching to the limit (10%) for change in drug policy (S. Das et al. 2012, Sabyasachi Das et al. 2012).

India is endemic to malaria and accounts for about 52% of the total malaria morbidity in Southeast Asia (Pradhan et al. 2016). Interestingly, majority of the malaria morbidity (about 26.9%) and mortality (about 17.6%) is contributed by Odisha state alone, although it comprises about 3% of Indian population (including some aboriginal tribes) (Pradhan et al. 2016). Intense and stable malaria has been reported from tribal areas of Odisha and neighbouring states (http://www.malariasite.com/tag/orissa/) (Nanda et al. 2000, Kumar et al. 2007, A. Das et al. 2012, Kumar et al. 2012). The state of Odisha consists of two highly malarious clusters; the North-Western (comprising of five districts, *viz.* Deogarh surrounded by Keonjhar, Sundergarh, Anugul and Sambalpur) and the South-Western (comprising of seven districts, *viz*.

Koraput, surrounded by Malkangiri, Nawarangpur, Kalahandi, Raygada, Nuapada, and Kandhamal (Mohanty et al. 2009, Sahu et al. 2013, Rao et al. 2015, Pradhan et al. 2016), although other districts too contribute to the total malaria cases. Interestingly, the districts in both the clusters are rich in hills and forests and home for aboriginal tribes (Sahu et al. 2013, Ramar et al. 2014, Pradhan et al. 2016). The Deogarh district is one of the epicentres of high malaria endemicity (Pradhan et al. 2016); comprising of two distinct ecotypes [Hilly-Forest (HF) and Riverine-Plain (RP)], and therefore can serve as a model to understand the influence of micro eco-typical habitats on malaria epidemiological outcome (in this case mutational pattern in different genes conferring drug resistance in the malaria parasite, *Pf*). This is important, as treatment failure in case of CQ and SP has now become very common in almost all malaria endemic regions of the globe including India (Klein 2013, Cui et al. 2015). In Odisha, high level of resistance to both CQ and SP has been reported from many malarious districts including Keonjhar, Sundargarh, Anugul and Sambalpur districts (Peterson et al. 1988, Mohanty et al. 2009, Sutar et al. 2011, Srivastava et al. 2013), with no report from the Deogarh district.

In the present study, DNA sequencing of four genes conferring drug-resistance in *Pf* field isolates collected from two different ecotypes in the Deogarh district of Odisha state were performed. SNPs in the four genes that are differentially segregating in these two populations and compared both the occurrence and distributional prevalence of different SNPs between the high endemic (HF) and moderately endemic (RP) areas were identified. Pattern of genetic diversity between populations for each gene and linkage disequilibrium (LD) between SNPs of different genes were also estimated. The results were interpreted in term of ongoing and past use of CQ and SP on evolution of drug resistance genotype of Indian *Pf* in the two different malaria ecotypes in Deogarh district of Odisha, India.

2. Methodology

2.1. Study area, sample collection and malaria species diagnosis

Plasmodium falciparum isolates were collected from villages representing two ecotypes (HF and RP) that are under the two primary health centres (PHCs) located at about 40-kilometre from each other (PHC Tileibani - HF and PHC Bampada – RP). The village wise malaria prevalence and treatment data were collected from Chief District Medical Officer (CDMO) [National Vector-Borne Disease Control Programme (NVBDCP) consultant reports] through proper channel during the field visits. Malaria transmission was perennial in both HF and RP and the intensity was much higher in former ecotype i.e. hyper-endemic. The malaria transmission peaks after monsoon in HF whereas in RP it peaks during monsoon (Figure 7). As per the annual PHC report, both HF and RP ecotype was *Plasmodium falciparum* dominated as only about 10% *Plasmodium vivax* cases were reported and the average API of HF was about 7 times higher than RP during 2006-2013. The detailed ecotype wise history of malaria transmission pattern is presented in Chapter I.

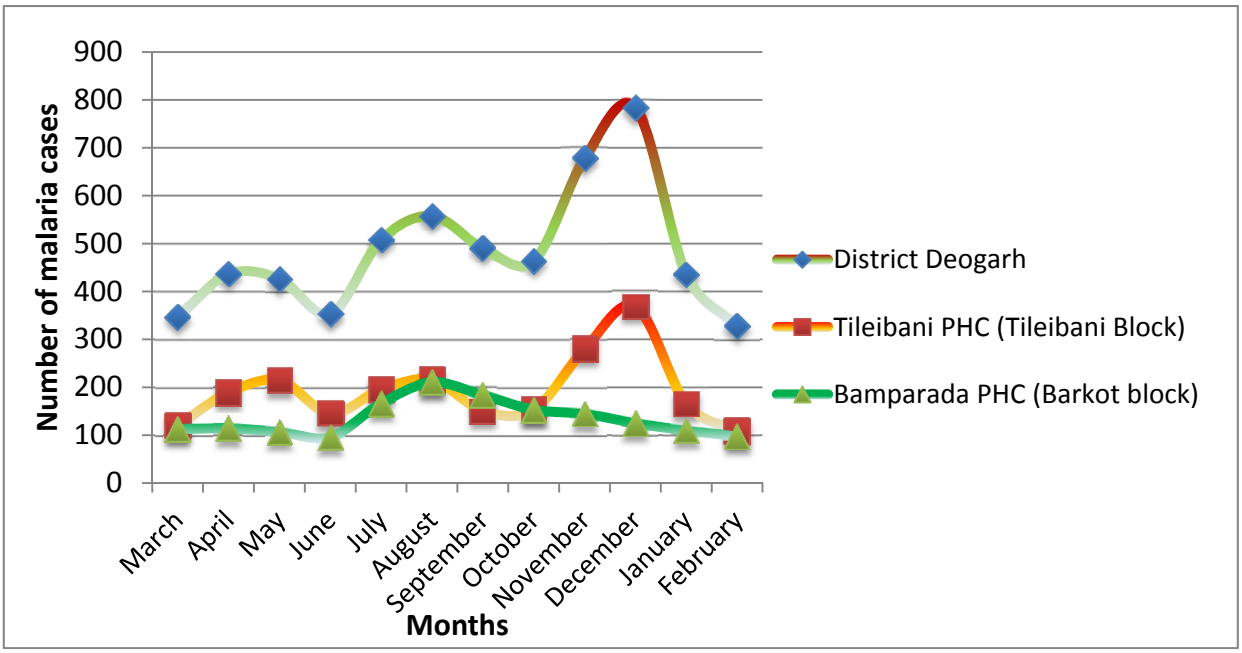


Figure 7: Seasonal pattern of malaria incidence in study areas

Since malaria transmission in the Deogarh district occurs almost throughout the year, *P. falciparum* and *P. vivax* samples were collected through active field collection during three different transmission seasons [pre-monsoon (February-March), monsoon (July-August) and post-monsoon (September-November)] in the years 2011 and 2012 (Table 1). *P. vivax* was only 8% of total positive malaria cases.

Table 1 (a): Malaria parasites in the samples detected by three methods

Detection method	Total examined	*P. falciparum*/*P. vivax* positive				Gametocyte carriers
		Total	Falciparum	Vivax	Vivax + Falciparum	
Microscopy	1000	180	169	19	8	30 (*Pf*) 5 (*Pv*)
RDT	1000	240	229	19	8	--
PCR	1000	240	229	20	9	--

Table 1 (b): Sampling detail of *P. falciparum* and *P. vivax* isolates in HF and RP ecotypes

Months	Feb.-Mar.		Jul.-Aug		Sep.& Nov.		Total		Grand total
Ecotype	Forest	Plain	Forest	Plain	Forest	Plain	Forest	Plain	
Transmission	High	Low	High	Low	High	Low	High	Low	
P. falciparum samples collected	20	24	29	40	69	47	118	111	229
P. vivax samples collected	0	2	3	4	5	6	8	12	20

Figure 8: Active collection of blood samples in a study village of RP ecotype

Individuals interested in free rapid malaria checkup and treatment due to feeling unwell or having a history of malaria were approached for participation in the present study. District health authorities (Chief District Medical Officer/ Chief Malaria Officer) were previously informed and permission was obtained for the study. The study was approved by the Human Ethics Committee of the National Institute of Malaria Research, New Delhi, India and written informed consents (in "*Odia"* language) were obtained from each adult participant and from parents/guardians of patients below 18 years of age. The consent form was read out by local volunteers/researcher whose "*Odia"* pronunciation was easily understandable to participants. The volunteers also signed the consent form as witnesses. Patients with complications and children less than one year old were excluded from the study. Fever was detected by measuring oral temperature with a thermometer. Individuals having fever at that time or a history of fever within one week were considered febrile and beyond one week as afebrile. Malaria-positive individuals were treated with appropriate antimalarials provided by district malaria authorities.

Individuals were finger-pricked and six drops of blood were collected from each patient. While three drops were used for diagnosis by (i) microscopic examination, preparation of thick and thin films and stained with Giemsa and (ii) rapid diagnostic test with bivalent kit [*"FalciVax"* rapid detection kits for *P. falciparum* and *P. vivax* infection (Zephyer Biomedicals, Goa)], the rest three drops were put on a Whatman filter paper (for DNA isolation in the lab). The blood samples in the slides and the filter paper (after dried) were brought to the laboratory in New Delhi for further analyses. In the lab, both the thick and thin films were stained with Giemsa and the results were matched with the observations on the rapid diagnostic test. Since diagnostic tests by PCR is considered to be highly sensitive (Johnston et al. 2006, Gupta et al. 2010), DNA was isolated from all the field collected samples using "Qiagen DNA mini kit" (Plowe et al. 1995). Considering high mixed parasite infections prevalent in India (Gupta et al. 2010), we used PCR diagnostic assay using published primers (Gupta et al. 2010) for determination of mono infection by *Pf* in the collected samples. Monoclonality of *Pf* infections was confirmed on the basis of determination of a single haplotype of each of the four genes conferring drug resistance (see below). In total, 1,000 samples have initially been collected from two ecological zones (354 from HF and 646 from RP); out of which only 229 were pure *Pf* infections (118 from HF and 111 from RP) were used for DNA sequencing of the four genes conferring drug resistance in *Pf* (Table 1).

2.2. PCR Amplification of the four drug-resistant genes

In this study, we have considered four genes (*Pfcrt*, *Pfmdr1*, *Pfdhfr,* and *Pfdhps*) that are reported to be associated with resistance to antimalarials, chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) in *Pf*. For all the four genes, the genetic regions containing SNPs that are associated with *in vitro* drug-resistance in *Pf* were only sequenced (for details of the genetic regions, primers for each gene fragment are given in Table 2).

Table 2: Primer sequence and protocol adopted for desired SNPs sequences

Gene/ Locus		Primer's sequence	Reference for primers and cycling conditions.	PCR amplification conditions (Temp in °C / Time in Seconds)					Variations in annealing temperature (°C)	PCR product size (bp)	Consensus sequence used	SNPs covered (Wild type amino acids with positions)
Pfcrt	Primary	ccgttaataataaat acaggc ttttaaaaatggaa gggtgta	Mallick et al.,2012	95/ 420	94/ 50	52/ 50	72/ 90	72/ 420	52,53, 54	264	72 bp (nucleotide positions 199-270)	C72V73M74 N75K76
				--40 cycles--								
	Nested	ggctcacgtttagg tgga tgaatttccctttttat ttccaaa		95/ 420	94/ 50	58.9/ 50	72/ 60	72/ 420	No			
				--40 cycles--								
Pfmdr1	Primary	atgggtaaagagc agaaaga aacgcaagtaatac ataaagtca	Tinto et al., 2003	95/ 420	94/ 30	58.8/ 30	72/ 90	72/ 420	No	702	388 bp (nucleotide positions 95-482)	N86
				--40 cycles--								
	Nested	tggtaacctcagta tcaaagaa ataaacctaaaaag gaactgg		95/ 420	94/ 30	55/ 45	72/ 90	72/ 420	No			
				--40 cycles--								
Pfdhfr	Primary	ccaacattttcaag attgatacataa acatcgctaacaga aataatttga		95/ 420	95/ 30	64/ 30	72/ 90	72/ 420	63, 64, 65	611	292 bp (nucleotide positions 87-378)	A16N51C59 S108I164
				--40 cycles--								
	Nested	gcgacgttttcgat atttatg gatactcattttcatt tatttctgga		95/ 420	95/ 30	52/ 30	72/ 90	72/ 420	51, 52, 53			
			Andriantsoan irina et al., 2009	--40 cycles--								
Pfdhps	Primary	ttgttgaacctaaac gtgctg ttgatccttgtctttc ctcatgt		95/ 420	95/ 30	57/ 30	72/ 90	72/ 420	No	600	537 bp (nucleotide positions 1304-1840)	S436A437K540 A581A613
				--40 cycles--								
	Nested	tttgaaatgataaat gaaggtgct tccaattgtgtgattt gtcca		95/ 420	95/ 30	57/ 30	72/ 60	72/ 420	No			
				--40 cycles--								

For this, nested PCR protocols have been followed (for details of PCR protocols for each individual gene, given in Table 2). For example, two different reaction volumes (15µl and 30µl) were used in primary and nested PCR. All PCR amplifications were performed by using "AmpliTaq Gold™" polymerase (PE Applied Bio Systems, Foster City, CA) and for visual quantification, only 3µl PCR products were used in 1.5% Agarose with 100bp DNA ladder (Bangalore Genei, Bengaluru, India).

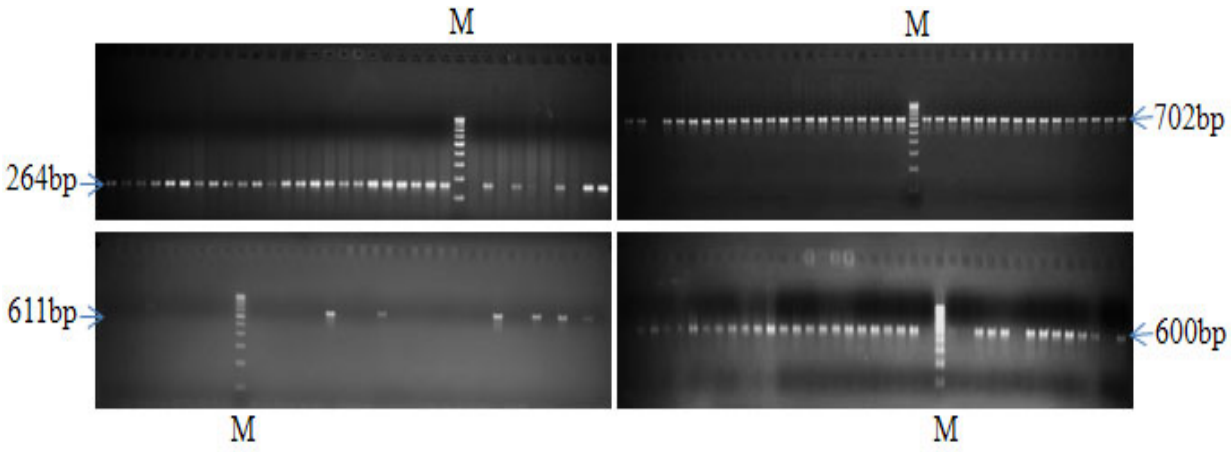


Figure 9: Visualisation of desired amplicon for qualitative and quantitative analysis

Four gel pictures presenting 264, 702, 611, and 600bp length amplicon corresponding to *Pfcrt*, *Pfmdr 1*, *Pfdhfr* and *Pfdhps* markers of *P. falciparum* analysed with 0.5 µg of Bangalore Genei 100 bp DNA ladder.

2.3. DNA Sequencing and population genetic analyses

All PCR products were purified using shrimp alkaline phosphatase (*SAP)* and exonuclease I (*Exo I)* enzymes (Fermentas, USA) before processing for DNA sequencing. For each 25µl PCR product, one unit of *Exo I* and one unit of *SAP* with 10X SAP buffer were used and final reaction volume was made up to 30.0 µl with autoclaved, nuclease-free water (Ambion, Life Technologies). The reaction mixtures were incubated in Eppendorf Master Cycler Pro gradient thermal cycler for 50 min at 37 °C (digestion) and then for 20 min at 85 °C (inactivation of enzymes). Purified PCR products were sequenced commercially (Macrogen Inc., Seoul, Korea, http://dna.macrogen.com/english).

Multiple DNA sequences from each gene were imported collectively to the DNADynamo computer program (Blue Tractor Software, North Wales, United Kingdom; (http://www.bluetractorsoftware.co.uk/) along with respective reference sequence of the wild type (*Pf*3D7) for viewing the sequence chromatogram, manual editing, and multiple sequence

alignment. The edited sequences were deposited in GenBank and DDBJ respectively with accessions KU923387-KU923572 and LC137682-LC137745.

Single nucleotide polymorphisms (SNPs) were spotted by scanning mismatch highlights from the split window of DNADynamo base-call alignment window and reconfirmed by referring aligned chromatograms in lower split window. Since all the four sequenced DNA fragments are located in the coding regions (exon) of the genes, the aligned sequences were translated to amino acid sequences and synonymous and non-synonymous mutations, if any, were spotted. Any change in the amino acid sequence was ascertained by looking at the responsible SNP in comparison to the standard reference sequence. Since specified SNPs in designated genetic regions have been implicated with drug-resistance phenotype of *Pf*, SNPs located in those specific regions were specifically looked at. For example, SNPs causing amino acid changes in the *Pfcrt*-$C_{72}V_{73}M_{74}N_{75}K_{76}$ (capital letters representing wild type amino acids (AA) with their positions in the gene subscripts), *Pfmdr1*-N_{86}, *Pfdhfr*-$A_{16}N_{51}C_{59}S_{108}I_{164,}$ and *Pfdhps*-$S_{436}A_{437}K_{540}A_{581}A_{613}$ were noted. Similarly, mutations in other regions of the sequenced DNA fragments of each gene and corresponding changes in amino acid were also noted (Figure 10).

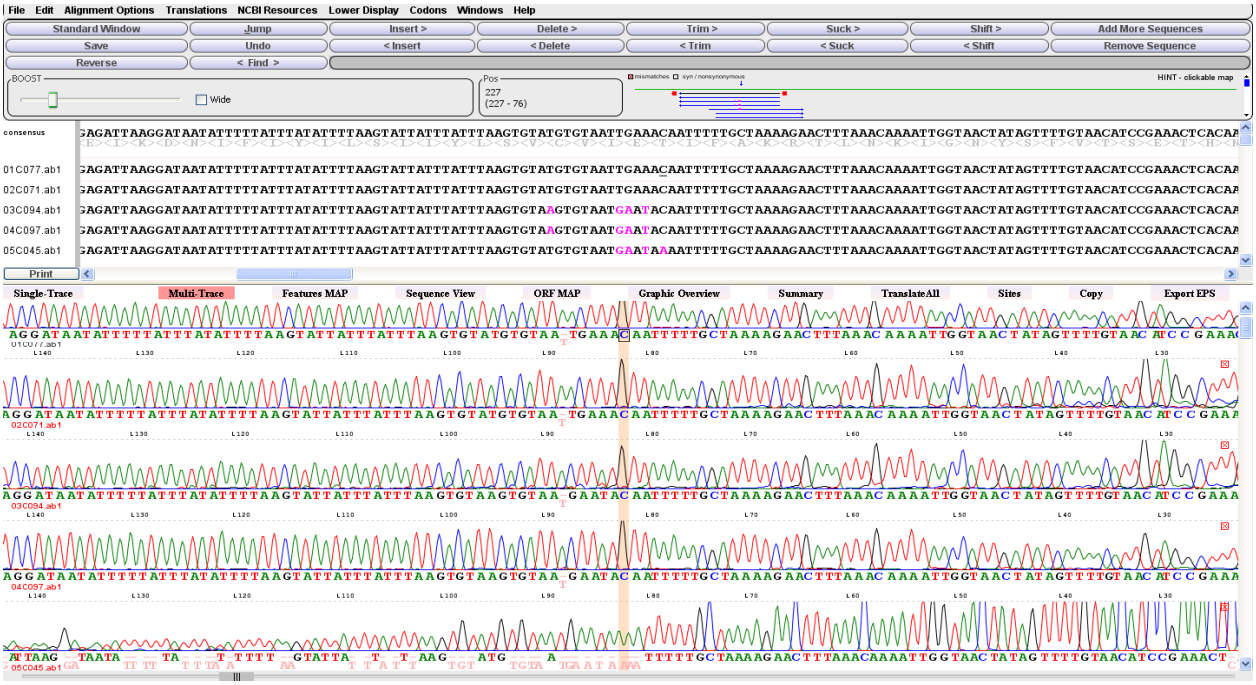


Figure 10: Aligned view of raw base call chromatograms of exon II of *Pfcrt* gene

Pfcrt gene's exon II sequence aligned and highlighted at nucleotide position 227 (amino acid position 76) in vertical middle.

Differential arrangements of SNPs for each gene in every *Pf* isolate form a particular haplotype. In order to know genetic differences between the *Pf* isolate collected from HF and RP ecotypes for each independent gene, first the frequencies of different haplotypes were calculated using the Statistical Package for Social Sciences 16.0 (SPSS, Inc., Chicago, IL, USA) for each ecotype separately. Further, in order to know if differential distribution of different haplotype in a particular gene exists between isolates from HF and RP ecotypes, chi-square tests were performed independently for each gene. In addition, haplotype diversity (Nei 1987) and two measures of nucleotide diversity (θ_w and π) were estimated for each of the *Pf* isolate from HF and RP ecotypes. While the nucleotide diversity parameter π is estimated based on average number of pair wise nucleotide difference per site, (Tajima 1989) the θ_w estimate is dependent on the number of segregating sites (Watterson 1975). All these parameters were estimated using the DnaSP 5.02 computer program (Librado and Rozas 2009). In order to ascertain if the four genes conferring drug resistance in *Pf* follow neutral equilibrium model of molecular evolution, the Tajima's *D* test was performed for each gene for the isolates from each ecotypes separately using the DnaSP 5.02 computer program (Librado and Rozas 2009). The Tajima's *D* (Tajima 1989) statistic calculates the normalized differences between the two measures of nucleotide diversity (θ_w and π) for isolates from each ecotype. Whereas an excess of low frequency polymorphism generates negative value of TD indicating directional selection or population size expansion, low level of high frequency polymorphism generates positive value indicating balancing selection or population size reduction (Das et al. 2004).

Moreover, in order to determine if differential association exists among SNPs segregating in all the four gene for isolates from a particular ecotype (either HF or RP), linkage disequilibrium (LD) tests were performed for each possible pair-wise SNP implicated as drug-resistant marker in the four genes by calculating the r^2 values using the Haploview computer program (Barrett 2005).

3. Results

Out of the total 1000 malaria positive blood samples collected (354 from HF and 646 from RP), only 229 isolates (118 from HF and 111 from RP) were found to be infected with *Pf* employing three different types of malaria diagnosis, *viz.* microscopy, RDT and PCR. PCR was found to be the most sensitive as one isolate which was detected as only *Pf* positive by both microscopy and RDT was found to be mixed positive for *Pf* and *Pv*. The microscopy only revealed 30 (*Pf*) 5 (*Pv*) gametocyte positivity. RDT was found to be more sensitive than microscopy and slightly less sensitive to identify *Pv* as RDT failed to identify *Pv* positivity in one mixed positive isolate for *Pf* and *Pv* [Table 1 (a)].

