आईसीएमआर-राष्ट्रीय मलेरिया अनुसंधान संस्थान की सटिप्पण ग्रंथ सूची

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(Publications: 2011-2015)

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Annotated Bibliography of National Institute of Malaria Research Publications (2011-2015)

Contents

Master List

Titles: A-Z

2011

1. Mathur A, Singh R, Yousuf S, Bhardwaj A, Verma SK, Babu P, Gupta V, Prasad GBKS, **Dua VK**. Antifungal activity of some plant extracts against clinical pathogens. ***Adv Appl Sci Res* 2011; *2*(2): 260-4.**

ABSTRACT

The antifungal activity and minimum inhibitory concentration (MIC) of various plant extracts in different solvents such as hydro-alcohol (50 % v/v) and hexane of plants traditionally used as medicines as *Valeriana jatamansi* (Sugandhbala), *Coleus barbatus* (Pathar choor), *Berberis aristata* (Kingore), *Asparagus racemosus* (Satrawal), *Andrographis paniculata* (Kalmegha), *Achyranthes aspera* (Latjiri), *Tinospora cordifolia* (Giloei), *Plantago depressa* (Isabgol) were evaluated against *Aspergillus niger* and *Candida albicans*. Hydro-alcoholic extracts of all the plants were found to have maximum antifungal activity in comparison to hexane extracts. Hydroalcoholic extracts of *Andrographis paniculata* and *Achyranthes aspera* showed maximum potency against *Aspergillus niger* and *Candida albicans* at highest MIC value of 0.5 and 0.3 mg/ml respectively. Hexane extracts of *Andrographis paniculata* showed highest MIC value of 0.7 mg/ml against *Aspergillus niger*.

KEY WORDS: Hydroalcoholic extracts, hexane extracts, clinical pathogens, antifungal activity.

2. **Prajapati SK**, **Joshi H**, **Dua VK**. Antigeneic repertoire of *Plasmodium vivax* transmission blocking vaccine candidates from the Indian subcontinent. ***Malar J* 2011; *10*: 111.**

ABSTRACT

Background: Genetic polymorphism is an inevitable component of a multistage infectious organism, such as the malaria parasite. By means of genetic polymorphism, parasite opts

particular polymorph and reveals survival advantage. *Pvs25* and *pvs28* are sexual stage antigen genes, expressed at the ookinete stage inside the mosquito gut, and considered as potential transmission-blocking vaccine candidates. This study presents sequence variations in two important transmission blocking antigen genes *pvs25* and *pvs28* in the field isolates of *P. vivax* from the Indian subcontinent. **Methods:** One hundred microscopically diagnosed *P. vivax* isolates were collected from five geographical regions of India. *Pvs25* and *pvs28* genes were PCR amplified and sequenced to assess sequence variation among field isolates. **Results:** A total of 26 amino acid substitutions were observed in *Pvs25* (10) and *Pvs28* (16) among field isolates of *P. vivax*. Tandem repeat polymorphism observed in pvs28 shows 3-6 tandem repeats in the field isolates. Seven and eight novel amino acid substitutions were observed in *Pvs25* and *Pvs28*, respectively in Indian isolates. Comparison of amino acid substitutions suggests that majority of substitutions observed in global isolates were also present in Indian subcontinent. A single haplotype was observed to be major haplotype among isolates of Delhi, Nadiad, Chennai and Panna except in isolates of Kamrup. Further, population comparison analyses suggest that *P. vivax* isolates inhabiting in north-eastern region (Kamrup) were distantly related with the isolates from remaining parts of the country. Majority of the amino acid substitutions observed in Indian isolates were more identical to the substitutions reported from isolates of Thailand and Bangladesh. **Conclusion:** Study uncovered many new amino acid substitutions as well as a predominance of single haplotype in Indian subcontinent except in north-eastern region of the country. The amino acid substitutions data generated in this study from different geographical regions of the Indian subcontinent could be helpful in designing a more effective anti-malarial transmission-blocking vaccine.

3. Verma G, **Dua VK**, Agarwal DD, **Atul PK**. Antimalarial activity of *Holarrhena anti- dysenterica* and *Viola canesxcens*, plants traditionally used against malaria in the Garhwal region of northwest Himalaya. ***Malar J* 2011; *10*: 20**.

ABSTRACT

Background: The increasing number of multidrug-resistant *Plasmodium* strains warrants exploration of new anti-malarials. Medicinal plant research has become more important, particularly after the development of Chinese anti-malarial drug artemisnin from *Artemisia annua*.

The present study shows evaluation of anti-malarial effects of two plants commonly used against malaria in the Garhwal region of north-west Himalaya, in order to discover the herbal-based medicine. **Methods**: *In vitro* anti-plasmodial sensitivity of plant extracts was assessed using schizont maturation and parasite lactate dehydrogenase (pLDH) assay. Cytotoxic activities of the examined extracts were determined on L-6 cells of rat skeletal muscle myoblast. The 4-day test for anti-malarial activity against a chloroquine sensitive*Plasmodium berghei* NK65 strain in Swiss albino mice was used for monitoring *in vivo* activity of plant extracts. **Results:** Chloroform extract of *H. antidysenterica* (HA-2) and petroleum ether extract of *V. canescens* (VC-1) plants significantly reduced parasitaemia in *P. berghei* infected mice. The extract HA-2 showed*in vitro* anti-plasmodial activity with its IC_{50} value 5.5 μg/ml using pLDH assay and ED_{50} value 18.29 mg/kg in *P. berghei* infected Swiss albino mice. Similarly petroleum ether extract of *V. canescens*(VC-1) showed *in vitro* anti-plasmodial activity with its IC_{50} value 2.76 μg/ml using pLDH assay and ED_{50} 15.8 mg/kg in *P. berghei*infected mice. The extracts coded as HA-2 at 30 mg/kg and VC-1 at 20 mg/kg exhibited parasite inhibition in mice: 73.2% and 63.0% respectively. Of these two plant extracts, petroleum ether extract of*V. canescens* was found slightly cytotoxic. **Conclusion:** The present investigation reflects the use of these traditional medicinal plants against malaria and these plants may work as potential source in the development of variety of herbal formulations for the treatment of malaria.

4. Mathur A, Verma SK, Yousuf S, **Singh SK**, Prasad GBKS, **Dua VK**. Antimicrobia Potential of roots of *Riccinus communis* against pathogenic microorganisms. ***Int J Pharma Biosci* 2011; *2*(1): 545-8.**

ABSTRACT

Antimicrobial activity of various extracts of roots (200mg/ml) of *Ricinus communis* were screened against pathogenic microorganisms such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus vulgaris, Bacillus subtilis, Candida albicans* and *Aspergillus niger* using well diffusion method. Aqueous extracts don't show any significant antimicrobial properties. The hexane and methanol extracts revealed maximum antimicrobial activity (p<0001). These findings established the potential of the roots of *Ricinus*

communis as an effective antimicrobial agent. However, further studies are needed to evaluate active compounds and probable medicinal benefits in chemotherapy among humans.

KEYWORDS: *Ricinus communis*, pathogenic microorganisms, methanol extracts, hexane extracts and chemotherapy.

5. Mathur A, Verma SK, **Singh SK**, Prakash A, Prasad A, Prasad GBKS, **Dua VK**. Anti-inflammatory activity of earthworm extracts. ***Int J Pharmaceut Sci Res* 2011; *2*(2): 278-81.**

ABSTRACT

Earthworm plays a major role in the proper functioning of the soil ecosystem. It acts as scavenger and helps in recycling of dead and decayed plant material by feeding on them. Earthworm increases the soil fertility and is often referred to as a farmer's friend. Earthworms have been used in medicine for various remedies. In the present investigation, various solvent extracts of an earthworm, *Eudrilus eugeniae* were prepared and antiinflammatory activity of these extracts were determined. The petroleum ether fraction possessed maximum anti-inflammatory activity in carrageenan induced albino rats in comparison to 95% ethanol and 0.2 M phosphate buffer (pH, 7.0) extracts. The paw volume was determined and was compared with that of aspirin, a standard anti-inflammatory drug. The results indicates that petroleum ether fraction of earthworm extract possessed similar anti-inflammatory activity as that of aspirin.

KEYWORDS: *Eudrilus eugeniae*, anti-inflammatory activity, 95% ethanol, petroleum ether, 0.2 M phosphate buffer (pH, 7.0).

6. **Shah NK**, Dhillon GP, **Dash AP**, Arora U, Meshnick SR, **Valecha N**. Antimalarial drug resistance of *Plasmodium falciparum* in India: changes over time and space. ***Lancet Infect Dis* 2011; *11*(1): 57-64.**

ABSTRACT

After the launch of the National Malaria Control Programme in 1953, the number of malaria cases reported in India fell to an all-time low of 0•1 million in 1965. However, the initial success could not be maintained and a resurgence of malaria began in the late 1960s. Resistance of *Plasmodium falciparum* to chloroquine was first reported in 1973 and increases in antimalarial resistance, along

with rapid urbanisation and labour migration, complicated the challenge that India's large geographical area and population size already pose for malaria control. Although several institutions have done drug-resistance monitoring in India, a complete analysis of countrywide data across institutions does not exist. We did a systematic review of *P. falciparum* malaria drug-efficacy studies in India to summarise drug-resistance data and describe changes over the past 30 years to inform future policy. Continued use of chloroquine for treatment of *P. falciparum* malaria in India will likely be ineffective. Resistance to sulfa–pyrimethamine should be closely monitored to protect the effectiveness of treatment with artesunate plus sulfadoxine–pyrimethamine, which is the new first-line treatment for *P. falciparum* malaria. Strategies to reduce the emergence and spread of future drug resistance need to be proactive and supported by intensive monitoring.

7. **Dua VK**, Verma G, Agarwal DD, Kaiser M, Burn R. Antiprotozoal activities of Traditional medicinal plants from the Garhwal region of northwest Himalaya, India. ***J Ethnopharmacol* 2011; *136*(1): 123-8.**

ABSTRACT

Ethnopharmacological Relevance: In a search for new plant-derived biologically active compounds against protozoan parasites, an ethnopharmacological study was carried out to evaluate extracts from selected 17 traditional medicinal plants which were used by healers from the Garhwal region of North West Himalaya for the treatment of protozoal infections and fever including malaria. **Materials and Methods**: *In vitro* activity against erythrocytic stages of *Plasmodium falciparum* was determined using a modified [3H]-hypoxanthine incorporation assay with the chloroquine- and pyrimethamine-resistant K1 strain. Activity against *Trypanosoma brucei rhodesiense* was performed on the STIB 900 strain and activity against *Trypanosoma cruzi* on infected rat skeletal myoblasts (L6 cells) seeded in 96-well microtitre plates while amastigotes of *Leishmania donovani* strain MHOM/ET/67/L82 were used to assess activity against *Leishmania donovani*. Cytotoxicity assays were performed against rat skeletal myoblasts (L6-cells). **Results and Conclusions**: Extracts of *Artemisia roxburghiana*, *Roylea cinerea*, *Leucas cephalotes*, *Nepeta hindostana* and *Viola canescens* showed good antiplasmodial activity (IC50<5 μg/ml). The chloroform extract of *Artemisia roxburghiana* was the most active (IC50 value of 0.42 μg/ml) and the most selective (SI=78) extract for *Plasmodium falciparum* among all plants

extracts examined. The chloroform extract of *Leucas cephalotes* and the petroleum ether extract of *Viola canescens* exhibited substantial activities against *Leishmania donovani* with IC50 values of 3.61 μg/ml (SI=8) and 0.40 μg/ml (SI=30), respectively. The petroleum ether extract of *Viola canescens* exhibited activity against *Trypanosoma cruzi* with an IC50 value of 1.86 μg/ml (SI=7). Methanol and water extracts from all plants under investigation were found inactive against all parasites tested. These results support investigation of components of traditional medicines as potential new antiprotozoal agents. On the other hand since herbalism has become the main stream throughout the world, investigation demonstrates that these non-polar plant extracts of six of the plants examined in this study could play an important role in herbal formulations for the treatment of vector borne protozoal diseases.

KEYWORDS: traditional medicinal plants; Garhwal region; antiprotozoal activity; *Plasmodium falciparum*; *Trypanosoma brucei rhodesiense*; *Leishmania donovani*; *Trypanosoma cruzi*; selectivity index; herbal formulation

8. Mathur A, Gupta V, Verma SK, **Singh SK**, Arachana P, Prasad GBKS, **Dua VK**. Anti–inflammatory activity of different fractions of *Leucas cephalotes* leaves extract. ***Int J Curr Pharmaceut Rev Res*** **2011; *1*(3): 28-32.**

ABSTRACT

Leucas cephalotes (Labiatae) is an annual herb found in Uttarakhand and throughout India as a weed in cultivated fields, wastelands and roadsides. In the present investigation the phytochemical screening of the crude methanolic extract of leaves was done by conventional methods and anti-inflammatory activity of crude, alkaloid, aqueous, hexane, petroleum ether and non alkaloid fractions of the leaves were investigated using carrageenan induced rat paw edema method. The results showed that alkaloidal fractions of the leaves causes significant reduction in inflammation *i.e* 80 % (100 mg/kg) followed by crude methanol extract *i.e* 61 % (100 mg/kg) and aqueous extract *i.e.*, 58 % (100 mg/kg) as compared to standard anti-inflammatory drug aspirin *i.e* 68.62% (25mg/kg). However, non alkaloidal, hexane and petroleum ether fractions did not show any anti-inflammatory activity irrespective of the time intervals. Thus crude methanolic extract and alkaloidal fractions of leaves of the plant can be fully explored for its anti-inflammatory potential.

KEYWORDS: *Leucas cephalotes*, anti-inflammatory activity, phytochemical screening.

9. Mathur A, Prasad GBKS, **Dua VK**. Antiinflammatory activity of leaves extracts of *Murraya koenigii* L. ***Int J Pharma Biosci 2011*; *2*(1): 541-4.**

ABSTRACT

This work has been done for the investigation of the anti-inflammatory activity of solvent extracts of dried leaves of *Murraya koenigii* Linn. by oral administration at dose of 100, 200 and 400 mg/kg body weight in healthy albino rats. Extracts were studied for its anti-inflammatory activity by using carrageenan- induced hind paw edema in albino rats and the mean increase in paw volume and % inhibition in paw volume were measured plethysmometrically at different time intervals after carrageenan (1% w/v) injection. The methanol extract showed significant ($P < 0.001$) reduction in the carrageenan-induced paw edema in comparison to aqueous extracts. Petroleum ether and hexane extracts showed no reduction in paw edema. The methanol extract showed anti-inflammatory effect in dose dependent manner when compared with the control and standard drug, Aspirin (10mg/kg, p.o). These inhibitions were statistically significant ($p < 0.05$). Thus our investigation suggests a potential benefit of methanol and aqueous extracts of leaves of *Murraya koenigii* in treating conditions associated with inflammation. This study illustrates about the presence of some active polar compounds in the leaves extracts which might be responsible for anti-inflammatory activity.

KEYWORDS: *Murraya koenigii*, anti-inflammatory activity, methanol extract, aqueous extract, paw volume, aspirin.

10. Mudiam MK, Jain R, **Dua VK**, Singh AK, **Sharma VP**, Murthy RC. Application of ethyl microextraction-gas chromatography-mass Spectrometric determination of bisphenol-A in water and milk samples. ***Anal Bioanal Chem* 2011; *401*(5): 1695-701.**

ABSTRACT

A simple and rapid analytical method based on in-matrix ethyl chloroformate (ECF) derivatization has been developed for the quantitative determination of bisphenol-A (BPA) in milk and water samples. The samples containing BPA were derivatised with ECF in the presence of pyridine for 20 s at room temperature, and the non-polar derivative thus formed was extracted using

polydimethylsiloxane solid-phase microextraction (SPME) fibres with thicknesses of 100 μm followed by analysis using gas chromatography-mass spectrometry. Three alkyl chloroformates (methyl, ethyl and isobutyl chloroformate) were tested for optimum derivatisation yields, and ECF has been found to be optimum for the derivatisation of BPA. Several parameters such as amount of ECF, pyridine and reaction time as well as SPME parameters were studied and optimised in the present work. The limit of detection for BPA in milk and water samples was found to be 0.1 and 0.01 μg L(-1), respectively, with a signal-to-noise ratio of 3:1. The limit of quantitation for BPA in milk and water was found to be 0.38 and 0.052 μg L(-1), respectively, with a signal-to-noise ratio of 10:1. In conclusion, the method developed was found to be rapid, reliable and cost-effective in comparison to silylation and highly suitable for the routine analysis of BPA by various food and environmental laboratories.

KEYWORDS: Bisphenol-A, GC–MS, Ethyl chloroformate, SPME, Water Milk.

11. Patil RR, **Tiwari SN**, **Ghosh SK**. Assessing perceptions about malaria among the elected representatives in rural India. ***Trop Parasitol*** **2011; *2*: 83-7**.

ABSTRACT

Objective: The short-term objective of our endeavour was to understand the perception of Grama panchayat presidents and secretaries on the issues related to malaria and its control, being the key leaders of the Panchayat Raj Institutions (PRIs) at a Grama panchayat level. This was necessary to achieve the long-term objective of the role of PRIs in malaria control and their enhanced participation/partnership with the public health sector. **Materials and Methods**: Grama panchayat presidents and secretaries representing all the 28 Grama panchayats of Chikkanayakanahalli taluk Tumkur district in Karnataka were invited for a 1-day workshop. Deliberations with the participants (*n* = 32) shed light on their perceptions with respect to knowledge, attitude and practice vis-a-vis malaria and its control strategies. **Results**: Their knowledge of malaria as a disease was fairly good as they were well aware of it being a communicable disease and its transmission by mosquitoes. However, knowledge about the breeding sources of malaria mosquitoes (*Anophelines*) was very poor. Many practices in vogue to control mosquitoes at the community level were unscientific. There was a general negative attitude toward the government's handling of the malaria problem and the credibility of the health care system. **Conclusion**:

Existence of health committees in every Grama panchayat coupled with their jurisdiction and responsibilities toward sanitation, water supply and health care resources makes PRIs a natural partner to the health sector. While health education and public health intervention strategies should be based on generic principles of science, the implementation and operational specifics should definitely be based on a sociological perspective of the stakeholders.

KEYWORDS: Grama panchayat, KAP, intersectoral, malaria, rural governance, social factors.

12. Tiwari HK, Upadhyay S, Upadhyay RK, **Rawat M**, **Sharma A**. Chalcones induced inhibition of plasmepsin ll, a hemoglobin degrading malarial parasite protease from *Plasmodium falciparum*. ***J Phama Res* 2011; *4*(4): 1253-8.**

ABSTRACT

A series of ten novel chalcones (2a-2j) have been synthesized, purified and characterized by elemental analyses, and IR, Mass and 1H NMR spectroscopy. All the products were evaluated for their in-vitro antimalarial activity against a chloroquine-sensitive isolate of *Plasmodium falciparum* (MRC 2) and IC_{50} values for cell growth inhibition calculated were found from 6 to 39 µg/ml. The interactions between these chalcones and plasmepsin II (an aspartic protease and plausible novel target for antimalarial drug development) being essential for hemoglobin degradation by the parasite have been assayed by UV-vis spectroscopy. The IC 50 values for inhibition of plasmepsin II by these chalcones were found from 3 to 57 µM. The pivotal mechanism of antimalarial activity of these compounds via plasmepsin II inhibition in the *P. falciparum* malaria parasite has been demonstrated.

KEYWORDS: Chalcones, antimalarial activity, malaria, *Plasmodium falciparum*, hemoglobin degradation, plasmepsin II

13. Khan N, Pandey V, **Das A**. Characterization, comparative genomics, and evolutionary Inferences of a human drug metabolizing (NAT2) gene. ***Interventional Med Appl Sci* 2011; *3*: 65-73.**

ABSTRACT

Aim: The present-day genetic architecture of a species bears much significance to its closely related species. In recent availability of whole genome sequence data for closely related species, it

is possible to detect genetic similarities/differences in specific lineages and infer the role of evolutionary forces in bringing such similarities/differences. In this respect, NAT2 gene, responsible for drug metabolism, is conserved across a few taxa and, thus, comparative genomic studies could be useful for better pharmacogenetic realization. **Methods**: DNA sequences of human NAT2 gene were retrieved from NCBI and characterized. Comparative and evolutionary analyses were performed with sequences from four mammalian taxa and one avian taxon with different statistical algorithms. **Results**: The observed genetic architecture of NAT2 gene was different across the taxa. Phylogenetic inferences revealed that human and chimpanzee are diverged recently and fowl was found to be diverged from rest of the taxa significantly. Also, gene length, microsatellites, *Ka/Ks*, secondary structure, and distribution of CpG islands were observed across taxa. **Conclusions**: The detail architecture of NAT2 gene and its evolutionary history in different taxa show relationships with other taxa. Future population-based study in NAT2 would unravel the correlation between nucleotide changes and differential ability of drug metabolization in humans.

KEYWORDS: NAT2, human, drug metabolism, comparative genomics, evolution.

14. **Raghavendra K**, **Barik TK**, **Sharma P**, **Bhatt RM**, **Srivastava HC**, **Sreehari U**, **Dash AP**. Chlorfenapyr: a new insecticide with novel mode of action can control pyrethroid resistant malaria vectors. ***Malar J*** **2011; *10*(1):16.**

ABSTRACT

Background: Malaria vectors have acquired widespread resistance to many of the currently used insecticides, including synthetic pyrethroids. Hence, there is an urgent need to develop alternative insecticides for effective management of insecticide resistance in malaria vectors. In the present study, chlorfenapyr was evaluated against *Anopheles culicifacies* and *Anopheles stephensi* for its possible use in vector control. **Methods**: Efficacy of chlorfenapyr against *An. culicifacies* and *An. stephensi* was assessed using adult bioassay tests. In the laboratory, determination of diagnostic dose, assessment of residual activity on different substrates, cross-resistance pattern with different insecticides and potentiation studies using piperonyl butoxide were undertaken by following standard procedures. Potential cross-resistance patterns were assessed on field populations of *An. culicifacies*. **Results**: A dose of 5.0% chlorfenapyr was determined as the diagnostic concentration

for assessing susceptibility applying the WHO tube test method in *anopheline* mosquitoes with 2 h exposure and 48 h holding period. The DDT-resistant/malathion-deltamethrin-susceptible strain of *An. culicifacies* species C showed higher LD50 and LD99 (0.67 and 2.39% respectively) values than the DDT-malathion-deltamethrin susceptible *An. culicifacies* species A (0.41 and 2.0% respectively) and *An. stephensi* strains (0.43 and 2.13% respectively) and there was no statistically significant difference in mortalities among the three mosquito species tested ($p > 0.05$). Residual activity of chlorfenapyr a.i. of 400 mg/m2 on five fabricated substrates, namely wood, mud, mud+lime, cement and cement + distemper was found to be effective up to 24 weeks against *A*n. *culicifacies* and up to 34 weeks against *An. stephensi*. No cross-resistance to DDT, malathion, bendiocarb and deltamethrin was observed with chlorfenapyr in laboratory-reared strains of *An. stephensi* and field-caught *An. culicifacies*. Potentiation studies demonstrated the antagonistic effect of PBO. **Conclusion**: Laboratory studies with susceptible and resistant strains of *An. culicifacies* and *An. stephensi*, coupled with limited field studies with multiple insecticide-resistant *An. culicifacies* have shown that chlorfenapyr can be a suitable insecticide for malaria vector control, in multiple-insecticide-resistant mosquitoes especially in areas with pyrethroid resistant mosquitoes.

15. Acharya P, Pallaavi R, Chandavate V, Sayeed SK Acharya J, Middha S, Kochar S, Kochar D, Subudhi A, oopathi AP, Garg S, **Das A**, **Ghosh SK**, Tatu U. Clinical Proteomics of the neglected human malarial parasite *Plasmodium vivax*. ***PLoS One* 2011; *6*(10): e26623**.

ABSTRACT

Recent reports highlight the severity and the morbidity of disease caused by the long neglected malaria parasite *Plasmodium vivax*. Due to inherent difficulties in the laboratory-propagation of *P. vivax*, the biology of this parasite has not been adequately explored. While the proteome of *P. falciparum*, the causative agent of cerebral malaria, has been extensively explored from several sources, there is limited information on the proteome of *P. vivax*. We have, for the first time, examined the proteome of *P. vivax* isolated directly from patients without adaptation to laboratory conditions. We have identified 153 proteins from clinical *P. vivax*, majority of which do not show homology to any previously known gene products. We also report 29 new proteins that were found to be expressed in *P. vivax* for the first time. In addition, several proteins previously implicated as

anti-malarial targets, were also found in our analysis. Most importantly, we found several unique proteins expressed by *P. vivax*.This study is an important step in providing insight into physiology of the parasite under clinical settings.

16. Baeza A, Bouma Menno J, Dobson A, **Dhiman R**, **Srivastava HC**, Pascual M. Climate forcing and desert malaria: the effect of irrigation. ***Malar J* 2011; *10*: 190**.

ABSTRACT

Background: Rainfall variability and associated remote sensing indices for vegetation are central to the development of early warning systems for epidemic malaria in arid regions. The considerable change in land-use practices resulting from increasing irrigation in recent decades raises important questions on concomitant change in malaria dynamics and its coupling to climate forcing. Here, the consequences of irrigation level for malaria epidemics are addressed with extensive time series data for confirmed *Plasmodium falciparum* monthly cases, spanning over two decades for five districts in north-west India. The work specifically focuses on the response of malaria epidemics to rainfall forcing and how this response is affected by increasing irrigation. **Methods and Findings**: Remote sensing data for the Normalized Difference Vegetation Index (NDVI) are used as an integrated measure of rainfall to examine correlation maps within the districts and at regional scales. The analyses specifically address whether irrigation has decreased the coupling between malaria incidence and climate variability, and whether this reflects (1) a breakdown of NDVI as a useful indicator of risk, (2) a weakening of rainfall forcing and a concomitant decrease in epidemic risk, or (3) an increase in the control of malaria transmission. The predictive power of NDVI is compared against that of rainfall, using simple linear models and wavelet analysis to study the association of NDVI and malaria variability in the time and in the frequency domain respectively. **Conclusions**: The results show that irrigation dampens the influence of climate forcing on the magnitude and frequency of malaria epidemics and, therefore, reduces their predictability. At low irrigation levels, this decoupling reflects a breakdown of local but not regional NDVI as an indicator of rainfall forcing. At higher levels of irrigation, the weakened role of climate variability may be compounded by increased levels of control; nevertheless this leads to no significant decrease in the actual risk of disease. This implies that irrigation can lead to more endemic conditions for malaria, creating the potential for unexpectedly

large epidemics in response to excess rainfall if these climatic events coincide with a relaxation of control over time. The implications of our findings for control policies of epidemic malaria in arid regions are discussed.

17. **Ghosh SK**, Chakravarthy P, Panch Sandhya R, Pushpalata K, **Tiwari SN**, **Ojha OP**. Manjushree R, **Dash AP**. Comparative efficacy of two poeciliid fish in indoor Cement tanks against chikungunya vector *Aedes aegypti* in villages in Karnataka, India. ***BMC Public Health* 2011; *11*: 599**.

ABSTRACT

Background: In 2006, severe outbreaks of *Aedes aegypti*-transmitted chikungunya occurred in villages in Karnataka, South India. We evaluated the effectiveness of combined information, education and communication (IEC) campaigns using two potential poeciliid larvivorous fish guppy (*Poecilia reticulata*) and mosquitofish (*Gambusia affinis*), in indoor cement tanks for *Aedes* larval control. **Methods**: Trials were conducted in two villages (Domatmari and Srinivaspura) in Tumkur District from March to May 2006 for *Poecilia* and one village (Balmanda) in Kolar District from July to October 2006 for *Gambusia*. A survey on knowledge, attitude and practice (KAP) on chikungunya was initially conducted and IEC campaigns were performed before and after fish release in Domatmari (IEC alone, followed by IEC + *Poecilia*) and Balmanda (IEC + *Gambusia*). In Srinivaspura, IEC was not conducted. Larval surveys were conducted at the baseline followed by one-week and one-month post-intervention periods. The impact of fish on *Aedes* larvae and disease was assessed based on baseline and post-intervention observations. **Results**: Only 18% of respondents knew of the role of mosquitoes in fever outbreaks, while almost all (n = 50 each) gained new knowledge from the IEC campaigns. In Domatmari, IEC alone was not effective (OR 0.54; p = 0.067). Indoor cement tanks were the most preferred *Ae. aegypti*breeding habitat (86.9%), and had a significant impact on *Aedes* breeding (Breteau Index) in all villages in the one-week period ($p < 0.001$). In the one-month period, the impact was most sustained in Domatmari (OR 1.58, $p < 0.001$) then Srinivaspura (OR 0.45, p = 0.063) and Balmanda (OR 0.51, p = 0.067). After fish introductions, chikungunya cases were reduced by 99.87% in Domatmari, 65.48% in Srinivaspura and 68.51% in Balmanda. **Conclusions**: *Poecilia* exhibited greater survival rates than *Gambusia* (86.04 *vs*.16.03%) in cement

tanks. Neither IEC nor *Poecilia* alone was effective against *Aedes* ($p > 0.05$). We conclude that *Poecilia* + IEC is an effective intervention strategy. The operational cost was 0.50 (US$ 0.011, 1 US$= 47) per capita per application. Proper water storage practices, focused IEC with *Poecilia* introductions and vector sanitation involving the local administration and community, is suggested as the best strategy for *Aedes* control.