In addition, in order to ascertain if all the 229 isolates are monoclonal infections, sequence chromatograms of the four genes conferring drug resistance (*Pfcrt*, *Pfmdr1*, *Pfdhfr*, and *Pfdhps*) of all the 229 isolates were carefully checked for double peaks. For every gene, mono-clonality was established based on single peaks, resulting fractions of the 229 isolates independently in each of the genes (*Pfcrt*-64/229; *Pfmdr1*-73/229; *Pfdhfr*-29/229 and *Pfdhps*-84/229) to be monoclonal. The distribution of single clonal infections was however not found to be significantly different between the isolates from HF and RP ecotypes based on the data of single peak in the four genes (for HF; *Pfcrt*-34, *Pfmdr1*-36, *Pfdhfr*-13 and *Pfdhps*-41 and for RP; *Pfcrt*-30, *Pfmdr1*-37, *Pfdhfr*-16 and *Pfdhps*-43). In total, only 24 isolates (12 from HF and 12 from RP) were found to be monoclonal based on single peak of nucleotide chromatogram when all the four genes were considered.

All the mutations implicated in conferring resistance to different antimalarials in *Pf* were sequenced in multiple isolates from both HF and RP ecotypes. In addition, with multiple sequence alignment, additional mutations (some reported and some novel) were also detected. The distribution of different haplotypes (due to combinations of different mutations implicated in drug resistance in four genes) in both the HF and RP is depicted in Table 3.

Table 3: Frequency (%) of different drug resistant marker haplotypes in four genes implicated in drug resistance in *P. falciparum* in two ecotypes

Ecotypes	Genes							
	Pfcrt		*Pfmdr*		*Pfdhfr*		*Pfdhps*	
Hilly-Forest	$C_{72}V_{73}I_{74}E_{75}T_{76}$	25 (73.5%)	N_{86}	8 (22.2%)	$C_{59}S_{108}$	4 (30.8%)	$A_{436}G_{437}E_{540}A_{581}$	3 (7.3%)
	$C_{72}V_{73}M_{74}N_{75}K_{76}$	7 (20.6%)	Y_{86}	28 (77.8%)	$R_{59}N_{108}$	9 (69.2%)	$A_{436}G_{437}K_{540}A_{581}$	1 (2.4%)
	$S_{72}V_{73}M_{74}N_{75}T_{76}$	2 (5.9%)					$S_{436}A_{437}K_{540}A_{581}$	28 (68.3%)
							$S_{436}A_{437}K_{540}G_{581}$	1 (2.4%)
							$S_{436}G_{437}K_{540}A_{581}$	1 (2.4%)
							$S_{436}G_{437}K_{540}G_{581}$	7 (17.1%)
	Total	34		36		13		41
Riverine-Plain	$C_{72}V_{73}I_{74}E_{75}T_{76}$	15 (50.0%)	N_{86}	10 (27.0%)	$C_{59}S_{108}$	7 (43.8%)	$A_{436}G_{437}E_{540}A_{581}$	4 (9.3%)
	$C_{72}V_{73}M_{74}N_{75}K_{76}$	6 (20.0%)	Y_{86}	27 (73.0%)	$R_{59}N_{108}$	9 (56.3%)	$S_{436}A_{437}K_{540}A_{581}$	33 (76.7%)
	$S_{72}V_{73}M_{74}N_{75}T_{76}$	9 (30.0%)					$S_{436}A_{437}K_{540}G_{581}$	1 (2.3%)
							$S_{436}G_{437}K_{540}G_{581}$	5 (11.6%)
	Total	34		37		16		43

For *Pfcrt*: χ^2=6.808*, P=0.033; *Pfmdr*: χ^2=0.227, P=0.634; *Pfdhfr*: χ^2=0.513, P=0.474;

Pfdhps: χ^2=2.840, P=0.725

* Statistically significant at 0.05 level.

Whereas in three genes similar haplotypes (*Pfcrt* three haplotypes, in *Pfmdr1* two and on *Pfdhfr* two haplotypes) were found between the HF and RP ecotypes, in case *Pfdhps*, six haplotypes in HF and four in RP were detected (Table 3). Although the frequency of different haplotypes of the four genes was different between isolates from two ecotypes, but no marked difference could be seen (Table 3).

The distribution of the number of haplotypes were subjected to chi-square test for all the four genes between the isolates from HF and RP ecotypes, which indicate statistically significantly deviation from the expected distribution only in case of *Pfcrt* gene. Therefore, the data in general on the frequency distribution of different haplotypes that are associated with phenotype drug resistance in *Pf* indicate no significant difference between the isolates from two ecotypes (Figure 11).

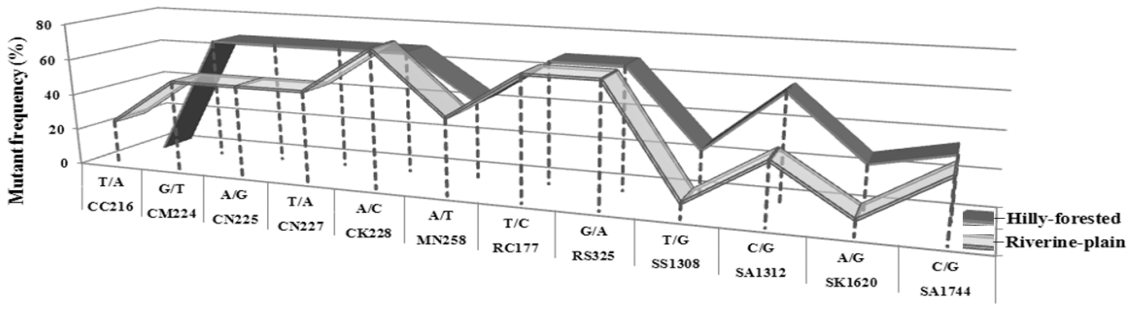


Figure 11: Nucleotide mutation pattern in *Pfcrt*, *Pfmdr 1*, *Pfdhfr*, and *Pfdhps* genes due to both CQ and SP drug pressure in RP and HF ecotypes

Interestingly, many other mutations (apart from the ones implicated in drug resistance) were found to be segregated in the two (*Pfmdr1* and *Pfdhps*) out of the four genes. Whereas the *Pfmdr1* gene harbours four mutations (one in HF and three in RP), only one mutation (C228T) was common between the isolates of two ecotypes and the rest two were confined to RP ecotype (Table 4).

Table 4: List of novel mutations (other than the mutations implicated in drug resistance) in the *Pfmdr1* and *Pfdhps* genes of *P. falciparum* in HF and RP ecotypes

Genes	Nucleotide mutations and resulting changes in amino acids (in parentheses)	
	Hilly Forest	**Riverine-plain**
Pfmdr 1	$C_{228}T$ ($S_{76}S$)	$C_{228}T$ ($S_{76}S$)
		$A_{269}G$ ($D_{90}G$)*$^{\$}$
		$T_{317}C$ ($F_{106}S$)$^{\$}$
	$T_{1632}G$ ($Y_{544}Z$)	$T_{1632}G$ ($Y_{544}Z$)
	$C_{1305}A$ ($S_{435}S$)**$^{\#}$	$C_{1378}T$ ($Q_{460}Z$)$^{\$}$
Pfdhps	$A_{1335}G$ ($K_{445}K$)$^{\#}$	$A_{1454}G$ ($N_{485}S$)$^{\$}$
	$T_{1373}C$ ($L_{458}S$)$^{\#}$	$A_{1508}G$ ($D_{503}G$)$^{\$}$
	$A_{1375}C$ ($F_{459}L$)$^{\#}$	$A_{1537}G$ ($I_{513}V$)$^{\$}$
	$A_{1426}I$ ($K_{476}Z$)$^{\#}$	$T_{1685}C$ ($L_{562}P$)$^{\$}$

No such novel mutations could be detected in the *Pfcrt* and *Pfdhfr* genes in the present study

*Reported in earlier studies *(Okombo et al. 2014) **(Kumar et al. 2015), $^{\#}$Unique to HF; $^{\$}$Unique to RP; 'Z' represents a stop codon.

While the A269G mutation (found in isolates from the RP ecotype) has been reported elsewhere, the other two mutations were novel (Table 4). On the other hand, the *Pfdhps* gene possessed as many as 11 mutations with one mutation common to the isolates from two

ecotypes and the rest were unique to an ecotype (five in HF and five in RP) (Table 4). Surprisingly, the mutation that is common in both the ecotypes (T1632Z) was found in multiple isolates (seven in each ecotype) and identified to be a stop codon. The rest 10 mutations were found in individual *Pf* isolates in two ecotypes of Deogarh district (Table 4).

In this study, Single Nucleotide Polymorphisms (SNPs) were identified by direct DNA sequencing of the DNA fragments in the four genes conferring drug resistance in *Pf*. In order to ascertain if genetic differentiation exists between the isolates from two ecotypes, we segregated the DNA sequences of the four genes according to ecotypes and performed different population genetic analyses of DNA sequence variation. In general, the *Pfdhps* gene was found to be highly polymorphic among the four genes (Table 5).

Table 5: Summary statistics of DNA sequence polymorphism in four different genes conferring drug resistance in *P. falciparum* in two ecotypes.

Ecotypes	Genes	No. of isolates	Number of haplotypes	Haplotype diversity	Nucleotide diversity		Test of neutrality
					π	θ	Tajima's *D*
Hilly-Forest	*Pfcrt*	34	3	0.426	0.02297	0.01698	0.93263
	Pfmdr 1	36	3	0.367	0.00106	0.00124	-0.28681
	Pfdhfr	13	3	0.5	0.0029	0.00221	1.89943
	Pfdhps	41	13	0.745	0.00307	0.00479	-1.08329
	Average			0.5095	0.0075	0.00630	
Riverine-Plain	*Pfcrt*	30	3	0.641	0.03218	0.01753	2.28081*
	Pfmdr 1	37	5	0.495	0.00146	0.00247	-0.9918
	Pfdhfr	16	2	0.525	0.0036	0.00206	1.89943
	Pfdhps	43	7	0.607	0.0026	0.00387	-0.94829
	Average			0.567	0.00996	0.00648	

* Statistically significant at 0.05 level.

This pattern was more pronounced in the HF than in the RP ecotype. The TD values were found to be variable across the genes and between the ecotypes. Surprisingly, for *Pfdhfr*, high and positive values of TD were observed in isolates from both the ecotypes (Table 5). However, in no case statistically significant deviation from neutral expectation could be observed. However, in case of the *Pfcrt* gene, positive value of TD with statistically significant

deviation from neutral model of molecular evolution could be observed in isolates from RP ecotype [Figure 12 (a) and (b)].

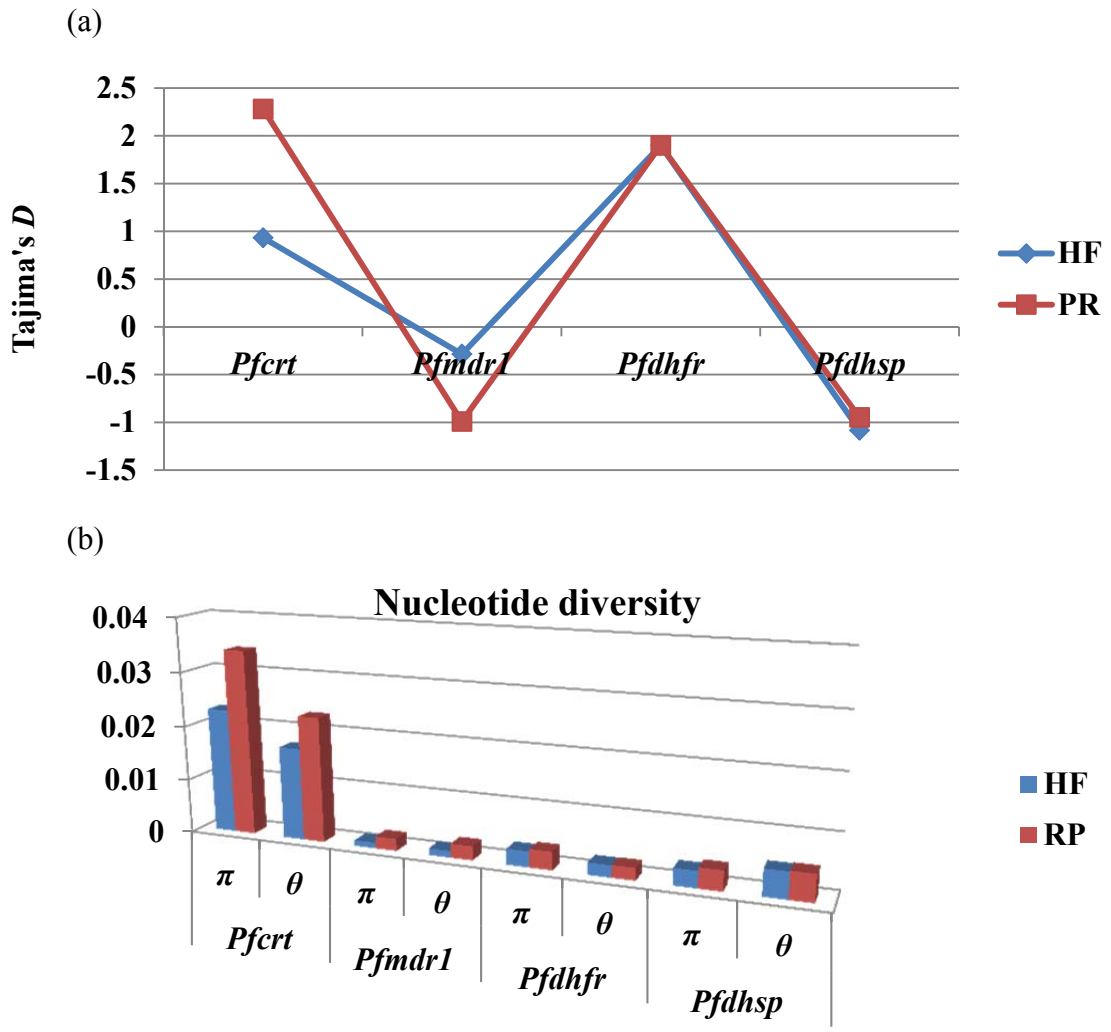
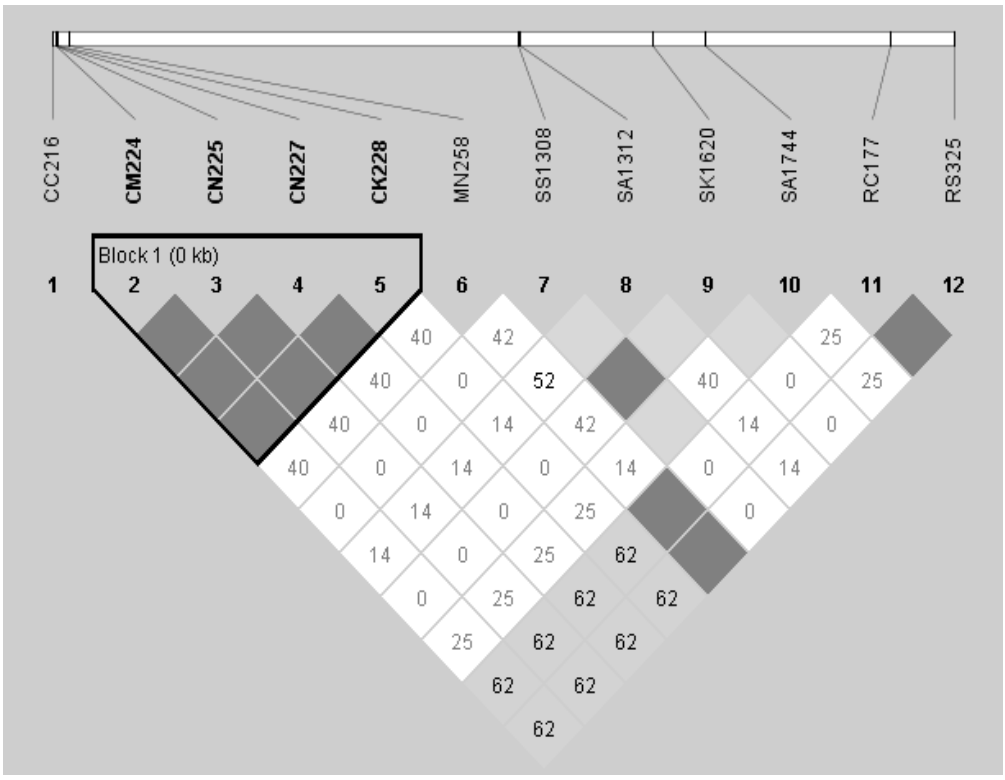


Figure 12: Deviation from neutral model of molecular evolution in isolates from HF and RP ecotypes in *Pfcrt*, *Pfmdr 1*, *Pfdhfr*, and *Pfdhps* genes [(a) Tajima's *D* graph and (b) Nucleotide diversity]

The data therefore suggest that DNA sequence polymorphisms do exist among different genes conferring drug resistance in *Pf* and between the isolates from two different ecotypes.

Finding of 12 isolates from each of the two ecotypes to be monoclonal in all the four genes and determination of 12 SNPs (variably present in four genes) to be segregating in the two ecotypes provides an opportunity to determine if any two SNPs either present in a single gene or in two different genes are associated with each other. For this, we performed LD test and determined the R^2 value of each SNP pair-wise association (Figure 13).

(a)


CC216
CM224
CN225
CN227
CK228
MN258
SS1308
SA1312
SK1620
SA1744
RC177
RS325
Block 1 (0 kb)
1 2 3 4 5 6 7 8 9 10 11 12
40 42 25
40 0 52 40 0 25
40 0 14 42 14 0
40 0 14 0 14 0 14
0 14 0 25 0
14 0 25 62
0 25 62 62
25 62 62
62 62
62

(b)

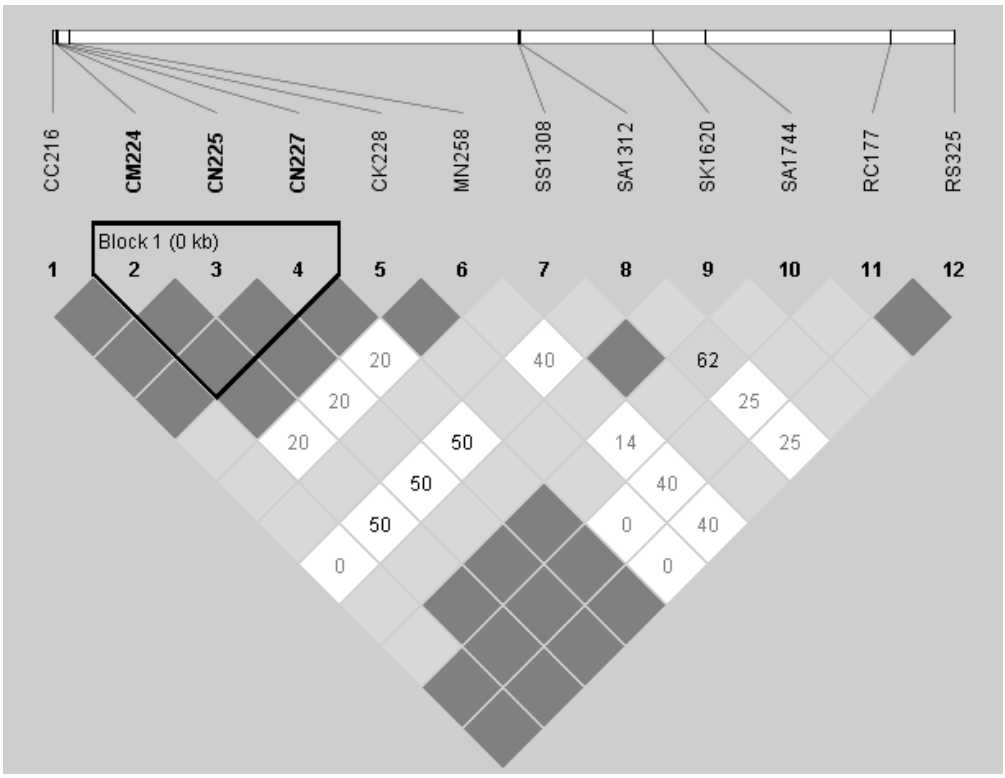

CC216
CM224
CN225
CN227
CK228
MN258
SS1308
SA1312
SK1620
SA1744
RC177
RS325
Block 1 (0 kb)
1 2 3 4 5 6 7 8 9 10 11 12
20 40 62
20 25
20 50 14 25
50 40
50 0 40
0 0

Figure 13: Linkage Disequilibrium (LD) between pairs of SNPs located in four different genes (*Pfcrt*, *Pfmdr 1*, *Pfdhfr* and *Pfdhps*) implicated in drug resistance in *P. falciparum* in (a) Hilly-Forest (HF) and (b) Riverine-Plain (RP)

In general, more number of statistically significant associations between SNP pairs was found in the isolates from RP in comparison to those from the HF ecotype. As expected, several statistically significant associations were found among SNPs located in the *Pfcrt* gene in isolates from both the ecotypes (Figure 13). Similarly, significant associations were found between SNPs located in both *Pfdhfr* and *Pfdhps* genes. Surprisingly, several cases of statistically significant associations were found between the SNPs present inside the *Pfcrt* gene and *Pfdhfr* gene and *Pfdhps* in isolates from RP ecotype. However, no such association could be detected in the isolates from HF ecotype. In contrast, in isolates from HF ecotype, the sole SNP of the *Pfmdr1* gene was significantly associated with both the SNPs of the *Pfdhfr* gene; and no such association was observed in the isolates from RP ecotype. The result on the whole thus indicate that while association of SNPs present in a particular gene is a common phenomenon in isolates from both the ecotypes, association of SNPs between different genes seems to be ecotype-specific.

District, Deogarh is among most malarious areas of India, so it has been under the Global Fund Supported Project "Intensified Malaria Control Project (IMCP)" which is being implemented from July 2005 hence, there is provision of Artemisinin Combination Therapy (ACT) for *Pf* cases. The questionnaire study of 4th chapter of this thesis revealed indiscriminate or injudicious usage of antimalarials by inhabitants of RP and HF ecotypes. Unfortunately, despite changes in drug policy from CQ to ASP by NVBDCP, a large proportion (44.5%) of the population surveyed (N=274) was found still using CQ in both the ecotypes to treat *Pf* malaria by local practitioners, quacks or self-medication, attributable to the easy availability and affordability of CQ. Between the ecotypes, use of CQ was found more among the choices of antimalarial in RP (54.4%) than in HF (39.1%). Only 4.4% of the total population surveyed, admitted contemporaneous consumption of antimalarial by themselves without any prescription attributing 6% and 2.6% of the HF and PR populations correspondingly.

4. Discussion

In district Deogarh intense malaria transmission occurs in the Hilly-Forest eco-epidemiological setting (HF), whereas the Riverine-Plain ecotype (RP) is moderately endemic for malaria. Therefore, the amount of total antimalarial consumption is manifold in HF as compared to that in RP. Despite drug policy change from CQ to ASP for *Pf* cases since 2005, the use of CQ has been found to be uncontrolled and high. ACT is being used as the first line drug for falciparum malaria since 2007, whereas prior to that SP was being used since 1982 (Sutar et al. 2013).

Considering malaria as a highly local and focal disease, local ecological conditions play vital role in malaria transmission (Rath 2004, Dash et al. 2008, Conn et al. 2015, Das 2015). To this extent, what extent the evolution and spread of drug resistance in micro ecological settings influence malaria epidemiological outcome is poorly understood. We herewith have considered differential prevalence, frequency, and evolutionary pattern of mutations in the four genes that are known to confer drug resistance in the most dreadful malaria parasite, *Pf* prevalent and predominant in two ecotypes (HF and RP). We have chosen these two ecotypes placed very closely (about 40 kilometres apart) to each other but with different ecological and topographical settings in a single district of Odisha state of India, which significantly contributes (about 26.9%) to malaria in India (Pradhan et al. 2016). Furthermore, it is reported that intense malaria transmission occurs in the HF, whereas the RP is moderately endemic for malaria (Kar et al. 2014, Pradhan et al. 2016). Since (i) resistance to different antimalarials is highly prevalent in India (and in Odisha), (ii) such conditions contribute considerably to malaria epidemiology, and (iii) genetic basis of drug resistance in malaria is widely established, we attempted to retrieve epidemiological information from population genetic studies of the genes conferring resistance and compared with ecological and other micro-variables between the two different ecotypes (HF and RP).