18. Rajamma AJ, Dubey S, Sateesha SB, **Tiwari SN**, **Ghosh SK**. Comparative larvicidal activity of different species of *Ocimum* against *Culex quinquefasciatus*. ***Nat Prod Res*** **2011; *25*(20): 1-7.**

ABSTRACT

Ocimum is a genus of aromatic herbs, undershurbs or shrubs distributed in the tropical and warm temperate regions of the world. Larvicidal activity of essential oils and different extracts of *O. sanctum, O. basilicum and O. gratissimum* were compared on laboratory reared and field collected larvae of *Culex quinquefasciatus*. Thin layer chromatographic analysis revealed that all the three species have similar components and results showed the presence of steroids and triterpenoids. The larvicidal activity was determined in terms of LD_{50} value on late third or early fourth instar larvae for a period of 24h. A comparison of LD_{50} value has shown that *O. basilicum* is more active than the other two species. The LD_{50} value of *O. basilicum* and *O. sanctum* oil were 39.31 and 40.02 on laboratory reared larvae and 129.53 and 139.49 on field collected larvae. Laboratory reared larvae were more sensitive than field collected larvae.

19. **Singh RK**, **Mittal PK**, Yadav NK, Gehlot OP, **Dhiman RC**. Cross-check of *Aedes aegypti* indices and KAP study in Sangam Vihar area during XIX Commonwealth Games Meet in 2010 at New Delhi. ***Dengue Bull*** **2011; *35*: 131-40**.

ABSTRACT

Dengue fever (DF) cases were reported in Delhi during August 2010. As the XIXth Commonwealth Games were to be held in Delhi in October 2010, entomological and community knowledge, attitude and practices (KAP) studies were carried out to assist the Municipal Corporation of Delhi (MCD) for better implementation of vector control activities in the city. A total of 495 houses were searched for *Aedes aegypti* breeding in all kinds of temporary and

permanent water receptacles in both indoors and outdoors in a thickly-populated, illegally-constructed locality, named Sangam Vihar, in south Delhi. The overall House Index (HI), Container Index (CI) and Breteau Index (BI) were 44.44%, 19.01% and 91.92 respectively. For KAP, a pre-tested, structured questionnaire was used for data collection. Out of the 384 households surveyed, 156 were aware about dengue and only 12 households knew that virus was the causative agent for DF. A majority (378) of the households practised water storage and 48 of them stored water for more than one week. No preventive/control measures were adopted to prevent mosquito breeding in the water-holding containers by a majority of the households (45.57%). 57% of them did not know the biting habits of dengue vector mosquitoes. The results of the study indicated that the community's knowledge about dengue fever, its transmission, vector breeding sources, biting habits and preventive measures was poor.

KEYWORDS: Dengue, *Aedes aegypti* indices, Knowledge Attitude and Practices (KAP), Delhi.

20. Wilson NO, **Jain V**, Robert CE, Naomi L, Joel PK, **Singh MP**, Nagpal AC, **Dash AP**, Udhayakumar V, **Singh N**, Stiles JK. CXCL 4 and CXCL 10 predict risk of fatal cerebral malaria. ***Dis Markers* 2011; *30*: 39-49.**

ABSTRACT

Plasmodium falciparum in a subset of patients can lead to a diffuse encephalopathy known as cerebral malaria (CM). Despite treatment, mortality caused by CM can be as high as 30% while 10% of survivors of the disease may experience short- and long-term neurological complications. The pathogenesis of CM involves alterations in cytokine and chemokine expression, local inflammation, vascular injury and repair processes. These diverse factors have limited the rate of discovery of prognostic predictors of fatal CM. Identification of reliable early predictors of CM severity will enable clinicians to adjust this risk with appropriate management of CM. Recent studies revealed that elevated levels of CXCL10 expression in cerebrospinal fluid and peripheral blood plasma independently predicted severe and fatal CM. CXCR3, a promiscuous receptor of CXCL10, plays an important role in pathogenesis of mouse model of CM. In this study the role of corresponding CXCR3 ligands (CXCL11, CXCL10, CXCL9 & CXCL4) in fatal or severe CM was evaluated by comparing their levels in 16 healthy control (HC), 26 mild malaria (MM), 26 cerebral malaria survivors (CMS) and 12 non-survivors (CMNS) using enzyme linked immunosorbent

assay (ELISA). Levels of CXCL4 and CXCL10 were significantly elevated in CMNS patients ($p < 0.05$) when compared with HC, MM and CMS. Elevated plasma levels of CXCL10 and CXCL4 were tightly associated with CM mortality. Receiver Operating Characteristic (ROC) curve analysis revealed that CXCL4 and CXCL10 can discriminate CMNS from MM ($p < 0.0001$) and CMS ($p < 0.0001$) with an area under the curve (AUC) = 1. These results suggest that CXCL4 and CXCL10 play a prominent role in pathogenesis of CM associated death and may be used as functional or surrogate biomarkers for predicting CM severity.

KEYWORDS: CXCL4, CXCL10, PF4, IP-10, malaria, cerebral malaria, *Plasmodium falciparum*, ROC curve

21. Liu M, Guo S, Hibbert JM, **Jain V**, **Singh N**, Wilson NO, Stiles JK. CXCL10/IP-10 In infectious diseases pathogenesis and potential therapeutic implications. ***Cytokine Growth Factor Rev* 2011; *22*(3): 121-30.**

ABSTRACT

C-X-C motif chemokine 10 (CXCL10) also known as interferon γ-induced protein 10 kDa (IP-10) or small-inducible cytokine B10 is a cytokine belonging to the CXC chemokine family. CXCL10 binds CXCR3 receptor to induce chemotaxis, apoptosis, cell growth and angiostasis. Alterations in CXCL10 expression levels have been associated with inflammatory diseases including infectious diseases, immune dysfunction and tumor development. CXCL10 is also recognized as a biomarker that predicts severity of various diseases. A review of the emerging role of CXCL10 in pathogenesis of infectious diseases revealed diverse roles of CXCL10 in disease initiation and progression. The potential utilization of CXCL10 as a therapeutic target for infectious diseases is discussed.

KEYWORDS: Chemokine, CXCL10, CXCR3, infectious diseases

22. **Pandey KC**. Cysteine proteases of human malaria parasites (Invited Review Article/ Centenary celebrations article). ***J Parasit Dis* 2011; *35*(2): 94-103**.

ABSTRACT

There is an urgent need for new drugs against malaria, which takes millions of lives annually. Cysteine proteases are potential new drug targets, especially when current drugs are showing resistance. Falcipains and vivapains are well characterized cysteine proteases of *P. falciparum* and *P. vivax*, respectively. Studies with cysteine protease inhibitors and manipulating cysteine proteases specific genes have suggested their roles in hemoglobin hydrolysis. In *P. falciparum*, falcipain-2 and falcipain-3 are major hemoglobinases that hydrolyze host erythrocyte hemoglobin in the parasite food vacuole. It is confirmed that disruption of the falcipain-2 gene led to a transient block in hemoglobin hydrolysis, and disruption of falcipain-3 gene was not possible, suggesting that protease is essential for erythrocytic parasites. On the other hand, vivapain-2, vivapain-3 and vivapain-4 are important cysteine proteases of *P. vivax*, which shared a number of features with falcipain-2 and falcipain-3. A recent study indicates that vivapains and aspartic protease of *P. vivax* works collaboratively to enhance the parasites' ability to hydrolyze host erythrocyte hemoglobin. Studies also indicate that falcipains and vivapains also hydrolyse the erythrocyte cytoskeleton proteins and involved in rupture of red blood cell. Structural and biochemical analysis of falcipains and vivapains showed that they have unique domains for specific functions. Overall, the complexes of cysteine proteases with small and macromolecular inhibitors provide structural insight to facilitate the drug design. Therefore, giving due importance to the cysteine proteases, this review will briefly focus the recent advancement in the field of cysteine proteases of human malaria parasites.

KEYWORDS: malaria, cysteine protease, hemoglobin hydrolysis.

23. Chhakchhuak L, **Ravindran KJ**, William J. Effect of plant oils on ovipositional activity Of the vector mosquito *Aedes aegypti* (Diptera: Culicidae). ***Hexapoda* 2011; *18*(1): 42-4**.

24. **Tiwari SN**, **Ghosh SK**, **Mittal PK**, **Dash AP**. Effectiveness of a new granular formulation of biolarvicide *Bacillus thuringiensis* var. *israelensis* (Bti) against larvae of malaria vector in India. ***Vector Borne Zoon Dis* 2011; *11*(1): 69-75.**

ABSTRACT

Control of vector(s) or mosquitoes, in general, through biolarvicide as an alternate biocontrol agent is a greatest desire. We evaluated a water-dispersible granular formulation biolarvicide *Bacillus*

thuringiensis var. israelensis (Bti, H-14 serotype; VectoBac(®) WDG) in the laboratory and also in the field against two principal malaria vectors, *Anopheles culicifacies* and *Anopheles stephensi*. Laboratory evaluations against laboratory-reared immature of the two species were carried out at a temperature of 28°C± 2°C and 70%-80% relative humidity. Field trials were conducted in a rural area and in Bangalore city, Karnataka, South India. First trial against the rural vector *An. culicifacies* was carried out in stone quarry pits at dosages of 0.05, 0.2, and 1 g/m(2). The second trial against urban vector *An. stephensi* was carried out in ring wells at 0.05, 0.1, 0.2, 0.5, and 1 g/m(2) dosages. Laboratory tests revealed increased efficacy against *An. stephensi*. The fifty percent lethal concentration (LC(50)) and LC(90) values against *An. culicifacies* and *An. stephensi* were 0.348 and 1.008 mg/L ($\chi(2) = 8.49$; $p > 0.05$) and 0.245 and 0.533 mg/L ($\chi(2) = 4.67$; $p < 0.05$), respectively. Based on the findings of no pupal production in the field, the formulation was effective up to 14 days at 0.2 g/m(2) or more appropriately at 0.25 g/m(2) dose for both the species under field conditions. We discuss how this new formulation was evaluated against *An. culicifacies* and *An. stephensi* under laboratory and field conditions. No adverse effects were observed on the nontarget species such as frogs, their tadpoles, small local fish, Notonectid bugs, and water scatters. We conclude that VectoBac WDG is effective at 0.25 g/m(2) and be recommended for its use in the vector-borne disease control program under integrated vector management concept.

25. **Mittal PK**, **Sreehari U**, **Razdan RK**, **Dash AP**, **Ansari MA**. Efficacy of advancedOdomos repellent cream (N, N-Diethyl-benzamide) against mosquito vectors. ***Indian J Med Res* 2011; *133*: 426-30**.

ABSTRACT

Background & objectives: Repellents are commonly used personal protection measures to avoid mosquito bites. In the present study, Advanced Odomos cream (12% N, N-diethyl-benzamide) was tested for its efficacy against mosquitoes in comparison to DEET (N,N-diethyl-3-methyl benzamide). **Methods**: Bioassays were conducted to assess the repellency of Advanced Odomos and DEET creams against *Anopheles stephensi* and *Aedes aegypti*. Their efficacy was tested on human volunteers applied with different concentrations of test creams ranging from 1 to 12

mg/cm2 and by exposing them to mosquitoes at hourly intervals. Field evaluation was also carried out to test the duration of protection of the test creams against *Anopheles* and *Aedes* mosquitoes during whole night and day time collections, respectively on human volunteers. Mosquito collections were done using torch light and aspirator. **Results**: Complete (100%) protection was achieved at 10 mg/cm2 cream formulation of Advanced Odomos (1.2 mg a.i/cm2) dose against *An. stephensi* and 12 mg/cm2 (1.44 mg a.i./cm2) against *Ae. aegypti* on human baits. There was no statistically significant differences in per cent protection against mosquito bites between Advanced odomos and DEET cream (P>0.05) in respective doses. Complete protection up to 11 h was observed against *Anopheles mosquitoes* during whole night collections and up to 6 h against *Ae. aegypti* in day time collections. No adverse reactions such as itching, irritation, vomiting, nausea, etc. were reported by the volunteers. **Interpretation & conclusions:** Advanced odomos cream applied at 10 mg/cm2 concentration provided 100% protection from *Anopheles mosquitoes* up to 11 h whereas about 6 h protection was recorded against *Ae. aegypti*. The laboratory and field trials indicate that for longer protection against *Anopheles* mosquitoes 10 mg/cm2 will be appropriate and in case of *Ae. aegypti* more than 10 mg/cm2 application is required for complete protection. In conclusion, the Advanced Odomos cream was comparable to the known repellent cream DEET for prolonged protection against malaria and dengue vectors.

KEYWORDS Advanced odomos, *Anopheles culicifacies*, *An. Stephensi*, *Aedes aegypti*, DEET, repellency.

26. **Singh N**, **Shukla MM**, Chand G, **Bharti PK**, **Singh MP**, Shukla MK, Mehra RK, Sharma RK, **Dash AP**. Epidemic of *Plasmodium falciparum* malaria in central India, an area where chloroquine has been replaced by artemisinin-based combination therapy. ***Trans R Soc Trop Med Hyg.* 2011; *105*(3): 133-9.**

ABSTRACT

India contributes greatly to the global incidence of malaria. The factors influencing malaria in India are highly diverse and vary greatly from the epidemiological setting of any other country. Central India is the most vulnerable area to malaria in India. This study was carried out in three community health centres in Dindori District, Madhya Pradesh (Central India). Dindori District is mesoendemic for malaria, with both *Plasmodium falciparum* and *P. vivax* being present in all age groups. *Anopheles culicifacies* and *A. fluviatilis* are highly efficient vectors of malaria. In this

study, an epidemic of malaria among indigenous ethnic group Baigas was investigated to determine the causes of the epidemic and the population involved in order to aid in disease containment. The existence of sporozoite-positive *A. culicifacies* and *A. fluviatilis* indicates either that spraying had not been done properly or the presence of insecticide resistance. A combination of factors propagated the epidemic. Evidence suggests that the non-availability of artemisinin-based combination therapy and rapid diagnostic tests along with an immunogenically vulnerable population each played an important role. As the global prevalence of malaria decreases owing to initiatives to control or eliminate the disease, more areas will become mesoendemic or hypoendemic for malaria. Detection and control of epidemics requires greater attention, and mechanisms to ensure the quality of interventions are essential.

27. **Mathur D, Singh SK,** Singh S**.** Evaluation of *in vitro* antimicrobial and antioxidant activities of peel and pulp of some citrus fruits. ***J Biotechnol Biotherapect*** **2011; *1*(2): 1-17.**

ABSTRACT

Citrus fruits are rich source of vitamin-C (Ascorbic acid). In the present study the antimicrobial and antioxidant activities of the peel and pulp of some of the citrus fruits has been investigated. The results indicate that the aqueous extracts from citrus fruit peel and pulp contains significant antimicrobial activity. *In vitro* antioxidant activity was determined by various procedures and it has been determined that aqueous and ethanolic fraction of peels and pulps of citrus fruits possessed maximum antioxidant activity in reference to standard antioxidant. Total polyphenolic content was found to be maximum in ethanolic extract of peels and chloroform extract of pulps, thus these have remarkable antioxidant property. The study thus revealed that peel and pulp of citrus fruits are useful for consumption and are beneficial for health. This study may thus lead to the formulation of an antimicrobial drug and can be used as a potent natural antioxidant additive or food products and as a dietary supplement.

28. **Mittal PK, Sreehari U, Razdan RK**, **Dash AP**. Evaluation of the impact of ZeroFly®, An insecticide incorporated plastic sheeting on malaria incidence in two temporary labour shelters in India. ***J Vector Borne Dis*** **2011; *48*: 138-43.**

ABSTRACT

Background & Objectives: Prevention of malaria is a major technical and operational problem in displaced and mobile populations such as refugee camps and temporary labour settlements. Insecticide incorporated plastic sheeting is a new technology to control mosquitoes in emergency shelters and also temporary habitations at different locations. In view of this, efficacy of ZeroFly® , an insecticide incorporated plastic sheeting (factory treated with deltamethrin 2.0 g/kg or 265 mg/m2) was evaluated for its efficacy against malaria vectors and its impact on malaria incidence in temporary labour settlements in two urban areas in India. **Methods**: This trial was conducted in two labour settlements in two urban areas, Delhi and Noida (U.P.), India with ~ 250 populations. In an area, two localities were selected for intervention with ZeroFly and untreated plastic sheets (control). Entomological and epidemiological data were collected using standard methods for one year. **Results**: Baseline studies on the susceptibility of mosquitoes in Delhi and Noida areas revealed 100% susceptibility of the malaria vector species *Anopheles culicifacies* and *An. stephensi* to deltamethrin. Cone bioassay tests performed against *An. culicifacies* and *An. stephensi* to determine the efficacy of ZeroFly sheets showed 100% mortality against *An. culicifacies* and *An. stephensi* with 3 min exposure and after 24 h recovery period. Against *Culex quinquefasciatus* and housefly 100% mortality was obtained after 30 min of exposure period. Intervention with the ZeroFly plastic sheets resulted in almost complete reduction in the resting density of *An. culicifacies* and *An. stephensi*, the two major malaria vectors and also in the reduction of malaria cases in ZeroFly camps as compared to control camps. The ZeroFly plastic sheeting was found to be safe for human. Barring some complaints of skin irritation and itching, which were temporary in nature, no adverse health effects were reported by the users. The community acceptance was high. **Conclusion**: Results of the present study revealed that ZeroFly® plastic sheeting is highly effective in reducing the indoor resting density of mosquitoes, man -vector contact and malaria incidences in labour populations living in temporary shelters.

29. **Raghavendra K**, **Barik TK**, **Bhatt RM**, **Srivastava HC**, **Sreehari U**, **Dash AP**. Evaluation of the pyrrole insecticide chlorfenapyr for the control of *Culex Quinquefaciatus* say**.** ***Acta Trop*** **2011; *118*(1): 50-5.**

ABSTRACT

Culex quinquefasciatus Say (Diptera: Culicidae) is a widely distributed mosquito vector species in India and also in other tropical regions of the world. This species is implicated in the transmission of lymphatic filariasis in many countries. This species is reported to be widely resistant to insecticides of different classes in current use. In the present study, bio-efficacy ofchlorfenapyr, an insecticide of pyrrole class with a novel mode of action was tested for thecontrol of *Cx. quinquefasciatus*. Studies were performed to determine the diagnostic dosage; residual efficacy on different artificially fabricated substrates, namely wood, mud, mud+lime, cement and cement+distemper; to assess cross-resistance with different insecticides; and synergism/antagonism using piperonyl butoxide (PBO). A dosage of 5.0% chlorfenapyr was determined as diagnostic dosage with 2 h exposure and 48 h holding period for assessing the susceptibility of mosquitoes. The residual efficacy was observed up to 34 weeks on wood and mud+lime substrates while on other substrates, it was about 15 weeks at a dosage of 400mg a.i./m(2). Laboratory-reared strains of *Cx. quinquefasciatus* showed cross-resistance, whereas field-collected mosquitoes showed absence of cross-resistance to chlorfenapyr. Potentiation bioassays showed antagonistic effect of PBO to chlorfenapyr toxicity owing to the involvement of oxidases in the initial step of a conversion of pro-insecticide chlorfenapyr to toxic form CL 303268. The present study results have shown that chlorfenapyr can be a potential insecticide for the control of multiple insecticide resistant strains of *Cx. quinquefasciatus*. However, in countries where indoor residual spray (IRS) is not targeted for the control of this species, like in India,chlorfenapyr used in IRS for the control of malaria vectors in rural and peri-urban areas can additionally provide control of *Cx. quinquefasciatus* also.

30. **Raghavendra K, Ghosh SK, Eapen A**, **Tiwari SN**, **Satyanarayan TS**, **Ravindran J**, **Sreehari U**, **Dash AP**. Field evaluation of lambda-cyhalothrin (ICON 10 CS) indoor residual spraying against *Anopheles culicifacies* in India. ***J Vector Borne Dis* 2011; *48*(1):18-26**.

ABSTRACT

Background & Objectives: Field trials of lambda-cyhalothrin 10 CS (ICON 10 CS) in indoor residual spraying (IRS) with 25 mg a.i./m2 against *Anopheles culicifacies* was undertaken vs malathion IRS (25% WP–2 g a.i./ m2) in Tumkur district, Karnataka; vs deltamethrin IRS (2.5%

WP–20 mg a.i./m2) in Dharmapuri district; and vs lambda-cyhalothrin (10 WP–25 mg a.i./m2) in Ramanathapuram district, Tamil Nadu, India. **Methods**: Spray operations in the experimental villages were done by the National Institute of Malaria Research (NIMR) and in the control villages by the respective State Health Department staff. Persistence of efficacy of insecticide sprayed in villages was assessed by contact bioassays against vector mosquitoes. Entomological indicators such as per structure density, parity rates of vector mosquitoes and sporozoite rates were measured in all the three study areas using standard procedures. Mass blood surveys and active fever case detections were carried out in experimental and control villages to study the impact of IRS on malaria transmission. **Results**: Persistence of effectiveness of ICON 10 CS was observed up to 2–3 months in all the three study areas. ICON 10 CS was found effective at par with or better than the insecticides used in the national programme in reducing the mosquito densities and in interrupting malaria transmission in the study villages. Vector density, parity rates and malaria cases considerably reduced in the ICON 10 CS-sprayed villages. **Conclusion**: Field trials at three sites have established that ICON 10 CS formulation was relatively more effective than malathion 25% WP, deltamethrin 2.5% WP and lambda-cyhalothrin 10% WP in some evaluation parameters like indoor resting mosquitoes, parity rates in vector mosquitoes and persistence of effectiveness. It can be used for IRS for malaria vector control with two rounds of spray at an interval of 3 months for curtailing the malaria transmission and an additional round is recommended in perennial malaria transmission areas.

KEYWORDS: *Anopheles culicifacies*, India, indoor residual spraying, lambda-cyhalothrin (ICON 10 CS), malaria control.

31. Dhamodharan R, Hoti SL, Sharma R, **Das MK**. Influence of antifilarial chemotherapy strategies on the genetic structure of *Wuchereria bancrofti* populations. ***Mem Inst Oswaldo Cruz* 2011; *106*(2): 240-7.**

ABSTRACT

Lymphatic filarial (LF) parasites have been under anti-filarial drug pressure for more than half a century. Currently, annual mass drug administration (MDA) of diethylcarbamazine (DEC) or ivermectin in combination with albendazole (ALB) have been used globally to eliminate LF. Long-term chemotherapies exert significant pressure on the genetic structure of parasitic

populations. We investigated the genetic variation among 210 Wuchereria bancrofti populations that were under three different chemotherapy strategies, namely MDA with DEC alone (group I, *n* = 74), MDA with DEC and ALB (group II, *n* = 60) and selective therapy (ST) with DEC (group III, *n* = 34) to understand the impact of these three drug regimens on the parasite genetic structure. Randomly amplified polymorphic DNA profiles were generated for the three groups of parasite populations; the gene diversity, gene flow and genetic distance values were determined and phylogenetic trees were constructed. Analysis of these parameters indicated that parasite populations under ST with a standard dose of DEC (group III) were genetically more diverse (0.2660) than parasite populations under MDA with DEC alone (group I, H = 0.2197) or with DEC + ALB (group II, H = 0.2317). These results indicate that the MDA may reduce the genetic diversity of *W. bancrofti* populations when compared to the genetic diversity of parasite populations under ST.

KEYWORDS: *Wuchereria bancrofti*, chemotherapy, genetic diversity.

32. **Reddy BP**, Prasad GB, **Raghavendra K**. *In silico* analysis of glutathione S-transferase supergene family revealed hitherto unreported insect specific δ- and ε-GSTs and mammalian specific μ-GSTs in *Ixodes scapularis* (Acari: Ixodidae). ***Comput Biol Chem* 2011; *35*(2): 114-20.**

ABSTRACT

The availability of whole genome sequence information of *Ixodes scapularis* (Acari: Ixodidae), an important disease vector of veterinary and public health importance, has opened up new opportunities to explore the vector species at genomic level. Use of acaricides is the mainstay in controlling the disease vector, as effective vaccines are not available for most of the diseases that are transmitted by ticks. The glutathione *S*-transferase (GST) enzymes are one of the important supergene families that are involved in protecting the organism from oxidative stress and xenobiotics including the acaricides. The analysis of GST supergene family from *Ixodes* identified all the three broad GST classes, *viz*. canonical, mitochondrial, and microsomal forms. In total, 35 GST genes belong to five different canonical GST classes, namely Delta (7 genes), Epsilon (5), Mu (14), Omega (3), and Zeta (3 genes) GST classes, and two mitochondrial Kappa class GST genes, and a single microsomal GST gene were found. Interestingly, Delta- and Epsilon-class members, which are thought to be specific to the class Insecta, were also identified in *Ixodes*.

Further, vertebrate/mammalian specific Mu-GSTs (14 genes) were also identified in *Ixodes*. Analyses of the intron–exon organization revealed higher frequency of phase '0' and phase '2' introns. The comprehensive listing of the GST supergene family members from *Ixodes* may help in understanding molecular mechanisms of the acaricide resistance in mites and ticks. Cumulatively, these findings may provide an in-depth understanding of the complex evolution of GST supergene family, one of the oldest supergene families that exist in all the domains of life.

KEYWORDS: Acari, *Ixodes scapularis*, Glutathione *S*-transferase, Kappa GSTs, Exon–intron organization.

33. **Reddy BP**, Prasad GB, **Raghavendra K**. *In silico* characterization and comparative genomic analysis of the *Culex quinquefasciatus* glutathione S-transferase (GST) supergene family. ***Parasitol Res* 2011; *109*(4): 1165-77.**

ABSTRACT

The glutathione *S*-transferases (GSTs) are phase II class of detoxification enzymes that are involved both directly and indirectly in insecticide resistance mechanisms. The *Culex quinquefasciatus* GST superfamily was analyzed by utilizing the public domain *Culex* genome sequence. In total, 35 cytosolic (seven classes) and 5 microsomal putatively active GSTs were retrieved, classified, and annotated. The study revealed the presence of three unclassified GSTs. Of 35 cytosolic GSTs, 65% contributed by insect specific Delta–Epsilon classes. Gene cluster analysis revealed that most of the genes of Delta, Epsilon, and Theta classes were organized into gene clusters. The gene organization analysis revealed the dominance of phase "0" introns in the*Culex* GST family. The studies on intron loss and gain events revealed that the Delta GSTs have experienced a higher number of loss and gains during their evolution. A positive correlation was observed between the phylogenetic relationship of members of the GST superfamily and their corresponding exon–intron organization. In addition, the genes within the gene clusters revealed the monophyletic phylogenetic relationship implying the importance of gene duplication events in the gene families' evolution. Finally, the comparative genomic analysis has shown a complex evolutionary scenario associated with the GST supergene family evolution in insects.