A general observation on the prevalence and distribution of different mutations associated with drug resistance in the four genes (*Pfcrt*, *Pfmdr1*, *Pfdhfr,* and *Pfdhps*) of *Pf* is the presence of all the mutations (that too in appreciable frequency) in samples from both the HF and RP ecotypes. However, some deviations exist; for example, *Pfdhps* gene harbours high diversity in both the ecotype and number of mutations and therefore the corresponding haplotypes.

All the four commonly reported amino acid mutations that confer resistance to sulfadoxine-pyrimethamine ($S_{436}A_{437}K_{540}A_{581}$) could be found in the present study, except one ($A_{613}S$) that is rare in India (Biswas et al. 2000, Kumar et al. 2015). The present findings therefore substantiate similar outcomes from other Indian states including Odisha with respect to the mutational pattern at the *Pfdhps* gene (D. Sharma et al. 2015). For the *Pfcrt* gene, three different haplotypes were found including the wild type (chloroquine sensitive) haplotype ($C_{72}V_{73}M_{74}N_{75}K_{76}$), which reconfirm its prevalence in Odisha. However, in other Indian states this haplotype is very rarely found (Mishra et al. 2006, Mixson-Hayden et al. 2010). Similarly, the $C_{72}V_{73}I_{74}E_{75}T_{76}$ haplotype is highly prevalent, and the frequency of the $S_{72}V_{73}M_{74}N_{75}T_{76}$ haplotype is comparatively low (Table 3), reconfirming previous observations from Odisha (Sutar et al. 2011, Sutar et al. 2013, Okombo et al. 2014, Ramani et al. 2016).

The skewed distributional prevalence of the $C_{72}V_{73}I_{74}E_{75}T_{76}$ haplotype is reflected by the observation of statistically significant χ^2 value (Table 3). To be noted that the $C_{72}V_{73}I_{74}E_{75}T_{76}$ type is known to confer higher resistance to CQ than the $S_{72}V_{73}M_{74}N_{75}T_{76}$ type (Mittra et al. 2006), suggesting high level of CQ resistance in *Pf* in Odisha (Ramani et al. 2016). The principal mutation conferring resistance to antimalarial at the *Pfmdr1* gene (Y_{86}) could be found in high frequency (73% in RP and 77.8% in HF; Table 3). Interestingly, not all the four amino acid substitutions ($I_{51}R_{59}N_{108}L_{164}$) of the *Pfdhfr* gene associated with drug resistance could be found in the present study, whereas, these have been reported from India including Odisha (D. Sharma et al. 2015). In the present study, we could find higher prevalence of the $R_{59}N_{108}$ combination (haplotype) in comparison to the $C_{59}S_{108}$ (drug sensitive type). These two mutations are considered to be highly dominant ones across many malaria endemic populations of the globe including India (D. Sharma et al. 2015) and the other two mutations ($I_{51}L_{164}$) are reported to be surfacing in India relatively recently (D. Sharma et al. 2015). Since it has been argued that *Pf* parasites with triple and quadruple mutations in the *Pfdhfr* gene are highly resistant to SP, and currently sulfadoxine-pyrimethamine is used in a combination therapy with Artemisinin, it can be noted that *Pf* isolates in Deogarh district of Odisha are less resistant to SP. However, recent finding on the prevalence of triple mutations [($I_{51}R_{59}N_{108}$) or ($R_{59}N_{108}L_{164}$)] in Odisha (D. Sharma et al. 2015) indicate that resistance to SP is emerging in this highly malaria endemic state.

The overall pattern of mutations associated with resistance to different antimalarials in *Pf* in Deogarh district of Odisha indicates that (i) haplotypes associated with drug resistance in all the four genes are prevalent in isolates from both HF and RP ecotypes, (ii) the *Pfdhps* gene harbours a comparatively larger number of haplotypes than other three genes and (iii) no significant differences could be observed for the patterns of mutations associated with drug resistance (and their corresponding haplotypes) between the HF and RP ecotypes.

Interestingly, only two (*Pfmdr1* and *Pfdhps*) out of the four genes harbour mutations other than the ones that are associated with drug resistance (Table 4). Based on the observed pattern of mutations in the *Pfcrt* gene in Indian *Pf*, it has been previously demonstrated that this gene is under massive genetic reconstruction (Das and Dash 2007, Chauhan et al. 2013). For the *Pfdhfr* gene, although we have sequenced a larger DNA fragment in comparison to the *Pfcrt* gene, occurrence of mutations other than the ones conferring to SP resistance is reported to be minimal (D. Sharma et al. 2015). For the *Pfmdr1* gene, three amino acid substitutions (other than the mutations associated with drug resistance) could be found; one common in isolates from both the HF and RP ecotypes, and two only confined to RP (Table 4). While the common one ($C_{228}T$) and the $T_{317}C$ (confined to RP) are entirely novel, the $A_{269}G$ (confined to RP) has been reported earlier in Kenya (Okombo et al. 2014). For the *Pfdhps* gene, as many as 11 nucleotide substitutions (resulting in two synonymous substitutions and nine non-synonymous, including three stop codons) could be found. Out of these two synonymous substitutions one has been recently reported from Odisha (Kumar et al. 2015). On the whole, the isolates from HF contain two stop codons; two synonymous and three non-synonymous substitutions and the RP isolates comprise two stop codons (one common with HF) and six non-synonymous substitutions. All the three non-synonymous mutations, five (out of six) non-synonymous substitutions and the three stop codons are novel and unique to the HF ecotype (Table 4); whereas with the $T_{544}Z$ non-synonymous substitution is common between the isolates from two ecotypes. Such an observation indicates that (i) pattern of mutations (other than the ones associated with drug resistance) in the four genes (*Pfcrt*, *Pfmdr1*, *Pfdhfr* and *Pfdhps*) are highly gene-specific, and (ii) no significant differentiation in the overall pattern could be observed between the isolates from HF and RP ecotypes.

In order to know if differential patterns of nucleotide diversity and signature of molecular evolution and association of commonly occurring SNPs in the four genes implicated in providing drug resistance in *Pf* exist between the isolates from HF and RP ecotypes, we have conducted population genetic analyses of DNA sequence data. As found in case number and prevalence of different mutations, the *Pfdhps* gene harbours the highest number of haplotypes (13 in HF and 7 in RP) as well as haplotype diversity among the other genes (Table 5). However, the *Pfcrt* gene displays the highest nucleotide diversity as measured by π (0.02297 in HF and 0.03218 in RP) among the four genes. High and positive TD values could be observed in case of the *Pfcrt* and *Pfdhfr* genes in both the ecotypes, with highest values of 2.28081 (*Pfcrt*-RP) (Table 5). This value is statistically significantly deviated from the expectation under neutral model of molecular evolution. Since positive TD values indicate evidence of balancing selection (Chauhan et al. 2014), it seems that alleles of both the *Pfcrt* and the *Pfdhfr* genes are maintained in stable frequencies. In case of *Pfdhps*, the TD values are high and negative in both the ecotypes, but not statistically significantly deviated from the neutral equilibrium model. This might indicate an initial genetic hitchhiking in the presence of large effective population size of *Pf* (due to high malaria transmission in Odisha) as observed earlier in *Pfmdr1* gene in Odisha (Chauhan et al. 2014) and in case of microsatellite polymorphisms in case of *Pfcrt* gene in India (Chauhan et al. 2013). To be noted that such evolutionary events (invoked by both natural selection and demography) might have created high haplotype diversity in the *Pfdhps* gene, as suggested by Chauhan et al. 2013.

The test of linkage disequilibrium between a pair of SNP yielded interesting results (Figure 13). As expected, all the four mutations in the *Pfcrt* gene were found to be strongly linked in *Pf* isolates sampled from both the HF and RP ecotypes. However, in the RP ecotype, all the four mutations of the *Pfcrt* gene were statistically significantly associated with mutations of the *Pfmdr1*, *Pfdhfr,* and *Pfdhps* genes. In the HF ecotype, however, significant LD was observed between SNPs of the *Pfmdr1* and *Pfdhfr* genes. The $N_{86}Y$ mutation is widely prevalent in almost all malaria endemic countries of the globe including India (Mita et al. 2009, Thomsen et al. 2013) and often found to be associated with mutations in the *Pfcrt* gene (Chauhan et al. 2014). Therefore, differential associations of mutations among the four genes implicated in drug resistance in the two ecotypes seem to be highly ecotype-specific. Different

ecological and topographical conditions prevailing in the two different ecotypes might have contributed to the observed differential association of SNPs present in different genes. However, distributional prevalence of different species of *Anopheles* (vectors to malaria parasites) (*An. fluviatilis* in HF and *An. culicifacies* in RP) might also have contributed to the observation (Nanda et al. 2000, Sharma et al. 2006, Sorosjinda-Nunthawarasilp and Bhumiratana 2014). It is known that individuals adjust their genomes through new genetic associations for adaptation to adverse eco-environmental conditions (Van Tyne et al. 2011). The presence of antimalarial resistance genotypes delays clearance of malaria parasites from individuals, which lengthens the period of malaria gametocyte seeding, ultimately acting as a reservoir, which consequently increases chances of malaria transmission. Such nomadic groups are known to play significant role in malaria transmission in areas of their prevalence (Service 1989, Erhart et al. 2005, Lokki et al. 2011). Furthermore, neglectful health seeking behaviour among tribal population of the HF group propagates drug resistance and high malaria transmission. High malaria episodes in the HF population reduce affordability and strength for care seeking and elevate resistance, which in turn confers high transmission in a cyclic process. The collective influence of these factors, along with a conducive HF environment fuels rapid evolution of antimalarial drug resistance and high malaria transmission vice versa. The frontward evolution centring HF was indicative. Whether the observed patterns of mutations and their evolution in the isolates from HF and RP ecotypes are propelled by adaptation by natural selection could not be established from the present study, but it could be ascertained that differential evolutionary forces (exerted by drug pressure in the field) are in operation at the four different genes implicated in drug resistance in the malaria parasite *Pf* in the Deogarh district of Odisha.

ASP combination therapy is safe and life-saving medicine for pregnant woman with malaria. After several years of ASP introduction and official withdrawal of CQ in the studied areas, it is still being used unrestricted. The high use of ASP and CQ resulted in a high accumulation of drug resistant genotypes in the case of both drugs. Manifold frequent usage of both antimalarials was apparent in the high transmission setting of HF. The linkage between CQ resistant markers with SP markers indicates the concurrent evolution of these two inter-loci antimalarial drug resistant genotypes in such high transmission areas with unrestricted use of

antimalarials. This increasing trend of CQ resistance obviates the possibility of re-introduction of CQ or similar drugs in place of SP in ACT.

In conclusion, the results of the present study, although limited to a single district of a state that contributes the highest number of cases and deaths due to malaria in India, provide many meaningful insights into the patterns of mutations in the four genes implicated in drug resistance in the malaria parasite, *Pf*. Although in many aspects, there were no significant differences between the two ecotypes (HF and RP), but some underlying differences could be noted. Considering the amount of genetic diversity associated with the intensity of malaria transmission in India (Chauhan et al. 2014, D. Sharma et al. 2015), the isolates from HF ecotype harbours more average genetic diversity (as estimated by nucleotide diversities, π and θ) than those from the RP. Further, the observed associations between pairs of SNPs present in four different genes could be due to different drug pressure applied in the field due to different treatment practices. The results from this population genetic studies indicate that CQ resistant genotypes are approaching fixation level and SP resistant genotypes are evolving very fast, it is therefore proposed that the existing drug policy needs to be reviewed, as SP is a partner drug administered with artemisinin. However, genetic studies of this kind in wide areas (*e.g.* the whole Odisha state) including different micro ecological settings with different malaria transmission patterns are needed before a conclusive decision on change in drug policy is made.

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CHAPTER IV

Assessment of behavioural responses of people of hilly-forest and riverine-plain eco-epidemiological regions against malaria intervention measures

1. Introduction

The question why malaria is disproportionately distributed in certain places and concentrated in the developing countries has been well debated and documented. Malaria is currently endemic in resource-limited countries and further predominant among the backward population. Rational implementation of control measures and better utilization of limited resources is very much necessary (Basu 1994, Sharma et al. 2000, Gallup and Sachs 2001, Sharma 2003). Policy-makers must pay attention to the basics of utilization of public as well as individual care to improve the campaign for operational service delivery to the beneficiaries (Smith et al. 2001, Williams et al. 2004, Paul et al. 2015, Watkins et al. 2015). It is reported that utilization of malaria control facilities is least among the backward populations (Sabin et al. 2010, Singh et al. 2013, Ravendra K. Sharma et al. 2015). The utilization of any malaria control intervention is solely dependent upon the access of people to the intervention. The care access is indirectly proportional to the distance, both physical (comprising travel time and cost of seeking treatment) and psychological (Noor et al. 2003, O'Meara et al. 2009, Sundararajan et al. 2013, Kar et al. 2014). The reasons behind under-utilization of the facilities are mainly due to wrong or inadequate knowledge, perception and cultural beliefs. It is also reported in many places that facilities are under-utilized or misused (Obrist et al. 2007, Eisele et al. 2011, Chaki et al. 2014). Efficient management of resources for standard malaria care warrants careful consideration of five dimensions viz., availability, accessibility, affordability, acceptability, and adequacy. These five dimensions influence awareness and perception which together influence treatment/care seeking practice and attitude of the people. The participation of the community in control interventions (practice) along with the existence of malaria infected population and suitable mosquitoes (vectors) together accelerate malaria transmission in the endemic settings (Figure 1).

Figure 1: Parameters influencing malaria control-related behavioural practices of population.

All the factors are overriding and influence each other

The burden of malaria negatively impacts economic condition which further reduces care seeking which ultimately increases malaria burden (Gallup and Sachs 2001, Amexo et al. 2004, Bates 2004, Vijayakumar et al. 2009).

In a given area, inhabitants' knowledge and practices related to malaria are important for the implementation of culturally appropriate, sustainable and effective interventions (Williams et al. 2002, Vijayakumar et al. 2009). The lack of availability, accessibility and adequacy is not always the obvious reason behind highly malarious regions. When high malaria incidences are reported from regions, regional authorities galvanize efforts on these

three parameters in such areas (Watkins et al. 2015). The limited resources can be better utilized provided that the information on behaviour, attitude and practices is generated / available at a regular interval to modify policy if necessary. Therefore a detailed study of community's adherence to drug regime, access to health care, use of bednets and facilitating indoor residual spray that largely influence malaria transmission has been carried out. In addition, behavioural heterogeneity in use of alternative malaria treatments and prevention practices were evaluated between the communities in HF and RP ecotypes. We assume that the combined assessment of response to all the malaria intervention access in these two contrasting eco-epidemiological settings in multidimensional analysis will shed light on the complete picture of malaria in typical rural settings of most malarious areas of India. This will enable to visualize ground realities of the highly vulnerable and disadvantaged populations and the loopholes in malaria control interventions. The covariate factors in the process of care, access and malaria transmission were analysed in three dimensions in this study viz.

1) Influence of geography and demography on access, awareness and perception.
2) Influence of access, awareness, and perception on practices and attitude.
3) Influence of practices and attitude on malaria transmission.

All the important factors studied together in two eco-epidemiologically different areas will help curb malaria by providing opportunities to remove obstacles to malaria control interventions.

1.1. Review of literature

Intense stable malaria is reported from tribal dominated areas of Odisha and neighbouring states. The tribal areas are rich in hills and forests (Kumar et al. 2007, Sahu et al. 2013, Ramar et al. 2014, Pradhan et al. 2016). The Deogarh district is one of the epicentres of high malaria endemicity (http://www.malariasite.com/tag/orissa/) (Pradhan et al. 2016). Two of its PHCs viz. Tileibani and Bamparada represent two distinct ecotypes, Hilly-forest (HF) and Riverine-Plain (RP) respectively (http://www.odishasampad.in/) and therefore can serve as a model to understand the influence of community's knowledge, attitude and perceptions in above mentioned eco-typical habitats on malaria epidemiology.

The health service hierarchy, health centres, organization and distribution of work up to the village level for specially curbing malaria in Odisha are dealt in a comprehensive study

(Vijayakumar et al. 2009). As per this study current malaria control strategies in Odisha consist of all three major malaria control interventions:

1. Diagnosis and treatment of clinical cases
2. Distribution and promotion of insecticide treated nets (ITNs) or long lasting insecticide-treated nets (LLINs) at subsidized rate
3. Insecticide residual spraying (IRS) in high malaria affected areas

The success of any malaria control intervention is mainly dependent upon the acceptance and utilization of intervention by the people (Malisa and Ndukai 2009). Awareness plays an important role besides perceptions, affordability and access in proper utilization of malaria control interventions (Muthwii et al. 2002, Nyamongo 2002, Malisa and Ndukai 2009). The diagnosis of malaria fever and other symptoms and assumption of malaria as a curable disease with allopathic medicines only help in enhancing acceptability of malaria care. Dissimilar perceptual experiences about malaria have been accounted among tribals globally. Certainly, the tribes of India and southwest Ethiopia believe that malaria is caused by the spirits, angry deities, black magic, or consider it a self-limiting fever (Dhillon and Kar 1963, Yadav et al. 2007). According to these workers, the reasons behind under-utilization of the available facilities also include misconception and cultural beliefs. For risk mitigation, interventions such as treatment, IRS and ITNs are to be backed by community's education on the link between mosquitoes and malaria (Malisa and Ndukai 2009). Inexpensive treatments with traditional medications, from quacks are opted to the highest degree in distant forest regions in rural Ethiopia (Deressa et al. 2008). Health seeking behaviour was directly related to culture, trust and the affordability of the health care in the forest regions (Adera 2003, Ribera and Hausmann-Muela 2011). According to Feyisetan et al. 1997 "health services may be underutilized and several health care instructions may be ineffective or ignored in traditional and transitional societies where people's ideas and behaviour patterns conflict with the knowledge being passed to them" (Feyisetan et al. 1997). More often than not, impoverishment is the next most important factor in approaching a distant healthcare facility just like illiteracy, superstitious notion, and cultural belief among the indigenous populations (Hossain et al. 2001, Onwujekwe et al. 2011, Kar et al. 2014).

The awareness about the availability of malaria treatment facilities and their cost and quality of treatment opens trial options of treatment for the natives (Barat et al. 2004). The knowledge of mosquito bite is precursor to influencing for bednet use, cooperation for IRS and self-protection measures to avoid mosquitoes at the household or workplace. Knowledge of mosquito breeding sites helps in breeding source reduction efforts (Stephens et al. 1995, Yasuoka et al. 2006). Awareness of peak malaria seasons makes individual cautious against malaria in those seasons (Alaii et al. 2003). If the community is not inclined towards regular use of preventive measures like ITNs in such a situation malaria can only be treated but not prevented (Malisa and Ndukai 2009). In Odisha, India it was reported that micro loan to purchase bednets increased bednet sale (Tarozzi et al. 2011) and the deprived populations do not possess a bednet mostly due to non-availability of low-cost nets in spite of the fact that they know the benefits of bednets and would want to use them (Elphick and Elphick 2003, Pulford et al. 2011). So not having bednets, the people cover their body and face with blankets, burn firewood and shrubs to ward off mosquitoes, and due to lack of low-priced modern medical facility they use indigenous amends (Konradsen et al. 1997, Esse et al. 2008). Surveillance is poor in distant regions (Moore et al. 2008) and Indiscriminate use of antimalarials not conforming to national guidelines on antimalarial use speeds up the evolution of antimalarial resistance (Talisuna et al. 2002, Hastings and Watkins 2005, Gosling et al. 2008, Sahu et al. 2015). All these above discussed factors are influenced by demographic and socio-cultural features of the inhabitants of an area (Ghebreyesus et al. 2000, Biran et al. 2007, Kar et al. 2014). Distances of houses from vector breeding habitats and vegetation indirectly influence malaria (Staedke et al. 2003, Kar et al. 2014). Zoo prophylaxis pulls malaria vectors towards domesticated animals (Kumari et al. 2009, Iwashita et al. 2014, Donnelly et al. 2015). Resettlements in malarious areas often fuels severe malaria (Castilla and Sawyer 1993, Woube 1997, Kar et al. 2014) and nomads exchange malaria through areas they traverse through (Service 1989, Bouma et al. 1996, Faulde et al. 2009). The nomads roam from place to place to explore pastures, seeking forest produce and trade with villagers, which exposes them to many malariogenic conditions (Pichainarong and Chaveepojnkamjorn 2004, Erhart et al. 2005, Lokki et al. 2011). Such a nomadic group also subsists through hunting and gathering in the woods of district Deogarh with very poor socioeconomic condition (Pati and Dash 2002, Nayak 2010). Malaria morbidity and asymptomatic malaria are often under reported among these groups (Erhart et al. 2005, Lokki et al. 2011).

2. Method

2.1. Study area

2.1.1. Study area and study period

In the present study, highly endemic hilly-forest (HF) villages from Tileibani block and moderately endemic Riverine-Plain (RP) villages in Barkot block were selected in district Deogarh, Odisha. The geography and environment of the selected areas have been described in Chapter 1. Surveys were carried out during March 2011 (the pre monsoon period), September 2011 (the post monsoon period), November 2011 (the winter period), and July 2012 (the monsoon period) from both the ecotypes.

2.1.2. Description of study sites

Surveys were carried out in 9 and 8 selected villages from typically HF and RP ecotypes respectively (Figure 2). The study sites of HF and RP were about 40 Km apart (http://www.odishasampad.in/). Both HF and RP population were socio-economically backward. The degree of remoteness and backwardness was relatively higher in HF than RP. The HF ecotype was sparsely populated with predominance of the scheduled tribes (ST) followed by the Scheduled Caste (SC), the other backward casts (OBC) and few general caste people. The RP ecotype on the other hand had predominance of SC followed by ST, OBC and few general caste people (http://www.odishasampad.in/). Detailed demography and malaria parasite prevalence in these selected study sites have been described in Chapters I, and III, respectively. Socioeconomic status of the populations of these study sites is described in later section of this chapter.

2.2. Collection of relevant data from district health department

The village wise malaria prevalence and treatment data were collected from Chief District Medical Officer (CDMO) [National Vector-Borne Disease Control Programme (NVBDCP) consultant reports] through proper channel during the field visits. Malaria transmission appeared perennial in both HF and RP and the intensity was much higher in the former ecotype which is hyper-endemic. The malaria transmission peaks after the monsoon season in HF ecotype whereas in the RP ecotype, it peaks during the monsoons. As per the annual PHC report, the average API of the HF ecotype was about 7 times higher than that of the RP ecotype in the last 5 years. The detailed ecotype wise history of malaria transmission pattern is presented in Chapter I.

Indoor residual spraying (IRS) of DDT is routinely carried out as the chief vector control measure. As per NVBDCP 2013 report of district Deogarh, The1st round of DDT 4% spray was done during June-July in HF and RP and second round of DDT 4% spray was restricted to HF and carried out during November-December. Since 2002, with the support of World Bank, the Enhanced Malaria Control Program (EMCP) introduced Insecticide Treated Nets (ITNs) in 100 most malaria endemic districts in India including Deogarh (Patil and Kumar 2011) and later since 2008 ITNs and Long Lasting Insecticidal Nets (LLINs) are distributed through state initiative (Pradhan et al. 2016) followed by Information Education and Communication (IEC) activities.

2.3. Sample size

2.3.1. Comprising HF and PR local inhabitants of study villages

The HF and RP areas were sampled for households having experience of episodes of malaria within 1 year. Sample sizes of both the zones were almost equal. Screening of representative households for the survey is tabulated in Table 1 (A) and (B) and schematically represented in Figure 2.

Out of total 17 villages in the two ecotypes i.e. 9 villages were in the HF ecotype (645 families having 3289 members) and 8 were in the RP ecotype (1350 families having 6226 members). These 17 villages therefore had a population of 9500 living in 1995 households, out of which only 274 representative households had recent malaria episodes and, thus, were included in the study. Thus, there were 138 and 136 representative respondents formally enrolled from HF and RP ecotypes respectively. Either head of the family or a member who makes decision for the family for treatment and preventive measures represented every household for answering the questionnaire. The average family size of the respondents was 5±2 and 6±2 in HF and RP respectively and the proportion of males and females in the family were almost equal.

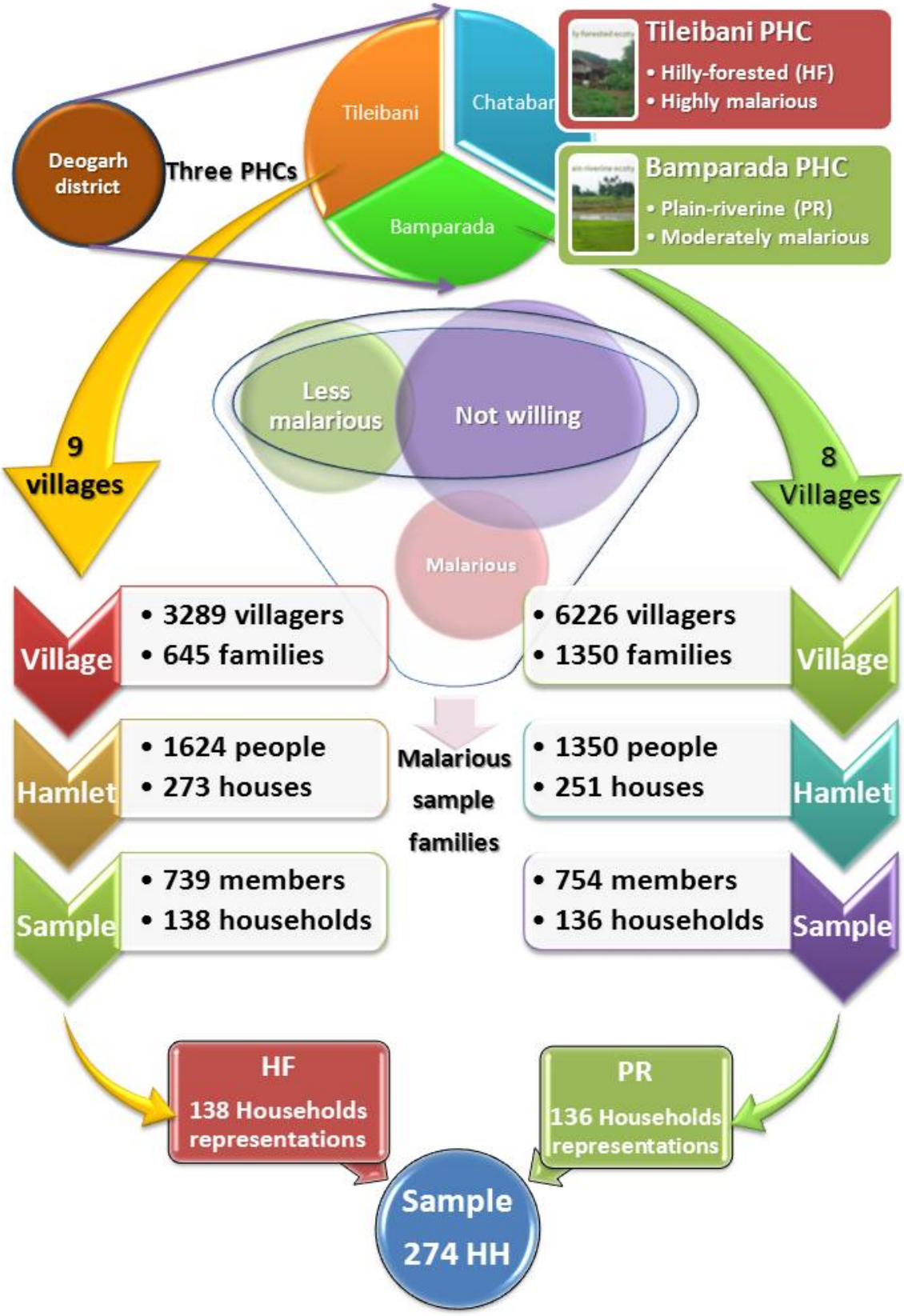

Figure 2: Representative sampling of surveyed population from HF and RP

Table 1: Profile of the study houses and Populations

(A) Demographic features of the HF and RP ecotypes

Hamlet description	Hilly-forest Ecotype Sum (%)	Riverine-Plain Ecotype Sum (%)	Total
Number of selected study villages	9	8	17
Number of people in the selected villages	3289 (34.6%)	6226 (65.4%)	9515
Total number of households in the villages	645 (32.3%)	1350 (67.7%)	1995
The sample households studied	138 (50.4%)	136 (49.6%)	274

(B) Family Profile of the selected households in HF and RP ecotypes

Family profile	Ecotype				Total
	Hilly-forest		Riverine-plain		
	Mean± Standard Deviation	Sum (%)	Mean± Standard Deviation	Sum (%)	
Number of males in the family	3±1	378 (50.4%)	3±1	371 (49.6%)	749
Number of females in the family	3±1	361 (50.4%)	3±1	383 (49.6%)	744
Total members in the family	5±2	739 (50.4%)	6±2	754 (49.6%)	1493

2.3.2. Nomadic families encountered in Hilly-Forest area

In the HF ecotype a nomadic group called 'Mankidia' was encountered camping in the outskirt of some study villages. Of this group one hundred responsible respondents from individual families were interviewed after their consent was obtained (59 and 41 during November 2011 and July 2012 surveys respectively). The interviewees represented 30, 38, and 14 temporarily camping families at the outskirts of villages 'Mankidiabasa', 'Kenduchappal' and 'Jhaliamara' respectively and 14 and 4 families were found in temporary shelters inside HF and RP study villages respectively.

Figure 3: Mankidias camping under trees in the outskirt of a village in district Deogarh

Figure 4: Temporary shelter of Mankidias called "Khumba"

'Mankidias' were observed living mostly in nuclear families, commonly five members per family. The surveyed population (421) comprised 213 males and 208 females.

2.4. KAP (Knowledge Attitude and Practices) data collection

The KAP survey consisted of interviewing inhabitants of HF and RP parts of district using a questionnaire. The questionnaire was prepared, pretested and used in this survey. The questionnaire was put to the head of the family which experienced episode(s) of malaria in the past one year. The survey was carried out in morning hours so that the head of families can be interviewed before leaving for work. Before interview, the participants were informed about the objectives of the study. After obtaining consent, questions were asked related to socio-demographic features of the household members, knowledge, and practices regarding malaria prevention and control, treatment-seeking behaviour and use of antimalarial drugs. Interviewers also completed an observational checklist at each of the households where people were interviewed. Both qualitative and quantitative data were collected.

Both quantitative and qualitative data from primary sources were edited (Checked for completeness) and coded. The data were entered directly into IBM-SPSS statistical software (Statistical Package for Social Sciences 16.0: SPSS, Inc., Chicago, IL, USA) twice and both the entry sheets were crosschecked for identities and discrepancies, and statistical analysis was done in the same software. Analyses of the responses related to the KAP aspects were presented in the following flowchart.

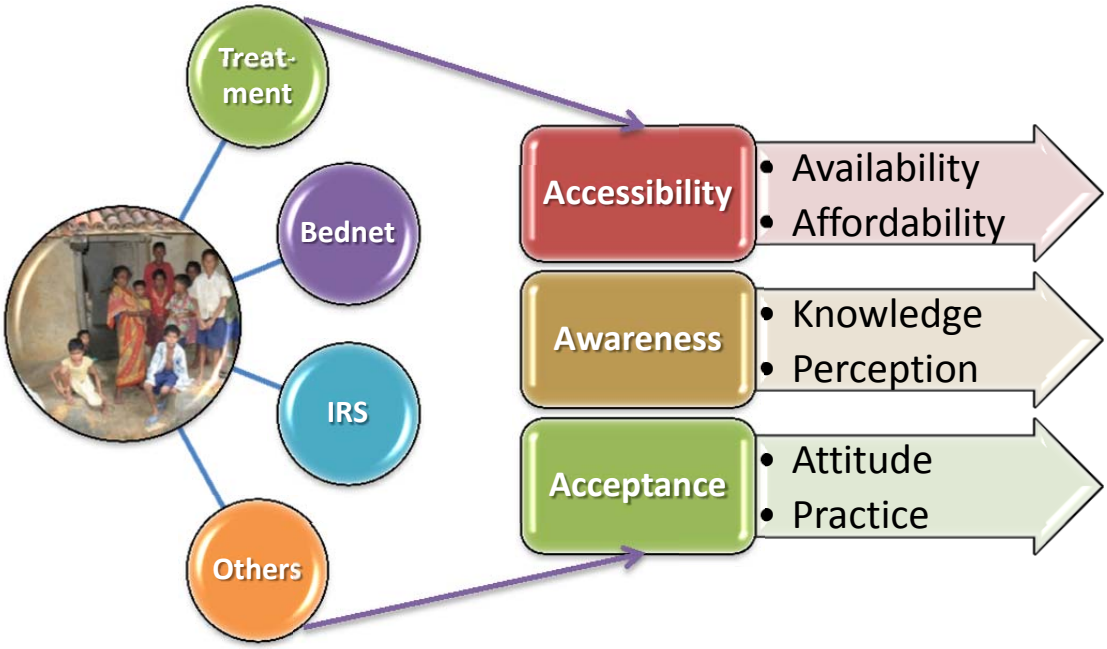

Fig. 5 Schematic flowchart of questionnaire based study (Knowledge Attitude and Practice)

In the questionnaire most of the aspects were covered related to malaria

Table 2: Questions regarding malaria related parameters concerning ecotypes

Aspect	Information sought/ Research Question 'code'
Demography	1. Total number of households in the village 'HouVil' 2. Sex of the respondent 'SexRes' 3. No of males 'Males' 4. No of females 'Femls' 5. Total members in household 'TotMem' 6. Caste/ tribe of the family 'Caste'
Household construction	7. Distance of households from each other 'Dis_H' 8. No of sleeping rooms 'SlpR' 9. No of veranda 'Vndh' 10. Separate store room 'StrR' 11. Kitchen in sleeping place 'Kitch' 12. Granary in sleeping room 'Gnry' 13. Closed wall sleeping place 'Closd' 14. No of windows 'WinNo' 15. Living place wall plaster 'Pls_H' shelter 16. Livestock wall plaster 'Pls_C' 17. Open space between wall and roof of living place 'Opn_H' 18. Open space between wall and roof of livestock shelter 'Opn_C' 19. Living place wall height 'Hit_H' 20. Livestock wall height 'Hit_C' 21. Living place roof type 'Roof' 22. Secure ventilation =Closed wall sleeping place 'Closd'+living place wall plaster covered 'Plaster'+ open space between wall and roof of living place≤1ft 'slit' 23. Fissure ventilation = Closed wall sleeping place+living place wall plastered+ open space between wall and roof of living place>1ft 24. Vulnerable ventilation = open space between wall and roof of living place=exposed or No closed wall sleeping place or no living place wall plastered 25. KACHHA=Non Cemented 'Pls_H' 26. PAKKA=Cemented 'Pls_H' 27. Kitchen in sleeping place 'Kitch'
Vector habitat	28. Stagnant clear water distance from household 'Dis_W' 29. Forest distance from household 'Dis_F' 30. Stream distance from household: 'Dis_S' 31. Cultivation land distance from household: 'Dis_C' 32. River distance from household: 'Dis_R'
Zooprophylaxis	33. Cows numbers 'Cows' 34. Cows distance from bed rooms 'Dist' 35. Bull numbers 'BullN' 36. Bull distance from bed rooms 'BullD' 37. Total number of cattle 'CatNo' 38. Goat numbers 'goatN' 39. Goat distance from bed rooms 'GoatD' 40. Pig numbers 'PigN' 41. Pig distance from bed rooms 'PigD'

	42. Hen numbers 'HenN' 43. Hen distance from bed rooms 'HenD' 44. No. Cattle 'CatNo'=No of cows+No of bulls 45. man/cattle ratio of household='TotMem'/'CatNo' 46. man/cattle ratio of village=sum of 'TotMem'/ sum 'CatNo' 47. No of livestock= No. Cattle+ No of goats+ pigs+ hens ('CatNo'+'HenN'+'goatN'+'PigN') 48. Strong zoo prophylaxis=low man/cattle ratio of household and village + less distance from the cattle 49. Weak zoo prophylaxis=High man/cattle ratio of household /village + more distance from the cattle
Income	50. Occupation / Living'Occu' 51. Labor place distance 'Dis_L' 52. Card holder 'Card' 53. Cultivation land possessed 'LndPos' 54. Cultivated land 'LndUsd' 55. No of times cultivated 'Times' 56. Average harvested 'AvgHar' 57. Extra labor type 'Ex_Lb' 58. No of labourers in the family 'No_Lb' 59. Average no of months laboured 'AvgMnt' 60. Average wage 'AvgWag' 61. Gathering fruits, flowers, leaf, mushroom and wood 'Gath' 62. Latex/ tree skin 'Latex' 63. How many members collect from forest/fields 'M_No' 64. Income from gathering 'GatInc' 65. Approximate annual income (Reported) 'Anul' 66. Per capita yearly family earnings= Estimated annual family income/ Total members in household 'TotMem' **Income category** **Despondent= Per capita yearly family earnings<8000** **Deficient= Per capita yearly family earnings<12000** **Competent = Per capita yearly family earnings>12000** 67. BPL (deficient/despondent)= reported BPL'Card'/Arnapurna card holder 'Card'/ Estimated annual income and reported annual income 'Anul'≤12000 68. APL (sufficient/competent)=reported APL 'Card'/ Estimated annual income or reported annual income'Anul'>12000
Malaria	69. Examined total cases 'ExaTot' 70. Member (s) with fever since 'Fever' 71. Fever positive found 'FevPos' 72. Asymptomatic *Pf* cases 'AsyPf' 73. Total malaria cases detected 'TotMal' 74. Number of *falciparum* positive individual 'PfPCR' 75. Falciparum (symptomatic only) 'PFsym' 76. Vivax (symptomatic only) 'PVsym' 77. Mixed (*falciparum* with *vivax* infection) 'MixPv'

Table 3: Questions regarding treatment seeking parameters concerning malaria

Aspect	Information sought/ Research Question 'code'
Availability	1. DHH distance from household 'D_DHH' near =0-25Km Medium Far =25-40Km High Very far=>40Km PHC/CHC/ Sub Centre distance from household 'D_PHC' Low Very near =<3001m Medium Near =3001-10000m High Far=>10Km Malaria test provider in the village 'MTesP' 2. Cost of test for malaria: 'CostT' 3. The result of malaria positivity test 'MPos' 4. Diagnosis method 'Methd' 5. Report generation time for malaria test 'RepTm' 6. Delay in report giving time 'Delay' 7. Number of antimalarials given for malaria 'NumMed' 8. Cost of the medicine 'CostMd' 9. Cost of the administration if any 'CostAd' 10. Cost of consultancy for malaria 'CostC' 11. Medicine provided by ASHA for Malaria 'AshaMd' 12. Free malaria treatment in vicinity of 3 Km 'TesFV' Available free malaria treatment in the village / within 3km radius =Malaria test provided in the village by ASHA 'MTesP'/distance of DHH 'D_DHH'/PHC/CHC 'D_PHC'≤3km +Free test 'CostT'+ free medicine 'CostMd' Non availability of free malaria treatment in the village / within the vicinity of the 3km radius=No malaria test provided in the village 'MTesP'/distance of DHH/PHC/CHC 'D_PHC' >3km/paid test 'CostT'/paid medicine'CostMd'
Accessibility	13. Distance travelled by walk for treatment 'Walked' Easy=≤2km Difficult=>2km 14. Distance travelled by vehicle for treatment 'ByVehi' Very expedient =<10km: Expedient =10-40 Km Inexpedient =>40 km 15. Pitch road distance 'D_Rod' expedient =≤2km Difficult=>2km Very difficult=>4Km 16. Road type to the village 'RodTy' Easy =concrete Tough=others 17. Someone took the patient for treatment 'TookU' Easy =somebody Difficult=nobody 18. Vehicle ownership 'OwnVeh'

	Easy =any vehicle Difficult=no 19. Out of pocket expenditure on going for treatment Fare cost to visit care. 'CostGo' Convenient =≤Rs.50 Inconvenient=> Rs.50 20. Telephone convenience 'TeliFs' Easy =yes Difficult=no 21. Access to private taxi/ ambulance facility 'CalCab' Easy =yes Difficult=no 22. The approximate cost of hiring taxi / ambulance 'CostHi' Easy =≤ Rs.200 Difficult=> Rs.200 23. Accessibility= Convenience = Convenient transportation Difficult to access Govt. Health Centre= Sum of above Difficulties ≥ 4 Ease to access Govt. Health Centre= Sum of above Difficulties <4
Awareness	24. Awareness about cause of malaria 'HowMal' Causative of malaria Apt ans=mosquito bite/female anopheles bite Inapt ans=rest 25. Awareness about cause of malaria 'MalSym' Apt ans=including fever/ blood check=rest Inapt ans=other signs of malaria recognition "Marks" 26. Awareness about malarious month in the village 'MalMon' Apt ans=winter/rainy Inapt ans=others 27. Does the doctor warn you to complete the malaria medicine course? 'WrnCom' 28. Positive awareness of existing fever in family
Perception	29. Perception that malaria is curable with medicines. 'CurMed' 30. Perception that malaria is self-limiting and can be cured without treatment. 'SlfLim' 31. Reason of preferring the test provider 'RsnWy' 32. Reason of preferring the treatment provider 'ConWhy' 33. Reason of not preferring going to ASHA / Anganbari? 'NotASH' 34. Reason of not preferring going to PHC/CHC/DHH 'NotPHC'
Practices	35. Provider of last fever diagnosis 'DiaBy' 36. Self-medication 'SlfMed' 37. No of days suffered from fever before seeking treatment 'DaySuf' 38. Severity of suffering when treatment was pursued 'Sevrty'
Attitude	39. Taking complete course of antimalarials 'DoUcom' 40. Time since onset of fever 'Fever' 41. Interested in a free blood test for malaria without an ailment 'TesInt'

Table 4: Questions regarding bednet use parameters

Aspect	Information sought/ Research Question 'code'
Availability	1. Number of free/Subsidized treated nets'Fre_Pd' 2. Subsidized cost of treated net 'SubCos' 3. Money paid for untreated nets 'UnNcos' 4. Size of bednet (double/single bed net) 'SizNet' 5. Physical condition of the bednet 'ConNet' 6. Number of untreated mosquito nets 'NetsUn' 7. Maximum number of nets available 8. Usefulness of the nets against mosquitoes'AblNet' 9. Number of mosquitoes found inside the nets during survey by the researcher 'VecFnd' 10. Protection adequacy by bednet 'SufNet'
Accessibility	11. Pitch road distance 'D_Rod' 12. Type of road to the village 'RodTy'
Awareness	13. Awareness about cause of malaria 'HowMal' 14. Awareness about symptoms of malaria 'MalSym' 15. Awareness about malarious months in their villages 'MalMon'
Perception	16. Prefer to use/not use mosquito nets 'LikNet'
Practices	17. Members slept under mosquito-net last night 'MemUsd' 18. Preferred months of sleeping under bednets 'MonNet' 19. Frequency of guests spending nights in the house 'GstNit' 20. Frequency of family members spending nights outside the house 'MemOut' 21. No of persons sleeping in closed place 'defined above' 22. Frequency of washing bednets 'WashNt' 23. Wash nets with cold/hot water 'HotWsh'
Attitude	24. Interest to buy mosquito nets 'MorNet'

Table 5: Questions regarding facilitating IRS parameters

Aspect	Information sought/ Research Question 'code'
Availability	1. Number of rounds of IRS in a year 'InsSpr' 2. Places of household sprayed 'PlsSpr' 3. Uniform spray 'UniSpr'
Knowledge	4. Instruction not to re-plaster after spray 'NotPlas'
Perception	5. Reason for not allowing IRS in certain places 'WhyNot'
Practices	6. Did you allow all places to be sprayed 'AlwSpr' 7. Do you remove all materials from walls to facilitate spray? 'RemMet' 8. Remove all livestock to facilitate spray 'MovCtl' 9. Frequency of plastering over the walls 'PstOfn'
Attitude	10. Facilitate insecticide spray operation 'PstTim'

Table 6: Questions regarding self-protection practices concerning malaria

Aspect	Information sought/ Research Question 'code'
Knowledge	1. Knowledge of mosquito breeding sites 'breed'
Practices	2. Practice to avoid mosquitoes in the living place 'AvoidH' 3. Number of persons sleeps in verandah 'Slp_V' 4. Distance between cooking and sleeping places 'Kitch' 5. Place of night halt done outside home 'NitAt' 6. No of persons doing night outs 'NitNo' 7. Place visited for taking animals for grazing 'Grz_F' 8. Time of visiting forest for taking animals for grazing 'Gz_Tm' 9. Protection against mosquito during work 'Prot' 10. Practice of collection from forest/ fields 'C_frm' 11. Months of collection from the forest/fields 'Mont' 12. Time of collected from the forest/fields 'Dur' 13. Vulnarability of family members to vector bite while sleeping 'Vn_Sl' 14. Self-protective practices 'SelfProt' COMPUTE 'SelfProt=Vulnarability*TimForest* During * Collected * Protection *Forstime*Forseason* Grazing * Out * Night * Cooking * Avoid. Good=Good self-protective practices against malaria=avoid mosquito in the living place'AvoidH'+No night out 'NitAt'+ No forest visit 'F_Tim'/Protection taken against mosquito during work'Prot' Average=Typical self-protective practices against malaria= avoid mosquito in living place/ No night out / No forest visit/Protection taken against mosquito during work / take presumptive medicine 'PreMed' (practicing any 2) Poor=Malariogenic practices= do nothing to avoid mosquito in living place/ night out / forest visit/Protection hasn't taken against mosquito during work (practicing >1)
Attitude	1. Attitude to take any presumptive medicine for malaria 'PreMed' 2. Attitude to drain or fill stagnant water bodies to control malaria 'DrnFil' Enthusiastic attitude for community-protection =Drain and fill water bodies 'DrnFil' Half-hearted attitude of community-protection = Don't Drain or fill water bodies 'DrnFil'

2.5. Data analysis

The responses were analysed for (1) Awareness about malaria, causes, and symptoms, (2) Health seeking behaviour, (3) Personal protection measures against malaria, (4) Occupation and economic status, (5) Response to malaria control activity. Chi-square test was used to compare the proportions and the results were considered significant at rejection of null hypothesis to less than 5% ($P < 0.05$). The questionnaire consisted of both closed and open-ended questions. For open-ended questions, equivalent narrations were pooled into different categories during analysis. Each question was analysed individually. Statistical relationships were sought between HF and RP and selected socio-demographic conditions and practices of the respondents. Results were recorded as frequencies, chi-square, degree of freedom (df) and significance (P) p-values.

Many required parameters were derived from the raw data by logical calculations using recode and compute option of the SPSS. The questions, the 'short name', and derived parameters are listed in the Table 2 to 6.

Note: Values within a set of comparison not "a" as subscript are significantly different at p< .05 in the two-sided test of equality for column proportions. Cells with no subscript are not included in the test. Tests assume equal variances 0.3.
[1]. This category was not used in comparisons because the sum of case weights is less than two.
[2]. This category was not used in comparisons because its column proportion is equal to zero or one.
3. Tests were adjusted for all pairwise comparisons within a set of each innermost subtable using the Bonferroni correction.
*. The Chi-square statistic is significant at the .05 level (the finding has a five percent (.05) chance of not being true, which means a 95% chance of being true).