34. Mathur A, Verma SK, **Singh SK**, **Mathur D**, Prasad GBKS, **Dua VK**. Investigation of anti-inflammatory properties of *Swertia chirayta* and *Gloriosa* superb. ***Recent Res Sci Technol* 2011; *3*(3): 40-3.**

ABSTRACT

Gloriosa superba (Liliaceae) is one of the oldest ingredients of species from ancient time. Tubers roots and seeds are two most important part of glory lily used for variety of purpose. *Swertia chirata* (Gentianaceae) is widely used in India to treat fever and malaria. It is also used to treat liver diseases. The whole plant methanol and aqueous extracts of *Swertia chirayta* possessed maximum anti-inflammatory activity in a dose dependent manner *i.e* 50 mg/kg and 100 mg/kg in carrageenan induced animal models. Further screening of the potent extracts of the plant confirmed the presence of xanthone (1, 5- dihydroxy-3, 8-dimethoxy xanthone, m.p.1850C, yellowish crystalline needles from methanol) was obtained. The structure of the fraction was confirmed by spectral analysis (UV, IR, NMR). The methanol and aqueous extracts of tubers of Gloriosa superba also possessed good anti-inflammatory in a dose dependent manner *i.e* 100 mg/kg and 200 mg/kg in carrageenan induced animal models. Further screening of the extracts confirmed the presence of colchicines in the extracts. The present study thus revealed the presence of potent anti-inflammatory drugs in these plants.

KEYWORDS: *Gloriosa superba*, *Swertia chirayta*, methanol extracts, xanthones, colchicines, anti-inflammatory activity.

35. Mathur A, Verma SK, **Singh SK,** Prasad GBKS, **Dua VK**. Investigation of the anti- Microbial antioxidant and anti-inflammatory compound isolated from *Murraya koenigii*. ***Int J App Biol Pharmaceut Technol* 2011; *2*(1): 470-7.**

ABSTRACT

In the present investigation, the compound responsible for antioxidant, antimicrobial and anti-inflammatory properties in methanolic extract of leaves of *Murraya koenigii* L. was determined by Perkin- Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer GC/MS technique. GC-MS analysis of methanol extract of the leaves of the plant revealed the existence of 1-Methyl-pyrrolidine-2-carboxylic acid (69.00%), Ethyl α-d glucopyranoside

(13.36%), Isolongifolene, Isolongifolene (3.68%), c-Himachalene (2.88%), 1,2-Ethanediol, monoacetate (2.79%), 1,2-Benzenedicarboxylic acid, di-isooctyl ester (2.55%). The pure compounds were separated using a Shimadzo LC 2010 HPLC system (Kyoto, Japan), equipped with a Shimadzo LC 2010 UV-VIS detector with a thermostated flow cell and a selectable two wavelengths of 190 - 370 nm or 371–600nm. These were further screened for their antimicrobial, antioxidant and anti-inflammatory properties. All the compounds possessed some or the other activity. It was found that the compound 9, 12 octadecadienoic acid having the retention time 18.81 and the peak area 0.60 % had potent antioxidant, antimicrobial and anti-inflammatory properties. The compound showed potent antimicrobial activity against *Bacillus subtilis, E.coli, Proteus vulgaris, Salmonella typhimurium, Staphylococcus aureus, Candida albicans, Saccharomyces cerevisae, Aspergillus niger* and *Penicillium notatum* at MIC value from 0.05-0.56 µg/ml. The compound showed less activity against *Pseudomonas aeruginosa* in comparison to other pathogens. The compound possessed to have strong antioxidant activity with IC50 value of 45.65 µg/ml as measured by DPPH assay. The compound possessed 85 % reduction in paw edema at a dose of 150 µg/ml in reference to standard anti-inflammatory drug, aspirin which showed 68.62 % reduction. The compound was further assayed for cellular toxicity to fresh sheep erythrocytes and found to have no cellular toxicity.

KEYWORDS: *Murraya koenigii*, methanol extract, compound 9, 12 octadecadienoic acid.

36. Tennyson S, Balaraju K, Kyungseok P, Raja AK, **Ravindran KJ**, **Eapen A**, William J. *In vitro* antioxidant activity of *Ageratum houstonianum* Mill. (Asteraceae). ***Elixir Food Sci* 2011; *35*: 2897-900**.

ABSTRACT

The present study was conducted in three different solvent extracts of leaves of *Ageratum houstonianum (Mill.) (Asteraceae)* and were screened against reference cultures, clinical isolates and fungal strains under in vitro condition. Among three solvent extracts of *A. houstonianum* tested against selected reference cultures, ethyl acetate extract was found to exhibit the highest activity (19 mm) against *P. aeruginosa*, followed by a second higher activity (18 mm) against *S. typhi*. In the case of *K. pneumoniae*, moderate inhibition was observed by ethyl acetate extract. The minimum inhibitory concentration for *P. aerugenosa* and *S. typhi* was found as 0.312 mg/ml of

ethyl acetate extract. In the case of clinical isolates also, ethyl acetate extract of *A. houstonianum* was found to show higher activity (17 mm) against methiciline Resistant *S. aureus* (ICMR-5) which is found to be better than commercially available streptomycin, followed by a second higher activity using hexane extract (16 mm) against the same clinical isolate, whereas methanol extract showed only a moderate activity. Streptomycin was used as the positive control.

37. Mathur A, Verma SK, **Singh SK**, Prakash A, Prasad GBKS, **Dua VK**. Isolation and determination of biochemical nature of anticoagulant from earthworm. ***Enviro Conserv J* 2011; *12*(1&2): 75-7.**

ABSTRACT

Earthworms are commonly known as farmer's friend. Various previous studies have confirmed the anti-inflammatory, analgesic, antipyretic and anticancer effects of earthworm extract. In the present study selected species of earthworm was used to study the anticoagulative activity of earthworm extract by APTT test. The study involves the extraction and isolation of anticoagulant from earthworm which was found to be in the form of DNA. In order to uncover the biochemical nature of this molecule, the anticoagulant was processed with various hydrolases such as Proteinase-K, Dnase, Rnase and lysozyme. Simultaneously APTT test and agarose gel electrophoresis were performed to confirm the results. Standard herring sperm DNA and yeast RNA were also used to compare the anticoagulative activities with that of anticoagulant purified from earthworm. Individual components of nucleotide were also checked which might be responsible for the anticoagulative capability.

38. Mathur A, Prasad GBKS, Rao N, Babu P, **Dua VK**. Isolation and identification ofAnti-microbial compound from *Mentha piperita* L. ***Rasayan J Chem* 2011; *4*(1): 36-42**.

ABSTRACT

Mentha piperita L. (Lamiaceae) leaves have been traditionally implemented in the treatment of minor sore throat and minor mouth or throat irritation by the indigenous people of India, although the compounds responsible for the medicinal properties have not been identified. In the present study, an antimicrobial compound was isolated and characterized, and its biological activity was assessed. The compound was isolated and characterized from the extracted essential oil using

different spectral techniques: TLC, FTIR spectra and HPLC. Antimicrobial activity of the compound was assessed using both well diffusion and microdilution method in 96 multi-well microtiter plates. The isolated compound was investigated for its antimicrobial activity against seven selected pathogenic and nonpathogenic microorganisms: *Staphylococcus aureus, Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenes, Lactobacillus acidophilus, Pseudomonas aeruginosa, E.coli K-12, Bacillus subtilis, Salmonella typhimurium* and the fungal strains *Candida albicans, Aspergillus niger, Penicillium notatum and Saccharomyces cerevisae*. Menthol at different concentrations (1:1, 1:5, 1:10, and 1:20) was active against all tested bacteria except for *S.aureus,* and the highest inhibitory effect was observed against *S. mutans* using the well diffusion method. Furthermore, menthol achieved considerable antifungal activity against all the fungal strains except *A.niger*. The isolation of an antimicrobial compound from *M. piperita* leaves validates the use of this plant in the treatment of minor sore throat and minor mouth or throat irritation as well as diseases such as typhoid.

KEYWORDS: *M.piperita,* antimicrobial compound, menthol, antimicrobial activity.

39. Mathur A, Rawat A, Bhatt G, Baweja S, Ahmad F, Grover A, Madhav K, Dhand M, **Mathur D**, Verma SK, **Dua VK**. Isolation of *Bacillus* producing Chitinase from soil: Prodution and purification of chito- oligosaccharides from chitin Extracted from fresh water crustaceans and antimicrobial activity of chitinase. ***Recent Res Sci Technol* 2011; *3*(11): 1-6**.

ABSTRACT

In the present investigation Bacillus sp. strain was isolated and screened from the red soil collected from Doiwala region of Dehradun (U.K), India. Serial dilution technique was adopted to isolate the organism and was screened for its chitinolytic activity. The biochemical tests were performed to prove its validity. The microorganism was also screened by inoculating a loop full of the isolated strain in basic cresol red dye and incubated for about 18- 24 h. The conversion of colour of the red dye into purple (pH, 6.5- 8.8) was taken as an indication for the presence of *Bacillus sp. Amylase* production by the organism was also screened by introduction of iodine in the broth/agar culture having starch. The broth/agar medium having starch but no bacterial strain was used as the control. The disappearance of color confirmed the presence of Bacillus strain producing amylase which degrades the starch. The chitinous wastes were collected from fresh water crustaceans *viz*. fresh water crab (*Potamon sp*.) and fresh water prawn (*Palaemon sp*.) and the chitin extracted was used

as the substrate for chitinase. The yield of chitin extracted from fresh water prawn (*Palaemon sp*.) was found to be comparatively higher than that of chitin extracted from fresh water crab (*Potamon* sp.). Standard colloidal chitin was used as the reference control. The enzyme activity of chitinase for degradation of chitin extracted from crab and prawn was compared. The results confirmed that chitinase activity for degradation of crab chitin was comparatively higher than that of degradation of prawn chitin. The enzyme activities were found to be 0.11 µg/ml/minute and 0.09 µg/ml/minute for degradation of crab and prawn chitin respectively. The antimicrobial activity of chitinase extracted was determined against the bacterial and fungal cultures. Potent antibacterial activity of chitinase was observed against the bacterial cultures but no antifungal activity was observed. The chitinase produced by the species was able to degrade the chitin and chito-oligosaccharides produced was separated by TLC and purified by HPLC.

KEYWORDS: bacillus, amylase producing strain, chitin, chito-oligosaccharides, chitinase, fresh water crustaceans, TLC, HPLC.

40. **Singh OP**, **Dykes CL**, Lather M, Aggrawal OP, **Adak T**. Knockdown resistance (*kdr*)-like mutations in the voltage-gated sodium channel of a malaria vector *Anopheles stephensi* and PCR assays for their detection. ***Malar J* 2011; *10*: 59.**

ABSTRACT

Background: Knockdown resistance (*kdr*) in insects, resulting from mutation(s) in the voltage-gated sodium channel (vgsc) gene is one of the mechanisms of resistance against DDT and pyrethroid-group of insecticides. The most common mutation(s) associated with knockdown resistance in insects, including anophelines, has been reported to be present at residue Leu1014 in the IIS6 transmembrane segment of the vgsc gene. This study reports the presence of two alternative *kdr*-like mutations, L1014S and L1014F, at this residue in a major malaria vector *Anopheles stephensi* and describes new PCR assays for their detection. **Methods**: Part of the vgsc (IIS4-S5 linker-to-IIS6 transmembrane segment) of *An. stephensi* collected from Alwar (Rajasthan, India) was PCR-amplified from genomic DNA, sequenced and analysed for the presence of deduced amino acid substitution(s). **Results**: Analysis of DNA sequences revealed the presence of two alternative non-synonymous point mutations at L1014 residue in the IIS6 transmembrane segment of vgsc, *i.e.,* T>C mutation on the second position and A>T mutation on the third position of the codon, leading to Leu (TTA)-to-Ser (TC A) and -Phe (TTT) amino acid

substitutions, respectively. Polymerase chain reaction (PCR) assays were developed for identification of each of these two point mutations. Genotyping of *An. stephensi* mosquitoes from Alwar by PCR assays revealed the presence of both mutations, with a high frequency of L1014S. The PCR assays developed for detection of the *kdr*mutations were specific as confirmed by DNA sequencing of PCR-genotyped samples. **Conclusions**: Two alternative *kdr*- like mutations, L1014S and L1014F, were detected in *An. stephensi* with a high allelic frequency of L1014S. The occurrence of L1014S is being reported for the first time in *An. stephensi*. Two specific PCR assays were developed for detection of two *kdr*-like mutations in *An. stephensi*.

41. **Mehrunnisa A**, **Adak T**, **Singh OP**, **Nanda N, Dua VK**, **Hardev P**, Khan W. Laboratory colonization of *Anopheles fluviatilis* species T and U. ***Med J Entomol*** **2011; *48*(2) : 395-7**.

ABSTRACT

The populations of *Anopheles fluviatilis* James (1902), a foothill vector, collected from village Tilpuri of district Udham Singh Nagar and from villages Auspur, Ismailpur, and Durgapur of district Hardwar were maintained at National Institute of Malaria Research Insectory at 28 ± 1°C and 80–85% RH. *Anopheles fluviatilis* sensu lato was identified for two sibling species T and U. A total of 94% of the females of both species T and U oviposited by day 4 after the blood meal. Maximum hatching, that is, 80 and 62% of the eggs of species T and U, was observed on the second and third day, respectively. For species T, mortality in second and third instars was recorded to be 144 ± 9 (N = 1,600) and 48 ± 6 (N = 1,200), whereas in species U, it was 196 ± 13 (N = 1,400) nd 70 ± 8 (N = 1,000), respectively. Mortalities in second instars of species T and U were significantly higher than third instars (P = 0.05). The female and male ratio in pupal stage of species T and U was found to be 53:47 and 58:42, respectively.

42. **Dev V**, **Phookan S**, **Padhan K**, **Tewari GG**, Khound K. Laboratory wash-resistance and field evaluation of deltamethrin incorporated long-lasting polyethylene netting (NetProtect®) against malaria transmission in Assam, northeast India. ***Acta Trop*** **2011; *119*: 172-7.**

ABSTRACT

North-east India is co-endemic for *Plasmodium falciparum* and *P. vivax* malaria, and disease transmission is perennial and persistent. This study reports the results of a field-based village scale trial of deltamethrin incorporated long-lasting polyethylene netting (Netprotect®) conducted in *P. falciparum* predominant pocket of Assam, north-east India to assess operational feasibility, acceptability and sustainability against disease vectors and malaria transmission. The study monitored the residual efficacy of the long-lasting net in relation to serial washings in the laboratory and malaria prevalence in experimental villages for the first year of investigations from September 2008 to June 2009. The mosquito vector populations of *Anopheles minimus* were observed to be highly susceptible to deltamethrin (0.05%), and follow up investigations revealed that the vector mosquito had virtually disappeared in Netprotect® intervention villages. Concurrently, there was consistent decline in malaria cases in Netprotect® villages and transmission reduction was statistically significant compared to untreated net (net without insecticide) and no-net control villages for the corresponding study period. The contact cone-bioassay investigations against malaria transmitting mosquito species revealed that the bioavailability of the insecticide on the net fiber was persistent up to 20th serial wash resulting in ≥80% mortality. Community compliance and acceptance were high, and users reported decreased nuisance due to biting mosquitoes. It was concluded that deltamethrin incorporated polyethylene long-lasting netting was safe, wash-resistant, and assessed to be an operationally feasible, community-based intervention for sustainable management of disease vectors to prevent malaria transmission.

KEYWORDS: malaria control, *Anopheles minimus*, long-lasting insecticidal net, Wash-resistance, *Plasmodium falciparum*, north-east India.

43. Swathi S, Muruganathan G, **Ghosh SK**, Pradeep AS. Larvicidal and repellent activities of ethanolic extract of *Pongamia pinnata* leaves against mosquitoes. ***RGUHS J Pharmaceut Sci* 2011; *1*(1): 55-7.**

ABSTRACT

Mosquitoes are responsible for spread of many diseases than any other group of arthropods. Diseases such as malaria, filariasis, dengue haemorrhagic fever and chikunguinya are real threat to

mankind. In the present study ethanolic extract of leaves of *Pongamia pinnata* was evaluated for larvicidal and repellent activities against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The *Pongamia pinnata* leaves were collected and authenticated from Gandhi Krishi Vignan Kendra, Bangalore. The leaves were shade-dried, powdered and extracted using ethanol. The ethanolic extract of *Pongamia pinnata* leaves showed LD (346.94, 124.20, 359.48 ppm) against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. 50 The ethanolic extract of *Pongamia pinnata* leaves provided complete protection time (Mosquito repellency) of 99.96, 141.35, 144.73 minutes against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* at higher concentration (1%).

KEYWORDS: *Pongamia pinnata*, Larvicidal, Repellent, Mosquitoes.

44. **Kumar A, Dua VK**, Rathod P. Malaria attributed death rates in India. ***Lancet* 2011; *337*: 991-2**.

45. **Valecha N**, Staedke S, Filler S, Mpimbaza A, Greenwood B, Chandramohan D. Malaria – attributed death rates in India. ***The Lancet* 2011; *337*: 992.**

46. Bhadra A, lonides EL, Laneri K, Pascual K, Bouma MJ, **Dhiman RC**. Malaria in Northwest India: Data analysis via partially observed stochastic differential equation models driven by Levy noise. ***J Am Stat Assoc* 2011; *106*(494): 440-51.**

ABSTRACT

Many biological systems are appropriately described by partially observed Markov process (POMP) models, also known as state space models. Such models also arise throughout the physical and social sciences, in engineering, and in finance. Statistical challenges arise in carrying out inference on nonlinear, nonstationary, vector-valued POMP models. Methodologies that depend on the Markov process model only through numerical solution of sample paths are said to have the plug-and-play property. This property enables consideration of models for which the evaluation of transition densities is problematic. Our case study employs plug-and-play methodology to investigate malaria transmission in Northwest India. We address the scientific question of the respective roles of environmental factors, immunity, and nonlinear disease transmission dynamics in epidemic malaria. Previous debates on this question have been hindered by the lack of a statistical investigation that gives simultaneous consideration to the roles of human immunity and

the fluctations in mosquito abundance associated with environmental or ecological covariates. We present the first time series analysis integrating these various components into a single vector-valued dynamic model. We are led to investigate a POMP involving a system of stochastic differential equations driven by Lévy noise. We find a clear role for rainfall and evidence to support models featuring the possibility of clinical immunity. An online supplement presents details of the methodology implemented and two additional figures.

KEYWORDS: Iterated filtering, partially observed Markov process, *Plasmodium falciparum*, Sequential Monte Carlo.

47. **Srivastava HC**, **Chandrashekar P**, Kurien G, **Sreehari U**, **Yadav RS**. Malaria in seasonal migrant population in Southern Gujarat, India. ***Trop Biomed* 2011 Dec; *28*(3): 638-45.**

ABSTRACT

Malaria in migrant workers is always a major problem to control due to their temporary stay in shelters, and other operational constraints. Hence, a study was undertaken in brick kilns in Bharuch district, Gujarat state, India to study the problem of malaria in the work force. Mass blood surveys were carried out in 15 brick kilns. Blood slides were collected from both febrile and afebrile cases. Positive cases were treated as per the national drug policy and were followed up. Mosquito collections were carried out by pyrethrum spray collection in early morning hours. Human blood index and sporozoite rates were determined as per standard procedures. All age groups were found affected with malaria at brick kilns. Prevalence of malaria was significantly higher in < 14 years of age-group as compared to adults. Post treatment follow up examination of patients revealed high malaria infection due to non-compliance of chloroquine. The appearance of parasitaemia among *Plasmodium falciparum* treated cases indicate the possibility of chloroquine resistance. The proportion of *P. falciparum* was >50% in migrant population. In stable population in villages, overall decline in malaria cases was observed in 2008-2010. The sporozoite rate of 4.2% in *Anopheles culicifacies* indicates active malaria transmission at brick kilns. The investigation demonstrated that suitable microclimatic conditions for malaria transmission exist in these areas during hottest period. The district health department should consider these factors in planning

malaria surveillance and control. As current magnitude and diversity of population movements in rural as well as in urban areas are unprecedented, this issue is worthy of attention.

48. **Raghavendra K**, **Barik TK**, Reddy BPN, **Sharma P**, **Dash AP.** Malaria vector control: From past to future. ***Parasitol Res*** **2011; *108*(4): 757-79.**

ABSTRACT

Malaria is one of the most common vector-borne diseases widespread in the tropical and subtropical regions. Despite considerable success of malaria control programs in the past, malaria still continues as a major public health problem in several countries. Vector control is an essential part for reducing malaria transmission and became less effective in recent years, due to many technical and administrative reasons, including poor or no adoption of alternative tools. Of the different strategies available for vector control, the most successful are indoor residual spraying and insecticide-treated nets (ITNs), including long-lasting ITNs and materials. Earlier DDT spray has shown spectacular success in decimating disease vectors but resulted in development of insecticide resistance, and to control the resistant mosquitoes, organophosphates, carbamates, and synthetic pyrethroids were introduced in indoor residual spraying with needed success but subsequently resulted in the development of widespread multiple insecticide resistance in vectors. Vector control in many countries still use insecticides in the absence of viable alternatives. Few developments for vector control, using ovitraps, space spray, biological control agents, etc., were encouraging when used in limited scale. Likewise, recent introduction of safer vector control agents, such as insect growth regulators, biocontrol agents, and natural plant products have yet to gain the needed scale of utility for vector control. Bacterial pesticides are promising and are effective in many countries. Environmental management has shown sufficient promise for vector control and disease management but still needs advocacy for inter-sectoral coordination and sometimes are very work-intensive. The more recent genetic manipulation and sterile insect techniques are under development and consideration for use in routine vector control and for these, standardized procedures and methods are available but need thorough understanding of biology, ethical considerations, and sufficiently trained manpower for implementation being technically intensive methods. All the methods mentioned in the review that are being implemented or proposed for implementation needs effective inter-sectoral coordination and community

participation. The latest strategy is evolution-proof insecticides that include fungal biopesticides, Wolbachia, and Denso virus that essentially manipulate the life cycle of the mosquitoes were found effective but needs more research. However, for effective vector control, integrated vector management methods, involving use of combination of effective tools, is needed and is also suggested by Global Malaria Control Strategy. This review article raises issues associated with the present-day vector control strategies and state opportunities with a focus on ongoing research and recent advances to enable to sustain the gains achieved so far.

49. Mathur A, Mathur D, Prasad GBKS, **Dua VK**. Microwave solvent extraction (MSE) as An effective technique against traditional solvent extraction (TSE) for screening different Plant extracts for antioxidant activity. ***Asian J Biochem Pharmaceut Res*** **2011; *2*(1): 410-8.**

ABSTRACT

In the present investigation 18 plants belonging to 12 families were randomly selected and different solvent extracts were prepared both by MSE and TSE of different plants and were evaluated for their in vitro antioxidant activity. The MSE of the each of the plant material was performed at 80 0 C in Teflon vessels of the Ethos E. Microwave Extraction System (Milestone, Inc. Monroe, CT) while TSE was performed by conventional method. The methanol extracts prepared by MSE method showed maximum yield in comparison to other solvent extracts prepared by MSE and TSE. The plant extracts were then after evaluated for their in vitro antioxidant activity by DPPH assay, Superoxide Anion radical scavenging Activity and Total Phenolic Content (TPC). The results indicate that extracts prepared by MSE technique showed potent antioxidant activity in comparison to the extracts prepared by TSE method.

50. **Das MK**, Dhamodharan R, Hoti SL, **Dash AP**. Molecular differentiation of periodic and subperiodic *Wuchereria bancrofti* and *Brugia malayi* by randomly amplified polymorphic DNA (RAPD) markers. ***World J Microbiol Biotechnol*** **2011; *27*(6): 1525-30. 10.1007/s 11274-010-0601-6.**

ABSTRACT

Wuchereria bancrofti, a nematode parasite, is responsible for causing 90% of lymphatic filariasis infection in the world. In India, *W. bancrofti* exists in two physiological forms, nocturnally periodic in the main land and diurnally sub-periodic in Car Nicobar group of islands. Differentiation of these two parasitic forms by conventional microscopic methods difficult requiring good skill and hence tedious. Therefore, we developed a simple and rapid Random Amplified Polymorphic DNA (RAPD) assay to differentiate these parasitic forms. Also, the phylogenetic relationship between periodic and sub-periodic *W. bancrofti* and also *Brugia malayi* populations was analyzed using RAPD profile generated. Distinct RAPD profiles were observed among the three parasites with the formation of three distinct clusters in the phylogenetic tree. Substantial genetic diversity (Nei's genetic diversity H) was observed among periodic (H = 0.0577) and sub-periodic (H = 0.1415) *W. bancrofti* populations.

51. **Prajapati SK**, **Joshi H**, **Dev V**, **Dua VK**. Molecular epidemiology of *Plasmodium Vivax* antifolate resistance in India. ***Malar J* 2011; *10*: 102**.

ABSTRACT

Background: Sulphadoxine and pyrimethamine are anti-folate drugs that show synergistic anti-malarial effect. Point mutations in *dihydrofolate reductase* (*dhfr*) and *dihydropteorate synthatase* (*dhps*) cause anti-folate drug resistance phenotype in human malaria parasites. This study presents pattern of point mutations in *dhfr/dhps* genes among Indian sub-continent. **Methods:** Microscopically diagnosed one hundred *Plasmodium vivax* field isolates were collected from five widely separated geographical regions of India. *Dhfr* and *dhps* genes were PCR amplified and sequenced. Previously published mutations data were collected and analyzed using Chi square test to identify geographical cluster of mutant/wild type genotypes. **Results:** Sequence analysis revealed single (S58R), double (S58R/S117N) and quadruple (F57L/S58R/T61M/S117T/) point mutations at *dhfr* and single (A383G) to double (A383G/A553G) mutations at *dhps* in *P. vivax* field isolates. In addition, three new mutations were also observed at *dhfr*. Both, *dhfr* and *dhps* genes revealed tandem repeat variations in field isolates. *Dhps* revealed very low mutation frequency (14.0%) compared to *dhfr* (50.70%). Comparative analysis revealed a

progressive increase in frequency of quadruple mutant *dhfr* genotype ($p < 0.001$) within five years in north-eastern state (Kamrup, Assam). Frequency of *dhfr* genotypes revealed three distinct geographical clusters of wild (northern India), double mutant (southern India), and quadruple mutant (north-eastern and island regions of India) on the Indian sub-continent. **Conclusion:** Study suggests that SP may be susceptible to *P. vivax* in India, except Andaman and nor th-eastern state. The distinction of geographical regions with sensitive and resistant parasite phenotypes would be highly useful for designing and administering national anti-malarial drug policy.

52. **Dixit J, Srivastava H, Singh OP,** Saksena DN**, Das A**. Multilocus nuclear DNA Markers and genetic parameters in an Indian *Anopheles mininus* population**.** ***Infect Genet Evol*** **2011; *11*(3): 572-9.**

ABSTRACT

Estimation of population genetic parameters is highly dependent on the choice of genetic markers. Furthermore, inferences based on single genes could lead to erroneous conclusions and population genetic outcomes, thus usage of multiple loci is suggested. Considering malaria is a highly fatal vector-borne infectious disease, inference on population genetic structure and demography could be of help in the long run for malaria vector management and control. Using the published genome sequence information of *Anopheles gambiae* we designed EPIC primers to amplify DNA fragments in *An. minimus* nuclear genome. Eight such DNA fragments could be successfully amplified and sequenced and homology to corresponding genes of *An. gambiae* was established. All the eight DNA fragments were found to be polymorphic for single nucleotide polymorphisms (SNPs) in a population sample of *An. minimus* from India. Several tests of neutrality confirmed that all the eight fragments evolve under a standard neutral model of molecular evolution. Furthermore, multilocus linkage disequilibrium studies revealed that the DNA fragments were not genetically linked to each other and thus are independently evolving. Tests of past population demographic events clearly revealed that this Indian population of *An. minimus* follows demographic equilibrium model, without any significant recent population bottleneck or expansion. The eight multilocus nuclear DNA fragments thus could be considered as 'putatively neutral' and be used to infer population structure and demographic history of *An. minimus*, a major malaria vector in the Southeast Asia and India. Moreover, the estimations of population

demography using these putatively neutral markers can provide a baseline against which, test for the role of natural selection in functionally relevant genes of *An. minimus*would be possible.