3. Results

3.1. Demographic characteristics of the household study

3.1.1. Background profile

The percentage of male respondents was high (69%) than the female respondents in both the ecotypes (Table 7). The high proportion of males is attributed to the fact that most of the households had males as heads of the family. ST followed by OBC were dominant castes in Hilly-Forest (HF) ecotype, whereas, SC followed by OBC was dominant castes in Riverine-Plain (RP) ecotype (Table 7). Malaria is largely considered as disease of the poor. Economic status of both the ecotypes was thus, surveyed (summarized in Table 8). However, no differences were found in the economic status of both the ecotypes. More proportion of people residing in the HF ecotype owned lands as compared with the people residing in the RP ecotype (Table 7). Literacy is another important factor that indirectly influences the malaria status of a region. However, the literacy rate was low and similar in both the settings (Table 8).

Table 7: Sex, caste, landowning, and nativity ratio of the respondents in HF and RP

		Households frequency		
		Hilly-forest	**Riverine-plain**	**Total**
Sex of the respondent: χ^2=.177, *df*=1, *p*=.732	Male	97_a (70.3%)	93_a (68.4%)	190 (69.3%)
	Female	41_a (29.7%)	43_a (31.6%)	84 (30.7%)
	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**
Caste/ tribe of the family: χ^2=87.001, *df*=4, *p*=.000*,b,c.	ST	79_a (57.2%)	18_a (13.2%)	97 (35.4%)
	SC	12_b (8.7%)	69_b (50.7%)	81 (29.6%)
	OBC	43_c (31.2%)	38_c (27.9%)	81 (29.6%)
	Gen	$2_{b,c}$ (1.4%)	$11_{b,c}$ (8.1%)	13 (4.7%)
	ST-Christian	2^1 (1.4%)	0^1 (0.0%)	2 (0.7%)
	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**
Landowning status: χ^2=50.254, *df*=1, *p*=.000*	Landless	36_a (26.9%)	94_a (70.1%)	130 (48.5%)
	Landed	98_b (73.1%)	40_b (29.9%)	138 (51.5%)
	Total	**134 (100%)**	**134 (100%)**	**268 (100%)**
Reason of displacement: χ^2=7.961, *df*=1, *p*=.005*	Government dam project	34_a (64.2%)	44_a (88.0%)	78 (75.7%)
	Occupational or	19_b (35.8%)	6_b (12.0%)	25 (24.3%)

	agricultural opportunity		
	Total	**53 (100%)**	**50 (100%)**	**103 (100%)**
Nativity status: χ^2=10.882, *df*=2, *p*=.004*	Native	120_a (87.0%)	103_a (75.7%)	223 (81.4%)
	Resettled	9_b (6.5%)	27_b (19.9%)	36 (13.1%)
	Not revealed	$9_{a,b}$ (6.5%)	$6_{a,b}$ (4.4%)	15 (5.5%)
	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**

Table 8: Occupation and literacy rate of the respondents in HF and RP settings

		Ecotype					
		Hilly-forest		**Riverine-plain**		**Total**	
Occupation: χ^2=91.392, *df*=4, *p*=.000*[b]	Farming	121_a	(89.6%)	51_a	(37.8%)	172	(63.7%)
	Fishing	0[1]	(0.0%)	54[1]	(40.0%)	54	(20.0%)
	Any job	$4_{a,b}$	(3.0%)	$4_{a,b}$	(3.0%)	8	(3.0%)
	Daily labour	10_b	(7.4%)	21_b	(15.6%)	31	(11.5%)
	Miscellaneous	0[1]	(0.0%)	5[1]	(3.7%)	5	(1.9%)
	Total	**135**	**(100%)**	**135**	**(100%)**	**270**	**(100%)**
Per capita income category: χ^2=.571, *df*=2, *p*=.752	Despondent <15	40_a	(29.6%)	42_a	(31.8%)	82	(30.7%)
	Deficient <15-30	70_a	(51.9%)	70_a	(53.0%)	140	(52.4%)
	Competent >30	25_a	(18.5%)	20_a	(15.2%)	45	(16.9%)
	Total	**135**	**(100%)**	**132**	**(100%)**	**267**	**(100%)**
Literacy: χ^2=3.788, *df*=3,*p*=.285	Can write	29_a	(21.0%)	27_a	(19.9%)	56	(20.4%)
	Can read only	13_a	(9.4%)	6_a	(4.4%)	19	(6.9%)
	Can sign only	23_a	(16.7%)	19_a	(14.0%)	42	(15.3%)
	Illiterate	73_a	(52.9%)	84_a	(61.8%)	157	(57.3%)
	Total	**138**	**(100%)**	**136**	**(100%)**	**274**	**(100%)**

3.1.2. Zoo prophylaxis parameters between HF and RP ecotypes

Different malaria vectors have varying affinity for animal or human blood, known as zoophagic or anthropophagic index respectively. The main vectors in the North-West Odisha region are *Anopheles fluviatilis* and *An. culicifacies*. Former is mainly anthropophilic (prefers to feed on humans) while latter is zoophilic (prefers to feed on animals than human beings) and thus, man: cattle ratio is another important determinant of the epidemiological status of malaria in the region. The man cattle ratio was insignificantly higher in riverine-plain ecotype than Hilly-forest ecotype (Table 9). More than half of the households surveyed were found without cattle in both the ecotypes (59.4% and 53.7% respectively in HF and RP). Among families with cattle, there was significantly high proportion (16.9%) of RP families with low cattle number (i.e. more than double man/cattle proportion) than in HF families (4.3%). On the other hand less number of families was having high cattle proportion in RP (29.4%) than HF (36.2%) (Table 9). Man/cattle proportion is calculated by adding number of cows, bulls and cattle and then dividing by total family members. These animals were incidentally kept in considerably distant places away from the sleeping area in significantly high proportion in the surveyed malarious households in both HF and RP ecotypes. The houses in HF ecotype were in close vicinity to each other, whereas in Riverine-plain areas 32 houses (23.5%) were constructed far from the hamlet. The housing pattern and construction details are summarized in Table 10. Closely located houses of highly malarious HF ecotype seem to be indicative of more transmission due to higher chances of an infective mosquito reaching other houses and biting the inhabitants.

Table 9: Zoo prophylaxis parameters of the households surveyed in HF and RP

		Ecotype					
		Hilly-forest		**Riverine-plain**		**Total**	
Man/Cattle proportion: χ^2=11.585, *df*=2, p=.003*	Lowest thru 2 (>0-2)	50_a	(36.2%)	40_a	(29.4%)	90	(32.8%)
	High >double	$\mathbf{6_b}$	(4.3%)	$\mathbf{23_b}$	(16.9%)	29	(10.6%)
	No cattle	82_a	(59.4%)	73_a	(53.7%)	155	(56.6%)
	Total	138	(100%)	136	(100%)	274	(100%)

Table 10: Household distribution and differences in architecture of the rural inhabitations in HF and RP ecotypes

		Ecotype					
		Hilly-forest		**Riverine-plain**		**Total**	
Distance of households from each other (Far/Less): χ^2=33.627, *df*=1,*p*=.000*	In close vicinity	137_a	(99.3%)	104_a	(76.5%)	241	(88.0%)
	Far	**1_b**	(0.7%)	**32_b**	(23.5%)	33	(12.0%)
	Total	**138**	**(100%)**	**136**	**(100%)**	**274**	**(100%)**
Cooking place/ kitchen location: χ^2=2.958, *df*=2, *p*=.228	Separate kitchen	15_a	(10.9%)	24_a	(17.6%)	39	(14.2%)
	In sleeping place	89_a	(64.5%)	85_a	(62.5%)	174	(63.5%)
	In veranda	34_a	(24.6%)	27_a	(19.9%)	61	(22.3%)
	Total	**138**	**(100%)**	**136**	**(100%)**	**274**	**(100%)**
Slit between roof and walls: χ^2=.009, *df*=1, *p*=.922	≤1 ft.	112_a	(81.2%)	111_a	(81.6%)	223	(81.4%)
	≥1 ft.	26_a	(18.8%)	25_a	(18.4%)	51	(18.6%)
	Total	**138**	**(100%)**	**136**	**(100%)**	**274**	**(100%)**
Vulnerability to mosquito bite: χ^2=.526, *df*=1, *p*=.468	Fortified	72_a	(52.2%)	65_a	(47.8%)	137	(50.0%)
	Vulnerable	66_a	(47.8%)	71_a	(52.2%)	137	(50.0%)
	Total	**138**	**(100%)**	**136**	**(100%)**	**274**	**(100%)**

3.1.3. Topographical factors with reference to malaria in two ecotypes

Forest ecotype consists of hills with perennial streams. Streams are considered important breeding sites for malaria vector *Anopheles fluviatilis* (Nanda et al. 2000). In addition, stagnant water pools are also found that serve as breeding ground for other malaria vectors such as *An. culicifacies*. In Riverine-Plains, stagnant water in various ponds and puddles serves as major breeding sites. Stagnant water pools were found more near HF households than RP households. Most of the families in both the ecotypes were having farming fields within half a kilometre from their residence. However, households were found closer to

streams and other water bodies in HF than in RP (Table 11). Between both the ecotypes Hilly-forest terrain had poor road infrastructure, which is difficult to reach by health workers for malaria control interventions (Table 12).

Table 11: Topographical features with reference to malaria in two ecotypes

		Ecotype		
		Hilly-forest	**Riverine-plain**	**Total**
Forest distance: χ^2=274, *df*=2, p=.000*	Very near (<501m)	104[1] (75.4%)	0[1] (0.0%)	104 (38.0%)
	Near (501-2000m)	34[1] (24.6%)	0[1] (0.0%)	34 (12.4%)
	Far (>2000m)	0[1] (0.0%)	136[1] (100%)	136 (49.6%)
	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**
Stream distance: χ^2=274, *df*=2, p=.000*	Very near (<501m)	115[1] (83.3%)	0[1] (0.0%)	115 (42.0%)
	Near (501-2000m)	23[1] (16.7%)	0[1] (0.0%)	23 (8.4%)
	Far (>2000m)	0[1] (0.0%)	136[1] (100%)	136 (49.6%)
	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**
Stagnant water distance: χ^2=165.315, *df*=2, p=.000*	Very near (<501m)	23_a (16.7%)	14_a (10.3%)	37 (13.5%)
	Near (501-2000m)	115_b (83.3%)	22_b (16.2%)	137 (50.0%)
	Far (>2000m)	0[1] (0.0%)	100[1] (73.5%)	100 (36.5%)
	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**
Farm distance from home: χ^2=38.872, *df*=1, p=.000*	Very near (<501m)	1_a (0.7%)	36_a (26.5%)	37 (13.5%)
	Near (501-2000m)	137_b (99.3%)	100_b (73.5%)	237 (86.5%)
	Total	138 (100%)	136 (100%)	274 (100%)
River distance: χ^2=223.155, *df*=2, p=.000*	Very near (<501m)	0[1] (0.0%)	22[1] (16.2%)	22 (8.0%)
	Near (501-2000m)	0[1] (0.0%)	100[1] (73.5%)	100 (36.5%)
	Far (>2000m)	138_a (100%)	14_a (10.3%)	152 (55.5%)
	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**
Vector breeding site distance: χ^2=163.315, *df*=2, p=.000*	Very near (<501m)	23_a (16.7%)	14_a (10.3%)	37 (13.5%)
	Near (501-2000m)	115_b (83.3%)	22_b (16.2%)	137 (50.0%)
	Far (2000-3000m)	0[1] (0.0%)	100[1] (73.5%)	100 (36.5%)

	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**

Table 12: Difficulty in reaching households for malaria control intervention in HF and RP ecotypes respectively

		Ecotype		
		Hilly-forest	**Riverine-plain**	**Total**
Difficulty of reaching households: χ^2=119.155, *df*=2, *p*=.000*.	Easily accessible	23_a (16.7%)	108_a (79.4%)	131 (47.8%)
	Accessible with difficulty	67_b (48.6%)	28_b (20.6%)	95 (34.7%)
	Quite difficult to approach	48_1 (34.8%)	0_1 (0.0%)	48 (17.5%)
	Total	**138 (100%)**	**136 (100%)**	**274 (100%)**

3.2. Malaria treatment related parameters

3.2.1. Health care provisions within villages of HF and RP ecotypes

Free malaria diagnosis as well as drug delivery were significantly different between the two ecotypes (Table 13). Very high malaria incidence (as high as 60 API) in HF draws attention of district malaria control authority and delivery of both the services was found significantly high in HF (65%) as compared to RP (35%). The location of houses with malaria infection was not quite far from Health Centres and District Headquarter Hospital (DHH) in HF ecotype. However, roads leading to heath facilities in undulated HF terrain are not easily approachable by the inhabitants. The houses in the RP were located far from the Health Centres and the District Headquarter Hospital (DHH) but the road connectivity was better than that in HF (Table 13). Although, both the ecotypes had equal access to telephones and private transport but the hiring of the vehicle is costlier for the residents of HF ecotype than for the RP residents. Overall, the difficulty in reaching healthcare facilities was found significantly high for people residing in HF than in RP.

Table 13: Healthcare accessibility aspects within HF and RP ecotypes

		Ecotype		
		Hilly-forest	**Riverine-plain**	**Total**
Free malaria test	Provided	83_a (64.3%)	44_a (33.8%)	127 (49.0%)

available in the villages: χ^2=24.094, *df*=1, *p*=.000*.	Not provided	46_b (35.7%)	86_b (66.2%)	132 (51.0%)
	Total	129 (100%)	130 (100%)	259 (100%)
Free malaria treatment in the vicinity (< 3Km): χ^2= 10.355, *df*=1, *p*=.001*.	Provided	86_a (65.2%)	59_a (45.4%)	145 (55.3%)
	Not provided	**46_b** (34.8%)	**71_b** (54.6%)	117 (44.7%)
	Total	132 (100%)	130 (100%)	262 (100%)
PHC distance: χ^2=17.832, *df* =2, *p*=.000*.	Very near (<3001 m)	0^1 (0.0%)	14^1 (10.3%)	14 (5.1%)
	Near (3001m-10 Km)	90_a (65.2%)	67_a (49.3%)	157 (57.3%)
	Far (>10 Km)	48_a (34.8%)	55_a (40.4%)	103 (37.6%)
	Total	138 (100%)	136 (100%)	274 (100%)
DHH distance: χ^2=274.000, *df*=2, *p*=.000*.	Near (<25Km)	138^1 (100%)	0^1 (0.0%)	138 (50.4%)
	Far (25-40Km)	0^1 (0.0%)	14^1 (10.3%)	14 (5.1%)
	Very far (>40Km)	0^1 (0.0%)	122^1 (89.7%)	122 (44.5%)
	Total	138 (100%)	136 (100%)	274 (100%)
Pitch road distance from village: χ^2=85.752, *df*=2, *p*=.000*.	Proximate (<2001 m)	23_a (16.7%)	85_a (62.5%)	108 (39.4%)
	Far (2001 m-4 Km)	67_b (48.6%)	51_b (37.5%)	118 (43.1%)
	Remote (>4 Km)	48^1 (34.8%)	0^1 (0.0%)	48 (17.5%)
	Total	138 (100%)	136 (100%)	274 (100%)
Road accessibility and approachability to the village: χ^2=39.792, *df*=1, *p*=.000*.	Easy	23_a (16.7%)	72_a (52.9%)	95 (34.7%)
	Tough	115_b (83.3%)	64_b (47.1%)	179 (65.3%)
	Total	138 (100%)	136 (100%)	274 (100%)
What distance travelled by walk for treatment? : χ^2=.542, *df*=1, *p*=.462b.	<2001 m	72_a (97.3%)	76_a (95.0%)	148 (96.1%)
	>2000 m	2_a (2.7%)	4_a (5.0%)	6 (3.9%)
	Total	74 (100%)	80 (100%)	154 (100%)
Out of Pocket Expenses (OPE) for treatment χ^2=17.033, *df*=1, *p*=.000*.	Affordable	6_a (24.0%)	37_a (74.0%)	43 (57.3%)
	Inconvenient	19_b (76.0%)	13_b (26.0%)	32 (42.7%)
	Total	25 (100%)	50 (100%)	75 (100%)
Vehicle owned χ^2=.044, *df*=1,	Cycle	37_a (26.8%)	38_a (27.9%)	75 (27.4%)
	No vehicle	101_a (73.2%)	98_a (72.1%)	199 (72.6%)

p=0.833.	Total	138 (100%)	136 (100%)	274 (100%)
Telephone facility: χ^2=1.108, df=1, p=0.292.	Available	108_a (78.3%)	99_a (72.8%)	207 (75.5%)
	Un available	30_a (21.7%)	37_a (27.2%)	67 (24.5%)
	Total	138 (100%)	136 (100%)	274 (100%)
Facility to arrange a taxi telephonically: χ^2=14.137, df=1, p=.000*.	Available	130_a (94.2%)	107_a (78.7%)	237 (86.5%)
	Un available	8_b (5.8%)	29_b (21.3%)	37 (13.5%)
	Total	138 (100%)	136 (100%)	274 (100%)
The approximate cost of hiring a private vehicle/ ambulance (in Rupees): χ^2=25.790, df=3, p=.000*.	Can't say	7_a (5.1%)	20_a (14.7%)	27 (9.9%)
	<500	$45_{a,b}$ (32.6%)	$64_{a,b}$ (47.1%)	109 (39.8%)
	<800	67_b (48.6%)	50_b (36.8%)	117 (42.7%)
	<1300	19_c (13.8%)	2_c (1.5%)	21 (7.7%)
	Total	138 (100%)	136 (100%)	274 (100%)
Overall accessibility to health care facilities: χ^2=10.681, df=2, p=.005*.	Convenient	21_a (15.2%)	32_a (23.5%)	53 (19.3%)
	Inconvenient	62_a (44.9%)	74_a (54.4%)	136 (49.6%)
	Very inconvenient	55_b (39.9%)	30_b (22.1%)	85 (31.0%)
	Total	138 (100%)	136 (100%)	274 (100%)

3.2.2. Predictors of knowledge of malaria

There were no significant differences between the families surveyed in both the ecotypes regarding the general awareness about malaria say, malaria symptoms, malarious months in the village and importance of compliance to antimalarials treatments (Table 14). Most of the respondents from both the ecotypes were aware that malaria is curable with medicine and it cannot be cured on its own. Respondents of both the ecotypes were fairly aware of the malaria and its transmission season. However ~90% respondents of each ecotype were not informed that they should not plaster their wall following IRS. Regarding knowledge about malaria vector breeding sites most of the malarious family representative (71% and 80 % in HF and RP respectively) gave inapt answers (Table 15). Additionally, a significant proportion of the respondents in both the ecotypes had positive views regarding the of the Government Health Care system (ASHA/PHC/DHH etc.) and was primary choice for fever test (70% and 64% in HF and RP respectively) and check-up (97% and 77% in HF and RP respectively) during early stages of the appearance of the symptoms (Table 16).

Table 14: Predictors of knowledge of malaria regarding its transmission and clinical features according to respondants of HF and RP ecotypes

		Ecotype		
		Hilly-forest	**Riverine-plain**	**Total**
Transmission of malaria: χ^2=1.875, *df*=1, *p*=.171.	Apt	68_a (51.9%)	79_a (60.3%)	147 (56.1%)
	Inapt	63_a (48.1%)	52_a (39.7%)	115 (43.9%)
	Total	131 (100%)	131 (100%)	262 (100%)
Signs of malaria infection: χ^2=.371, *df*=1, *p*=.542.	Apt	117_a (88.6%)	120_a (90.9%)	237 (89.8%)
	Inapt	15_a (11.4%)	12_a (9.1%)	27 (10.2%)
	Total	132 (100%)	132 (100%)	264 (100%)
Malarious months: χ^2=.197, *df*=1, *p*=.657.	Apt	113_a (84.3%)	113_a (86.3%)	226 (85.3%)
	Inapt	21_a (15.7%)	18_a (13.7%)	39 (14.7%)
	Total	134 (100%)	131 (100%)	265 (100%)
Instructed to take full course of antimalarials: χ^2=.737, *df*=1, *p*=.391a.	Advised	125_a (97.7%)	109_a (99.1%)	234 (98.3%)
	Not advised	3_a (2.3%)	1_a (0.9%)	4 (1.7%)
	Total	128 (100%)	110 (100%)	238 (100%)
Water bodies where malaria vectors can breed. χ^2=3.193, df=1, p=.074.	Apt answer	39a (29.3%)	26a (19.8%)	65 (24.6%)
	Inapt answer	94a (70.7%)	105a (80.2%)	199 (75.4%)
	Total	133 (100%)	131 (100%)	264 (100%)
Overall awareness about malaria and its treatment: χ^2=.187, *df*=2, *p*=.911.	High	30_a (21.9%)	28_a (20.6%)	58 (21.2%)
	Reasonable	56_a (40.9%)	54_a (39.7%)	110 (40.3%)
	Low	51_a (37.2%)	54_a (39.7%)	105 (38.5%)
	Total	137 (100%)	136 (100%)	273 (100%)

Table 15: Perception of malaria healthcare between the respondents of HF and RP

		Ecotype					
		Hilly-forest		**Riverine-plain**		**Total**	
Malaria is curable with medicines: χ^2=9.388, *df*=2, *p*=.009[*,b,c].	Sure	118_a	(88.1%)	110_a	(98.2%)	228	(92.7%)
	Tentative	2^1	(1.5%)	0^1	(0.0%)	2	(0.8%)
	Unsure	14_b	(10.4%)	2_b	(1.8%)	16	(6.5%)
	Total	134	(100%)	112	(100%)	246	(100%)
Malaria is self-limiting and can be cured without treatment: χ^2=17.198, *df*=2, *p*=.000*.	Disbelieves	86_a	(64.7%)	107_a	(85.6%)	193	(74.8%)
	Inconsistent	36_b	(27.1%)	17_b	(13.6%)	53	(20.5%)
	Insistent	11_b	(8.3%)	1_b	(0.8%)	12	(4.7%)
	Total	133	(100%)	125	(100%)	258	(100%)
Judging malaria test provider: χ^2=16.102, *df*=1, *p*=.000*.	Decent perception	138_a	(100%)	121_a	(89.0%)	259	(94.5%)
	Mediocre perception	0^1	(0.0%)	15^1	(11.0%)	15	(5.5%)
	Total	138	(100%)	136	(100%)	274	(100%)
Evaluation of malaria care provider: χ^2=8.751, *df*=1, *p*=.003*.	Decent perception	135_a	(97.8%)	121_a	(89.0%)	256	(93.4%)
	Mediocre perception	3_b	(2.2%)	15_b	(11.0%)	18	(6.6%)
	Total	138	(100%)	136	(100%)	274	(100%)
Reason of preferring/ not preferring ASHA: χ^2=47.426, *df*=2, *p*=.000*.	Malaria test and or medicine provided	31_a	(26.3%)	13_a	(10.4%)	44	(18.1%)
	Malaria test and or medicine inadequately provided	87_a	(73.7%)	73_a	(58.4%)	160	(65.8%)
	Absence of ASHA in the village	0^1	(0.0%)	39^1	(31.2%)	39	(16.0%)
	Total	118	(100%)	125	(100%)	243	(100%)
Reason of preferring/ not preferring PHC: χ^2=45.053, *df*=2, *p*=.000*.	Services up to expectations	39_a	(32.5%)	12_a	(9.9%)	51	(21.2%)
	Services not up to expectations	8_b	(6.7%)	49_b	(40.5%)	57	(23.7%)
	Situated far away	73_c	(60.8%)	60_c	(49.6%)	133	(55.2%)
	Total	120	(100%)	121	(100%)	241	(100%)

Overall perception about government health care facility: χ^2=1.168, *df*=1, *p*=.280.	Satisfactory	60_a	(59.4%)	47_a	(51.6%)	107	(55.7%)
	Not satisfactory	41_a	(40.6%)	44_a	(48.4%)	85	(44.3%)
	Total	101	(100%)	91	(100%)	192	(100%)

Table 16: Treatment seeking practices in respondents of HF and RP

		Ecotype					
		Hilly-forest		**Riverine-plain**		**Total**	
Choice of fever/malaria test: χ^2=.997, *df*=1, *p*=.318.	Government setup	87_a	(69.6%)	75_a	(63.6%)	162	(66.7%)
	Non-government setup	38_a	(30.4%)	43_a	(36.4%)	81	(33.3%)
	Total	125	(100%)	118	(100%)	243	(100%)
Practice of self-medication of antimalarials: χ^2=1.725, *df*=1, *p*=.189.	Not often	8_a	(6.0%)	3_a	(2.6%)	11	(4.4%)
	Not practiced	125_a	(94.0%)	113_a	(97.4%)	238	(95.6%)
	Total	133	(100%)	116	(100%)	249	(100%)
Delay in treatment seeking: χ^2=1.877, *df*=2, *p*=.391.	Prompt (within 3 days)	67_a	(51.1%)	68_a	(58.6%)	135	(54.7%)
	Restrained (4-7 days)	56_a	(42.7%)	44_a	(37.9%)	100	(40.5%)
	Delayed (more than a week)	8_a	(6.1%)	4_a	(3.4%)	12	(4.9%)
	Total	131	(100%)	116	(100%)	247	(100%)
Severity of suffering when treatment was pursued: χ^2=9.220, *df*=1, *p*=.002*.	Moderate	74_a	(57.4%)	90_a	(75.6%)	164	(66.1%)
	Severe	55_b	(42.6%)	29_b	(24.4%)	84	(33.9%)
	Total	129	(100%)	119	(100%)	248	(100%)
Overall health-seeking to government setups: χ^2=2.323, *df*=2, *p*=.313.	Prompt	37_a	(26.8%)	26_a	(19.1%)	63	(23.0%)
	Restrained	60_a	(43.5%)	64_a	(47.1%)	124	(45.3%)
	Neglect	41_a	(29.7%)	46_a	(33.8%)	87	(31.8%)
	Total	138	(100%)	136	(100%)	274	(100%)

3.2.3. Treatment attitude and practices in HF and RP

Drug compliance was very good among the surveyed populations of both the ecotypes (Table 17). Nearly half of the HF and 37% of the RP families showed interest in a free diagnostic test for malaria without an ailment. The majority of the surveyed head of the families were affirmative regarding giving few drops of blood (51% and 55% respectively in HF and RP) for malaria research purpose.

Attitude for treatment was categorized into three groups viz., excellent, decent and inadequate. Health seeking behaviour was found independent of economic status and accessibility of households. However, literacy was found to positively affect the health seeking behaviour since, most literates had decent attitude towards malaria treatment in HF ecotype, whereas in RP ecotype most literates had excellent attitude (Table 18). Similarly, person with high awareness of malaria were found to have decent attitude towards malaria treatment in HF ecotype and excellent attitude in RP ecotype.

Table 17: Treatment attitude concerns of the respondents of HF and RP

		Ecotype					
		Hilly-forest		**Riverine-plain**		**Total**	
Complete the full course of antimalarials.	Yes	130[1]	(100%)	112[1]	(100%)	242	(100%)
	No	0	(0%)	0	(0%)	0	(0%)
	Total	130	(100%)	112	(100%)	242	(100%)
Interested in a free blood test for malaria: χ^2=4.348, *df*=1, *p*=.037*.	Intent	69_a	(50.0%)	51_a	(37.5%)	120	(43.8%)
	Impassive	69_b	(50.0%)	85_b	(62.5%)	154	(56.2%)
	Total	138	(100%)	136	(100%)	274	(100%)

Table 18: Background parameters influencing family's attitude for seeking treatment from local government setup

	Attitude for treatment					
	Hilly-forest			**Riverine-plain**		
	Excellent	**Decent**	**Inadequate**	**Excellent**	**Decent**	**Inadequate**

Per capita income category (RP: χ^2=19.495, *df*=4, *p*=.001*, b.)												
Despondent <`15	7_a	(17.1%)	15_a	(36.6%)	19_a	(46.3%)	$10_{a,b}$	(17.2%)	26_a	(44.8%)	22_b	(37.9%)
Deficient <`15-30	15_a	(21.1%)	23_a	(32.4%)	33_a	(46.5%)	15_a	(23.1%)	12_a	(18.5%)	38_a	(58.5%)
Competent >`30	4_a	(15.4%)	7_a	(26.9%)	15_a	(57.7%)	0^1	(0%)	1_a	(7.7%)	12_b	(92.3%)
Total	26	(18.8%)	45	(32.6%)	67	(48.6%)	25	(18.4%)	39	(28.7%)	72	(52.9%)
Literacy (HF: χ^2=14.151, *df*=6, *p*=.028*, b, RP: χ^2=32.001, *df*=6, *p*=.000*, b.)												
Can write	$7_{a,b}$	(24.1%)	14_a	(48.3%)	8_b	(27.6%)	14_a	(51.9%)	7_b	(25.9%)	6_b	(22.2%)
Can read only	3_a	(23.1%)	6_a	(46.2%)	4_a	(30.8%)	0^1	(0%)	0^1	(0%)	6_a	(100%)
Can sign only	6_a	(26.1%)	8_a	(34.8%)	9_a	(39.1%)	3_a	(15.8%)	4_a	(21.1%)	12_a	(63.2%)
Illiterate	10_a	(13.7%)	17_a	(23.3%)	46_b	(63.0%)	8_a	(9.5%)	28_b	(33.3%)	48_b	(57.1%)
Total	26	(18.8%)	45	(32.6%)	67	(48.6%)	25	(18.4%)	39	(28.7%)	72	(52.9%)
Accessibility												
Easily accessible	7_a	(33.3%)	5_a	(23.8%)	9_a	(42.9%)	6_a	(18.8%)	7_a	(21.9%)	19_a	(59.4%)
Accessible with difficulty	8_a	(12.9%)	22_a	(35.5%)	32_a	(51.6%)	14_a	(18.9%)	22_a	(29.7%)	38_a	(51.4%)
Quite difficult to approach	11_a	(20.0%)	18_a	(32.7%)	26_a	(47.3%)	5_a	(16.7%)	10_a	(33.3%)	15_a	(50.0%)
Total	26	(18.8%)	45	(32.6%)	67	(48.6%)	25	(18.4%)	39	(28.7%)	72	(52.9%)
Awareness about malaria and its treatment (RP: χ^2=19.127, *df*=4, *p*=.001*.)												
High	8_a	(26.7%)	13_a	(43.3%)	9_a	(30.0%)	13_a	(46.4%)	4_b	(14.3%)	11_b	(39.3%)
Reasonable	10_a	(17.9%)	19_a	(33.9%)	27_a	(48.2%)	7_a	(13.0%)	17_a	(31.5%)	30_a	(55.6%)
Low	8_a	(15.7%)	12_a	(23.5%)	31_a	(60.8%)	5_a	(9.3%)	18_a	(33.3%)	31_a	(57.4%)
Total	26	(19.0%)	44	(32.1%)	67	(48.9%)	25	(18.4%)	39	(28.7%)	72	(52.9%)
Free malaria treatment in vicinity of 3 Km												
Delivered	20_a	(23.3%)	25_a	(29.1%)	41_a	(47.7%)	8_a	(13.6%)	15_a	(25.4%)	36_a	(61.0%)
Undelivered	6_a	(13.0%)	17_a	(37.0%)	23_a	(50.0%)	16_a	(22.5%)	21_a	(29.6%)	34_a	(47.9%)

Total	26	(19.7%)	42	(31.8%)	64	(48.5%)	24	(18.5%)	36	(27.7%)	70 (53.8%)
Overall perception about HC											
Good	17_a	(28.3%)	16_a	(26.7%)	27_a	(45.0%)	10_a	(21.3%)	13_a	(27.7%)	24_a (51.1%)
Bad	4_a	(9.8%)	13_a	(31.7%)	24_a	(58.5%)	11_a	(25.0%)	8_a	(18.2%)	25_a (56.8%)
Total	21	(20.8%)	29	(28.7%)	51	(50.5%)	21	(23.1%)	21	(23.1%)	49 (53.8%)

3.3. Bednet related parameters in HF and RP ecotypes

3.3.1. Bednet availability in HF and RP ecotypes

Quantitative and qualitative availability of bed nets is summarized in Table 19. Most of the families surveyed had two bed nets for a family averaging 5-6 persons. Only 10% in HF and 8% in RP had bednets in good conditions. The net number was not adequate for 79% and 97% households in HF and RP respectively. There were significantly more families were deprived of bednets in HF (65%) than in RP (41%).

Table 19: Bednet adequacy issues among respondents of HF and RP

		Ecotype		
		Hilly-forest	**Riverine-plain**	**Total**
Number of nets available in the household: χ^2=3.251, *df*=5, *p*=.661$_{a,b}$.	One	1_a (1.9%)	5_a (6.0%)	6 (4.4%)
	Two	44_a (84.6%)	69_a (83.1%)	113 (83.7%)
	Three	6_a (11.5%)	6_a (7.2%)	12 (8.9%)
	Four	$0^{1,2}$ (0.0%)	$1^{1,2}$ (1.2%)	1 (0.7%)
	Five	1_a (1.9%)	1_a (1.2%)	2 (1.5%)
	Seven	$0^{1,2}$ (0.0%)	$1^{1,2}$ (1.2%)	1 (0.7%)
	Total	52 (100%)	83 (100%)	135 (100%)
Usefulness of the nets against mosquitoes: χ^2=11.980, *df*=1, *p*=.001*.	In good condition	11_a (21.2%)	2_a (2.6%)	13 (10.0%)
	Not in proper condition	41_b (78.8%)	76_b (97.4%)	117 (90.0%)
	Total	52 (100%)	78 (100%)	130 (100%)
Maximum numbers of good condition bednets:	One	$0^{1,2}$ (0.0%)	$1^{1,2}$ (1.4%)	1 (0.9%)
	Two	38_a (86.4%)	63_a (87.5%)	101 (87.1%)

χ^2=2.580, *df*=4, *p*=.630$_{a,b}$.	Three	6$_a$ (13.6%)	6$_a$ (8.3%)	12 (10.3%)
	Four	0^{1,2} (0.0%)	1^{1,2} (1.4%)	1 (0.9%)
	Five	0^{1,2} (0.0%)	1^{1,2} (1.4%)	1 (0.9%)
	Total	44 (100%)	72 (100%)	116 (100%)
Adequacy of bednets per household: χ^2=.058, *df*=1, *p*=.810.	Manageable	34$_a$ (77.3%)	57$_a$ (79.2%)	91 (78.4%)
	Unmanageable	10$_a$ (22.7%)	15$_a$ (20.8%)	25 (21.6%)
	Total	44 (100%)	72 (100%)	116 (100%)
Overall adequacy of protection by bednet: χ^2=14.947, *df*=2, *p*=.001*.	Adequate	5$_{a,b}$ (3.6%)	8$_{a,b}$ (5.9%)	13 (4.7%)
	Inadequate	44$_a$ (31.9%)	72$_a$ (52.9%)	116 (42.3%)
	Deprived	89$_b$ (64.5%)	56$_b$ (41.2%)	145 (52.9%)
	Total	138 (100%)	136 (100%)	274 (100%)

3.3.2. Bednet use experiences between respondents of HF and RP

Most of the families, which had used bednets felt sleeping under bednet was safer than otherwise and believed that bednets protected them from mosquitoes as well as malaria (Table 20). Almost all the families which had usable, effective bednets used them the previous night of the survey. The bednets were in use throughout the year in both the ecotypes. Most of the families (63% in HF and 68% in RP ecotype) were keen on utilizing bednets (Table 21).

Table 20: Bednet use aspects among respondents in HF and RP ecotypes

		Ecotype		
		Hilly-forest	**Riverine-plain**	**Total**
Why you like or dislike mosquito nets? χ^2=14.978, *df*=7, *p*=.036*,b,c.	It protects from mosquitoes	9$_a$ (6.8%)	11$_a$ (8.4%)	20 (7.6%)
	It protects from Insects	20$_a$ (15.0%)	10$_a$ (7.6%)	30 (11.4%)
	It protects from malaria+mosquito	30$_a$ (22.6%)	48$_a$ (36.6%)	78 (29.5%)
	Malaria protection	10$_a$ (7.5%)	9$_a$ (6.9%)	19 (7.2%)
	skin itching	0^{1,2} (0.0%)	1^{1,2} (0.8%)	1 (0.4%)
	NA	61$_a$ (45.9%)	44$_a$ (33.6%)	105 (39.8%)

	Sneezing	0^{2} (0.0%)	3^{2} (2.3%)	3 (1.1%)
	It causes discomfort	3_{a} (2.3%)	5_{a} (3.8%)	8 (3.0%)
	Total	133 (100%)	131 (100%)	264 (100%)
Perception about bednet use: χ^2=1.489, *df*=1, *p*=.222.	Positive	69_{a} (50.0%)	78_{a} (57.4%)	147 (53.6%)
	Negative	69_{a} (50.0%)	58_{a} (42.6%)	127 (46.4%)
	Total	138 (100%)	136 (100%)	274 (100%)

Table 21: Bednet usage among respondents in HF and RP ecotypes

		Ecotype		
		Hilly-forest	**Riverine-plain**	**Total**
Good bednet and last night usage ratio: χ^2=1.603, *df*=1, *p*=.205a.	Max utilized	40_{a} (97.6%)	65_{a} (91.5%)	105 (93.8%)
	Underutilized	1_{a} (2.4%)	6_{a} (8.5%)	7 (6.3%)
	Total	41 (100%)	71 (100%)	112 (100%)
Season of bednet use: χ^2=.528, *df*=1, *p*=.467.	Always	33_{a} (63.5%)	57_{a} (69.5%)	90 (67.2%)
	Seasonal	19_{a} (36.5%)	25_{a} (30.5%)	44 (32.8%)
	Total	52 (100%)	82 (100%)	134 (100%)
Guest visit in the household: χ^2=.200, *df*=2, *p*=.905.	Never	27_{a} (20.0%)	29_{a} (21.6%)	56 (20.8%)
	Seldom (1-3 times)	39_{a} (28.9%)	40_{a} (29.9%)	79 (29.4%)
	Often	69_{a} (51.1%)	65_{a} (48.5%)	134 (49.8%)
	Total	135 (100%)	134 (100%)	269 (100%)
Frequency of family members spending the night outside home: χ^2=.134, *df*=2, *p*=.935.	Never	21_{a} (15.6%)	22_{a} (16.8%)	43 (16.2%)
	Seldom (1-3 times)	51_{a} (37.8%)	47_{a} (35.9%)	98 (36.8%)
	Often	63_{a} (46.7%)	62_{a} (47.3%)	125 (47.0%)
	Total	135 (100%)	131 (100%)	266 (100%)
Overall utilization of bednets: χ^2=.304, *df*=2, *p*=.$859_{a,b}$.	Keen	26_{a} (63.4%)	48_{a} (67.6%)	74 (66.1%)
	Moderate	1_{a} (2.4%)	1_{a} (1.4%)	2 (1.8%)
	Inadequate	14_{a} (34.1%)	22_{a} (31.0%)	36 (32.1%)
	Total	41 (100%)	71 (100%)	112 (100%)

3.3.1. Bednet maintenance within respondents in HF and RP ecotypes

Proper maintenance of bednets is important for their effective use in malaria control program. In both the ecotypes bednets were washed at reasonable intervals (not more than once in 3months) (around 80% in HF and 90% in RP ecotypes) with cold/ lukewarm water (Table 22). However, more than 80% of the families in both the ecotypes used detergent to wash the bednets, which deteriorates the efficacy of insecticide treated bednets. Additionally more than 85% of the families kept bednets for drying in direct sunlight that further reduces their efficacy. Overall the maintenance of bednets was not as per norms and thus, the inhabitants need to be properly educated for maintenance of the ITNs.

Table 22: Bednet maintenance within respondents in HF and RP ecotypes

		Ecotype					
		Hilly-forest		Riverine-plain		Total	
Frequency of washing nets: χ^2=8.356, *df*=1, *p*=.004*.	Reasonable (not more than once in 3months)	42_a	(80.8%)	76_a	(96.2%)	118	(90.1%)
	Excess (more than once in 3months)	10_b	(19.2%)	3_b	(3.8%)	13	(9.9%)
	Total	52	(100%)	79	(100%)	131	(100%)
Use of cold/hot water for washing nets: χ^2=1.316, *df*=1, *p*=.251.	Cold/ Luke warm	44_a	(84.6%)	72_a	(91.1%)	116	(88.5%)
	Hot	8_a	(15.4%)	7_a	(8.9%)	15	(11.5%)
	Total	52	(100%)	79	(100%)	131	(100%)
Use of soap/detergent for washing nets: χ^2=1.129, *df*=1, *p*=.288.	Mild(soap/nothing)	6_a	(11.8%)	15_a	(18.8%)	21	(16.0%)
	Hard (detergent)	45_a	(88.2%)	65_a	(81.3%)	110	(84.0%)
	Total	51	(100%)	80	(100%)	131	(100%)
Drying of nets in Shade/sunlight: χ^2=3.366, *df*=1, *p*=.067.	Shade	2_a	(3.9%)	11_a	(13.8%)	13	(9.9%)
	Sunlight	49_a	(96.1%)	69_a	(86.3%)	118	(90.1%)
	Total	51	(100%)	80	(100%)	131	(100%)
Overall maintenance of bednet: χ^2=7.995,	Good	4_a	(8.0%)	17_a	(23.3%)	21	(17.1%)
	Bad	$33_{a,b}$	(66.0%)	$48_{a,b}$	(65.8%)	81	(65.9%)

df=2, p=.018*.	Worst	13_b	(26.0%)	8_b	(11.0%)	21	(17.1%)
	Total	50	(100%)	73	(100%)	123	(100%)

3.3.2. Attitude to purchase bednets

Only 8% and 13% respondents from HF and RP respectively were not willing to have bednets. In HF and RP (respectively 43% and 44%) respondents willing to buy bednet and most of them preferred to have bednets free or at subsidized cost (Table 23). However, 24% of responders in HF ecotype preferred to pay in instalments in case they do not get the nets free of cost, whereas, only 1% of the responders in RP ecotype opted for paying the full money in instalments. Background parameters that might influence family's attitude towards purchase of bednets are summarized in Table 24. The per capita income and literacy were not found significantly associated with purchase of bednets whereas the other parameters like awareness about malaria, prior experience of using bednets and the positive perceptions of the respondents showed significant correlation (Table 24).

Table 23: Attitude about bednet use within respondents in HF and RP ecotypes

		Ecotype					
		Hilly-forest		Riverine-plain		Total	
Bednet purchase preference. χ^2=39.724, df=6, p=.000*,b,c.	Treated and free	1_a	(0.8%)	6_a	(4.5%)	7	(2.6%)
	No	11_a	(8.3%)	18_a	(13.5%)	29	(10.9%)
	Free/subsidized/untreated/treated	$1^{1,2}$	(0.8%)	$0^{1,2}$	(0.0%)	1	(0.4%)
	Paid/Instalment/subsidized/ free/treated	5_a	(3.8%)	10_a	(7.5%)	15	(5.7%)
	Instalment/subsidized/free/treated	32_b	(24.2%)	1_b	(0.8%)	33	(12.5%)
	Paid/Instalment/subsidized/ free/treated/untreated	26_a	(19.7%)	39_a	(29.3%)	65	(24.5%)
	Free/subsidized/treated	56_a	(42.4%)	59_a	(44.4%)	115	(43.4%)
	Total	132	(100%)	133	(100%)	265	(100%)
Interest to have bednets: χ^2=2.301, df=2,	Enthusiastic/Keen	63_a	(45.7%)	50_a	(36.8%)	113	(41.2%)
	Willing	58_a	(42.0%)	65_a	(47.8%)	123	(44.9%)

p=.317.	Not willing	17_a	(12.3%)	21_a	(15.4%)	38	(13.9%)
	Total	138	(100%)	136	(100%)	274	(100%)

Table 24: Background parameters influencing family's purchase of bed nets

	Interested to buy bed nets					
	Hilly-forest			**Riverine-plain**		
	Keen	**Willing**	**Not willing**	**Keen**	**Willing**	**Not willing**
Per capita income category						
Despondent <`15	15_a (36.6%)	20_a (48.8%)	6_a (14.6%)	18_a (31.0%)	29_a (50.0%)	11_a (19.0%)
Deficient <`15-30	35_a (49.3%)	28_a (39.4%)	8_a (11.3%)	29_a (44.6%)	28_a (43.1%)	8_a (12.3%)
Competent >`30	13_a (50.0%)	10_a (38.5%)	3_a (11.5%)	3_a (23.1%)	8_a (61.5%)	2_a (15.4%)
Total	63 (45.7%)	58 (42.0%)	17 (12.3%)	50 (36.8%)	65 (47.8%)	21 (15.4%)
Literateness						
Can write	11_a (37.9%)	13_a (44.8%)	5_a (17.2%)	10_a (37.0%)	12_a (44.4%)	5_a (18.5%)
Can read only	5_a (38.5%)	5_a (38.5%)	3_a (23.1%)	3_a (50.0%)	2_a (33.3%)	1_a (16.7%)
Can sign only	13_a (56.5%)	7_a (30.4%)	3_a (13.0%)	8_a (42.1%)	9_a (47.4%)	2_a (10.5%)
Illiterate	34_a (46.6%)	33_a (45.2%)	6_a (8.2%)	29_a (34.5%)	42_a (50.0%)	13_a (15.5%)
Total	63 (45.7%)	58 (42.0%)	17 (12.3%)	50 (36.8%)	65 (47.8%)	21 (15.4%)
Adequate protection by bednet (HF: χ^2=51.764, df=4, p=.000[a,*, c], RP: χ^2=38.851, df=4, p=.000[a,*].)						
Adequate	3_a (60.0%)	0^1 (0%)	2_a (40.0%)	3_a (37.5%)	5_a (62.5%)	0^1 (0%)
Inadequate	34_a (77.3%)	1_b (2.3%)	9_a (20.5%)	36_a (50.0%)	17_b (23.6%)	19_a (26.4%)
Deprived	26_a (29.2%)	57_b (64.0%)	6_a (6.7%)	11_a (19.6%)	43_b (76.8%)	2_a (3.6%)
Total	63 (45.7%)	58 (42.0%)	17 (12.3%)	50 (36.8%)	65 (47.8%)	21 (15.4%)
Awareness about how malaria is caused, its symptoms and malarious months (HF: χ^2=11.243, df=4, p=.024*.)						

Best	15_a	(31.9%)	$21_{a,b}$	(44.7%)	11_b	(23.4%)	15_a	(26.3%)	30_a	(52.6%)	12_a	(21.1%)
Good	33_a	(50.0%)	29_a	(43.9%)	4_a	(6.1%)	24_a	(42.9%)	28_a	(50.0%)	4_a	(7.1%)
Worse	15_a	(60.0%)	8_a	(32.0%)	2_a	(8.0%)	11_a	(47.8%)	7_a	(30.4%)	5_a	(21.7%)
Total	63	(45.7%)	58	(42.0%)	17	(12.3%)	50	(36.8%)	65	(47.8%)	21	(15.4%)
Perception about bednet use (HF: χ^2=39.069, *df*=2, *p*=.000*, RP: χ^2=28.216, *df*=2, *p*=.000*.)												
Positive	47_a	(68.1%)	11_b	(15.9%)	11_a	(15.9%)	40_a	(51.3%)	22_b	(28.2%)	16_a	(20.5%)
Negative	16_a	(23.2%)	47_b	(68.1%)	6_a	(8.7%)	10_a	(17.2%)	43_b	(74.1%)	5_a	(8.6%)
Total	63	(45.7%)	58	(42.0%)	17	(12.3%)	50	(36.8%)	65	(47.8%)	21	(15.4%)

3.4. IRS related parameters in HF and RP

3.4.1. IRS coverage in HF and RP

Among the household surveyed in HF, 51% were sprayed twice a year, 33% were sprayed once a year and 16% were sprayed were not sprayed even once a year (Table 25). Whereas in RP ecotype 35% households were sprayed rest 65% were not sprayed even one a year. Among the sprayed houses, 78% and 70% houses in HF and RP respectively received complete IRS coverage, while rest were partially sprayed.