KEYWORDS: malaria; *Anopheles minimus*; putatively neutral markers; SNPs; population genetics; India

53. Lumb V, **Das MK, Singh N, Dev V**, Khan W, Sharma YD. Multiple origin of *Plasmodium falciparum* dihydropteroate synthetase mutant alleles associated with Sulfadoxine resistance in India. ***Antimicrob Chemother* 2011; *55*(6): 2813-7.**

ABSTRACT

With the spread of chloroquine (CQ)-resistant malaria in India, sulfadoxine-pyrimethamine (SP) alone or in combination with artesunate is used as an alternative antimalarial drug. Due to continuous drug pressure, the *Plasmodium falciparum* parasite is exhibiting resistance to antifolates because of mutations in candidate genes dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*). Our earlier study on flanking microsatellite markers of dhfr mutant alleles from India had shown a single origin of the pyrimethamine resistance and some minor haplotypes which shared haplotypes with Southeast Asian (Thailand) strains. In the present study, we have analyzed 193 of these Indian *P. falciparum* isolates for 15 microsatellite loci around dhps to investigate the genetic lineages of the mutant dhps alleles in different parts of the country. Eighty-one of these samples had mutant dhps alleles, of which 62 were from Andaman and Nicobar Islands and the remaining 19 were from mainland India. Of 112 isolates with a wild-type dhpsallele, 109 were from mainland India and only 3 were from Andaman and Nicobar Islands. Consistent with the model of selection, the mean expected heterozygosity (He) around mutant dhps alleles (*He* = 0.55; *n* = 81) associated with sulfadoxine resistance was lower ($P \leq 0.05$) than the mean He around the wild-type dhps allele (*He* = 0.80; *n* = 112). There was more genetic diversity in flanking microsatellites of dhps than dhfr among these isolates, which confirms the assertion that dhpsmutations are at a very early stage of fixation in the parasite population. Microsatellite haplotypes around various mutant dhps alleles suggest that the resistant dhps alleles have multiple independent origins in India, especially in Andaman and Nicobar Islands. Determining the genetic lineages of the resistantdhps alleles on Andaman and Nicobar Islands and

mainland India is significant, given the role of Asia in the intercontinental spread of chloroquine- and pyrimethamine-resistant parasites in the past.

54. **Dhiman RC**, Chavan L, Pant M, Pahwa S. National and regional impacts of climate Change on malaria by 2030. ***Curr Sci*** **2011; *101*(93): 372-83.**

ABSTRACT

The article reports projection of malaria by 2030 using A1B scenario of PRECIS model basically derived from HadRM3. Malaria scenario has been defined in terms of opening of months of malaria transmission based on minimum required temperature and relative humidity for baseline (1961–1990) and by 2030. Detailed analysis has been done for four vulnerable sectors, *viz*. Himalayan region, northeast, the Western Ghats and coastal region. Some parts of Uttarakhand, Jammu and Kashmir and Arunachal Pradesh are likely to open transmission windows in new districts with increase in 4–6 months category of transmission. In the northeastern states, intensity of transmission is projected to increase from 7–9 months to 10–12 months. The Western Ghats is projected to be affected to a minimum, whereas in the east coastal districts, reduction in transmission months is likely due to increased temperature. As malaria transmission dynamics is multi-factorial, driven by agricultural practices, water availability, urbanization, migration, socioeconomic conditions and intervention measures, projections based on climatic parameters alone should not be viewed with certainty rather they are for guidelines for preparedness in vulnerable areas and strengthen health infrastructure, effective health education and use of best available tools of intervention to cope with the threat of climate change.

KEYWORDS: Climate change, malaria, relative humidity, transmission window, temperature.

55. Chhakchhuak L, **Ravindran KJ**, William SJ. Ovicidal activity of eucalyptus oil against the dengue vector *Aedes aegypti* (Diptera:Culicidae). ***Hexapoda*** **2011; *18*(1): 45-6.**

56. Mathur A, Sharma V, Bhardwaj A, Yousuf S, Verma SK, **Singh SK**, **Dua VK.** Pectin Content as an index for screening different varieties of apple (*Pyrus malus* L.) on the basis of antimicrobial activity. ***J Chem Pharmaceut Res*** **2011; *3*(2): 886-91.**

ABSTRACT

In the present investigation, pectin, the polysaccharide content in fruits is used as a basis for screening different varieties of Apple (*Pyrus malus* L.) of the same season of Kashmir (J&K). Different varieties of apple fruit of Kashmir (J&K) of the same season *viz* American, Delicious and Maharaj-ji were collected from the local gardens of Kashmir and pectin content present was extracted. The yield of pectin content was found to be maximum in Maharaj-ji (20.60 %) followed by Delicious (14.40 %) and American (11.60 %). The pectin extracted was then evaluated for its in vitro antibacterial activity against different pathogenic bacterial cultures. The results investigated that pectin extracted from Delicious variety showed potent antibacterial activity against *Klebsiella pneumoniae* (MIC value: 0.8 mg/ml) followed by *Streptococcus pyogenes* (MIC value: 0.3 mg/ml), *E.coli* (MIC value: 0.7 mg/ml) and *Lactococcus* sp. (MIC value: 0.7 mg/ml). The pectin extracted from other varieties showed no potency against any of the bacterial cultures. Further the pectin extracted from each of the variety was evaluated for in vitro antifungal activity against *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisae*. It was observed that pectin extracted from any of the variety showed no potency against any of the test fungal cultures. The results of the antimicrobial activity of the pectin extracted from each of the varieties were compared with that of standard pectin and Azithromycin. It was observed that standard pectin also not showed antifungal activity similarly to that of extracted pectin. The results thus confirmed that pectin can be utilized as a potent antibacterial agent and can be utilized as an index for screening different apple varieties of Kashmir (J & K). Furthermore our study validates the use of Delicious variety for the treatment of the infections borne by the tested organisms. The pectin extracted was further assayed for cellular toxicity against sheep fresh blood erythrocytes but no hemolysis against sheep fresh blood erythrocytes was observed.

KEYWORDS: *Pyrus malus* L., antimicrobial activity, pectin content, Delicious American, Maharaj-ji.

57. Mathur A, Purohit R, Mathur D, Prasad GBKS, **Dua VK**. Pharmacologial investigation of *Mentha piperita* L. roots on the basis of anti- microbial, antioxidant and anti- inflammatory properties. ***Der Pharmacia Sinica*** **2011; *2*(1): 208-16.**

ABSTRACT

Mentha piperita L. (Lamiaceae) leaves have been traditionally implemented in the treatment of minor sore throat and minor mouth or throat irritation by the indigenous people of India, although the compounds responsible for the medicinal properties have not been identified. The plant has been used in the Ayurvedic system of medicine for centuries. The methanol root extract of the plant was evaluated for antioxidant, antimicrobial and anti-inflammatory properties. The extract was found to possess maximum potency against infectious pathogens. Besides the antimicrobial effect maximum antioxidant capacity was observed in methanol extracts. The methanol extract of the roots of the plant also possessed maximum anti-inflammatory activity in carrageenan induced animal model in dose dependent manner at a dose of 50 mg/kg. The values $p < 0.05$ were found to be significant. Different other extracts showed no potency in comparison to other solvent extracts of the plant. These active crude methanol extracts were also assayed for cellular toxicity to fresh sheep erythrocytes and found to have no cellular toxicity.

KEYWORDS: *Mentha piperita* root, antimicrobial activity, antioxidant activity, anti-inflammatory activity, methanol extract.

58. Mathur A, Purohit R, Mathur D, Prasad GBKS, **Dua VK**. Pharmacologial investigation of Menthnol extract of *Syzigum cuminii* seeds and *Crateva nurvula* back on the basis of Anti-microbial, antioxidant and anti- inflammatory properties . ***Der Chemica Sinica*** **2011; *2*(1): 174-81.**

59. **Rawat M**, **Vijay S**, **Adak T, Dixit R, Nanda N, Srivastava H,** Sharma JK, Prasad GBKS, **Sharma A**. Parasite killing in malaria non-vector mosquito *Anopheles culicifacies* species B: implication of nitric oxide synthase upregulation. ***PLoS ONE*** **2011; *6*(4): e18400. doi: 10.1371/journal.pone.0018400.**

ABSTRACT

Background: *Anopheles culicifacies*, the main vector of human malaria in rural India, is a complex of five sibling species. Despite being phylogenetically related, a naturally selected subgroup species B of this sibling species complex is found to be a poor vector of malaria. We have attempted to understand the differences between vector and non-vector *Anopheles culicifacies* mosquitoes in terms of transcriptionally activated nitric oxide synthase (AcNOS) physiologies to

elucidate the mechanism of refractoriness. Identification of the differences between genes and gene products that may impart refractory phenotype can facilitate development of novel malaria transmission blocking strategies. **Methodology/Principal Findings:** We conducted a study on phylogenetically related susceptible (species A) and refractory (species B) sibling species of *An. culicifacies* mosquitoes to characterize biochemical and molecular differences in AcNOS gene and gene elements and their ability to inhibit oocyst growth. We demonstrate that in species B, AcNOS specific activity and nitrite/nitrates in mid-guts and haemolymph were higher as compared to species A after invasion of the mid-gut by *P. vivax* at the beginning and during the course of blood feeding. Semiquantitative RT-PCR and real time PCR data of AcNOSconcluded that this gene is more abundantly expressed in midgut of species B than in species A and is transcriptionally upregulated post blood meals. Dietary feeding of L-NAME along with blood meals significantly inhibited midgut AcNOS activity leading to an increase in oocyst production in *An. culicifacies* species B. **Conclusions/Significance:** We hypothesize that upregulation of mosquito innate cytotoxicity due to NOS in refractory strain to *Plasmodium vivax* infection may contribute to natural refractoriness in *An. culicifacies* mosquito population. This innate capacity of refractory mosquitoes could represent the ancestral function of the mosquito immune system against the parasite and could be utilized to understand the molecular basis of refractoriness in planning effective vector control strategies.

60. Mathur A, Singh GK, Verma SK, Yousuf S, Bhardwaj A, **Singh SK**, Prasad GBKS, **Dua VK**. Phytochemical investigation and *in vitro* antimicrobial activity of different parts of *Ficus racemosa* L. ***Der Pharmacia Sinica* 2011; *2*(2): 270-5.**

ABSTRACT

In the present investigation different parts of Ficus racemosa L. were screened for their in vitro antimicrobial activity by well diffusion method and phytochemical screening by different conventional methods. The hydro-alcoholic extracts (50 % v/v) were prepared according to cold percolation method. The extracts were assayed against different pathogenic microorganisms. The hydro-alcoholic extracts of different parts of the tree showed maximum activity against bacterial strains in comparison to fungal strains. It was found that hydro-alcoholic extracts of leaves of the tree showed maximum potency against Lactococcus sp. at MIC value 0.5 mg/ml, but showed almost similar activity against *E.coli* and *Aspergillus niger*. The leaves extract showed no potency

against *Klebsiella pneumoniae, Streptococcus pyogenes* and *Saccharomyces cerevisae*. The hydro-alcoholic extracts of the bark also showed potent activity against Lactococcus sp. at MIC value 0.3 mg/ml and showed minimum activity against Candida albicans. The extracts also showed no antimicrobial activity against *E.coli, Klebsiella pnemoniae, Streptococcus pyogenes, Aspergillus niger* and *Saccharomyces cerevisae*. The hydro-alcoholic extracts of the fruits of the plant showed maximum potency against *E.coli* at MIC value 0.6 mg/ml while moderate activity against *Lactococcus sp*. and minimum activity against *Aspergillus niger*. The extracts of the fruit showed no activity against *Klebsiella pnemoniae, Streptococcus pyogenes, Candida albicans and Saccharomyces cerevisae*. The extracts of different parts of the tree were further assayed for phytochemical screening and cellular toxicity. The extracts of different parts of the tree showed no cellular toxicity against sheep fresh blood erythrocytes.

KEYWORDS: *Ficus racemosa* L., antimicrobial activity, phytochemical screening, pathogens.

61. **Dua VK**, Mathur A, Prasad GBKS, Verma SK, **Singh SK**. Phytochemical investigation and *in vitro* antioxidant activity of some medicinally important plants. ***Int Res J Pharm*** **2011; *2*(6): 116-2.**

62. **Jain V**, Lucknow NW, Wilson NO, Blackstock AJ, Nagpal AC, Joel PK, **Singh MP**, Udhayakumar V, Stiles KJ, **Singh N**. Plasma levels of angiopoietin-1 and -2 predict cerebral malaria outcome in central India. ***Malar J*** **2011; *10*(1): 383**.

ABSTRACT

Background: The mechanisms underlying the pathogenesis of cerebral malaria (CM) syndrome are not well understood. Previous studies have shown a strong association of inflammatory chemokines, apoptotic markers and angiogenic molecules with CM associated mortality. Recognizing the importance of angiopoietins (ANG) in the pathogenesis of CM, a retrospective investigation was carried out in a hospital cohort of malaria patients with *Plasmodium* infection in central India to determine if these factors could be suitable markers of CM associated severity. **Methods:** Patients enrolled in the study were clinically characterized as healthy controls (HC), mild malaria (MM), CM survivors (CMS) and CM non-survivors (CMNS) based on their malaria status and hospital treatment outcome. Plasma ANG-1 and ANG-2 levels were assessed using sandwich ELISA. Receiver operating characteristic (ROC) curve analysis was used to calculate

area under the curve (AUC) for each biomarker in order to assess predictive accuracy of individual biomarkers. **Results:** The plasma levels of ANG-1 were lower in CMS and CMNS compared to control groups (mild malaria and healthy controls) at the time of hospital admission. On the contrary, ANG-2 levels positively correlated with malaria severity and were significantly higher in CMNS. The ratio of ANG-2/ANG-1 was highest in CMNS compared to other groups. Receiver operating characteristic curves revealed that compared to ANG-1 (AUC = 0.35), ANG-2 (AUC = 0.95) and ratio of ANG-2/ANG-1 (AUC = 0.90) were better markers to discriminate CMNS from MM cases. However, they were less specific in predicting fatal outcome amongst CM cases at the time of hospital admission. **Conclusion:** These results suggest that at the time of admission plasma levels of ANG-2 and ratio of ANG-2/ANG-1 are clinically informative biomarkers to predict fatal CM from MM cases while they have limited usefulness in discriminating fatal CM outcomes in a pool of CM cases in endemic settings of Central India.

KEYWORDS: angiopoietins, cerebral malaria, pathogenesis, biomarkers, receiver operating characteristic analysis

63. **Prajapathi SK**, **Joshi H**, **Shalini S**, Patarroya MA, Suwanarusk R, **Kumar A, Sharma SK, Eapen A, Dev V, Bhatt RM, Valecha N,** Nosten F, Rizvi MA, **Dash AP**. *Plasmodium vivax* lineages: Geographical distribution, tandem repeat polymorphism and phylo-genetic relationship. ***Malar J* 2011; *10*: 374.**

ABSTRACT

Background: Multi-drug resistance and severe/complicated cases are the emerging phenotypes of *vivax* malaria, which may deteriorate current anti-malarial control measures. The emergence of these phenotypes could be associated with either of the two *Plasmodium vivax* lineages. The two lineages had been categorized as Old World and New World, based on geographical sub-division and genetic and phenotypical markers. This study revisited the lineage hypothesis of *P. vivax* by typing the distribution of lineages among global isolates and evaluated their genetic relatedness using a panel of new mini-satellite markers. **Methods:** *18S SSU rRNA S-type* gene was amplified from 420 *Plasmodium vivax* field isolates collected from different geographical regions of India, Thailand and Colombia as well as four strains each of *P. vivax* originating from Nicaragua, Panama, Thailand (Pak Chang), and Vietnam (ONG). A mini-satellite marker panel was then

developed to understand the population genetic parameters and tested on a sample subset of both lineages. **Results:** *18S SSU rRNA S-type* gene typing revealed the distribution of both lineages (Old World and New World) in all geographical regions. However, distribution of *Plasmodium vivax* lineages was highly variable in every geographical region. The lack of geographical sub-division between lineages suggests that both lineages are globally distributed. Ten mini-satellites were scanned from the *P. vivax* genome sequence; these tandem repeats were located in eight of the chromosomes. Mini-satellites revealed substantial allelic diversity (7-21, $AE = 14.6 \pm 2.0$) and heterozygosity ($He = 0.697\text{-}0.924$, $AE = 0.857 \pm 0.033$) per locus. Mini-satellite comparison between the two lineages revealed high but similar pattern of genetic diversity, allele frequency, and high degree of allele sharing. A Neighbour-Joining phylogenetic tree derived from genetic distance data obtained from ten mini-satellites also placed both lineages together in every cluster. **Conclusions**: The global lineage distribution, lack of genetic distance, similar pattern of genetic diversity, and allele sharing strongly suggested that both lineages are a single species and thus new emerging phenotypes associated with *vivax* malaria could not be clearly classified as belonging to a particular lineage on basis of their geographical origin.

64. **Awasthi G**, Prasad GBKS, **Das A**. Population genetic analyses of *Plasmodium Falciparum* chloroquine receptor transporter gene haplotypes reveal the evolutionary history of chloroquine-resistant malaria in India. ***Int J Parasitol*** **2011; *41*: 705-9.**

ABSTRACT

Inferring the origin and dispersal of the chloroquine-resistant (CQR) malaria parasite, *Plasmodium falciparum*, is of academic and public health importance. The *Pfcrt* gene of *P. falciparum* is widely known as the CQR gene and two major haplotypes of this gene (CVIET and SVMNT) occur widely across CQR-endemic regions of the globe. In India, studies to date of the *Pfcrt* gene have indicated the widespread prevalence of the SVMNT haplotype (prevalent in the South America and Papua New Guinea), whereas the CVIET haplotype, primarily found in southeast Asia, was not detected at a high frequency in India. This distribution pattern of the two most common CQR-*Pfcrt* haplotypes in India is quite surprising. Thus, in order to understand probable evolutionary and migration patterns of the CQR-*Pfcrt* haplotypes into India, we generated new sequence data of exon 2 of the *Pfcrt* gene and collected published information on the CQR-*Pfcrt* haplotype data

from India, Papua New Guinea, southeast Asia and South America, and performed several population and evolutionary genetic analyses. Among several interesting findings, statistically significant longitudinal clines for the CVIET and SVMNT haplotypes (in opposite directions) in India, and the clustering of India and Papua New Guinea under the SVMNT-specific clade in the phylogenetic tree, are the two most remarkable aspects of the data. It also appears that both the SVMNT and CVIET haplotypes in India have migrated from southeast Asia. In particular, whereas the Indian CVIET haplotype has a southeast Asian origin, the SVMNT haplotype, prevalent in India, seems to have originated in Papua New Guinea and entered India through southeast Asia.

KEYWORDS: malaria, chloroquine resistance, *Plasmodium falciparum, Pfcrt* haplotypes, India.

65. Naomi WL, **Jain V**, Wilson NO, **Singh N,** Udhayakumar V, Stiles JK. Potential serological biomarkers of cerebral malaria. ***Dis Markers*** **2011; *31*(6): 327-35.**

ABSTRACT.

Biomarkers have been used to diagnose and prognosticate the progress and outcome of many chronic diseases such as neoplastic and non communicable diseases. However, only recently did the field of malaria research move in the direction of actively identifying biomarkers that can accurately discriminate the severe forms of malaria. Malaria continues to be a deadly disease, killing close to a million people (mostly children) every year. One life-threatening complication of malaria is cerebral malaria (CM). Studies carried out in Africa have demonstrated that even with the best treatment, as high as 15–30% of CM patients die and about 10–24% of CM survivors suffer short-or long-term neurological impairment. The transition from mild malaria to CM can be sudden and requires immediate intervention. Currently, there is no biological test available to confirm the diagnosis of CM and its complications. It is hoped that development of biomarkers to identify CM patients and potential risk for adverse outcomes would greatly enhance better intervention and clinical management to improve the outcomes. We review here what is currently known regarding biomarkers for CM outcomes. A Pub Med literature search was performed using the following search terms: “malaria,” “cerebral malaria,” “biomarkers,” “mortality” and “neurological sequelae.” This search revealed a paucity of usable biomarkers for CM management. We propose three main areas in which researchers can attempt to identify CM biomarkers: 1) early biomarkers, 2) diagnostic biomarkers and 3) prognostic biomarkers.

KEYWORDS: biomarkers, cerebral malaria, severe malaria, malaria.

66. **Mishra N**, **Anvikar AR**, Kamal VK, **Sharma SK**, **Srivastava HC**, **Das MK**, **Pradhan K**, Kumar H, Gupta YK, Gupta P, **Dash AP**, **Valecha N**. Prescription and availability of artemisinin monotherapy in India: Where do we stand? ***Malar J* 2011; *10*: 360. doi: 10.1186/1475-2875-10-360.**

ABSTRACT

Background: The World Health Organization has urged all member states to deploy artemisinin-based combination therapy and progressively withdraw oral artemisinin monotherapies from the market due to their high recrudescence rates and to reduce the risk of drug resistance. Prescription practices by physicians and the availability of oral artemisinin monotherapies with pharmacists directly affect the pattern of their use. Thus, treatment practices for malaria, with special reference to artemisinin monotherapy prescription, in selected states of India were evaluated. **Methods**: Structured, tested questionnaires were used to conduct convenience surveys of physicians and pharmacists in eleven purposively selected districts across six states in 2008. In addition, exit interviews of patients with a diagnosis of uncomplicated malaria or a prescription for an anti-malarial drug were also performed. Logistic regression was used to determine patient clinical care, and institutional factors associated with artemisinin monotherapy prescription. **Results**: Five hundred and eleven physicians from 196 health facilities, 530 pharmacists, and 1, 832 patients were interviewed. Artemisinin monotherapy was available in 72.6% of pharmacies and was prescribed by physicians for uncomplicated malaria in all study states. Exit interviews among patients confirmed the high rate of use of artemisinin monotherapy with 14.8% receiving such a prescription. Case management, *i.e*. method of diagnosis and overall treatment, varied by state and public or private sector. Treatment in the private sector (OR 8.0, 95%CI: 3.8, 17) was the strongest predictor of artemisinin monotherapy prescription when accounting for other factors. Use of the combination therapy recommended by the national drug policy, artesunate + sulphadoxine-pyrimethamine, was minimal (4.9%), with the exception of one state. **Conclusions:** Artemisinin monotherapy use was widespread across India in 2008. The accessible sale of oral artemisinin monotherapy in retail market and an inadequate supply of recommended drugs in the public sector health facilities promoted its prescription. This study resulted in notifications to all state drug

controllers in India to withdraw the oral artemisinin formulations from the market. In 2010, artesunate + sulphadoxine-pyrimethamine became the universal first-line treatment for confirmed*Plasmodium falciparum* malaria and was deployed at full scale.

67. **Singh RK, Dhiman RC, Dua VK**. Prevalence of *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse in Koderma, Jharkhand. ***J Commun Dis*** **2011; *43*(3): 223-8.**

ABSTRACT

Entomological survey was carried out in different localities of Koderma district of Jharkhand with a view to study the prevalence, distribution and stratification of areas for *Aedes* mosquito species. A total of 233 houses were covered during house to house larval and adult survey. *Aedes* breeding could be detected in 157 houses. In all, a total of 942 domestic water containers were searched, out of which 461 were found positive. The overall house index(HI) container index(CI) breteau index(B1) and pupal index(PI) were 67.38%, 48.94%, 197.85% and 79.4%, respectively. The survey revealed that *Aedes aegypti* Linnaeus and *Aedes albopictus* Skuse are well established in Koderma with most of the areas showing high adult and larval indices. The preventive strategy needs to be directed towards minimizing the breeding potential of *Aedes* and water management practice by individuals along with implementation of urban bye-laws as well as IEC activities to contain *Aedes* breeding in future.

KEYWORDS: prevalence, *Aedes mosquitoes*, Koderma, Jharkhand, India.

68. Islam MN, Zulkifle MS, Khan MA, **Ghosh SK, Tiwari SN**. Prevalence of malaria, dengue, and chikungunya significantly associated with mosquite breedings sites. ***J Islamic Med Assoc North Am*** **2011; *43*(2): 58-67.**

ABSTRACT

Objectives: To observe the prevalence of malaria, dengue, and chikungunya and their association with mosquito breeding sites. **Methods**: The study was observational and analytical. A total of 162 houses and 670 subjects were observed during the study period. One hundred forty-two febrile patients were eligible for the study. After obtaining informed consent from all febrile patients, 140 blood samples were collected to diagnose malaria, dengue, and chikungunya. Larval samples were

collected by the standard protocol that follows. Correlation of data was performed by Pearson correlation test. **Results**: Forty-seven blood samples were found positive: 33 for chikungunya, 3 for dengue, and 11 for malaria. Fifty-one out of 224 larval samples were found positive. Out of the 51 positive samples, 37 were positive for *Aedes*, 12 were positive for *Anopheles*, and two were positive for *Culex* larvae. **Interpretation and Conclusion**: Mosquito-borne fevers, especially malaria, dengue, and chikungunya, have shown a significant relationship with mosquito breeding sites.

KEYWORDS: Malaria, dengue, chikungunya, larvae, mosquito breeding sites

69. Charkady R, Kelkar DS, Muthusamy B, Kandasamy K, Dwivedi SB, Sahastrabudhe NB, Kim MS, Renuse S, Pinto SN, Sharma R, Pawar H, Sheker NR, **Mohanty AK**, Getnet D, Yong Y, Jhong J, **Dash AP**, McCallum RM, Delanghe B, Mlange G, **Kumar A**, Prasad KT, Okulate M, Kumar N, Pandey A. A proteogenomic analysis of *Anopheles gambiae* using high –resolution Fourier transform mass spectrometry. *Genome Res* 2011: *21*(11): 1872-81.

ABSTRACT

Anopheles gambiae is a major mosquito vector responsible for malaria transmission, whose genome sequence was reported in 2002. Genome annotation is a continuing effort, and many of the approximately 13,000 genes listed in VectorBase for *Anopheles gambiae* are predictions that have still not been validated by any other method. To identify protein-coding genes of *An. gambiae* based on its genomic sequence, we carried out a deep proteomic analysis using high-resolution Fourier transform mass spectrometry for both precursor and fragment ions. Based on peptide evidence, we were able to support or correct more than 6000 gene annotations including 80 novel gene structures and about 500 translational start sites. An additional validation by RT-PCR and cDNA sequencing was successfully performed for 105 selected genes. Our proteogenomic analysis led to the identification of 2682 genome search–specific peptides. Numerous cases of encoded proteins were documented in regions annotated as intergenic, introns, or untranslated regions. Using a database created to contain potential splice sites, we also identified 35 novel splice junctions. This is a first report to annotate the *An. gambiae* genome using high-accuracy mass spectrometry data as a complementary technology for genome annotation.

70. Poravuth Yi Socheat D, Rueangweerayut R, Uthaisin C, Phyo AP, **Valecha N**, Rao BHK, Tjitra E, Purnama A, Borghini- Fuhrer l, Duparc S, Shin Chang-Sik Fleckenstein L. Pyronaridine-Artesunate versus chloroquine in patients with acute *Plasmodium vivax* malaria: A randomized, double-blind, non- inferiority trial. ***PLoS One* 2011; *6*(1): e14501.**

ABSTRACT

Background: New antimalarials are needed for *P. vivax* and *P. falciparum* malaria. This study compared the efficacy and safety of pyronaridine-artesunate with that of chloroquine for the treatment of uncomplicated *P. vivax* malaria. **Methods and Findings**: This phase III randomized, double-blind, non-inferiority trial included five centers across Cambodia, Thailand, India, and Indonesia. In a double-dummy design, patients (aged .3–#60 years) with microscopically confirmed *P. vivax* mono-infection were randomized (1:1) to receive pyronaridine-artesunate (target dose 7.2:2.4 mg/kg to 13.8:4.6 mg/ kg) or chloroquine (standard dose) once daily for three days. Each treatment group included 228 randomized patients. Outcomes for the primary endpoint, Day-14 cure rate in the per-protocol population, were 99.5%, (217/218; 95%CI 97.5, 100) with pyronaridine-artesunate and 100% (209/209; 95%CI 98.3, 100) with chloroquine. Pyronaridine was non-inferior to chloroquine: treatment difference 20.5% (95%CI 22.6, 1.4), *i.e.*, the lower limit of the 2-sided 95%CI for the treatment difference was greater than 210%. Pyronaridine-artesunate cure rates were non-inferior to chloroquine for Days 21, 28, 35 and 42. Parasite clearance time was shorter with pyronaridine-artesunate (median 23.0 h) versus chloroquine (32.0 h; p,0.0001), as was fever clearance time (median 15.9 h and 23.8 h, respectively; $p = 0.0017$). Kaplan-Meier estimates of postbaseline *P. falciparum* infection incidence until Day 42 were 2.5% with pyronaridine-artesunate, 6.1% with chloroquine ($p = 0.048$, log-rank test). Post-baseline *P. vivax* or *P. falciparum* infection incidence until Day 42 was 6.8% and 12.4%, respectively ($p = 0.022$, log rank test). There were no deaths. Adverse events occurred in 92/228 (40.4%) patients with pyronaridine-artesunate and 72/228 (31.6%) with chloroquine. Mild and transient increases in hepatic enzymes were observed for pyronaridine-artesunate. **Conclusion**: Pyronaridine-artesunate efficacy in acute uncomplicated *P. vivax* malaria was at least that of chloroquine. As pyronaridine-artesunate is also efficacious against *P. falciparum* malaria, this combination has potential utility as a global antimalarial drug.