60% of HF respondents and 37% of RP respondents did not resist the NVBDCP IRS staff to enter their premises. Among those resisted, majority saw it as disturbance in their privacy (21% in HF ecotype and 28% in RP ecotype). Next major reason for resistance in RP (24%) was fear that IRS may harm their livestock; however this was not the case with HF respondents i.e. only 8% raised this concern. In HF ecotype 45.7% whole heartily supported procedural IRS, whereas in RP only 27% did. People that allow entry of IRS staff 71% in HF and 67% of RP allowed complete house to be sprayed. More than 80% of the respondents of each ecotype plaster their walls over the sprayed insecticide. The frequency of plastering was moderate in both the ecotypes and usually people prefer to plaster just before important festivals, such as nearly 57% plaster before 'Nua-khai' and 15% during 'Makar sankranti'. The information is very important for planning spray.

Table 25: IRS coverage aspects within respondents in HF and RP ecotypes

	Ecotype		
	Hilly-forest	**Riverine-plain**	**Total**

Frequency of IRS in the household surveyed: χ^2=105.824, *df*=2, *p*=.000*.	Twice a year	68[1]	(51.1%)	0[1]	(0.0%)	68	(25.9%)
	Once a year	44_a	(33.1%)	46_a	(35.4%)	90	(34.2%)
	< One per year	21_b	(15.8%)	84_b	(64.6%)	105	(39.9%)
	Total	133	(100%)	130	(100%)	263	(100%)
Extent of coverage: χ^2=1.526, *df*=1, *p*=.217.	Practically all places	86_a	(77.5%)	70_a	(70.0%)	156	(73.9%)
	Partial (certain places)	25_a	(22.5%)	30_a	(30.0%)	55	(26.1%)
	Total	111	(100%)	100	(100%)	211	(100%)
Overall IRS coverage: χ^2=56.294, *df*=2, *p*=.000*.	Excellent	39[1]	(28.3%)	0[1]	(0.0%)	39	(14.2%)
	Moderate	39_a	(28.3%)	26_a	(19.1%)	65	(23.7%)
	Neglected	60_b	(43.5%)	110_b	(80.9%)	170	(62.0%)
	Total	138	(100%)	136	(100%)	274	(100%)

3.5. Alternative practices related to malaria

3.5.1. Self-protective practices other than bednets

To protect themselves from mosquitoes, fumigation by burning of leaves was best choice after bednets. Lesser (14%) respondents of HF prefer fumigation using 'Jhun' against 34.6% of RP (Table 26). Whereas, majority (i.e., 58%) fumigate with any herb in HF and only 9% would prefer any herb in RP ecotype. Most of the respondents of both the ecotype (66%) prefer sleeping in their bed rooms. Use of *'Neem'* oil or herbs was adopted by 10.5% of HF respondents during work against only 2% of RP respondents. Overall personal protection is insalubrious in HF (91%), while it is healthier in RP (nearly 5% insalubrious).

Table 26: Factors like self-protective practices other than bednets between respondents in HF and RP ecotypes

		Ecotype					
		Hilly-forest		**Riverine-plain**		**Total**	
Methods to avoid mosquitoes in living place: χ^2=70.800, *df*=2, *p*=.000*.	Making smoke with Jhun	19_a	(14.2%)	46_a	(34.6%)	65	(24.3%)
	Fumigate with any herb/shrub	77_b	(57.5%)	12_b	(9.0%)	89	(33.3%)

	Do nothing	38_a	(28.4%)	75_a	(56.4%)	113	(42.3%)
	Total	134	(100%)	133	(100%)	267	(100%)
Persons sleep in bedroom (all/some/none): χ^2=4.247, *df*=2, *p*=.120b.	All	95_a	(68.8%)	87_a	(64.0%)	182	(66.4%)
	Some	40_a	(29.0%)	49_a	(36.0%)	89	(32.5%)
	None	3^1	(2.2%)	0^1	(0.0%)	3	(1.1%)
	Total	138	(100%)	136	(100%)	274	(100%)
Protection against mosquito during work: χ^2=8.900, *df*=4, *p*=.064b.	Use commercial repellent	$2_{a,b}$	(1.5%)	$1_{a,b}$	(0.8%)	3	(1.2%)
	Wear full sleeves	$27_{a,b}$	(20.3%)	$28_{a,b}$	(23.0%)	55	(21.6%)
	Use Neem oil or herbs	14_a	(10.5%)	2_a	(1.6%)	16	(6.3%)
	Drive off mosquitoes with hand	$11_{a,b}$	(8.3%)	$11_{a,b}$	(9.0%)	22	(8.6%)
	Do nothing	79_b	(59.4%)	80_b	(65.6%)	159	(62.4%)
	Total	133	(100%)	122	(100%)	255	(100%)
Overall self-protective practices: χ^2=205.390, *df*=2, *p*=.000*.	Wholesome	4_a	(2.9%)	88_a	(64.7%)	92	(33.6%)
	Sporadic	8_b	(5.8%)	41_b	(30.1%)	49	(17.9%)
	Not practicing	126_c	(91.3%)	7_c	(5.1%)	133	(48.5%)
	Total	138	(100%)	136	(100%)	274	(100%)

3.5.2. Self-protection attitude by respondents of HF and RP ecotypes towards malaria

Majority of respondents (92% of HF and 86% of RP) do not take any presumptive medicine for malaria (Table 27). Moreover ~95% of respondents of both the ecotype were uncommitted or not enthusiastic for source reduction. Although, only six and three percent of the families reported to take self-medication in HF and RP respectively.

Table 27: Self-protection practice of respondents by antimalarial in HF and RP ecotypes

		Ecotype					
		Hilly-forest		**Riverine-plain**		**Total**	
Taking presumptive medicine for malaria: χ^2=2.679, *df*=1,	None	125_a	(91.9%)	113_a	(85.6%)	238	(88.8%)
	Some	11_a	(8.1%)	19_a	(14.4%)	30	(11.2%)

p=.102.	Total	136	(100%)	132	(100%)	268	(100%)
Self-medication with antimalarials when sick χ^2=1.725, df=1, p=.189.	Yes	8_a	(6.0%)	3_a	(2.6%)	11	(4.4%)
	No	125_a	(94.0%)	113_a	(97.4%)	238	(95.6%)
	Total	133	(100%)	116	(100%)	249	(100%)

3.6. Knowledge, Attitude and Practices study in 'Mankidias' – a nomadic tribe

Mankidias are a nomadic tribe that roams around certain parts of Shimlipal forest of Odisha. They have not yet adapted agricultural practices and follow their ancient traditions. The KAP survey was extended to this tribe and 100 heads of the families were questioned in local language. The results of the survey are summarized in Table 28.

Among 100 respondents, who were head and make decisions for their families, 95% were illiterate, 3% could only sign and only 2% could both read and write. Questionnaire-based survey revealed that only 17% consider malaria to be a fatal disease and 20% believe that it is curable, but alarmingly, 84% believe that malaria is a self-limiting disease. Thirty-three percent thought the symptoms of malaria to be just fever, 4% mentioned fever along with headache and body pain, 17% indicated symptoms like vomiting, nausea, headache, and body pain, but 42% of Mankidias did not know the symptoms of malaria. Twenty-four percent recognized mosquito bites are the cause of malaria whereas others believed differently, i.e., bathing with cold water (17%) or bathing in streams (12%), or forest visits (12%) caused malaria. Regarding sources of mosquito breeding, 12% believed that mosquitoes breed in clean streams whereas others attributed it to stagnant dirty water (50%), pond (7%), stagnant clear water (4%), dirty stream (5%), leaves (3%), well (2%), or did not know (17%). Thirty-five percent knew about bednets (irrespective of possessing bednets) but only 26% believed that they could prevent malaria and only 24 % knew that bednets could prevent insect bites. Eleven percent cited winter as the high malaria season, 13% mentioned monsoon season, and 41% believed that malaria is high throughout the year. Besides foot-dragging in seeking healthcare (12%), the unavailability of government health service (PHC/CHC/DHH) within accessible distance and transport difficulties was cited by 18% and 5% for not availing government facilities. Among respondents with access to government health providers, only 21% would avail government facilities. Reliance on remedies such as curative herbs against malaria (59%), faith in an Ayurveda practitioner or the faith healer "*Gunia*" (14%), and preference for home visits from local mobile health providers (self-styled compounder) (25%) were also cited. The

number of days typically tolerated before seeking assistance differed among individuals; only 3% seek health care within 3 days of suffering, 24% within a week, 17% after a week, 27% whenever the ailment is intolerable, and surprisingly, 47 % hardly seek any treatment. In addition to other treatments 59% reported taking traditional herbs for prevention of malaria. To prevent mosquitoes in the temporary shelters 41% used any kind of smoke, 22% used herbal smoke, and 15% specified fumigation of a specific bark secretion, which sticks to mosquito's wings, called "*Jhuna*". For self-protection against mosquito bites, 19% covered their body with cloths, 18% applied herbals like neem oil topically, and 15% crushed or drove away mosquitoes. Only 23% possessed bednet and just 8% had usable/proper nets. Only 8% used bednets in all seasons but 10% did not use bednets during summer and just 4% used them only during winter (Table 28).

Table 28: Knowledge, Attitude, and Practice (KAP) with reference to malaria in Mankidia tribe (100 respondents)

Knowledge	**Frequency (%)**
General awareness about malaria	
Malaria is a killer disease	17
Malaria is curable by taking medicine	20
Malaria is self-limiting disease	84
Winter is the high malaria season	11
Malaria is high during monsoon season	13
Malaria is high all the months	41
Symptoms of malaria	
Merely fever	33
Fever along with headache and body pain	4
Symptoms like vomiting, nausea, headache	17
Cause of malaria	
Mosquito bites	24
Bathing with cold water	17
Bathing in streams in Hilly-Forests	12
Forest visit	12
Source of malaria vectors	
Clean stream	12
Stagnant dirty water	50

Pond or stagnant clear water	11
Dirty stream	5
Leaves	3
Wells	2
Did not know	17
Knowledge of bednets	
Knew about bednets	35
Bednet could prevent malaria	26
Bednet could avoid insect nuisance	24
Attitude and Practice	
Sources for treatment of malaria	
Government health providers	21
Local chemists	9
Faith-healers "*Gunia*"	7
self-styled compounder	6
Indigenous Ayurveda practitioner	7
Hesitant to visit any health service providers	47
Complimentary practice of self-recognized traditional herbs for preventing malaria	59
Time lag in seeking treatment	
Within 3 days of suffering	3
Within a week	24
After a week	17
Hardly seek any treatment	47
Self-protection against mosquito bites	
Cover their body	19
Apply herbals like Neem oil topically	18
Crush or drive away mosquitos	15
Do nothing	47
Prevention of mosquitos in temporary shelters	
Smoke of any kind	41
Make smoke of available herbs	22
Regularly use fumigation of particular bark secretion which sticks to mosquito's wings called "*Jhuna*"	15

Use of bednets	
Possessed bednets	23
Possessed usable bednets	8
Use nets in all seasons	8
Uses nets in all seasons except summer	10
Use nets only during winter	4

4. Discussion

Epidemiology of malaria is affected by the socio-economic and demographic factors such as, family size, gender, caste, resettlement of human populations, education and occupation of the people. In addition, the health seeking behaviour of the community and their response to intervention measures influence malaria transmission (Sanjana et al. 2006, Yadav et al. 2007, Imbahale et al. 2010, Weldegebreal et al. 2014). In present study the two epidemiologically different ecotypes viz., highly malarious Hilly-Forest (HF) and comparatively less malarious Riverine-Plains (RP) showed variation in socio-demography and 'Knowledge, Attitude and Practice' (KAP) in relation to malaria. At micro level, in a family settings decision of the head of the family influences the health of all the members of the family. The questionnaire was thus, put to the head of the families surveyed in the two ecologically as well as epidemiologically different ecotypes. The responders were mostly males, since most of the families were headed by male members.

The socio-demographic features varied, the HF ecotype was dominated by Scheduled Tribes (ST) and Other Backward Classes (OBC), whereas, RP ecotype was dominated by Scheduled Castes (SC) and OBCs. Literacy rate was very low in both the ecotypes. Lower literacy rates were reported to be one of the major limiting factors in the way of effective malaria control and intervention measures (Bashar et al. 2012). The economic status was also different; income from agriculture produce was higher in HF ecotype. Besides, HF respondents also make money by collecting forest produce thereby increasing their earnings. The other factors like type of household construction, distance of household from vegetation, livestock, and vector breeding sources significantly influence disease transmission (Peterson et al. 2009, Bashar et al. 2012, Iwashita et al. 2014), were also studied. The construction pattern of houses in both the ecotypes was almost similar, but the houses were in close vicinity to farm lands and the vector breeding sites in the highly malarious HF ecotype than those in RP ecotype.

Moreover, the houses are clustered in HF ecotype. All these factors make community more prone to the malaria transmission by vector mosquitoes in HF ecotype.

Cattle: Man ratio was higher in Riverine-Plain areas, where *Anopheles culicifacies* is major malaria vector (Nanda et al. 2000, Sharma et al. 2006). *An. culicifacies* has higher zoophagic index, i.e., it prefers to feed on animals than on humans (Sharma, 1999). One can correlate the higher cattle per human ratio in *An. culicifacies*-dominated RP with the lower malaria transmission in this region as a result of zooprophylaxis. On the other hand, *An. fluviatilis* which is dominant in HF ecotype during the malaria season has higher anthropophagic index (Nanda et al. 2000, Sharma et al. 2006). Therefore zooprophylaxis in HF may not be significant.

Overall drug delivery was inadequate in the study villages of both the ecotypes as observed in all districts of Odisha. On an average 71% of ANMs and 55% of ASHAs usually refer falciparum-positive patients to the PHC/sub centres for treatment, the major reason for referral being the non-availability of drugs at the ANM and ASHA level as reported in an earlier study (Hussain et al. 2013). "The key strategy in the 2010 malaria drug policy is to make RDTs and anti-malarials available to ANMs and ASHAs for early diagnosis and complete treatment of each malaria case. The finding that half (48%) of the facilities did not stock all strengths of ACT is worrisome" (Hussain et al. 2013). In highly malarious HF areas, free malaria diagnosis and drug delivery at village level was significantly higher than that in the RP areas. Moreover the PHCs were also in close proximity (<3 Km) in HF than in RP. It is believed that availability of free malaria treatment facility in near vicinity reduces the chances of resorting to other treatment practices, delay in treatment seeking that leads to severe or complicated malaria (Vijayakumar et al. 2009). However, poor transport infrastructure and poor roads were main obstacle in accessing healthcare facilities in HF ecotype. Despite these being closer, the health care facilities outside the village (Sub center/PHC/DHH) are not easily accessible. In addition, hiring private transport/ ambulance is costlier in the HF ecotype. Therefore improving the roads and transport infrastructure and by providing ambulances at cheaper rates, the treatment seeking in severe malaria cases can be improved. Early diagnosis and prompt treatment (EDPT) is a key issue for malaria control Programme of World Health Organization (WHO) as well as National Vector-Borne Disease Control Programme (NVBDCP) of Government of India. Since the people of both the ecotypes had general

awareness about malaria and more than 50% of the respondents of both the ecotypes were found willing to opt for early treatment and most of them reported to be taking full course of antimalarials in both the ecotypes (Table 17), an efficient drug delivery system if in place, would lead to complete parasite clearance, provided there is no drug resistance.

Low socio-economic and educational status were reported as strong marker of ignorance about malaria (Yadav et al. 2007, Weldegebreal et al. 2014). In both the ecotypes studied, the literacy and awareness about malaria were positively related to the health seeking behaviour (Table 18). Earlier also it was reported that malaria treatment choices were affected by knowledge of the malaria problem (Karanja et al. 2002, Nyamongo 2002, Malisa and Ndukai 2009). Though majority of respondents in two ecotypes believed that malaria cannot be cured on its own, but is curable only with medicine so they seek treatment. However, some (11%) (Table 15) of the HF respondents also believe that malaria can be cured without medicines. Therefore it would be appropriate to enhance the Information Education and Communication (IEC) activities in highly malarious HF ecotype for further improvement in their overall awareness about malaria and health seeking behaviour.

A primary driver for the health-seeking behaviour was found to be the governance of the healthcare system mentioning that the private health sector leans to attend to the wealthy and the public sector to the poor (Smith et al. 2001, Balarajan et al. 2011). In the present survey, the families in low income category in both ecotypes have shown better attitude towards government setups than those families who could afford treatment from alternative private sector. Lesser proportion of competent families from HF and RP had shown preference for local Government setup (Table 18). It is probable that the people's perceptions about treatment seeking from Government setups might be influenced due to their prior experience of less than optimal services in terms of malaria diagnosis and treatments available at the health units. In such situation people might prefer purchasing drugs from chemists and self-styled doctors rather than spending time and money for going to government health units as observed in other malarious regions (Obrist et al. 2007). In this study respondents from HF ecotype preferred ASHA and PHC as diagnosis and treatment services for malaria was satisfactory, whereas in RP ecotype the above mentioned services were poor and the people relied more on non-government setups (local chemists and private practitioners) (Table 18).

Personal protection by bednets not only protects the household but also benefits even nonusers in the surrounding community by achieving suppression of the malaria transmission intensity due to their mass effect provided their coverage is above 70% (Hawley et al. 2003, Killeen et al. 2007). In district Deogarh bednet distribution at subsidized rate started in 2002 but it has not yet reached to adequate levels among families in RP and HF. Very few families in HF (4%) and RP (6%) were adequately protected by bednets (Table 19). Bednet adequacy was measured by possession, condition, usefulness and family size in the household (Peterson et al. 2009). The older bednets were found to be ineffective in both the ecotypes and only the bednets distributed within last two-three years were in good condition and usable.

Collectively, only 37% of respondents of Hilly forest and 52% respondents of RP related bednets with mosquitoes, malaria or both (Table 20). Rest of the respondents were not aware of the fact that the bednets are very effective tool for protecting them against malaria in particular but believed that these are useful for preventing mosquito bites which in general sense is true. However, majority of the bed net holders in both the ecotypes were keen for using bednet to protect themselves from bites of mosquitoes and other insects (Table 20). Other studies also reported this phenomenon suggesting people are primarily concerned about mosquito bites rather than threat of malaria (Padonou et al. 2011). Similar observations were previously reported from South-Eastern Tanzania (Minja et al. 2001, Malisa and Ndukai 2009) and suggested ITNs to be backed by communities education (Malisa and Ndukai 2009). Willingness (regular or seasonal) to adopt malaria preventive measures including use of insecticide treated / untreated bednets influences malaria incidences among households (Erhun et al. 2006, Padonou et al. 2011, Mora-Ruíz et al. 2014).

Insecticide treated bednets need proper maintenance since the insecticidal properties of nets are reduced by using hot water and drying nets in direct sunlight (Odhiambo et al. 2013). The bednet maintenance in both the ecotypes was not as per norms. Majority of the people in two ecotypes washed bed nets using detergent and dried them in sunlight against standard practice of drying them in shade. Therefore educating the bednet holders about the maintenance of the bednets is crucial for their effectiveness in malaria control.

In the two ecotypes bednets are found to be highly attractive commodity once available at a low / subsidized price. Most of them were willing to buy ITNs at a subsidized cost (Table

23). They positively perceive bednets as the main protective measure but can't afford bednets due to inadequate distribution at subsidized cost or high cost of commercially available nets as reported in other such economically backward populations of other districts of Odisha, Tanzania, and Bangladesh (Malisa and Ndukai 2009, Tarozzi et al. 2011, Bashar et al. 2012). High cost of commercially available nets was found to be the most limiting factor affecting individual's willingness-to-pay for ITN in both HF and RP ecotypes (Table 24). Cost of bednets is one of the important reason that prevent residents form purchasing ITN (Fraser-Hurt and Lyimo 1998, Hanson and Jones 2000, Malisa and Ndukai 2009, Peeters Grietens et al. 2010, Gebresilassie and Mariam 2011), besides differences in malaria endemicity (Mboera et al. 2008), socioeconomic status and accessibility to health services (Mboera et al. 2008, Malisa and Ndukai 2009). One of the keys to malaria control program among ethnic minorities and in remote rural areas was found to be the free-of-charge distribution of insecticide-treated bednets (Schellenberg et al. 2001, Prakash et al. 2008). Micro loan to purchase bednets in studied ecotypes might be of help as people are eager to buy bednets in instalments as this has been shown to be useful in other regions of Odisha (Tarozzi et al. 2011).

The perceptions and acceptance of IRS are known to influence malaria epidemiology (Erhun et al. 2006, Padonou et al. 2011, Mora-Ruíz et al. 2014).Vector-control personnel's calibre is important for successful IRS (Hlongwana et al. 2013). Our survey revealed that the local health authorities sprayed two rounds of IRS per year in HF whereas the IRS were not carried out once a year or even at a greater interval in RP (Table 25). In HF the coverage under IRS operation was better than that in RP. IRS was carried out as per norms in the houses covered. In HF and RP ecotype most of the respondents were well aware of the importance of IRS in controlling mosquitoes / malaria and majority of them allowed IRS operations in the houses visited by the spray team. Reasons for obstructing spray in certain places of the house by respondents (34% in HF and 38% in RP) were disturbance in their personal affairs and for a lesser proportion (8-24%) it was fear of poisoning of cattle. Similar reasons for refusal to household spraying were cited in desert areas of Rajasthan as well (Yadav et al. 2007). Notwithstanding these restrictions by some families, the inhabitants of households sprayed were cooperative and helped in IRS operations by removing animals and materials from walls.

The villagers of both the ecotypes (50-57%) were well aware about malaria and importance of IRS. However, (95%) respondents were not aware of the fact that plastering

shortly after spray would make the IRS ineffective. Traditionally, locals plaster their walls at regular intervals especially during the festivals. Due to the ignorance and traditional practices they mask the insecticide by mud plaster and make the residual insecticide ineffective as reported in other areas (Yadav et al. 2007). Therefore the spray team is required to instruct the community regarding re-plastering on the sprayed surfaces and this can also be conveyed through IEC activities.

For most of the natives of both ecotypes, fumigation/ smoke was the second best choice after bednets to protect themselves from mosquito bites. Usually people practice fumigation in homes as it has become a tradition irrespective of the fact that it drives mosquitoes away and hence by doing so malaria may be prevented (Imbahale et al. 2010). '*Jhun*' was preferred for fumigation by respondents of RP but not for those residing in HF ecotype inhabitants use any kind of smoke to ward off mosquitoes. Willingness to select malaria preventive measures/combat mosquitoes and their perception varies according to localities and socioeconomic groups viz. fumigation, traditional / modern malaria prophylactics which influences malaria incidences among households (Erhun et al. 2006, Yadav et al. 2007, Padonou et al. 2011). A small fraction of the inhabitants of HF and RP resorts to taking presumptive medicine for malaria and self-medication with antimalarials when sick. During working outside, most of the respondents of two ecotypes relied on their long sleeves clothing or on Neem oil (Tables 26). In both the ecotypes majority of people (>60%) preferred sleeping in rooms but almost half of the rooms were with open eves (having gaps between roof and walls) and, thus, could not restrict mosquito entry. Though people know about some mosquito control measures at personal level or community level, not all of them practice these control measures (Imbahale et al. 2010).