71. **Dixit R**, **Rawat M**, Kumar S, **Pandey KC**, **Adak T**, **Sharma A.** Salivary Gland Transcriptome analysis in response to sugar feeding in malaria vector *Anopheles Stephensi*. ***J Insect Physiol* 2011; *57*(10): 1399-406.**

ABSTRACT

In this study, we analyzed a small scale transcriptome of salivary glands in sugar fed female mosquitoes. Thirty five percent of the transcripts could not be assigned a function. Some of them may code for salivary gland specific products involved in sugar feeding. We identified and characterized two new putative cDNAs encoding a sugar transporter and a cAMP generating DAPIT (Diabetes-Associated proteins in insulin sensitive tissues). Down regulation of these two cDNAs in response to blood feeding suggest that both *AsST* and *AsDAPIT* salivary genes may specifically be involved in the facilitation of sugar metabolism and energy production. The inability to absorb or digest sugar may cause organ failure, improper functioning of nervous system, behavioral disorder and death. Further functional characterization of theses putative transcripts is under investigation to examine their role in the mosquito salivary glands.

KEYWORDS: mosquito, salivary gland, sugar transporter, transcriptome.

72. Tennyson S, **Ravindr*an* KJ**, Arivoli S. Screening of plant extracts for ovicidal activity against *Culex quinquefasciatus* Say (Diptera Culicidae). ***Appl Botany* 2011; *40*: 5456-60.**

ABSTRACT

Hexane, diethyl ether, dichloromethane and ethyl acetate extracts of twenty five plant species, including fourteen varieties of leaves, five varieties of whole plants, two varieties of barks and one variety of flower, fruit, seed and root were tested for ovicidal activity against *Culex quinquefasciatus* at 1000 ppm. During preliminary screening, significant ovicidal activity was observed in all four solvent extracts of *Cleistanthus collinus, Hydrocotyle javanica, Leucas aspera, Murraya koeingii, Sphaeranthus indicus* and *Zanthophyllus limonella*. Solvent crude extracts of plants showing maximum ovicidal activity were selected and treated at 500 ppm. The percentage of egg hatchability significantly reduced in different solvent extracts of above mentioned plants and eggs treated with different plant extracts varied from 26.59 per cent in diethyl ether extract of Murraya koeingii to 82.61 per cent in hexane extract of *Leucas aspera*.

KEYWORDS: plant materials, *Culex quinquefasciatus*, statistical analysis.

73. **Rawat M**, **Vijay S**, Gupta Y, **Dixit R**, Tiwari PK, **Sharma A**. Sequence homology and structural analysis of plasmepsin 4 isolated from Indian *Plasmodium vivax* isolates. ***Infect Gen Evol.*** **2011; *11*(5): 924-33. doi: 10.1016/j.meegid.2011.02.024.**

ABSTRACT

Plasmodium vivax malaria is a globally widespread disease responsible for 50% of human malaria cases in Central and South America, South East Asia and Indian subcontinent. The rising severity of the disease and emerging resistance of the parasite has emphasized the need for the search of novel therapeutic targets to combat *P. vivax* malaria. Plasmepsin 4 (PM4) a food vacuole aspartic protease is essential in parasite functions and viability such as initiating hemoglobin digestion and processing of proteins and is being looked upon as potential drug target. Although the plasmepsins of *Plasmodium falciparum* have been extensively studied, the plasmepsins of *P. vivax* are not well characterized. This is the first report detailing complete PM4 gene analysis from Indian *P. vivax* isolates. Blast results of sequences of *P. vivax* plasmepsin 4 (*Pv*PM4) shows 100% homology among isolates of *P. vivax* collected from different geographical regions of India. All of the seven Indian isolates did not contain intron within the coding region. Interestingly, *Pv*PM4 sequence analysis showed a very high degree of homology with all other sequences of *Plasmodium* species available in the genebank. Our results strongly suggest that *Pv*PM4 are highly conserved except a small number of amino acid substitutions that did not modify key motifs at active site formation for the function or the structure of the enzymes. Furthermore, our study shows that *Pv*PM4 occupies unique phylogenetic status within *Plasmodium* group and sufficiently differ from the most closely related human aspartic protease, cathepsin D. The analysis of 3D model of PM4 showed a typical aspartic protease structure with bi-lobed, compact and distinct peptide binding cleft in both *P. vivax* and *P. falciparum*. In order to validate appropriate use of PM4 as potential anti-malarial drug target, studies on genetic and structural variations among *P. vivax* plasmepsins (*Pv*PMs) from different geographical regions are of utmost importance for drugs and vaccine designs for anti-malarial strategies.

KEYWORDS: malaria, *Plasmodium vivax*, India, Plasmepsins Aspartic proteases, genetic diversity, molecular modeling.

74. **Singh RK, Dhiman RC**, **Das MK**. Situation analysis of malaria in Godda district of Jharkhand during malaria epidemic. ***J Commun Dis.*** **2011; *43*(2): 135-42.**

ABSTRACT

The epidemiological and entomological investigations were carried out in 18 tribal villages of Godda district of Jharkhand state revealed average slide positivity rate (SPR) 9.9% and slide positivity rate for *Plasmodium falciparum* (SfR) 5.0%, respectively. *P. falciparum* was the dominant parasite accounting 51.2 per cent of the total infections. All the villages are situated in the deep forest and forest fringes. A total of 416 blood slides were collected and examined. Out of which 41 slides were found positive for malaria parasite (21 positive for *P. falciparum* and 20 positive for *P. vivax*). All the positive cases were treated with Blister Packs of anti-malarial of chloroquine and primaquine as per NBVDCP schedule to prevent further transmission of malaria, which were available. Rapid diagnostic Kits were used selectively only on the recommendation of the Physician/Medical Officer. Results of house to house fever survey indicated the presence of high percentage of symptomatic carriers of malaria parasites in the local population and in our study *Pf* % is more than reported data by District Malaria Office, Godda district, Jharkhand. In entomological studies, for mosquito fauna with reference to both *Anopheline*, 11 species belonging to one genus *Anopheles* were collected and identified during the survey; among them some species were most prevalent. A total of 599 mosquitoes were recorded and average density of mosquitoes recorded was 13.19 in human dwellings and 86.11 in cattle sheds.

KEYWORDS: Mosquitoes, epidemiological and entomological investigation, malaria situation, Godda district of Jharkhand state.

75. **Shukla M**, **Singh N**, **Singh MP**. Spleen rates and infant parasite rates as surveillance tool for malaria control in remote hard to reach areas of central India. ***Malar J*** **2011; *10*: 381**.

ABSTRACT

Background: Malaria due to both *Plasmodium falciparum* and *Plasmodium vivax* is a major public health problem in India. The quantification of malaria transmission for the classification of malaria risk has long been a concern for epidemiologists. Results are presented from 30 cross-

sectional surveys which measured spleen rates (SR) and infant parasite rates (IPR) in the forested districts of Madhya Pradesh during malaria outbreaks to assess whether both IPR and SR can still be used as indicators of malaria endemicity as spleen examination has lost much of its value as an epidemiological indicator in areas where anti-malarials drugs are widely used. **Methods**: Rapid fever surveys were carried out from door to door and all suspected malaria cases in the entire population of a village were screened for malaria parasites on the basis of clinical symptoms such as fever, chill, rigor, headache and body ache etc. Children between 2 and 9 years were examined for enlarged spleen according to Hacketts method. Finger prick blood smears were collected from all children with enlarged spleen with or without fever after obtaining written informed consent following institutional ethical guidelines. Infants less than 1 year were also screened for malaria with or without fever. **Results**: Since malaria is local and focal, in some areas the outbreak waned quickly in few months and in some areas continued for 3 to 4 years. The analysis of trend revealed that when IPR decline over the years as a result of malaria intervention measures, SR also decline. In case splenomegaly continues without diminution in size, it is probably due to recrudescence or relapse, although it is not possible to separate malaria parasite species on the basis of SR. **Conclusion**: Both the tools are of immense value in evaluating and assessing the malaria situation especially in remote areas where sophisticated molecular and serological techniques are difficult to establish. Therefore, in forested areas malaria surveillance system will require adoption of multiple approaches that have proven effective now or in the past.

76. **Das MK**, **Singh RK**, Lal RK, **Dhiman RC**. Susceptibility of *Aedes aegypti* to insecticides in Ranchi city, Jharkhand state, India. ***Dengue Bull* 2011; *35*: 194-8.**

ABSTRACT

A study was undertaken to find out the susceptibility status of the dengue vector, *Aedes aegypti*, to various insecticides in 2008 in Ranchi, capital of Jharkhand state, India, using the WHO standard susceptibility test kits. The susceptibility test showed that *Ae. aegypti* mosquitoes were resistant to DDT but susceptible to malathion, deltamethrin and cyfluthrin. The mortalities of adults, using diagnostic dosages of DDT (4.0%) were 19.5%; malathion (5.0%) 88.83%; deltamethrin (0.05%) 99.57%; and cyfluthrin (0.15%) 93.33%. For the larval susceptibility test on III and IV instar, *Ae. aegypti* larvae collected from the field were tested according to the WHO-recommended diagnostic dosages for *Aedes* spp against temephos (0.02 mg/L). The tests revealed that larvae of *Aedes*

aegypti species were susceptible to temephos and the mortality was 96.53% to 100% within 24 hours of treatment.

KEYWORDS: *Aedes aegypti*, Insecticide susceptibility, Ranchi city, Jharkhand.

77. **Singh RK**, **Dhiman RC**, **Mittal PK**, **Dua VK**. Susceptibility status of dengue vectors against various insecticides in Koderma (Jharkhand), India. ***J Vector Borne Dis*** **2011; *48* (2): 116-8.**

78. **Singh RK**, **Dhiman RC**, **Kumar G**, Sinha AT, **Dua VK**. Susceptibility status of malaria vectors to insecticides in Koderma, Jharkhand. ***J Commun Dis*** **2011; *43*(4): 273-6.**

79. Alam MF, Mohammed MS, Chopra AK, **Dua VK**. Toxicological properties of several Medicinal plants from the Himalayas (India) against vectors of malaria, filariasis and dengue. ***Trop Biomed*** **2011 Aug; *28*(2): 343-50.**

ABSTRACT

The leaves of five plants namely *Nyctanthes arbortistis* (Oleaceae), *Catharanthus roseus* (Apocynaceae), *Boeninghusenia albiflora* (Rutaceae), *Valeriana hardwickii* (Valerianaceae) and *Eupatorium odoratum* (Asteraceae) were selected for the first time from the Garhwal region of north west Himalaya to investigation its toxicological properties against mosquito vectors of malaria, filariasis and dengue. In a laboratory study, using different polarity solvents (petroleum ether, chloroform and methanol) were tested against important larvae of malaria, filariasis and dengue vectors in India. It was observed that petroleum ether fraction of all selected plant possess good larvicidal properties than other solvent fraction. The LC50 values of isolates from *Nyctanthes arbortistis* (HAR-1), *C. roseus* (CAT-1), *B. albiflora* (BOA-1), *V. hardwickii* (SUG-1) and *E. odoratum* (EUP-1) against *Anopheles stephensi* were 185 ppm, 150 ppm, 105 ppm, 225 ppm and 135 ppm, respectively. The results therefore suggest that the fraction code BOA-1 has excellent larvicidal properties and could be incorporated as botanical insecticides against mosquito vectors with high safety to nontarget organisms. The same fraction was tested against adult vectors of malaria, filariasis and dengue, but no mortality was observed.

80. **Sood RD**, **Mittal PK**, Kapoor N, **Razdan RK**, **Dash AP**. Wash resistance and efficacy of Olyset® net and Permanet® 2.0 against *Anopheles stephensi*.An urban malaria vector in India, as assessed by cone and tunnel boiassays. ***J Am Mosq Control Assoc*** **2011; *27*(4): 432-8.**

ABSTRACT

Long-lasting insecticidal nets (LLIN) have been developed for wash resistance and long-lasting effects against mosquito vectors. In this study we evaluated 2 LLIN products, Olyset® net and Permanet® 2.0, washed for 0, 5, 10, 15, and 20 times, against *Anopheles stephensi*, an urban malaria vector in India. We assessed the wash resistance and efficacy of these nets in relation to bloodfeeding inhibition and percent mortality in cone and tunnel test bioassays. Both Olyset and Permanet showed >80% mortality of *An. stephensi* in cone bioassays after 20 washes. In tunnel tests there was no significant difference between Olyset and Permanet 2.0 in causing total mosquito mortality (immediate and delayed) up to 10 washes and bloodfeeding inhibition and entry rate up to 15 washes. After the 20th wash, Permanet 2.0 was significantly more effective than the Olyset net in causing total mosquito mortality, whereas Olyset net showed less bloodfeeding and entry of mosquitoes as compared to Permanet 2.0. There was a gradual decline in efficacy of both LLIN products as the number of washings increased. Cone bioassays indicated a strong wash resistance in both the LLIN products after 20 washes. However, the tunnel tests demonstrated a gradual decline in efficacy of both products with the number of washings.

KEYWORDS: Long-lasting insecticidal nets, cone bioassays, tunnel tests, blood feeding inhibition, entry rate.

Titles: A-Z

2012

81. Pathak S, Rege M, Gogtay NJ, Aigal U, **Sharma SK**, **Valecha N**, Bhanot G, Kshirsagar NA, Sharma S. Age-dependent sex bias in clinical malarial disease in hypoendemic regions. ***Plos ONE* 2012; *7*(4): e35592.**

ABSTRACT

Background and Objectives: Experimental models show a male bias in murine malaria; however, extant literature on biases in human clinical malaria is inconclusive. Studies in hyperendemic areas document an absence of sexual dimorphism in clinical malaria. Data on sex bias in clinical malaria in hypoendemic areas is ambiguous—some reports note a male bias but do not investigate the role of differential mosquito exposure in that bias. Moreover, these studies do not examine whether the bias is age related. This study investigates whether clinical malaria in hypoendemic regions exhibits a sex bias and whether this bias is age-dependent. We also consider the role of vector exposure in this bias. **Methods:** Retrospective passive clinical malaria datasets (2002–2007) and active surveillance datasets (2000–2009) were captured for the hypoendemic Mumbai region in Western India. To validate findings, passive retrospective data was captured from a primary malaria clinic (2006–2007) in hypoendemic Rourkela (Eastern India). Data was normalized by determining percent slide-positivity rates (SPRs) for males and females, and parasite-positivity distributions were established across age groups. The Mann–Whitney test, Wilcoxon Signed Rank test, and Chi-square analysis were used to determine statistical significances. **Results:** In both the Mumbai and Rourkela regions, clinical malaria exhibited an adult male bias ($p<0.01$). A sex bias was not observed in children aged ≤10. Post -puberty, male SPRs were significantly greater than females SPRs ($p<0.01$). This adult male bias was observed for both *vivax* and *falciparum* clinical disease. Analysis of active surveillance data did not reveal an age or sex bias in the frequency of parasite positivity. **Conclusion:** This study demonstrates an age-dependent sex bias in clinical malaria in hypoendemic regions and enhanced incidence of clinical malaria in males following

puberty. Possible roles of sex hormones, vector exposure, co-infections, and other factors in this enhanced susceptibility are discussed.

82. **Niranjan Reddy BP**, **Raghavendra K**, Prasad GBKS. *Anopheles gambiae* (Diptera: Culicidae) Cytochrome P450 (P450) Supergene family: Phylogenetic analyses and exon-intron organization. ***Entomol Ornithol Herpetol*** **2012; *1*: 1.**

ABSTRACT

The cytochrome P450 superfamily is involved mainly in developmental processes and xenobiotic metabolism in insects. Analysis of the *Anopheles gambiae* genome has shown 105 putatively active P450 genes that are distributed in four major clans, namely mitochondrial, CYP2, CYP3, and CYP4. In the present study, phylogenetic analysis using multiple methodologies, exon-intron organization, correlation between genes in gene clusters and their gene organizations were analyzed. Further to this, usability of intronic positions in deciphering the evolutionary relatedness among the members of AgP450 supergene family was studied. The results show that the AgP450 supergene family is evolved through the complex process of duplications followed by structural-functional evolution. This supergene family might have undergone numerous intron-losses and gains during the process of evolution. However, this process is closely related with the evolutionary relationship among the members of the AgP450 supergene family. Furthermore, this study identifies the need of in-depth study to elucidate the functional importance of the conserved intron in CYP6 family.

KEYWORDS: Cytochrome P450 (P450), Phylogenetic analysis, Dollo parsimony, Exon-intron (gene) organization, Intron loss-gain.

83. **Valecha N**, Krudsood S, Tangpukdee N, Mohanty S, **Sharma SK**, **Tyagi PK**, **Anvikar AR**, Mohanty R, Rao BS, Jha AC, **Shahi B**, **Singh JPN**, Roy A, Kaur P, Kothari M, Mehta S, Gautam A, Paliwal JK, Arora S, Saha N. Arterolane maleate and piperaquine phosphate combination for the treatment of uncomplicated *Plasmodium falciparum* malaria: A comparative, multicentre randomized clinical trial. ***Clin Infect Dis*** **2012; *55*(5): 663–71.**

ABSTRACT

Background: Artemisinin-based combination therapy is the first-line treatment for uncomplicated *falciparum* malaria. This study assessed the antimalarial efficacy and safety of a combination of 150 mg of arterolane maleate and 750 mg of piperaquine phosphate (AM-PQP) in comparison to Coartem (artemether and lumefantrine) in patients with acute uncomplicated *P. falciparum* malaria. **Methods**: In this open-label, randomized, multicentric, parallel group clinical trial, 240 patients were randomized to receive AM-PQP (160 patients) or Coartem (80 patients). Patients with *P. falciparum* monoinfection and initial parasite densities ranging from 1000 to 100 000 asexual parasites/μL of blood were followed for 28 days. Polymerase chain reaction–corrected adequate clinical and parasitologic response on day 28, parasite clearance time, and fever clearance time were evaluated. **Results:** A total of 151 (94.4%) of 160 patients in the AM-PQP group completed the trial, while 77 (96.3%) of 80 patients in the Coartem group completed the trial. No treatment failure was noted in the AM-PQP group, while one patient receiving Coartem failed treatment on day 28. There was no difference in the median parasite clearance time (30 hours in both groups) or median fever clearance time (24 hours in both groups) after administration of the 2 study treatments. **Conclusions:** The available data support the evaluation of a drug combination in a larger population as a fixed-dose combination.

84. **Anvikar AR**, Sharma B, Shahi BH, **Tyagi PK**, Bose TK, **Sharma SK**, **Srivastava P**, **Srivastava B**, Kiechel JR, **Dash AP**, **Valecha N**. Artesunate-amodiaquine fixed dose combination for the treatment of *Plasmodium falciparum* malaria in India. ***Malar J* 2012; *11*: 97.**

ABSTRACT

Background: Artemisinin-based combination therapy (ACT) has been recommended for the treatment of *falciparum* malaria by the World Health Organization. Though India has already switched to ACT for treating *falciparum* malaria, there is need to have multiple options of alternative forms of ACT. A randomized trial was conducted to assess the safety and efficacy of the fixed dose combination of artesunate-amodiaquine (ASAQ) and amodiaquine (AQ) for the treatment of uncomplicated *falciparum* malaria for the first time in India. The study sites are located in malaria-endemic, chloroquine-resistant areas. **Methods:** This was an open label, randomized trial conducted at two sites in India from January 2007 to January 2008. Patients

between six months and 60 years of age having *Plasmodium falciparum* mono-infection were randomly allocated to ASAQ and AQ arms. The primary endpoint was 28-day PCR-corrected parasitological cure rate. **Results:** Three hundred patients were enrolled at two participating centres, Ranchi, Jharkhand and Rourkela, Odisha. Two patients in AQ arm had early treatment failure while there was no early treatment failure in ASAQ arm. Late treatment failures were seen in 13 and 12 patients in ASAQ and AQ arms, respectively. The PCR-corrected cure rates in intent-to-treat population were 97.51% (94.6-99.1%) in ASAQ and 88.65% (81.3-93.9%) in AQ arms. In per-protocol population, they were 97.47% (94.2-99.2%) and 88.30% (80-94%) in ASAQ and AQ arms respectively. Seven serious adverse events (SAEs) were reported in five patients, of which two were reported as related to the treatment. All SAEs resolved without sequel. **Conclusion:** The fixed dose combination of ASAQ was found to be efficacious and safe treatment for *P. falciparum* malaria. Amodiaquine also showed acceptable efficacy, making it a suitable partner of artesunate. The combination could prove to be a viable option in case India opts for fixed dose combination ACT.

KEYWORDS: Artesunate, Amodiaquine, *falciparum* malaria, India.

85. Gupta P, **Das A**, **Singh OP**, **Ghosh SK**, **Singh V**. Assessing the genetic diversity of the vir genes in Indian *Plasmodium vivax* population. ***ActaTrop*** **2012; *124* (2): 133–9**.

ABSTRACT

Variant surface antigens (VSAs) present on the surface of parasitized erythrocytes facilitate many *Plasmodium* spp. to escape the host immune system during infection. Multigene families coding for VSAs exist in several *Plasmodium* spp. and are located on telomeric and subtelomeric regions of the chromosomes. *P. vivax* genome also contains a multigene superfamily *vir* (variant interspersed repeats), present in the subtelomeric region with a possible role in immune evasion like the *var* gene in *P. falciparum*. Blood samples from 148 symptomatic malaria cases were collected from five different regions of India, *viz.* Mangalore, Rourkela, Goa, Delhi and Jabalpur. *P. vivax* isolates (74 single infections) were sequenced for four *vir* genes (*viz. vir* 27, *vir* 4, *vir* 12 and *vir* 21) and analyzed for the genetic variability existing in different populations of India. The results indicate that *vir* genes in different *P. vivax* populations in India are highly divergent both within and between the isolates. High levels of single nucleotide

polymorphisms (SNPs) were observed attributing to the existing polymorphism for all the four *vir* genes studied across the population. Detailed knowledge of the genetic variation among the *vir* genes will help in understanding the evolutionary aspects of *vir* genes and may also provide basis for understanding the disease chronicity.

86. **Rai S**, **Dua VK**, Chopra AK. Bio-monitoring of persistent organochlorines in human milk and blood samples from sub-himalayan region of India. ***Bull Environ Contam Toxicol*** **2012; *89*(3): 592–7.**

ABSTRACT

In the present study, concentrations of organochlorine pesticide residues *viz*. Dichlorodiphenyltrichloroethane and its metabolites (DDTs) and Hexachlorocyclohexane isomers (HCHs) in human breast milk and human blood samples, collected from several high altitude regions of Garhwal Himalaya in Uttarakhand, India *viz*. Devprayag, Chamoli, Uttarkashi, Joshimath, Bhatwari and Gangnani (altitude ranging from 472 to 1,982 m above sea level) were determined. Mean concentrations of HCH and DDT in human milk samples ranged from 4.53 to 34.32 mg/kg and 6.09 to 12.98 mg/kg, respectively. While the human blood showed mean values ranging from 6.64 to 281.7 μg/L and 12.37 to 104.10 μg/L for HCH and DDT, respectively. The study showed much higher concentrations of organochlorine residue contamination in the Garhwal region as compared to other parts of India. Risk assessments for infants were also calculated and were found within WHO limits.

KEYWORDS: DDT, HCH, Organochlorines, Persistent, Residues.

87. Khan MM, Chatterjee S, Dwivedi VP, Pandey NK, Singh Y, Tousif S, Bhavesh NS, Van Kaer L, **Das J**, Das G. CD4+ T-cell derived novel peptide Thp5 induces interleukin-4 production in CD4+ T-cells to direct T-helper 2 cell differentiation. ***J Biol Chem*** **2012; *287*(4): 2830–5.**

ABSTRACT

The differentiation of naïve $CD4^+$ T cells into T helper 2 (Th2) cells requires production of the cytokine IL-4 in the local microenvironment. It is evident that naïve/quiescently activated $CD4^+$ T cells produce the IL-4 that drives Th2 cell differentiation. Because early production of IL-4 in

naïve T cells leads to preferential Th2 cell differentiation, this process needs to be tightly regulated so as to avoid catastrophic and misdirected Th2 cell differentiation. Here, we show that Thp5, a novel peptide with structural similarity to vasoactive intestinal peptide, regulates production of early IL-4 in newly activated $CD4^+$ T cells. Induction of IL-4 in $CD4^+$ T cells by Thp5 is independent of the transcription factor STAT6 but dependent on ERK1/2 signaling. Furthermore, cytokines (IL-12 and TGF-β) that promote the differentiation of Th1 or Th17 cells inhibit Thp5 induction, thus suppressing Th2 cell differentiation. We further showed that Thp5 enhances Th2 responses and exacerbates allergic airway inflammation in mice. Taken together, our findings reveal that early activated $CD4^+$ T cells produce Thp5, which plays a critical role as a molecular switch in the differentiation of Th cells, biasing the response toward the Th2 cell phenotype.

KEYWORDS: Asthma, Cytokine, Immunology, NMR, T cell.

88. Singh PK, **Dhiman RC**. Climate change and human health: Indian context. ***J Vector Borne Dis* 2012; *49*(2): 55–60.**

ABSTRACT

The article reviews the issue of climate change and health in the Indian context. The importance of climate change leading to estimated loss of above 2.5 million DALYs in southeast Asia, mortality due to heat waves, and the importance of air quality related respiratory diseases, disasters due to excessive floods, malnutrition due to reduction in rice, maize and sorghum crops etc. Latest work undertaken in India, vis-a-vis current scenario and need for further work has been discussed. There is felt need of further studies on assessing the impact on dengue and chikungunya as the transmission dynamics of these diseases involve water availability, storage and life style, etc. Uncertainties and knowledge gaps identified in the studies undertaken so far have also been highlighted. As regards to vector borne diseases, there is a need to concentrate in the areas which are presently free from malaria and with use of best available tools of interventions in already disease endemic areas like northeastern states, the risk of climate change impacts can be minimized.

KEYWORDS: Chikungunya, climate change, dengue, human health, Japanese encephalitis malaria, vector-borne diseases.

89. Laishram DD, Sutton PL, **Nanda N**, **Sharma VL**, Sobti RC, Carlton JM, Joshi H. The complexities of malaria disease manifestations with a focus on asymptomatic malaria. ***Malar J* 2012; *11*: 29.**

ABSTRACT

Malaria is a serious parasitic disease in the developing world, causing high morbidity and mortality. The pathogenesis of malaria is complex, and the clinical presentation of disease ranges from severe and complicated, to mild and uncomplicated, to asymptomatic malaria. Despite a wealth of studies on the clinical severity of disease, asymptomatic malaria infections are still poorly understood. Asymptomatic malaria remains a challenge for malaria control programs as it significantly influences transmission dynamics. A thorough understanding of the interaction between hosts and parasites in the development of different clinical outcomes is required. In this review, the problems and obstacles to the study and control of asymptomatic malaria are discussed. The human and parasite factors associated with differential clinical outcomes are described and the management and treatment strategies for the control of the disease are outlined. Further, the crucial gaps in the knowledge of asymptomatic malaria that should be the focus of future research towards development of more effective malaria control strategies are highlighted.

KEYWORDS: asymptomatic malari,a host factors, parasite factors, transmission dynamics.

90. Jha P, Sinha S, Kanchan K, Qidwai T, Narang A, Singh PK, Pati Sudhanshu S, Mohanty S, Mishra Saroj K, **Sharma SK**, Awasthi S, Venkatesh V, Jain S, Basu A, Xu S. Indian genome variation consortium, Mukerji M, Habib S. Deletion of the APOBEC3B gene strongly impacts susceptibility to *falciparum* malaria. ***Infect Genet Evol* 2012; *12*: 142–8.**

ABSTRACT

APOBEC3B, a gene involved in innate response, exhibits insertion-deletion polymorphism across world populations. We observed the insertion allele to be nearly fixed in malaria endemic regions of sub-Saharan Africa as well as populations with high malaria incidence in the past. This prompted us to investigate the possible association of the polymorphism with *falciparum* malaria. We studied the distribution of APOBEC3B, in 25 diverse Indian populations comprising of 500

samples and 176 severe or non-severe *Plasmodium falciparum* patients and 174 ethnically-matched uninfected individuals from a *P. falciparum* endemic and a non-endemic region of India. The deletion frequencies ranged from 0% to 43% in the Indian populations. The frequency of the insertion allele strikingly correlated with the endemicity map of *P. falciparum* malaria in India. A strong association of the deletion allele with susceptibility to *falciparum* malaria in the endemic region (non-severe vs. control, Odds ratio=4.96, *P* value=9.5E(-06); severe vs. control, OR=4.36, *P* value=5.76E(-05)) was observed. Although the frequency of deletion allele was higher in the non-endemic region, there was a significant association of the homozygous deletion genotype with malaria (OR=3.17, 95% CI=1.10-10.32, *P* value=0.0177). Our study also presents a case formalaria as a positive selection force for the APOBEC3B insertion and suggests a major role for this gene in innate immunity against malaria.

KEYWORDS: APOBEC3B, *Plasmodium falciparum*, Malaria endemicity, innate response, Structural variation.

91. Lumb V, Madan R, **Das MK**, Rawat V, **Dev V**, Khan W, Khan H, Sharma YD. Differential genetic hitchhiking around mutant *pfcrt* alleles in the Indian *Plasmodium falciparum* population. ***J Antimicrob Chemother* 2012; *67*: 600–8.**

ABSTRACT

Objectives: To study the origin and spread of the chloroquine-resistant *Plasmodium falciparum* population in the Indian subcontinent. **Methods**: Fourteen microsatellites spanning a ~120 kb region, flanking the *P. falciparum chloroquine resistance transporter (pfcrt)* gene, were analysed in 185 parasite isolates. **Results:** The Indian *P. falciparum* population exhibited a selective valley of reduced genetic variation in the flanking microsatellites of the mutant *pfcrt* alleles (up to ±29 kb) as compared with the wild-type allele. This valley is much narrower than the ±200 kb valley reported from African and South-East Asian countries. The majority of the isolates showed asymmetry in the selective valley, where upstream microsatellites showed less genetic variation than the downstream microsatellites. Regional variation in the width and symmetry of the selective valley was noticed, which seems to be related to the number of *pfcrt* alleles present in the parasite population of a region. Forty-six different microsatellite haplotypes were observed among the *P. falciparum* isolates containing mutant *pfcrt* alleles. Parasite populations from different regions of

mainland India shared microsatellite haplotypes between them, but they shared none with the isolates from the Andaman and Nicobar Islands, and vice versa. Indian isolates shared microsatellite haplotypes with the isolates from Papua New Guinea and Thailand. **Conclusions:** With regard to chloroquine there is regional variation in the selection pressure on the *P. falciparum* population in India. These findings will help the regional implementation of drug policy in India's malaria control programme.

KEYWORDS: malaria, chloroquine resistance, microsatellite markers, population genetics.

92. **Khan N**, **Chittoria A**, **Pande V**, Jaiswal Y, **Das A**. Development of multilocus putatively neutral DNA markers in the X-chromosome for population genetic studies in humans. ***Ann Human Biol* 2012; *39*: 281–9.**

ABSTRACT

Background: It has now been well documented that the type (coding, non-coding) and location (nuclear, mitochondrial etc.) of genetic markers heavily influence evolutionary inferences; realistic assumptions can be drawn if multiple putatively neutral DNA fragments spread across the genome are used. **Aim**: To infer human population history, Single Nucleotide Polymorphisms (SNPs), located in the non-coding regions of different genes in the X-chromosome have been developed as 'putatively neutral markers'. **Subjects and methods**: A population sample consisting of 16 male individuals from the western part of India was utilized for sequencing eight DNA fragments located in introns of three genes (Duchenne muscular dystrophy, Factor IX and Pyruvate dehydrogenase E1 sub-unit) on the human X-chromosome. PCR amplification and DNA sequencing confirmed the polymorphic status of all the fragments. **Results**: Twenty nine SNPs were found to be segregating in the Western Indian population samples. Using these SNPs the nucleotide diversity and demographic parameters of the Western Indian population were estimated. Several tests of neutrality ascertained that all eight fragments evolve putatively neutrally. Further, linkage disequilibrium analyses confirmed this fact. **Conclusion**: All eight DNA fragments seem to bear the characteristics to be considered as 'putatively neutral genetic markers' and thus, could be utilized for inference of human population and demographic histories.

KEYWORDS: Multilocus, non-coding DNA, SNPs, evolution, India.

93. **Bhatt RM**, **Sharma SN**, **Shreehari U**, **Dash AP**, **Raghavendra K**. Effectiveness and durability of Interceptor® long-lasting insecticidal nets in a malaria endemic area of central India. ***Malar J* 2012; *11*: 189.**

ABSTRACT

Background: In the present study, Interceptor®, long-lasting polyester net, 75 denier and bursting strength of minimum 250kPa coated with alpha-cypermethrin @ 200mg/m^2 was evaluated for its efficacy in reducing the mosquito density, blood feeding inhibition and malaria incidence in a tribal dominated malaria endemic area in Chhattisgarh state, central India. Its durability, washing practices and usage pattern by the community was also assessed up to a period of three years. **Methods:** The study was carried out in two phases. In the first phase (September 2006 to August 2007), 16 malaria endemic villages in district Kanker were randomized into three groups, *viz*. Interceptor net (LN), untreated polyester net (100 denier) and without net. Malaria cases were detected by undertaking fortnightly surveillance by home visits and treated as per the national drug policy. Mosquito collections were made by hand catch and pyrethrum space spray methods from human dwellings once every month. Slide positivity rate (SPR) and malaria incidence per 1000 population (PI) were compared between the three study arms to assess the impact of use of Interceptor nets. Simultaneously, wash resistance studies were carried out in the laboratory by doing cone bioassays on Interceptor LNs washed up to 20 times. Activities undertaken in second Phase (April 2008 to October 2009) after an interval of about 18 months post-net distribution included questionnaire based surveys at every six months, *i.e*. 18, 24, 30 and 36 months to observe durability, usage pattern of LNs and washing practices by the community. After 36 months of field use, 30 nets were retrieved and sampled destructively for chemical analysis. **Results:** Interceptor nets were found effective in reducing the density, parity rate and blood feeding success rate of main malaria vector *Anopheles culicifacies* as compared to that in untreated net and no net villages. SPR in LN villages was 3.7% as compared to 6.5% in untreated and 11% in no net villages. PI in LN villages was 16.4 in comparison to 24.8 and 44.2 in untreated polyester net and no net villages respectively. In surveys carried out after three years of initial distribution, 78.7% (737/936) nets were still in possession with the households, of which 68% were used every night. *An. culicifacies* mortality was >80% in cone bioassays done on LNs washed up to 20 times in laboratory. Mean alpha-cypermethrin content was 43.5-51.7 mg/m^2 on Interceptor LNs

withdrawn after three years of household use against the baseline specification of 200 mg/m^2. A gradual increase in the proportion of holed nets was observed with the increased period of usage. **Conclusion:** Interceptor nets were highly effective in reducing vector densities as well as malaria incidence in the study villages. Availability of 78% nets with the households in usable condition clearly indicated durability of Interceptor LNs up to three years in the rural setting of India. The nets were found to contain an effective concentration of alpha-cypermethrin against malaria vector after three years of household use.

KEYWORDS: *Anopheles culicifacies*, Bioassay Density, Interceptor nets, Malaria Mosquitoes.

94. **Bharti PK**, **Chand SK**, **Singh MP**, Mishra S, **Shukla MM**, Singh R, **Singh N**. Emergence of a new focus of *Plasmodium malariae* in forest villages of District Balaghat, central India: Implications for the diagnosis of malaria and its control. ***Trop Med Int Health*** **2012; *18*(1): 12-7. doi: 10.1111/tmi.12005.**

ABSTRACT

Objective: During an epidemiological study (January–July 2012) on malaria in forest villages of Central India, *Plasmodium malariae*-like malaria parasites were observed in blood smears of fever cases. We aimed to confirm the presence of *P. malariae* using molecular tools *i.e.* species-specific nested polymerase chain reaction (PCR) and DNA sequencing. **Methods:** All fever cases or cases with history of fever in 25 villages of Balaghat district were screened for malaria parasite using bivalent rapid diagnostic test and microscopy after obtaining written informed consent. Nested PCR was employed on microscopically suspected*P. malariae* cases. DNA sequences in the target region for PCR diagnosis were analysed for all the suspected cases of *P. malariae*. **Results:** Among the 22 microscopy suspected *P. malariae* cases, nested PCR confirmed the identity of *P. malariae* in 19 cases. Among these 14 were mono *P. malariae* infections, three were mixed infection of *P. malariae* with *Plasmodium falciparum* and two were mixed infection of *P. malariae* with *Plasmodium vivax*. Clinically *P. malariae* subjects generally presented with fever and headache. However, the typical 3-day pattern of quantum malaria was not observed. The parasite density of *P. malariae* was significantly lower than that of *P. vivax*and *P. falciparum*. **Discussions:** *Plasmodium malariae* may have been in existence in forest villages of central India but escaped identification due to its close resemblance to *P. vivax*. The results re-affirm the

importance of molecular methods of testing on routine basis for efficacious control strategies against malaria.

KEYWORDS: malaria, *Plasmodium malariae* , Central India, malaria control.

95. Rodrigues J, Oliveira GA, Kotsyfakis M, **Dixit R**, Molina-Cruz A, Jochim R, Barillas-Mury C. An epithelial serine protease, AgESP, is required for *Plasmodium* invasion in the mosquito *Anopheles gambiae*. ***PloS One* 2012; *7*(4): e35210.**

ABSTRACT

Background: *Plasmodium* parasites need to cross the midgut and salivary gland epithelia to complete their life cycle in the mosquito. However, our understanding of the molecular mechanism and the mosquito genes that participate in this process is still very limited. **Methodology/Principal Findings**: We identified an *Anopheles gambiae* epithelial serine protease (AgESP) that is constitutively expressed in the submicrovillar region of mosquito midgut epithelial cells and in the basal side of the salivary glands that is critical for *Plasmodium* parasites to cross these two epithelial barriers. AgESP silencing greatly reduces *Plasmodium berghei* and *Plasmodium falciparum*midgut invasion and prevents the transcriptional activation of gelsolin, a key regulator of actin remodeling and a reported *Plasmodium* agonist. AgESP expression is highly induced in midgut cells invaded by *Plasmodium*, suggesting that this protease also participates in the apoptotic response to invasion. In salivary gland epithelial cells, AgESP is localized on the basal side–the surface with which sporozoites interact. AgESP expression in the salivary gland is also induced in response to *P. berghei* and *P. falciparum* sporozoite invasion, and AgESP silencing significantly reduces the number of sporozoites that invade this organ. **Conclusion**: Our findings indicate that AgESP is required for *Plasmodium* parasites to effectively traverse the midgut and salivary gland epithelial barriers. *Plasmodium* parasites need to modify the actin cytoskeleton of mosquito epithelial cells to successfully complete their life cycle in the mosquito and AgESP appears to be a major player in the regulation of this process.

96. **Singh RK**, **Das MK**, **Dhiman RC**, **Mittal PK**, **Dua VK**, **Sreehari U**, Prasad Shankar, Bora D. Evaluation of indoor residual spray and insecticide treated bed nets in a malaria endemic area of Santhal Pargana, Dumka district (Jharkhand). ***J Commun Dis* 2012; *44*(3): 169–79.**

ABSTRACT

The study was carried out for evaluation of various activities of malaria control programme in five different tribal and malaria endemic Primary Health Centres of Dumka district (Jharkhand) during 2007-08. A total of 321 houses of 18 villages were surveyed on use of indoor residual spray (IRS) and insecticide-treated bed nets (ITN) and other activities as tool for vector control and interrupting the transmission of malaria. Out of 690 living rooms and 343 verandahs examined, IRS with Dichloro-diphenyl-trichloro-ethane (DDT) was done only in 16.23% living rooms and 64.72% verandahs. Refusal rate of IRS in living rooms was 81.93% due to lack of knowledge regarding the importance of IRS, no prior information to villagers, houses locked, reluctance to remove domestic articles, dislike of smell of DDT spray. Compliance rate of ITN uses was 71.66% during the night, which might be a factor for decline in malaria cases in the study area. Various important components of the programme, *viz*. surveillance and compliance to treatment activities, use of rapid diagnostic test kits (RDKs), involvement of accredited social health activist (ASHA's) and fever treatment depots (FTDs), laboratory activities, adult mosquito collection, other activities like constitution of village health sanitation committee, information education and communication activities, capacity building, use of larvivorous fishes, upervision of IRS etc. require much strengthening. However, 100% community acceptance was recorded for ITN in the villages surveyed. In addition, an entomological study was carried out for information on prevalence of mosquitospecies in this area to find out ffectiveness of IRS activities. Eleven anopheline species, including three malaria vectors *i.e*., *An. culicifacies, An. stephensi, An. fluviatilis, An. annularis, An. subpictus, An. nigerrimus, An. pallidus, An. aconitus, An. vagus, An. jamsii* and *An. splendidus* were collected from cattle and human dwellings.

KEYWORDS: *Anopheline* species, indoor residual spray, insecticide-treated bed nets, vector control and malaria.

97. **Mittal PK**, **Sood RD**, Kapoor N, **Razdan RK**, **Dash AP**. Field evaluation of Icon®Life, a long-lasting insecticidal net (LLIN) against *Anopheles culicifacies* and transmission of malaria in District Gautam Budh Nagar (Uttar Pradesh), India. ***J Vector Borne Dis*** **2012; *49*(3): 181–7.**

ABSTRACT

Background & Objectives: In the present study, Icon®Life net, a long-lasting polyethylene net, 100 denier and bursting strength of minimum 280 kpa incorporated with deltamethrin @ 65 mg/m2 was evaluated for its efficacy in reducing the density of malaria vector *Anopheles culicifacies* and impact on malaria prevalence in a malaria endemic area of District Gautam Budh Nagar, India. **Methods**: Wash resistance of Icon® Life LLIN was determined up to 20 serial washings using *An. culicifacies* in cone bioassays under field conditions. Efficacy of Icon®Life LLIN was determined in the field in three sets of villages in District Gautam Budh Nagar (Uttar Pradesh), India, selected randomly for the intervention with Icon®Life LLIN, untreated nets and a control without any intervention for the period of August 2008–July 2009. Entomological and malariometric indices in all the three villages were compared during pre- and post-intervention periods for one year against *An. culicifacies*. A survey was also conducted in the village provided with Icon® Life LLIN to assessing the perception of community regarding acceptance of these nets by the community. **Results**: In cone bioassays on Icon®Life LLIN with *An. culicifacies*, >95% knockdown within 1 h and 100% mortality after 24 h exposure were reported even after 20× serial washings under field conditions. Results of the field study revealed reduced entry rate, resting density and parity rate of *An. culicifacies* in the village with Icon®Life LLIN when compared to no net and untreated net villages. Number of malaria cases reported were less in the Icon®Life LLIN used villages when compared to other two villages. The community compliance and acceptance was high and no adverse health events were reported by the households using these nets. **Conclusions**: Icon® Life LLIN is an effective intervention for the control of *An. culicifacies* transmitted malaria in India. Long-term studies are indicated for the duration of effectiveness and to ascertain the epidemiological impact of the use of Icon® Life nets.

KEYWORDS: *Anopheles culicifacies*, Icon® Life, India, long-lasting insecticidal nets, mosquito density, wash resistance,

98. Kumar N, Pande V, **Bhatt RM**, **Shah NK**, **Mishra N**, **Srivastava B**, **Valecha N**, **Anvikar AR**. Genetic deletion of HRP2 and HRP3 in Indian *Plasmodium falciparum* population and false negative malaria rapid diagnostic test. ***Acta Trop*** **2012; *125*(1): 119–21.**

ABSTRACT

Genetic polymorphisms in diagnostic antigens are important factors responsible for variable performance of rapid diagnostic tests. Additionally, the failure of antigen expression due to gene deletion may also contribute to variable performance. We report Indian *Plasmodium falciparum* field isolates lacking both *Pfhrp2* and *Pfhrp3* genes leading to false negative results of rapid diagnostic tests. The study highlights need to determine the prevalence of *P. falciparum* isolates lacking these genes in larger field populations in India.

KEYWORDS: malaria; *Pfhrp2*; *Pfhrp3*; genetic deletion; rapid diagnostic tests; India

99. **Bharti PK**, **Shukla MM**, Sharma YD, **Singh N**. Genetic diversity in the block 2 region of the merozoite surface protein-1 of *Plasmodium falciparum* in central India. ***Malar J* 2012; *11*: 78.**

ABSTRACT

Background: Malaria continues to be a significant health problem in India. Several of the intended *Plasmodium falciparum* vaccine candidate antigens are highly polymorphic. The genetic diversity of *P. falciparum* merozoite surface protein-1 (MSP-1) has been extensively studied from various parts of the world. However, limited data are available from India. The aim of the present study was a molecular characterization of block 2 region of MSP-1 gene from the tribal-dominated, forested region of Madhya Pradesh. **Methods:** DNA sequencing analysis was carried out in 71 field isolates collected between July 2005 to November 2005 and in 98 field isolates collected from July 2009 to December 2009. Alleles identified by DNA sequencing were aligned with the strain 3D7 and polymorphism analysis was done by using Edit Sequence tool (DNASTAR). **Results:** The malaria positivity was 26% in 2005, which rose to 29% in 2009 and *P. falciparum* prevalence was also increased from 72% in 2005 to 81% in 2009. The overall allelic prevalence was higher in K1 (51%) followed by MAD20 (28%) and RO33 (21%) in 2005 while in 2009, RO33 was highest (40%) followed by K1 (36%) and MAD20 (24%). **Conclusions:** The present study reports extensive genetic variations and dynamic evolution of block 2 region of MSP-1 in central India. Characterization of antigenic diversity in vaccine candidate antigens are valuable for future vaccine trials as well as understanding the population dynamics of *P. falciparum* parasites in this area.

100. **Kumar N**, **Singh JPN**, Pande V, **Mishra N**, **Srivastava B**, **Kapoor R**, **Valecha N**, **Anvikar AR**. Genetic variation in histidine rich proteins among Indian *Plasmodium falciparum* population: Possible cause of variable sensiti-vity of malaria rapid diagnostic tests. ***Malar J*** **2012; 11: 298.**

ABSTRACT

Background: Rapid diagnostic tests (RDTs) have revolutionized the diagnosis of malaria. Among the various factors affecting RDTs sensitivity is genetic variation of the antigen used. The genetic variation in *Pf*HRP2 and *PfHRP3* proteins was studied among the Indian *Plasmodium falciparum* isolates. **Methods:** One hundred and forty isolates of *P. falciparum* were collected from six geographical regions of India. Target genes encoding *Pf*HRP2 and *Pf*HRP3 antigens were sequenced to study genetic polymorphism. Minimum detection limit giving a positive rapid diagnostic test was also determined. **Results:** Extensive variations were observed in amino acid repeat types of *Pf*HRP2 and *Pf*HRP3. *Pf*HRP2 exhibited more polymorphism than *Pf*HRP3. Significant relation was observed between type 2 and type 7 repeats and RDT detection rate as higher number of these repeats showed better sensitivity with RDTs. **Conclusion:** The results provide insights into the genetic diversity of *Pfhrp2* and *Pfhrp3* genes among Indian *P. falciparum* population and its relation to RDT sensitivity.

KEYWORDS: *Plasmodium falciparum,* Histidine rich protein, *Plasmodium falciparum,* Histidine rich protein, Rapid Diagnostic Tests, Genetic polymorphism, India.

101. **Niranjan Reddy BP**, **Rao B Prasad**, Prasad GBKS, **Raghavendra K**. Identification and classification of detoxification enzymes from *Culex quinquefasciatus* (Diptera: Culicidae). ***Bioinformation*** **2012; *8*(9): 430–6.**

ABSTRACT

Molecular characterization of the insecticide resistance has become a hot research topic ever since the first disease transmitting arthropod (*Anopheles gambiae*) genome sequence has unveiled in 2002. A recent publication of the *Culex quinquefasciatus* genome sequence has opened up new opportunities for molecular and comparative genomic analysis of multiple mosquito genomes to characterize the insecticide resistance. Here, we utilized a whole genome sequence of *Cx*.

quinquefasciatus to identify putatively active members of the detoxification supergene families, namely cytochrome P450s (P450s), glutathione-S-transferases (GSTs), and choline/carboxylesterases (CCEs). The *Culex* genome analysis revealed 166 P450s, 40 GSTs, and 62 CCEs. Further, the comparative genomic analysis shows that these numbers are considerably higher than the other dipteran mosquitoes. These observed speciesspecific expansions of the detoxification super gene family members endorse the popular understanding of the involvement of these gene families in protecting the organism against multitudinous classes of toxic substances during its complex (aquatic and terrestrial) life cycle. Thus, the generated data set may provide an initial point to start with to characterize the insecticide resistance at a molecular level which could then lead the development of an easy to use molecular marker to monitor the incipient insecticide resistance in field environs.

KEYWORDS: *Culex quinquefasciatus*, detoxification enzymes, cytochrome P450 (P450), glutathione-S-transferase (GST), choline/carboxylesterase (CCE).

102. **Sundararaj S**, Singh D, Saxena AK, **Vashisht K**, Sijwali PS, **Dixit R**, **Pandey KC**. The ionic and hydrophobic interactions are required for the auto activation of cysteine proteases of *Plasmodium falciparum*. ***PloS ONE* 2012; 7(10): e47227**.

ABSTRACT

The *Plasmodium falciparum* cysteine proteases falcipain-2 and falcipain-3 are major hemoglobinases and potential antimalarial drug targets. Our previous studies demonstrated that these enzymes are equipped with specific domains for specific functions. Structural and functional analysis of falcipains showed that they have unique domains including a refolding domain and a hemoglobin binding domain. As with many proteases, falcipain-2 and falcipain-3 are synthesized as inactive zymogens. However, it is not known how these enzymes get activated for hemoglobin hydrolysis. In this study, we are presenting the first evidence that salt bridges and hydrophobic interactions are required for the auto activation of cysteine proteases of *P.falciparum*. To investigate the mechanism of activation of these enzymes, we expressed the wild type protein as well as different mutants in *E.coli*. Refolding was assessed by circular dichroism. Both CD and trans activation data showed that the wild type enzymes and mutants are rich in secondary structures with similar folds. Our study revealed that prodomain-mature domain of falcipain-2 and

falcipain-3 interacts via salt bridges and hydrophobic interactions. We mutated specific residues of falcipain-2 and falcipain-3, and evaluated their ability to undergo auto processing. Mutagenesis result showed that two salt bridges (Arg ^{185} - Glu ^{221}, Glu ^{210} - Lys ^{403}) in falcipain-2, and one salt bridge (Arg ^{202}-Glu ^{238}) in falcipain-3, play crucial roles in the activation of these enzymes. Further study revealed that hydrophobic interactions present both in falcipain-2 (Phe^{214,} Trp^{449} Trp ^{453}) and falcipain-3 (Phe ^{231} Trp ^{457} Trp ^{461}) also play important roles in the activation of these enzymes. Our results revealed the interactions involved in auto processing of two major hemoglobinases of malaria parasite.

103. **Sharma SK**, **Upadhyay AK**, **Haque MA**, **Tyagi PK**, Kindo BK. Impact of changing over of insecticide from synthetic pyrethroids to DDT for indoor residual spray in a malaria endemic area of Orissa, India. ***Indian J Med Res*** **2012; *135*: 382–8.**

ABSTRACT

Background & Objectives: Development of insecticide resistance in malaria vectors has been a major problem for achieving effective vector control. Due to limited availability of insecticides, the only option is management of resistance by judiciously using the insecticides and rotating them to maintain their effectiveness. This study was carried out in a malaria endemic area of Sundergarh district in Orissa where synthetic pyrethroids (SP) were in use for the last couple of years. The change-over from SP to DDT was done in one arm of study, and the other two arms remained on SP and insecticide-treated nets (ITN). Entomological and parasitological monitoring was done to assess the impact. **Methods:** The study design comprised of three arms (i) two rounds of indoor residual spraying (IRS) with DDT 1g/m [2] as a change-over insecticide in areas previously under synthetic pyrethroids; (ii) two rounds of IRS with synthetic pyrethroid (alphacypermethrin, ACM) @ 25 mg/m [2] ; and (iii) an unsprayed area under ITN/long lasting insecticide nets (LNs). Indoor residual spraying was undertaken under strict supervision to maintain quality and coverage. Contact bioassays were conducted to know the persistence of insecticide on sprayed surfaces and adult vector density was monitored in fixed and randomly selected houses. Malaria incidence was measured through fortnightly domiciliary surveillance under primary health care system in all the study villages. **Results:** The insecticide susceptibility tests showed that *An.culicifacies* was resistant to DDT but susceptible to malathion and ACM. However, *An. fluviatilis* was susceptible

to all the three insecticides. ACM was effective in killing *An. culicifacies* on mud and wooden sprayed surfaces and maintained effective bioefficacy ranging from 92 to 100 per cent up to five months, whereas DDT failed to achieve effective mortality in *An.culicifacies*. However, there was significant decline in the density of *An.culicifacies* in ACM and DDT areas in comparison to ITNs/LNs. There was 61 per cent reduction in the slide positivity rate in ACM area in comparison to 48 and 51 per cent in DDT and ITN/LNs areas, respectively. The adjusted incidence rate of malaria cases per 1000 population in three study areas also showed significant declines within each group. **Interpretation & Conclusions:** The present findings show that the change-over of insecticide from synthetic pyrethroids to DDT brings about the same epidemiological impact as envisaged from continuing SP spray or distributing insecticide treated nets/long-lasting insecticidal nets provided there is a good quality spray and house coverage.

KEYWORDS: Bioassays, DDT, indoor residual spraying, insecticide resistance, malaria incidence, rotation of insecticide, synthetic pyrethroid, vector density.

104. Jhajharia D, Chattopadhyay S, Choudhary RR, **Dev V**, Singh VP, Lal S. Influence of climate on incidences of malaria in the Thar Desert, northwest India. ***Int J Climatol* 2012; *33*(2): 312–25.**

ABSTRACT

Climatic variability and rise in temperature are considered as the key determinants to the transmission of malaria. In the present study, the trends in the cases of malaria caused by *Plasmodium falciparum* and *Plasmodium vivax* were investigated by using the nonparametric Mann-Kendall test after removing the effect of significant lag-1 serial correlation from the time series of cases of malaria incidence by pre-whitening in annual, seasonal, and monthly time scales at Bikaner, located in the Thar Desert of Rajasthan, in northwest India. Multi-collinearity within the datasets under consideration was investigated by means of correlation matrix, the Bartlett sphericity test, and the Kaiser-Meyer-Olkin measure of sampling adequacy, subsequent to which it was removed by using principal component analysis. Finally, artificial neural network models were employed to predict cases of malaria incidence caused by *P. falciparum* and *P. vivax* at various scales. During the last 34 years from 1975 to 2008, *P. falciparum* malaria incidence cases have been found to increase significantly corresponding to monthly (April and September) and seasonal (monsoon) time scales over Bikaner. On the other hand, no significant trends were

observed in *P. vivax* malaria cases at Bikaner. Concomitant increases in *P. falciparum*cases of malaria incidence and observed temperature increases at Bikaner hint that*P. falciparum* malaria may have grown significantly under the warming climate of the Thar Desert.

KEYWORDS: climate change, malaria, *Plasmodium vivax*, *Plasmodium falciparum*, Bikaner, Thar Desert, Rajasthan, principal component analysis, artificial neural network.