In this study KAP of nomadic tribe Mankidia prevalent in HF revealed that this tribe was largely illiterate having poor knowledge about causes and symptoms of malaria and reluctant to visit health service providers (Table 28). Such nomadic groups are known to play significant role in malaria transmission in areas of their prevalence (Service 1989, Erhart et al. 2005, Lokki et al. 2011). Such groups needs special attention of the government / health department and should be specially targeted for proper diagnosis and treatment of malaria.

Based on KAP study the key factors influencing malaria transmission in HF and RP ecotypes are depicted in Figure 5.

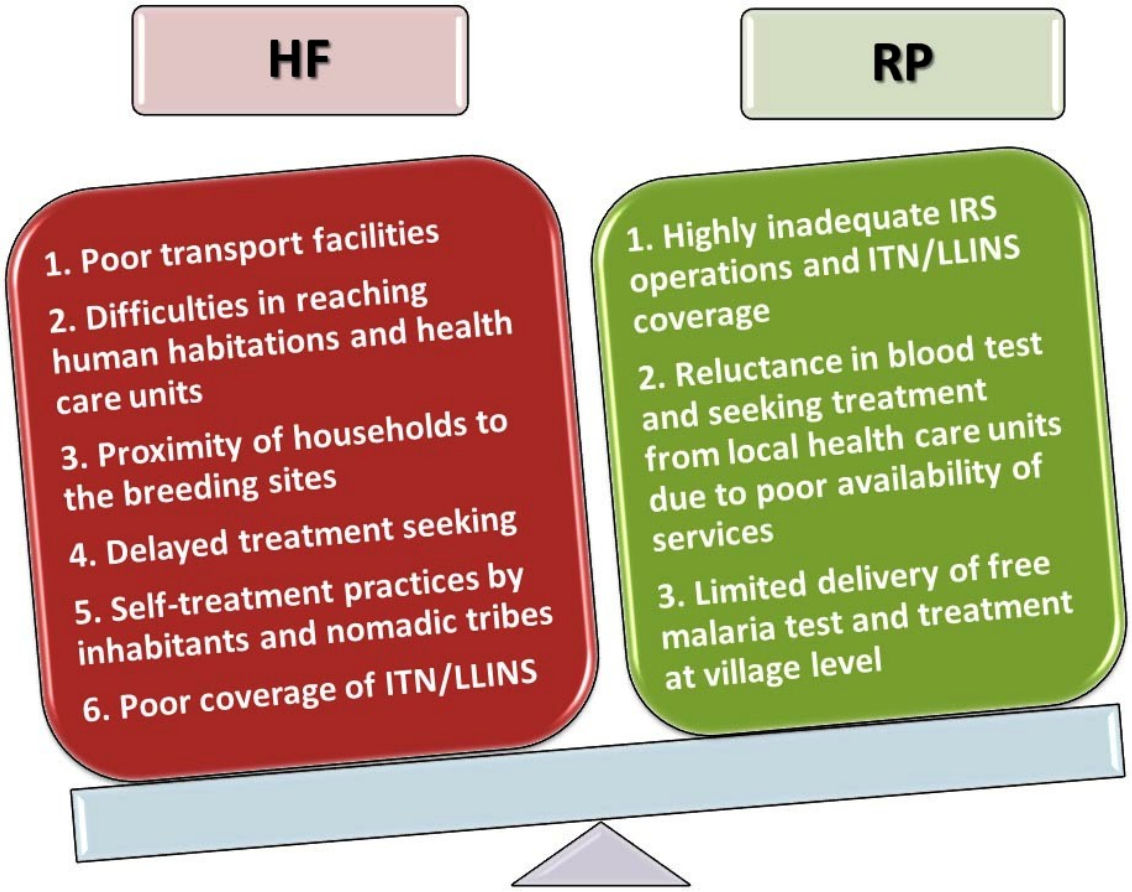

Figure 5: Key factors influencing malaria transmission in HF and RP ecotypes as revealed by questionnaire based survey

Low literacy, poor socioeconomic status, and prevalence of backward classes have strong association with malaria (Sabin et al. 2010, Singh et al. 2013, Ravendra K Sharma et al. 2015, Ravendra K. Sharma et al. 2015). In the present study the results are also in accordance with above facts. However there are some key factors which are distinct and influence malaria transmission in HF and RP ecotypes. In HF these include poor transport facilities, difficulties in reaching human habitations and health care units, proximity of households to the breeding sites, delayed treatment seeking, self-treatment practices by inhabitants and nomadic tribes and poor coverage of ITN/LLINS. Whereas in RP ecotype, highly inadequate IRS operations and ITN/LLINS coverage, reluctance in blood test and seeking treatment from local health care units due to poor availability of services and limited delivery of free malaria test and treatment at village level were the key factors. Therefore, strengthening of the basic infrastructure and

improvement in diagnosis and treatment services and vector control measures are needed to combat malaria transmission in different ecotypes of district Deogarh.

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CHAPTER V

Evidence based vector/ malaria control measures in hilly-forest and riverine-plain ecotypes

1. Introduction

This is the concluding chapter, where the salient features and risk factors that influence malaria transmission in both the ecotypes have been discussed. Situation specific and evidence based intervention measures have been suggested for effective malaria control in two ecotypes.

Malaria transmission is a complex process involving interplay between topographical, entomological, parasitological and human factors. Complex interlinks exist between different factors acting simultaneously to influence malaria transmission. No single malaria control strategy can be implemented worldwide. Considering malaria as a local and focal disease, epidemiological understanding of microhabitat ecotypes of malaria can help in devising effective control measures. Therefore an in-depth understanding and synthesis of the intricate relationship of the factors influencing transmission is essential for achieving better malaria control in various eco-epidemiological settings.

Most of the India's malaria burden is from rural parts of East and North-East regions which include majorly hilly forested and riverine-plain areas. District Deogarh of highly malarious state (Odisha) of the country is located in the core of the eastern malarious zone of India. Malaria is highly endemic and stable in this district in comparison to encircling districts. District Deogarh is less explored from malaria point of view due to lack of infrastructure and access problems. The health infrastructure in this district is inadequate and unlike in other districts there are no private hospitals. District Deogarh has rich forest cover (22% of the total area) and water resources comprising network of river and rivulets, perennial streams and stream channels, permanent ponds and irrigation canals. It is a less developed district primarily domiciled by SC and ST people in almost equal proportion. 79% of the rural population falls Below Poverty Line and 29% of the inhabitants have an earning less than Rs. 4000 per annum. The entire population of the district is at risk of malaria. Deogarh district can be broadly

divided in two eco-epidemiological settings, the hilly-forest (HF) and riverine-plain (RP) ecotypes which exhibit distinct topographical features and malaria incidence pattern. Based on the topography and epidemiological data collected from the district, villages under Tileibani PHC (Tileibani Block) representing HF ecotype and villages in Bamparada PHC (Barkot block) representing RP ecotype were investigated in order to understand the malaria transmission dynamics in two ecotypes and the interlinked factors were evaluated as follows.

i. Effect of environmental factors on vectors abundances and vector competence
ii. Effect of vectors abundances and vector competence on malaria transmission and antimalarial drug resistance
iii. Influence of parasite prevalence and their genetic makeup on evolution of drug resistance and transmission intensity
iv. Impact of people's knowledge, attitude, and practices related to intervention measures in shaping malaria transmission in two ecotypes

2. Salient findings of the study

Observations generated on various aspects like environment, vector bionomics, antimalarial drug resistance status, demographic and socioeconomic features of inhabitants and their responses against malaria control interventions have been analysed.

2.1. Geo-climatic and demographic studies

From analyses of the information generated, it was found that both the ecotypes were inhabited majorly by socioeconomically backward classes. Backward classes were predominant in HF comprised primarily by Scheduled Tribe (ST) followed by Scheduled Caste (SC) and Other Backward Classes (OBC) whereas in RP, there was dominance of SC followed by ST and OBC. This break up of communities was necessary to understand the practices and health seeking choices they make when suffering from malaria. The healthcare centres were distantly located and difficult to reach in HF as compared to RP due to poor roads and public transport facilities. In HF, houses were in close proximity to bodies of water (e.g. streams) which were covered with dense vegetation providing suitable breeding sites for malaria vectors, whereas in RP, the major vector breeding sites were riverbed water pools farther away from houses. In HF, the high humidity and lesser temperature extremities were observed which provide conducive climatic conditions for highly efficient sylvatic vectors like *An. fluviatilis*, and *An. minimus* along with the regional vector *An. culicifacies*. These demographic,

socioeconomic, and ecological features were found to influence malaria transmission and epidemiological variability in the two ecotypes.

2.2. Entomological investigations

Regarding entomological investigations carried out in two ecotypes, *Anopheles fluviatilis* was prevalent only in HF ecotype. Its population was found to build-up during post monsoon (September) and winter (November) and peak densities were observed in March. *Anopheles culicifacies* was found prevalent in both HF and RP ecotypes with peak densities in monsoon and post monsoon months. In HF *An. fluviatilis* comprised of sibling species S and T which were found almost in equal proportion. Both the sibling species viz. S and T of *An. fluviatilis* were endophilic and found resting mainly in mixed dwellings of human and cattle. Species S was predominantly anthropophagic and found positive for *P. falciparum* sporozoites in all seasons of its prevalence. In contrast, *An. fluviatilis* species T was primarily zoophagic and none of the samples were found positive for human malaria parasites. In both the ecotypes sibling species B and C of *An. culicifacies* were prevalent with predominance of species B. Species B and C were primarily zoophagic. *An. culicifacies* C was found positive for *P. falciparum* sporozoites in both HF and RP whereas species B was also incriminated in RP ecotype. These sibling species were incriminated during monsoon and post monsoon months. Assessment of susceptibility status of vector species against different insecticides used in public health revealed that *An. fluviatilis* was fully susceptible to malathion and deltamethrin but was in verification required category for DDT. *Anopheles culicifacies* was found highly resistant to DDT and malathion and percent mortality against deltamethrin ranged between 80-82% in two ecotypes hence making it tolerant / resistant to this synthetic pyrethroid as well.

The information generated provides better understanding of entomological factors influencing intensity of malaria transmission in two ecotypes and would be useful in planning effective vector control strategies in study areas.

2.3. Parasitological observations

There was predominance of *P. falciparum* (>95%) in both the ecotypes and *P. vivax* cases were very few. Molecular analysis revealed genetic variation at the Single Nucleotide Polymorphism (SNP) level in four different genes of *P. falciparum* (*Pfcrt*, *Pfmdr1*, *Pfdhfr,* and *Pfdhps*) that confer resistance to different antimalarials in two different eco-epidemiological settings, *i.e*. HF and RP ecotypes. Greater frequency of antimalarial resistance conferring SNPs

and haplotypes was observed in all four genes in *P. falciparum,* and *Pfdhps* was the most variable gene among the four. No significant genetic differentiation could be observed in isolates from HF and RP ecotypes. Twelve novel, hitherto unreported nucleotide mutations could be observed in the *Pfmdr1* and *Pfdhps* genes. While the *Pfdhps* gene presented highest haplotype diversity, the *Pfcrt* gene displayed the highest nucleotide diversity. When the data on all the four genes were complied, the isolates from HF ecotype were found to harbour higher average nucleotide diversity than those from RP ecotype. High and positive Tajima's *D* values were obtained for the *Pfcrt* and *Pfdhfr* genes in isolates from both the HF and RP ecotypes, with statistically significant deviation from neutrality in the RP ecotype. Different pattern of Linkage Disequilibrium (LD) among SNPs located in different drug-resistant genes was found in the isolates collected from HF and RP ecotypes. Whereas in the HF ecotype, SNPs in the *Pfmdr1* and *Pfdhfr* were significantly associated, in the RP ecotype, SNPs located in *Pfcrt* were associated with *Pfmdr1*, *Pfdhfr* and *Pfdhps*. These findings provide a baseline understanding on how different micro eco-epidemiological settings influence evolution and spread of different drug resistance alleles. These findings further suggest that drug resistance to chloroquine and sulfadoxine-pyrimethamine is approaching fixation level, which requires urgent attention of malaria control program in the study area. This study will be of great significance in devising appropriate drug policy for effective treatment of malaria patients and for halting the spread of multi-drug resistant *P. falciparum*.

2.4. KAP studies

The questionnaire based KAP survey conducted showed certain commonalities and few distinct and important differences in relation to malaria transmission in two ecotypes. The common factors that contribute to malaria transmission in two ecotypes are as follows.

Overall very low educational status was reported in the families of the respondents of two ecotypes. Economic status of the families surveyed in two ecotypes was poor as estimated mean income per capita per day was Rs. 19.5 and 22.5 in RP and HF respectively. Very few families in HF (4%) and RP (6%) were adequately protected with bednets and the available bednets with them were not maintained as per norms. High cost of commercially available nets was found to be the most limiting factor affecting individual's willingness-to-pay for ITN in both HF and RP ecotypes. Though the respondents were cooperative in IRS operations, 95% of them were not aware of the fact that plastering shortly after spray would make the IRS

ineffective. In the two ecotypes majority of respondents slept in the rooms but half of the rooms in both the ecotypes were found widely open facilitating entry of mosquitoes.

KAP study helped in identification of some key factors which were distinct and influenced malaria transmission in HF and RP ecotypes. In HF these included poor road infrastructure and transport facilities, proximity of households to the breeding sites, difficulties in reaching human habitations by the healthcare/ surveillance workers and health care units by the community, delayed treatment seeking, self-treatment practices by inhabitants and poor coverage of ITN/LLINS. A nomadic tribe prevalent in HF area was largely illiterate having poor knowledge about causes and symptoms of malaria and reluctant to visit health service providers. Whereas in RP ecotype, highly inadequate IRS operations and insufficient coverage with ITN/LLINS, reluctance in blood test and seeking treatment from local health units due to poor availability of services and limited delivery of free malaria test and treatment at village level were the key factors.

From the above findings it can be concluded that in district Deogarh HF areas are mainly contributing to the malaria transmission. This is so because in HF ecotype the presence of highly efficient forest vectors like *Anopheles fluviatilis* and *Anopheles minimus* and the favourable environment accelerate the malaria transmission. Moreover prevalence of nomadic tribe in HF acts as floating reservoir of malaria and contributed to high malaria incidence. The RP ecotype was found to be comparatively less malarious primarily due to geo-climatic and entomological features. In both the ecotypes improper/ inadequate use of antimalarials by the inhabitants has shown to increase drug resistance and transmission intensity (Kar et al. 2014, Kar et al. 2016). The malaria episodes experienced by the population in both the ecotypes reduce affordability and strength for care-seeking and again elevate resistance, which sets in vicious cycle and in turn confers high transmission in a cyclic process (Pattanayak et al. 2006, Chuma et al. 2009, Chuma et al. 2010). The vector / malaria intervention measures in form of IRS operation, bednet coverage, and health care delivery were found to be inadequate and insufficient in both the ecological settings though the degree varied. Based on the observations the key risk factors in malaria transmission in the two ecotypes are depicted in Figure 1. Therefore, strengthening the basic infrastructure including the healthcare delivery system and educating the community for improvement in their attitude and practices in respect of malaria intervention are some of the factors which require attention.

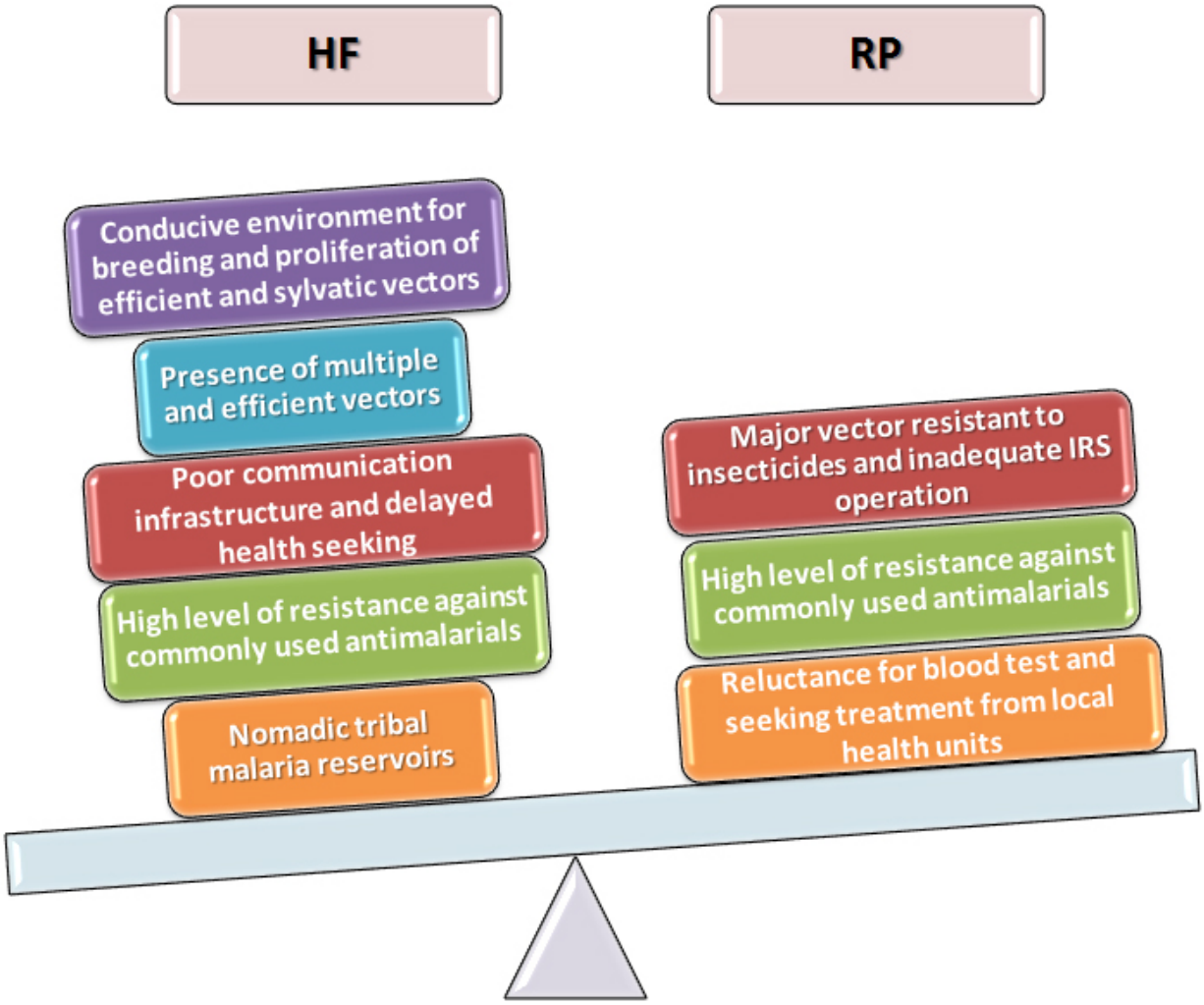

Figure 1: Key risk factors contributing to malaria incidence in HF and RP ecotypes

3. Evidence based suggested malaria control methods

Taking into account observations on topography, vector distribution and bionomics, vector susceptibility status to insecticides, parasite prevalence and resistance status against antimalarials, people's health seeking behaviour and attitude towards malaria control activities, the following evidence based intervention measures are suggested.

3.1. Anti-vector measures

Presently Indoor Residual Spray (IRS) with DDT is being carried out, two rounds in HF and only one round in RP. Based on susceptibility status of primary malaria vectors (increase in tolerance / resistance against DDT in *An. fluviatilis* and highly resistant status of *An. culicifacies* against DDT) and differences in their prevalence period, it would be appropriate to replace DDT with synthetic pyrethroids in IRS operations and a minimum of

two rounds of IRS are required in both ecotypes with good coverage of human and mixed dwellings. An additional round of IRS after winter in HF ecotype would be useful in reducing extended transmission by the highly efficient vector *An. fluviatilis* which is prevalent in good numbers in spring season.

Long lasting Insecticidal Nets (LLINS) are distributed at subsidized cost by the health authorities in both HF and RP ecotypes but the population coverage is very low. The KAP study revealed that the families possessing bednets were mostly having good perception and were keen to use bednets. Poor socioeconomic status of the communities limits them from paying the subsidized cost or buying these from markets. Therefore free/ subsidized distribution of LLIN by the Government / NGOs / international funding agencies needs to be increased so that good population coverage is ensured, particularly in HF ecotype where highly efficient and primarily anthropophagic malaria vectors viz. *An. fluviatilis* sibling species S and *An. minimus* sibling species A are prevalent. Provision to pay the subsidized cost of the bednets in instalments would also improve their usage as has been observed in the present study and also reported in neighbouring districts (Tarozzi et al. 2011).

3.2. Anti-parasitic measures

In this study *P. falciparum* was found to be predominant malaria parasite (>95%) prevalent in both ecotypes. Genetic characterization of *P. falciparum* has shown high prevalence of drug resistant genotypes in both HF and RP ecotypes. Higher prevalence in drug resistant genotypes can be attributed to indiscriminate use of antimalarials and different treatment practices. The findings indicate that CQ resistant genotypes are approaching fixation level and SP resistant genotypes are evolving very fast as reported from neighbouring regions (S. Das et al. 2012, Sabyasachi Das et al. 2012, Das Sutar et al. 2013).

Therefore, the existing drug policy needs to be reviewed as SP is a partner drug administered with artemisinin in the presently used artemisinin based combination therapy (ACT) against *P. falciparum* cases. Replacement of partner drug in ACT is suggested so as to restrict the use of SP composition for pregnant women (ASP combination therapy is only safe and life-saving medicine for pregnant malaria patient). The use of CQ should be restricted to treating vivax malaria. Efforts should be made to stop indiscriminate use of antimalarials in both HF and RP ecotypes and stricter implementation of national drug policies.

3.3. Additional steps required on part of government/ health department

As the study has revealed that delay in health seeking specifically in HF is primarily due to poor communication infrastructure viz. poor roads, and unavailability of public transport system/ ambulance facility. Therefore, efforts are required to improve roads and transport facilities in these areas so that accessibility to Government run health care units is facilitated. An important parameter of encouraging treatment seeking at village level ASHA and at PHC is the availability of free malaria test and medicines. Government health care units and village level ASHA workers should be made better equipped for free testing by Rapid Diagnostic Kits (RDTs) and treatment of malaria. This in turn would reduce treatment seeking from alternative sources.

IEC activities needs to be strengthened in study areas for educating the community on various aspects of malaria and seeking their cooperation so that mud plastering of the houses immediately after IRS operation is avoided and bednets are used properly. For safeguarding the effectiveness of bednets it would be important to educate villages on their proper maintenance. The pharmacists and self-styled doctors should be educated on national drug policy so that indiscriminate use of antimalarial is avoided.

Primarily Vulnerable Nomadic Tribes prevalent in forest areas are floating reservoirs of malaria infection. They are often asymptomatic and reluctant to seek treatment. Such groups should be specially targeted for proper diagnosis and treatment of malaria. Imparting knowledge and improving the socio-economic status of Mankidias can in the long run improve their health-seeking behaviour and malaria prevention practices.

This study has provided in-depth understanding of malaria transmission dynamics in the HF and RP settings, highlighted epidemiological risk factors influencing malaria transmission. It is envisaged that the suggested evidence based and situation specific control measures would be useful in curbing malaria transmission in study areas and in other similar eco-epidemiological settings.

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List of publications (articles enclosed)

Kar, N. P., K. Chauhan, N. Nanda, A. Kumar, J. M. Carlton and A. Das (2016). "Comparative assessment on the prevalence of mutation in the *Plasmodium falciparum* drug-resistant genes in two different ecotypes of Odisha state, India." Infect Genet Evo. **41**: 47-55.

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