105. **Mishra AK**, **Chand SK**, **Barik TK**, **Dua VK**, **Raghavendra K**. Insecticide resistance status in *Anopheles culicifacies* in Madhya Pradesh, central India. ***J Vector Borne Dis* 2012; *49*(1): 39–41.**

106. **Singh RK**, **Mittal PK**, **Dhiman RC.** Insecticide susceptibility status of *Phlebotomus argentipes*, a vector of visceral leishmaniasis in different foci in three states of India. ***J Vector Borne Dis* 2012; *49*(4): 254–7.**

ABSTRACT

Background & Objectives: *Phlebotomus argentipes* is the vector for visceral leishmaniasis in India. The development of resistance in kala-azar vector to DDT has been reported from various parts of India. The main objective of this study was to generate information on insecticides susceptibility status of *P. argentipes* to DDT, malathion and deltamethrin in different parts in three states of India. **Methods**: *Phlebotomus argentipes* were collected from different villages, identified and used to investigate the susceptibility status against DDT, malathion and deltamethrin as per the WHO standard methods. **Results**: *Phlebotomus argentipes* was resistant to DDT in different areas, *viz*. PHCs Murumgaon in Maharashtra; Ramgarh in Jharkhand; Kodah, Falka, Mahua and Lalganj in Bihar. In Phulwari Shareef PHC of Patna district in Bihar, DDT produced 89% mortality in *P. argentipes*, indicating resistant/tolerance (verification required) to DDT. The corrected percent mortality to malathion (5%) in different areas ranged between 98 and 100%; and to deltamethrin (0.05%) between 98.4 and 100%. The results showed that the tested *P. argentipes* are susceptible to malathion and deltamethrin. **Conclusion**: *Phlebotomus argentipes* are still susceptible to malathion and deltamethrin, but resistant to DDT. The susceptibility status of *P. argentipes* should be monitored regularly in diversified situations to ascertain the judicious use of insecticides being used for indoor residual spraying in the programme for rational use of appropriate insecticide.

KEYWORDS: Kala-azar, *Phlebotomus argentipes*, resistance, susceptibility.

107. **Anvikar AR**, Sharma B, **Sharma SK**, **Ghosh SK**, **Bhatt RM**, **Kumar A**, Mohanty SS, **Pillai CR**, **Dash AP**, **Valecha N**. In vitro assessment of drug resistance in *Plasmodium falciparum* in five states of India. ***Indian J Med Res* 2012; *135*: 494–9.**

ABSTRACT

Background & Objectives: In vitro assays are an important tool to assess baseline sensitivity and monitor the drug response of *Plasmodium falciparum* over time and place and, therefore, can provide background information for the development and evaluation of drug policies. This study was aimed at determining the in vitro sensitivity of *P. falciparum* isolates to antimalarials. **Methods**: The in vitro activity of 108 *P. falciparum* isolates obtained from five States of India was evaluated using WHO microtest (Mark III) to chloroquine, monodesethylamodiaquine, dihydroartesunate and mefloquine. Samples were collected from the States of Orissa, Jharkhand, Karnataka, Goa and Chhattisgarh from September 2007 to August 2009. In addition, representative samples from different States of India cryopreserved and culture adapted in the Malaria Parasite Bank of National Institute of Malaria Research, New Delhi, were also evaluated. **Results**: The proportion of isolates resistant to chloroquine and monodesethylamodiaquine was 44.4 and 25 per cent, respectively. Of the 27 isolates resistant to monodesethylamodiaquine, 16 (59.3%) were cross-resistant to chloroquine. No isolate showed resistance to dihydroartesunate and mefloquine. Isolates from Orissa showed the highest degree of resistance to chloroquine and amodiaquine followed by Jharkhand. Forty two isolates were genotyped for *pfcrt* T76K chloroquine resistant mutation; mutations were seen in 38 (90.47%) isolates. **Interpretation & Conclusions**: The Indian *P. falciparum* isolates showed a high degree of resistance to chloroquine followed by monodesethylamodiaquine. No resistance was recorded to mefloquine and dihydroartesunate.

KEYWORDS: In vitro sensitivity, India, malaria, *Plasmodium falciparum*.

108. Shreya N, Raghavendra NP, Mukherji V, Maria VR, Kumari N, **Pradeep AS**, **Ghosh SK**, Bindhu OS. Larvicidal activity of *Calotropis gigantea* (L.) R.Br. on dengue and chikungunya vector *Aedes aegypti*. ***Res J Pharm Biol Chem Sci* 2012; *3*: 118–21.**

ABSTRACT

Aedes aegypti is a major mosquito vector responsible for transmitting many viral diseases. Present status of insecticide resistant among vector populations towards exciting effective insecticides has paved the way to search for herbal larvicide as alternatives for mosquito control is important. *Calotropis sp*. has been recommended as a medicinally important plant by Ayurveda and has been in use for the prevention and treatment of many diseases. The present study was designed to screen the larvicidal activity of *Calotropis gigantea* (L.) R.Br. leaf extract on *Ae. aegypti* larvae. Larval bioassays were carried out with concentrations ranging from 100 to 1000 ppm of ethanolic extract and mortality was recorded after 24 hour exposure. The experiments were conducted under laboratory conditions at 27–28°C and 80–90% relative humidity. The leaf ethanolic extract showed a concentration dependent larvicidal activity with a LD50 value of 351.43 (95%CI: 345.64-345.51). The present report is the first preliminary study to show the larvicidal effect of *C. gigantea*.

KEYWORDS: *Calotropis gigantean, Aedes aegypti*, Larvicide, extract, dengue, chikungunya.

109. Swathi S, Murugananthan G, **Ghosh SK**, **Pradeep AS**. Larvicidal and repellent activities of ethanolic extract of *Datura stramonium* leaves against mosquitoes. *Int J Pharmacog Phytochem **Res*** **2012; 4: *25*(1): 25–7.**

ABSTRACT

Mosquitoes are responsible for spread of many diseases than any other group of arthropods. Diseases such as malaria, filariasis, dengue haemorrhagic fever (DHF) and chikunguinya are real threat to mankind. In the present study ethanolic extracts of leaves of *Datura stramonium* were evaluated for larvicidal and mosquito repellent activities against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. The LD50 values for larvicidal activity were found to be 86.25, 16.07 and 6.25 ppm against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* respectively. The ethanolic leaves extract of *Datura stramonium* provided complete protection time (Mosquito repellency) of 2.73, 71.66, 117.7 mins against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at higher concentration (1%).

KEYWORDS: *Datura stramonium*, larvicidal, mosquito repellent, mosquitoes.

110. Yangzom T, Gueye CS, Namgay R, Galappaththy GNL, Thimasarn KR, Murugasampillay GS, **Dev V**. Malaria control in Bhutan: Case study of a country embarking on elimination. ***Malar J* 2012; *11*: 9.**

ABSTRACT

Background: Bhutan has achieved a major reduction in malaria incidence amid multiple challenges. This case study seeks to characterize the Bhutan malaria control programme over the last 10 years. **Methods:** A review of the malaria epidemiology, control strategies, and elimination strategies employed in Bhutan was carried out through a literature review of peer-reviewed and grey national and international literature with the addition of reviewing the surveillance and vector control records of the Bhutan Vector-Borne Disease Control Programme (VDCP). Data triangulation was used to identify trends in epidemiology and key strategies and interventions through analysis of the VDCP surveillance and programme records and the literature review. Enabling and challenging factors were identified through analysis of socio-economic and health indicators, corroborated through a review of national and international reports and peer-review articles. **Findings:** Confirmed malaria cases in Bhutan declined by 98.7% from 1994 to 2010. The majority of indigenous cases were due to *Plasmodium vivax* (59.9%) and adult males are most at-risk of malaria. Imported cases, or those in foreign nationals, varied over the years, reaching 21.8% of all confirmed cases in 2006.Strategies implemented by the VDCP are likely to be related to the decline in cases over the last 10 years. Access to malaria diagnosis in treatment was expanded throughout the country and evidence-based case management, including the introduction of artemisinin-based combination therapy (ACT) for *P. falciparum*, increasing coverage of high risk areas with Indoor Residual Spraying, insecticide-treated bed nets, and long-lasting insecticidal nets are likely to have contributed to the decline alongside enabling factors such as economic development and increasing access to health services. **Conclusion:** Bhutan has made significant strides towards elimination and has adopted a goal of national elimination. A major challenge in the future will be prevention and management of imported malaria infections from neighbouring Indian states. Bhutan plans to implement screening at border points to prevent importation of malaria and to targeted prevention and surveillance efforts towards at-risk Bhutanese and migrant workers in construction sites.

KEYWORDS: Bhutan, malaria, elimination, control, migration, migrant, diagnosis, treatment, surveillance, vector.

111. Narayanasamy K, Chery L, Basu A, Duraisingh MT, Escalante A, Fowble J, Guler JL, Herricks T, **Kumar A**, Majumder P, Maki J, Mascarenhas A, Rodrigues J, Roy B, Sen S, Shastri J, Smith J, **Valecha N**, White J, Rathod PK. Malaria evolution in south Asia: Knowledge for control and elimination. ***Acta Trop*** **2012; *121*(3): 256–66.**

ABSTRACT

The study of malaria parasites on the Indian subcontinent should help us understand unexpected disease outbreaks and unpredictable disease presentations from*Plasmodium falciparum* and *Plasmodium vivax* infections. The Malaria Evolution in South Asia (MESA) research program is one of ten International Centers of Excellence for Malaria Research (ICEMR) sponsored by the US National Institutes of Health. In this second of two reviews, we describe why population structures of *Plasmodia* in India will be characterized and how we will determine their consequences on disease presentation, outcome and patterns. Specific projects will determine if genetic diversity, possibly driven by parasites with higher genetic plasticity, plays a role in changing epidemiology, pathogenesis, vector competence of parasite populations and whether innate human genetic traits protect Indians from malaria today. Deep local clinical knowledge of malaria in India will be supplemented by basic scientists who bring new research tools. Such tools will include whole genome sequencing and analysis methods;*in vitro* assays to measure genome plasticity, RBC cytoadhesion, invasion, and deformability; mosquito infectivity assays to evaluate changing parasite-vector compatibilities; and host genetics to understand protective traits in Indian populations. The MESA-ICEMR study sites span diagonally across India and include a mixture of very urban and rural hospitals, each with very different disease patterns and patient populations. Research partnerships include government-associated research institutes, private medical schools, city and state government hospitals, and hospitals with industry ties. Between 2012 and 2017, in addition to developing clinical research and basic science infrastructure at new clinical sites, our training workshops will engage new scientists and clinicians throughout South Asia in the malaria research field.

KEYWORDS: Malaria, *Plasmodium falciparum*, *Plasmodium vivax*, India, South Asia, Epidemiology, Drug resistance, ICEMR.

112. **Das A**, **Anvikar AR**, Cator LJ, **Dhiman RC**, **Eapen A**, **Mishra N**, **Nagpal BN**, **Nanda N**, **Raghavendra K**, Read AF, **Sharma SK**, **Singh OP**, **Singh V**, Sinnis P, **Srivastava HC**, Sullivan SA, Sutton PL, Thomas MB, Carlton JM, **Valecha N**. Malaria in India: The centre for study of complex malaria in India. ***Acta Trop*** **2012; *121*: 267–73**.

ABSTRACT

Malaria is a major public health problem in India and one which contributes significantly to the overall malaria burden in Southeast Asia. The National Vector Borne Disease Control Program of India reported ~1.6 million cases and ~1100 malaria deaths in 2009. Some experts argue that this is a serious underestimation and that the actual number of malaria cases per year is likely between 9 and 50 times greater, with an approximate 13-fold underestimation of malaria-related mortality. The difficulty in making these estimations is further exacerbated by (i) highly variable malaria eco-epidemiological profiles, (ii) the transmission and overlap of multiple *Plasmodium* species and *Anopheles*vectors, (iii) increasing antimalarial drug resistance and insecticide resistance, and (iv) the impact of climate change on each of these variables. Simply stated, the burden of malaria in India is complex. Here we describe plans for a Center for the Study of Complex Malaria in India (CSCMi), one of ten International Centers of Excellence in Malaria Research (ICEMRs) located in malarious regions of the world recently funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health. The CSCMi is a close partnership between Indian and United States scientists, and aims to address major gaps in our understanding of the complexity of malaria in India, including changing patterns of epidemiology, vector biology and control, drug resistance, and parasite genomics. We hope that such a multidisciplinary approach that integrates clinical and field studies with laboratory, molecular, and genomic methods will provide a powerful combination for malaria control and prevention in India.

KEYWORDS: malaria, *plasmodium*, *anopheles*, india, genomics, epidemiology.

113. **Kumar A**, Chery L, Biswas C, Dubhashi N, Dutta P, **Dua VK**, Kacchap M, Kakati S, Khandeparkar A, Kour D, Mahajan SN, Maji A, Majumder P, Mohanta J, Mohapatra PK,

Narayanasamy K, Roy K, Shastri J, **Valecha N**, Vikash R, Wani R, White J, Rathod PK. Malaria in south Asia: Prevalence and control. ***Acta Trop*** **2012; *121*(3): 246–55.**

ABSTRACT

The "Malaria Evolution in South Asia" (MESA) program project is an International Center of Excellence for Malaria Research (ICEMR) sponsored by the US National Institutes of Health. This US–India collaborative program will study the origin of genetic diversity of malaria parasites and their selection on the Indian subcontinent. This knowledge should contribute to a better understanding of unexpected disease outbreaks and unpredictable disease presentations from *Plasmodium falciparum* and *Plasmodium vivax* infections. In this first of two reviews, we highlight malaria prevalence in India. In particular, we draw attention to variations in distribution of different human-parasites and different vectors, variation in drug resistance traits, and multiple forms of clinical presentations. Uneven malaria severity in India is often attributed to large discrepancies in health care accessibility as well as human migrations within the country and across neighboring borders. Poor access to health care goes hand in hand with poor reporting from some of the same areas, combining to possibly distort disease prevalence and death from malaria in some parts of India. Corrections are underway in the form of increased resources for disease control, greater engagement of village-level health workers for early diagnosis and treatment, and possibly new public–private partnerships activities accompanying traditional national malaria control programs in the most severely affected areas. A second accompanying review raises the possibility that, beyond uneven health care, evolutionary pressures may alter malaria parasites in ways that contribute to severe disease in India, particularly in the NE corridor of India bordering Myanmar.

KEYWORDS: Malaria, *Plasmodium falciparum*, *Plasmodium vivax*, India, South Asia, Epidemiology, drug resistance ICEMR.

114. Neafsey DE, Galinsky K, Jiang RHY, Young L, Sykes SM, Saif S, Gujja S, Goldberg JM, Young S, Zeng Q, Chapman SB, **Dash AP**, **Anvikar AR**, Sutton PL, Birren BW, Escalante AA, Barnwell JW, Carlton JM. The malaria parasite *Plasmodium vivax* exhibits greater genetic diversity than *Plasmodium falciparum*. ***Nat Genet*** **2012; 44(9): 1046–50.**

ABSTRACT

We sequenced and annotated the genomes of four *P. vivax* strains collected from disparate geographic locations, tripling the number of genome sequences available for this understudied parasite and providing the first genome-wide perspective of global variability in this species. We observe approximately twice as much SNP diversity among these isolates as we do among a comparable collection of isolates of *P. falciparum*, a malaria-causing parasite that results in higher mortality. This indicates a distinct history of global colonization and/or a more stable demographic history for *P. vivax* relative to *P. falciparum*, which is thought to have undergone a recent population bottleneck. The SNP diversity, as well as additional microsatellite and gene family variability, suggests a capacity for greater functional variation in the global population of *P.vivax*. These findings warrant a deeper survey of variation in *P. vivax* to equip disease interventions targeting the distinctive biology of this neglected but major pathogen.

KEYWORDS: Bacterial genetics, malaria, population genetics.

115. **Prajapati SK**, **Kumari P**, **Singh OP**. Molecular analysis of reticulocyte binding protein-2 gene in *Plasmodium vivax* isolates from India. ***BMC Microbiol*** **2012; *12*: 243**.

ABSTRACT

Background: *Plasmodium vivax* reticulocyte binding protein-2 (*Pv*RBP-2) is a promising candidate for development of vaccine against parasite. DNA sequence polymorphism in *pvrbp-2* which may hamper the vaccine development program has been identified in laboratory strains. Therefore, unraveling genetic polymorphism in *pvrbp-2* from field isolates is a prerequisite for success in vaccine development. This study was designed with a primary aim to uncover genetic polymorphism in *pvrbp-2* among *P. vivax* field isolates. **Results:** Using virtual restriction mapping of *pvrbp-2* sequences, two restriction enzymes (*Alu*I and *Apo*I) were selected for the development of *pvrbp-2* as a PCR-RFLP marker. Restriction fragment length polymorphism (RFLP) analysis revealed a high degree of genetic polymorphism in the *pvrbp-2*gene among field isolates of *P. vivax*. *Apo*I-RFLP was found to be more efficient in identifying the extent of genetic polymorphism in *pvrbp-2*compared to *Alu*I-RFLP. Combined genotyping/haplotyping of RFLP pattern revealed a total of 36 distinct RFLP patterns among 83 *P. vivax*isolates analyzed. DNA sequence analysis also supports high degree of genetic polymorphism among field isolates of *P.*

vivax. *Pvrbp-2* PCR-RFLP method is able to distinguish multiple infection up to 16.86% and it revealed a low level of shared genetic pool between more than two populations. **Conclusion:** The study suggests that *pvrbp-2* is highly polymorphic genetic marker which can be used for population genetic analyses. RFLP analysis suggests presence of nearly similar proportion of *Sal-1* and *Belem* alleles in Indian *P. vivax* populations. The larger extent of genetic polymorphism identified from limited samples advocates to screen genetic polymorphism in *pvrbp-2* from malaria endemic geographical regions and countries for designing *pvrbp-2* based anti-malarial control measures.

116. **Mishra N**, **Singh JP**, **Srivastava B**, Arora U, **Shah NK**, **Ghosh SK**, **Bhatt RM**, **Sharma SK**, **Das MK**, **Kumar A**, **Anvikar AR**, Kaitholia K, Gupta R, Sonal GS, Dhariwal AC, **Valecha N**. Monitoring antimalarial drug resistance in India via sentinel sites: Outcomes and risk factors for treatment failure 2009–2010. ***Bull World Health Organ*** **2012; *90* (12): 895–904.**

ABSTRACT

Objective: To describe India's National Antimalarial Drug Resistance Monitoring System, measure the efficacy of first-line malaria treatments, and determine risk factors for treatment failure. **Methods:** In 2009–2010, prospective studies with 28 days of follow-up were conducted at 25 sentinel sites. Patients infected with *Plasmodium falciparum* were given artesunate plus sulfadoxine-pyrimethamine (AS+SP); those infected with *P. vivax* were given chloroquine. Polymerase chain reaction was used to distinguish post-treatment reinfection from treatment failure. Isolates of *P. falciparum* were checked for dhfr and dhps mutations. **Findings:** Overall, 1664 patients were enrolled. Kaplan–Meier survival analysis showed an efficacy of 98.8% for AS+SP. Most patients with *P. falciparum* parasitaemia cleared their parasitaemias within 24 hours of treatment initiation, but six, including four with treatment failure, remained parasitaemic after 72 hours. Double mutants in dhfrwere found in 68.4% of the genotyped isolates. Triple or quadruple mutants in dhfr and mutations in dhps were rare. A daily dose of artesunate of 3 mg per kg of body weight, age of less than 5 years, and fever at enrolment were associated with an increased risk of treatment failure. Chloroquine remained 100% efficacious and generally cleared *P. vivax* parasitaemias within 48 hours. Vomiting (seen in 47 patients) was the most common adverse event. **Conclusion:** India's National Antimalarial Drug Resistance Monitoring System

provides wide coverage. The first-line antimalarials used in the country remain safe and efficacious. The treatment of malaria in young children and the relative benefits of age- and weight-based dosing need further exploration.

117. Korgaonkar Nandini S, **Kumar A**, **Yadav RS**, Kabadi Dipak, **Dash AP**. Mosquitoes biting activity on human & detection of *Plasmodium falciparum* infection in *Anopheles stephensi* in Goa, India. ***Indian J Med Res* 2012; *135*(1): 120–6.**

ABSTRACT

Background & Objectives: Knowledge of the bionomics of mosquitoes, especially of disease vectors, is essential to plan appropriate vector avoidance and control strategies. Information on biting activity of vectors during the night hours in different seasons is important for choosing personal protection measures. This study was carried out to find out the composition of mosquito fauna biting on humans and seasonal biting trends in Goa, India. **Methods**: Biting activities of all mosquitoes including vectors were studied from 1800 to 0600 h during 85 nights using human volunteers in 14 different localities of three distinct ecotypes in Goa. Seasonal biting trends of vector species were analysed and compared. Seasonal biting periodicity during different phases of night was also studied using William's mean. **Results**: A total of 4,191 mosquitoes of five genera and 23 species were collected. Ten species belonged to *Anopheles*, eight to *Culex*, three to *Aedes* and one each to *Mansonia* and *Armigeres*. Eleven vector species had human hosts, including malaria vectors *Anopheles stephensi* (1.3%), *An. fluviatilis* (1.8%), and *An. culicifacies* (0.76%); filariasis vectors *Culex quinquefasciatus* (40.8%) and *Mansonia uniformis* (1.8%); Japanese encephalitis vectors *Cx. tritaeniorhynchus* (17.4%), *Cx. vishnui* (7.7%), *Cx. pseudovishnui*(0.1%), and *Cx. gelidus* (2.4%); and dengue and chikungunya vectors *Aedes albopictus* (0.9%) and *Ae. aegypti* (0.6%). Two *An. stephensi* of the total 831 female anophelines, were found positive for *P. falciparum* sporozoites. The entomological inoculation rate (EIR) of *P. falciparum* was 18.1 and 2.35 for Panaji city and Goa, respectively. **Interpretation & Conclusions:** Most of the mosquito vector species were collected in all seasons and throughout the scotophase. Biting rates of different vector species differed during different phases of night and seasons. Personal protection methods could be used to stop vector-host contact.

KEYWORDS: *Anopheles stephensi*, biting activity, circumsporozoite protein, entomological inoculation rate, landing rates, William's mean.

118. **Mallick PK**, **Joshi H**, **Valecha N**, **Sharma SK**, **Eapen A**, **Bhatt RM**, **Srivastava HC**, Sutton PL, **Dash AP**, Bhasin VK. Mutant *pfcrt* "SVMNT" haplotype and wild type *pfmdr1* "N86" are endemic in *Plasmodium vivax* dominated areas of India under high chloroquine exposure. ***Malar J* 2012; *11*: 16.**

ABSTRACT

Background: Chloroquine resistance (CQR) phenotype in *Plasmodium falciparum* is associated with mutations in *pfcrt* and *pfmdr*-1 genes. Mutations at amino acid position 72-76 of *pfcrt* gene, here defined as *pfcrt* haplotype are associated with the geographic origin ofchloroquine resistant parasite. Here, mutations at 72-76 and codon 220 of *pfcrt* gene and N86Y *pfmdr*-1 mutation were studied in blood samples collected across 11 field sites, inclusive of highand low *P. falciparum* prevalent areas in India. Any probable correlation between these mutations and clinical outcome of CQ treatment was also investigated. **Methods**: Finger pricked blood spotted on Whatman No.3 papers were collected from *falciparum* malaria patients of high and low *P. falciparum* prevalent areas. For *pfcrt* haplotypeinvestigation, the parasite DNA was extracted from blood samples and used for PCR amplification, followed by partial sequencing of the *pfcrt* gene. For *pfmdr*-1 N86Y mutation, the PCR product was subjected to restriction digestion with AflIII endonuclease enzyme. **Results**: In 240 *P. falciparum* isolates with reported in vivo CQ therapeutic efficacy, the analysis of mutations in *pfcrt* gene shows that mutant SVMNT-S (67.50%) and CVIET-S (23.75%) occurred irrespective of clinical outcome and wild type CVMNK-A (7.91%) occurred only in adequate clinical and parasitological response samples. Of 287 *P. falciparum* isolates, SVMNTS 192 (66.89%) prevailed in all study sites and showed almost monomorphic existence (98.42% isolates) in low *P. falciparum* prevalent areas. However, CVIETS-S (19.51%) and CVMNK-A (11.84%) occurrence was limited to high *P. falciparum* prevalent areas. Investigation of *pfmdr*-1 N86Y mutation shows no correlation with clinical outcomes. The wild type N86 was prevalent in all the low *P. falciparum* prevalent areas (94.48%). However, mutant N86Y was comparably higher in numbers at the high *P. falciparum* prevalent areas (42.76%). **Conclusions**: The wild type *pfcrt* gene is linked to chloroquine sensitivity; however, presence of mutation cannot explain the therapeutic efficacy of CQ in the current scenario of chloroquineresistance. The monomorphic existence

of mutant SVMNT haplotype, infer inbreeding and faster spread of CQR parasite in areas with higher *P. vivax* prevalance and chloroquine exposure, whereas, diversity is maintained in *pfcrt* gene at high *P. falciparum* prevalent areas.

119. **Chittoria A**, Mohanty S, Jaiswal Y, **Das A**. Natural selection mediated association of the Duffy (FY) gene polymorphisms with *Plasmodium vivax* malaria in India. ***PLoS One* 2012; *7*(9): e45219.**

ABSTRACT

The Duffy (Fy) antigens act as receptors for chemokines as well as for *Plasmodium vivax* to invade human RBCs. A recent study has correlated the occurrence of the *FY*A* allele of Duffy gene with decreased susceptibility to *vivax* malaria, but no epidemiological correlation between the distribution of FY*A allele and incidences of *vivax* malaria has been established so far. Furthermore, if such correlations exist, whether natural selection has mediated the association, is an important question. Since India is highly endemic to *P. vivax* malaria with variable eco-climatic and varying *vivax* malaria epidemiology across different regions, such a question could well be answered in Indians. For this, we have genotyped the *FY* gene at the -33^{rd} and the 125^{th} nucleotide positions in 250 Indians sampled from six different zonal plus one tribal population covering the whole of India and studied possible correlations with eco-climatic and *vivax* malaria incidences. No *FY*O* allele was found, however, both the *FY*A* and *FY*B* alleles forming *FY*A/FY*A*, *FY*A/FY*B* and *FY*B/FY*B* genotypes were widely distributed among Indians. Five out of seven population samples significantly deviated from the Hardy-Weinberg equilibrium expectation, and two alleles (*FY*A* and *FY*B*) and the homozygote genotype, *FY*B*/*FY*B* were clinally distributed over the population coordinates. Furthermore, *vivax* malaria incidences over the past five years were significantly negatively and positively associated with the frequencies of the *FY*A* and *FY*B* alleles, respectively. The Northern Indians were highly differentiated from the other zonal population samples at the *FY* gene, as evidenced from the reconstructed Neighbor-Joining phylogenetic tree. The results specify the role of natural selection in the distribution of *FY* gene polymorphism in India. Furthermore, the hypotheses on the part of the *FY*A* allele in conferring protection to *vivax* malaria could be validated following population genetic studies in a *vivax* malaria epidemiological setting, such as India.

120. **Shah NK**, **Kumar A**, **Valecha N**. New global estimates of malaria deaths. ***Lancet* 2012; *380*(9841): 560.**

121. **Nanda N**, **Bhatt RM**, **Sharma SN**, Rana PK, Kar NP, **Sharma A**, **Adak T**. Prevalence and incrimination of *Anopheles fluviatilis* species S (Diptera : Culicidae) in a malaria endemic forest area of Chhattisgarh state, central India. ***Parasit Vectors* 2012; *5*: 215.**

ABSTRACT

Background: Chhattisgarh state in central India is highly endemic for malaria and contributes about 13% of annually reported malaria cases in the country with predominance of *P. falciparum*. Entomological investigations were carried out in a tribal forested area of district Bastar located in the southern part of Chhattisgarh state to record the prevalence of sibling species of *Anopheles fluviatilis* and *An. culicifacies* complexes. The vector species complexes were investigated at sibling species level for their biology in terms of resting and feeding behavior and malaria transmission potential. **Methods:** Indoor resting vector mosquitoes collected during 2010–2011 were identified to sibling species by cytotaxonomy and polymerase chain reaction (PCR) assay. The blood meal source analysis and incrimination studies were done at sibling species level by counter current immunoelectrophoresis and enzyme linked immunosorbent assay (ELISA) respectively. **Results:** Analysis of sibling species composition revealed predominance of *An. fluviatilis* species S in the study area, which was found to be highly anthropophagic and rested in human dwellings whereas the sympatric species T was primarily zoophagic. Incrimination studies showed high sporozoite rate in species S, thereby confirming its vectorial efficiency. *An. culicifacies* was encountered in low numbers and comprised species B and C in almost equal proportion. Both these species were found to be exclusively zoophagic. **Conclusion:** The observations made strongly suggest that species S of *Fluviatilis* Complex is the principal vector of malaria in certain forest areas of district Bastar, Chhattisgarh state and should be the target species for vector control operation. Vector control strategies based on biological characteristics of *Fluviatilis S* will lead to substantial decline in malaria incidence in such areas.

KEYWORDS: *Anopheles fluviatilis*, *Anopheles culicifacies*, species complex, sibling species, anthropophagic, zoophagic, malaria vector.

122. Kaul V, Van Kaer L, Das G, **Das J**. Prostanoid receptor 2 signalling protects T-helper 2 cells from BALB/c mice against activation-induced cell death. ***J Biol Chem*** **2012; 287: 2543–9.**

ABSTRACT

T helper 2 (Th2) cells play a central role in the progression of many diseases such as allergic airway inflammation, autoimmune diseases, and infections caused by intracellular pathogens. Consequently, animals such as BALB/c mice, which exhibit a propensity for generating Th2 responses, are susceptible to allergic airway inflammation, type-II autoimmune diseases, and various infections induced by intracellular pathogens, namely, *Leishmania*. In contrast, C3H/OuJ mice have a tendency for generating T helper 1 (Th1) responses and show resistance to these diseases. Here, we show that prostaglandin endoperoxide E_2 selectively inhibits activation-induced cell death of Th2 cells by signaling through its receptor E-prostanoid receptor 2 (EP2). Consequently, Th2 cells derived from BALB/c mice expressed very high levels of EP2. On the other hand, Th2 cells derived from C3H/OuJ mice expressed very low levels of EP2, which failed to support the survival of Th2 cells. Furthermore, we found that this effect of EP2 on Th2 cells from BALB/c mice was executed by a granzyme B-mediated mechanism. EP2 belongs to a group of G-protein-coupled receptors that are amenable to therapeutic targeting. Our findings therefore identify EP2 as a promising target for small molecule-directed immunomodulation.

KEYWORDS: cell death, cytokine, immunology, prostaglandins, T cell, T cell biology, activation-induced cell death, E Prostanoid Receptor EP2.

123. Rueangweerayut R, Phyo AP, Uthaisin C, Poravuth Y, Binh TQ, Tinto H, Pénali LK, **Valecha N**, Tien NT, Abdulla S, BorghiniFuhrer I, Duparc S, Shin CS, Fleckenstein L. Pyronaridine-artesunate study team. Pyronaridine-artesunate versus mefloquine plus artesunate for malaria. ***N Engl J Med*** **2012; *366*(14): 1298–309.**

ABSTRACT

Background: Pyronaridine-artesunate is an artemisinin-based combination therapy under evaluation for the treatment of *Plasmodium falciparum* and *P. vivax* malaria. **Methods**: We conducted a phase 3, open-label, multicenter, noninferiority trial that included 1271 patients between 3 and 60 years of age from Asia (81.3%) or Africa (18.7%) with microscopically

confirmed, uncomplicated *P. falciparum* malaria. Patients underwent randomization for treatment with a fixed-dose combination of 180 mg of pyronaridine and 60 mg of artesunate or with 250 mg of mefloquine plus 100 mg of artesunate. Doses were calculated according to body weight and administered once daily for 3 days. **Results**: Pyronaridine-artesunate was noninferior to mefloquine plus artesunate for the primary outcome: adequate clinical and parasitologic response in the per-protocol population on day 28, corrected for reinfection with the use of polymerase-chain-reaction (PCR) genotyping. For this outcome, efficacy in the group receiving pyronaridine-artesunate was 99.2% (743 of 749 patients; 95% confidence interval [CI], 98.3 to 99.7) and that in the group receiving mefloquine plus artesunate was 97.8% (360 of 368 patients; 95% CI, 95.8 to 99.1), with a treatment difference of 1.4 percentage points (95% CI, 0.0 to 3.5; *P*=0.05). In the intention-to-treat population, efficacy on day 42 in the group receiving pyronaridine-artesunate was 83.1% (705 of 848 patients; 95% CI, 80.4 to 85.6) and that in the group receiving mefloquine plus artesunate was 83.9% (355 of 423 patients; 95% CI, 80.1 to 87.3). In Cambodia, where there were 211 study patients, the median parasite clearance time was prolonged for both treatments: 64 hours versus 16.0 to 38.9 hours in other countries (*P*<0.001, on the basis of Kaplan-Meier estimates). Kaplan-Meier estimates of the recrudescence rate in the intention-to-treat population in Cambodia until day 42 were higher with pyronaridine-artesunate than with mefloquine plus artesunate (10.2% [95% CI, 5.4 to 18.6] *vs.* 0%; *P*=0.04 as calculated with the log-rank test), but similar for the other countries combined (4.7% [95% CI, 3.3 to 6.7] and 2.8% [95% CI, 1.5 to 5.3], respectively; *P*=0.24). Elevated levels of aminotransferases were observed in those receiving pyronaridine-artesunate. Two patients receiving mefloquine plus artesunate had seizures. **Conclusions**: Fixed-dose pyronaridine-artesunate was efficacious in the treatment of uncomplicated *P. falciparum* malaria. In Cambodia, extended parasite clearance times were suggestive of in vivo resistance to artemisinin.

124. **Ghosh SK**, **Tiwari S**, **Ojha VP**. A renewed way of malaria control in Karnataka, south India. ***Front Physiol*** **2012; *3*: 194.**

125. **Nagpal BN**, **Saxena R**, **Srivastava A**, **Singh N**, **Ghosh SK**, **Sharma SK**, **Kumar A**, **Kumar H**, **Sharma AS**, **Chand SK**, **Ojha VP**, **Mohanty SS**, **Mohanty AK**, Dasgupta RK, Dhillon GPS,

Dash AP. Retrospective study of chikungunya outbreak in urban areas of India. ***Indian J Med Res* 2012; *135*: 351–8.**

ABSTRACT

Background & objectives: A retrospective study on chikungunya outbreak in India in five States *viz*. Delhi, Madhya Pradesh, Orissa, Maharashtra and Kerala was conducted in 2007-2008 to know the distribution and determinants of chikungunya fever outbreak in India. **Methods**: On the basis of high and low incidence of chikungunya fever, two districts from each State and two wards from the selected district were taken for random selection of 1000 households from 10 districts and 5 States. Semi-structured questionnaires were administered to individuals, patients, qualified health professionals and to stakeholders for collecting information. **Results**: The educational background and occupation of the respondents showed variations across the study States. Only in high incidence ward of Maharashtra, water storage period for 3-6 days and emptying, drying of water containers on weekly basis was noted. The study through knowledge, attitude, belief, practice (KABP) obtained individual's perception of chikungunya fever, its prevention and control. Patients' expenditure on treatment was mainly recorded less than `500 across study States. Health facility survey obtained an overview of the capacity of local health facilities. Stakeholders' perception regarding chikungunya fever was also noted. **Interpretation & conclusions:** The study revealed differences in awareness of chikungunya, cause of the disease, vector responsible, mode of transmission, biting time and elimination of breeding of mosquitoes statistically significant among high and low incidence wards of all the States. Expenditure on treatment was independent of economically active status and loss of man-days across all the States. Education and occupation did not have any relation with emptying/drying of water containers in high incidence wards. Strengthening of surveillance, information, education and communication (IEC) activities along with case management facilities may be provided by the State health department for prevention of chikungunya outbreaks in future. Stakeholders should be more involved in outbreak management and future planning.

KEYWORDS: *Aedes* mosquito, case management, chikungunya, health facility, retrospective study, stakeholders, urban areas.

126. **Sethi P**, **Dua VK**, Jain R. Sensitive and specific LC-MS/MS method for the simultaneous determination of chlorproguanil, dapsone and their metabolites in human plasma. ***J Liquid Chromatographic Sci*** **2012; *35*(18): 2584–601.**

ABSTRACT

A sensitive, specific, and rapid liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method for the determination of dapsone, chlorproguanil, and their metabolites was developed and validated over a concentration range of 2–2000 ng/mL using 200 µL of plasma. After a simple solvent precipitation procedure, the supernatant was analyzed directly by HPLC-MS/MS method using an XTerra RP18 (2.1 mm × 100 mm, 5.0 µm) column with mobile phase consisting of acetonitrile-0.1% formic acid in water (75:25, v/v, pH 3.0) in an isocratic mode at a flow rate of 0.3 mL/min. Mass detection was performed using a triple quadrupole mass spectrometer operating in positive electrospray ionization mode. The elution of chlorproguanil (CPG, 288 ->204), chlorcycloguanil (CCG, 286 ->229), dapsone (DDS, 249 ->156), monoacetyldapsone (MADDS, 291 ->156), and trimipramine-D3 (TMP-D3, 298 ->103) was monitored using multiple reaction monitoring. The lower limit of detection for CPG, CCG, DDS, and MADDS was 0.5 ng mL^{-1} while the limit of quantification was 2 ng mL^{-1} in human plasma. The extraction recovery of CPG, CCG, DDS, MADDS, and TMP-D3 from human plasma was higher than 87%. The method may find its application in therapeutic monitoring of these compounds in biological matrix.

127. **Valecha N**, Mohanty S, **Srivastava P**, **Sharma S**, **Tyagi P**, Bergqvist Y, Ringwald P. Short report: Efficacy of artemether-lumefantrine in area of high malaria endemicity in India and its correlation with blood concentration of lumefantrine. ***Am J Trop Med Hyg*** **2012; *86*(3): 395–7.**

ABSTRACT

This study was conducted to correlate blood concentrations of lumefantrine with treatment outcome for patients with *Plasmodium falciparum* malaria when the drug was given without specific instructions for administration with food. Patients with *P. falciparum* malaria in the highly endemic state of Orissa, India, were enrolled during 2008 and followed-up for 28 days after admistration of artemether-lumefantrine for three days according to a World Health Organization protocol. Drug concentration in whole blood was determined by using blood spots placed on filter

paper on day 7. The technology is suitable for field studies. One hundred percent of the patients had an adequate clinical and parasitological response.These results confirm the efficacy of artemether-lumefantrine in persons from poor tribal communities when given without specific instructions regarding co-administration with food, despite high inter-individual variability in blood concentrations of lumefantrine.

128. **Barik TK**, **Raghavendra K,** Goswami A. Silica nanoparticle: A potential new insecticide for mosquito vector control. ***Parasitol Res*** **2012; *111*(3): 1075–83.**

ABSTRACT

Presently, there is a need for increased efforts to develop newer and effective methods to control mosquito vectors as the existing chemical and biological methods are not as effective as in earlier period owing to different technical and operational reasons. The use of nanomaterial products in various sectors of science including health increased during the last decade. We tested three types of nanosilica, namely lipophilic, hydrophilic and hydrophobic, to assess their larvicidal, pupicidal and growth inhibitor properties and also their influence on oviposition behaviour (attraction/deterrence) of mosquito species that transmit human diseases, namely malaria (*Anopheles*), yellow fever, chickungunya and dengue (*Aedes*), lymphatic filariasis and encephalitis (*Culex* and *Aedes*). Application of hydrophobic nanosilica at 112.5 ppm was found effective against mosquito species tested. The larvicidal effect of hydrophobic nanosilica on mosquito species tested was in the order of*Anopheles stephensi* > *Aedes aegypti* > *Culex quinquefasciatus*, and the pupicidal effect was in the order of *A. stephensi* > *C. quinquefasciatus* > *Ae. aegypti*. Results of combined treatment of hydrophobic nanosilica with temephos in larvicidal test indicated independent toxic action without any additive effect. This is probably the first report that demonstrated that nanoparticles particularly nanosilica could be used in mosquito vector control.

129. **Saxena R**, **Nagpal BN**, **Das MK**, **Srivastava A**, **Gupta SK**, **Kumar A**, Jeyaseelan AT, Baraik VK. A spatial statistical approach to analyze malaria situation at micro level for priority control in Ranchi district, Jharkhand. ***Indian J Med Res*** **2012; *136*(5): 776–82.**

ABSTRACT

Background & Objectives: The presence of efficient malaria vectors namely *Anopeles culicifacies, An. fluviatilis* and *An. annularis* (Diptera: Culicidae), rapid industrialization causing large influx of population and poor health infrastructure are some of the factors that make malariaan important public health problem in Ranchi, the capital of Jharkhand State, India. A geographical information system (GIS) based retrospective study using spatial statistical tools was initiated in 328 subcentres of 14 primary health centres (PHCs) of the district using malaria epidemiological data of three years (2007-2009) to identify spatial distribution pattern of *Plasmodium vivax* (*Pv*) and *Plasmodium falciparum* (*Pf*) occurrence, delineation of hot spots and to map directional distribution trend of *Pf* spread to help formulate evidence-based policy and to prioritize control during 2011. **Methods**: Spatial statistics tools like Global Moran's I index, Getis-Ord Gi* and Standard Deviational Ellipse were used in GIS domain for analysis. **Results**: Spatial distribution pattern of *Pv* occurrence was found random while *Pf* distribution was significantly clustered. During 2007-2009, the number of subcentres under *Pf* hot spot category exhibited downward trend while high *Pf* risk subcentres exhibited upward trend. One consistent *Pf* hot spot consisting of five subcentres was identified in Silli PHC. During 2009, one *Pf* hot spot consisting of 20 subcentres and 18 subcentres under high *Pf* risk category were identified in Angara, Silli, Burmu and Kanke PHCs. A shifting trend in *Pf* spread was noticed from north-west to western direction from 2008 onwards. **Interpretation & Conclusions:** The study recommended priority control in 20 *Pf* hot spot and 18 high *Pf* risk reporting subcentres including five consistent *Pf* hot spot subcentres in Angara, Silli, Burmu and Kanke PHCs during 2011 to address grave malaria situation in thedistrict in a cost-effective manner.

KEYWORDS: cold spots, high risk, hot spots, spatial statistics, standard deviational ellipse.

130. **Ghosh SK**, Patil RR, **Tiwari SN**. Socio-economic-political-cultural aspects in malaria research papers published (January–December 2012) control programme implementation in southern India. ***J Parasitol Res*** **2012 April 30,** ***2012*****: 317908.**

ABSTRACT

Objective: A Socio-economic-political-cultural (SEPC) study was undertaken under the Roll Back Malaria (RBM) initiative to understand the process of programme implementation and how

far in the changing malaria context, the broader environment has been understood and programme components have undergone changes. **Material and Methods**: Two studies were carried out; first in four villages under the primary health unit (PHU) Banavaralu in Tiptur Taluka in September 2002 and the second one in April 2003 in four villages in Chitradurga district, namely, Kappagere, Kellodu in Hosadurga Taluka, and Vani Vilas Puram and Kathrikenhally in Hiriyur Taluka. Focus group discussion and key interviews were adopted to collect the qualitative data. **Results**: Gender discrimination and lack of empowerment of women came out strongly in social analysis. In the rural elected bodies called Panchayats, the concept of health committees was not known. Health committees as one of the important statutory committees under every Panchayat were nonexistent in reality in these villages. Financial difficulties at Grama Panchayat level and also meager budget allocation for health have led to indifferent attitude of Panchayat members towards health. It was observed that there were generally no specific cultural practices in relation to malaria cure. Cultural and traditional practices in malaria-related issues were not predominant in the community except for some sporadic instances. **Conclusion and Recommendation**: SEPC study is an important indicator in malaria control programme. It is ultimately the community that takes the major decision directly or indirectly and the health authority must guide them in right direction.

131. **Bhatt RM**, **Sharma SN**, **Barik TK**, **Raghavendra K**. Status of insecticide resistance in malaria vector, *Anopheles culicifacies* in Chhattisgarh state, India. ***J Vector Borne Dis*** **2012; *49*(1): 36–8.**

132. **Pandey KC**, **Dixit R**. Structure-function of falcipains: Malarial cysteine proteases. ***J Trop Med*** **2012; *2012*: 345195.**

ABSTRACT

Evidence indicates that cysteine proteases play essential role in malaria parasites; therefore an obvious area of investigation is the inhibition of these enzymes to treat malaria. Studies with cysteine protease inhibitors and manipulating cysteine proteases genes have suggested a role for cysteine proteases in hemoglobin hydrolysis. The best characterized *Plasmodium* cysteine proteases are falcipains, which are papain family enzymes. Falcipain-2 and falcipain-3 are major hemoglobinases of *P. falciparum*. Structural and functional analysis of falcipains showed that they

have unique domains including a refolding domain and a hemoglobin binding domain. Overall, the complexes of falcipain-2 and falcipain-3 with small and macromolecular inhibitors provide structural insight to facilitate the design or modification of effective drug treatment against malaria. Drug development targeting falcipains should be aided by a strong foundation of biochemical and structural studies.

133. **Das MK**, **Singh RK**, Lal RK, **Dhiman RC**. Susceptibility of *Aedes aegypti* to insecticides in Ranchi city, Jharkhand state, India. ***Dengue Bull WHO* 2012; *35*: 194–8.**

134. **Singh RK**, **Mittal PK**, Gourshettiwar MP, Pande SJ, **Dhiman RC**. Susceptibility of malaria vectors to insecticides in Gadchiroli district (Maharashtra), India. ***J Vector Borne Dis* 2012; *49*(1): 42–4.**

135. Gargano N, Ubben D, Tommasini S, Bacchieri A, Corsi M, Bhattacharyya PC, Rao BH, Dubashi N, **Dev V**, **Ghosh SK**, **Kumar A**, **Srivastava B**, **Valecha N**. Therapeutic efficacy and safety of dihydroartemisininpiperaquine versus artesunate-mefloquine in uncomplicated *Plasmodium falciparum* malaria in India. ***Malar J* 2012; *11*: 233.**

ABSTRACT

Background: Resistance in *Plasmodium falciparum* to commonly used anti-malarial drugs, especially chloroquine, is being increasingly documented in India. By 2007, the first-line treatment for uncomplicated malaria has been revised to recommend artemisinin-based combination therapy (ACT) for all confirmed *P. falciparum* cases. **Objective**: The objective of this study was to compare the efficacy, safety and tolerability between dihydroartemisinin-piperaquine (DP) and artesunate plus mefloquine (AM) drug combinations in the treatment of uncomplicated *P. falciparum* malaria in India. Methods: Between 2006 and 2007, 150 patients with acute uncomplicated *P. falciparum* malaria were enrolled, randomized to DP (101) or AM (49) and followed up for 63 days as part of an open-label, non-inferiority, randomized, phase III multicenter trial in Asia. **Results**: The heterogeneity analysis showed no statistically significant difference between India and the other countries involved in the phase III study, for both the PCR-corrected and uncorrected cure rates. As shown at the whole study level, both forms of ACT were highly

efficacious in India. In fact, in the per protocol population, the 63-day cure rates were 100% for A + M and 98.8% for DP. The DP combination exerted a significant post-treatment prophylactic effect, and compared with A+ M a significant reduction in the incidence of new infections for DP was observed (respectively 17.1% versus 7.5% of patients experienced new infection within follow up). Parasite and fever clearance was rapid in both treatment arms (median time to parasite clearance of one day for both groups). Both DP and A + M were well tolerated, with the majority of adverse events of mild or moderate severity. The frequencies of individual adverse events were generally similar between treatments, although the incidence of post treatment adverse events was slightly higher in patients who received A+ M with respect to those treated with DP. **Conclusion**: DP is a new ACT displaying high efficacy and safety in the treatment of uncomplicated *P. falciparum* malaria and could potentially be considered for the first-line treatment of uncomplicated *falciparum* malaria in India.

KEYWORDS: *Plasmodium falciparum*, Malaria, Artemisinin-based combination therapy (ACT), Dihydroartemisinin-piperaquine, Artesunate Mefloquine, India.

136. Dwivedi VP, Tousif S, Bhattacharya D, Prasad DV, Van Kaer L, **Das J**, Das G. Transforming growth factor-β protein inversely regulates in vivo differentiation of interleukin-17 (IL-17)-producing CD4+ and CD8+ T-cells. ***J Biol Chem* 2012; *287*(5): 2943–7.**

ABSTRACT

TGF-β is a pleiotropic cytokine that predominantly exerts inhibitory functions in the immune system. Unexpectedly, the *in vitro* differentiation of both Th17 and Tc17 cells requires TGF-β. However, animals that are impaired in TGF-β signaling (TGF-βRIIDN mice) display multiorgan autoimmune disorders. Here we show that $CD4^+$ T cells from TGF-βRIIDN mice are resistant to Th17 cell differentiation and, paradoxically, that $CD8^+$T cells from these animals spontaneously acquire an IL-17-producing phenotype. Neutralization of IL-17 or depletion of $CD8^+$ T cells dramatically inhibited inflammation in TGF-βRIIDN mice. Therefore, the absence of TGF-β triggers spontaneous differentiation of IL-17-producing $CD8^+$ T cells, suggesting that the *in vivo* and *in vitro*conditions that promote the differentiation of IL-17-producing $CD8^+$ T cells are distinct.

KEYWORDS: cytokine, inflammatory bowel disease, interleukin, T cell, Transforming Growth Factor Beta (TGFbeta), CD4 T Cell, CD8 T Cell, IL-17, IL-6.

Titles: A-Z

2013

137. **Dhiman RC**, **Yadav YK**, **Saraswat S**, **Singh P**. Altitude, temperature, and malaria vectors in Nainital and Udham Singh Nagar districts of Uttarakhand, India: An evidence-based study. ***J Vector Borne Dis*** **2013; *50*(3): 220–4.**

ABSTRACT

Background & Objectives: The relationship between altitude, temperature and malaria are poorly understood. Hence, a study was undertaken at three sites of Udham Singh Nagar (erstwhile Nainital district) and Nainital district (Uttarakhand) during 2010–11 for the generation of evidences in the context of potential threat of climate change. **Methods**: Data on temperature and relative humidity (RH) were recorded through data-logger device in study villages at the altitudes of 166, 226 and 609 m were selected for detailed work. Mosquito collections were made fortnightly during 0600–0800 hrs. Malaria incidence data were procured from concerned Primary Health Centres. **Results**: The study provides evidences of decrease in temperature with increase in altitude, even within a district resulting in variation in temporal distribution of malaria vector. With the increase of 67 m altitude between plains and foothill village, there was a reduction in temperature to the tune of 1.1°C and with further increase in altitude of 416 m between foothill and hilly villages, the temperature decreased by 0.27°C. The difference in temperature at three altitudes affects the Transmission windows (TWs) of both *Plasmodium vivax* (*Pv*) and *P. falciparum* (*Pf*), and opening of TWs are inversely proportional to altitude. In the plains, the TW for *Pv* and *Pf* were open for 11 and 10 months respectively, while 10 and 9 months in the foothills and 9 and 8 months, respectively for both the parasites at hilly altitude. Comparison of malaria vectors in plains, foothills, and hilly villages showed that the availability of *Anopheles culicifacies* and *An. fluviatilis* decreased with an increase in altitude from foothills to hilly areas. **Interpretation & Conclusion**: This study may be extrapolated to know the suitability of occurrence of malaria

vectors and transmission of parasites at different altitudes from the viewpoint of temperature as limiting factor in unknown areas.

KEYWORDS: altitude, malaria, man hour density, *Plasmodium falciparum*, *P. vivax*, temperature, transmission window.

138. **Chauhan K**, Pande V, **Das A**. Analyses of genetic variations at microsatellite loci present in-and-around the *Pfcrt* gene in Indian *Plasmodium falcipafrum*. ***Infect Genet Evol* 2013; *20*: 476–87**.

ABSTRACT

Evolution and spread of chloroquine resistant (CQR) malaria parasite *Plasmodium falciparum* have posed great threat in malaria intervention across the globe. The occurrence of K76T mutation in the *P. falciparum* chloroquine resistance transporter (*pfcrt*) gene has been widely attributed to CQR with four neighboring mutations providing compensatory fitness benefit to the parasite survival. Understanding evolutionary patterns of the *pfcrt* gene is of great relevance not only for devising new malaria control measures but also could serve as a model to understand evolution and spread of other human drug-resistant pathogens. Several studies, mainly based on differential patterns of diversities of the microsatellite loci placed in-and-around the *pfcrt* gene have indicated the role of positive natural selection under the 'hitchhiking' model of molecular evolution. However, the studies were restricted to limited number of microsatellite loci present inside the *pfcrt* gene. Moreover, comparatively higher level of diversities in microsatellite loci present inside the *pfcrt* gene than the loci flanking the *pfcrt* gene are hallmarks of Indian *P. falciparum*, presenting contrasting evolutionary models to global isolates. With a view to infer evolutionary patterns of the *pfcrt* gene in Indian *P. falciparum*, we have adopted a unique sampling scheme of two types of populations (cultured and field collected) and utilized 20 polymorphic microsatellite loci (16 located inside the *pfcrt* gene and four in the two flanking regions) to disentangle between genetic drift (inbred cultured isolates) and natural selection (field isolates). Data analyses employing different population genetic tests could not straightforwardly explain either the model invoking 'genetic hitchhiking' or 'genetic drift'. However, complex evolutionary models influenced by both demography and natural selection or an alternative model of natural

selection (*e.g*. diversifying/balancing selection) might better explain the observed microsatellite variation in-and-around the *pfcrt* gene in Indian *P. falciparum*.

KEYWORDS: malaria, *Plasmodium falciparum*, India, microsatellites, *Pfcrt*, genetic diversity.

139. **Sunil S**, **Singh OP**, **Nanda N**, **Raghavendra K**, **Reddy BP**, **Subbarao SK**. Analysis of population genetic structure of Indian *Anopheles culicifacies* species A using microsatellite markers. ***Parasit Vectors* 2013; *6*: 166.**

ABSTRACT

Background: *Anopheles culicifacies sensu lato* is an important vector of malaria in Southeast Asia contributing to almost 70% of malaria cases in India. It exists as morphologically similar sibling species A, B, C, D and E with varied geographical distribution patterns. Vector control measures have been difficult for this important vector as the sibling species have developed varying levels of resistance to the currently used insecticides. In view of the importance of this vector, we developed and validated a set of microsatellite markers and the same were used to analyze the population genetic structure of five different geographical populations of *An. culicifacies* A. **Methods**: *Anopheles culicifacies* A samples were collected from different localities across India, and genotyping was performed using eight microsatellite markers on ABI Prism 310 Genetic Analyzer. Several statistical analyses were performed to ascertain the genetic diversity that exists within and between the populations. **Results**: The markers were found to be moderately polymorphic in the populations. Genetic analysis indicated significant genetic differentiation between the majority of the population pairs analyzed and was not found to be related to the geographical distances between populations. **Conclusion**: This is the first and successful attempt to test the microsatellite markers developed for population genetic analysis of *An. culicifacies* A. Host feeding and breeding habits of species A suggest that factors other than ecological and geographical barriers were responsible for the genetic differentiation that has been observed between the populations.

KEYWORDS: *Anopheles culicifacies* complex, microsatellite markers, population genetics, hardy-weinberg equilibrium.

140. **Dua VK**, Verma G, Singh B, Rajan A, Bagai U, Agarwal DD, Gupta NC, Kumar S, Rastogi A. Anti-malarial property of steroidal alkaloid conessine isolated from the bark of *Holarrhena antidysenterica*. ***Malar J*** **2013; *12*: 194.**

ABSTRACT

Background: In the face of chronic and emerging resistance of parasites to currently available drugs and constant need for new anti-malarials, natural plant products have been the bastion of anti-malarials for thousands of years. Moreover natural plant products and their derivatives have traditionally been a common source of drugs, and represent more than 30% of the current pharmaceutical market. The present study shows evaluation of anti-malarial effects of compound conessine isolated from plant *Holarrhena antidysenterica* frequently used against malaria in the Garhwal region of north-west Himalaya. **Methods**: *In vitro* anti-plasmodial activity of compound was assessed using schizont maturation and parasite lactate dehydrogenase (pLDH) assay. Cytotoxic activities of the examined compound were determined on L-6 cells of rat skeletal muscle myoblast. The four-day test for anti-malarial activity against a chloroquine-sensitive *Plasmodium berghei* NK65 strain in BALB/c mice was used for monitoring *in vivo* activity of compound. In liver and kidney function test, the activity of alkaline phosphatase (ALP) was examined by p-NPP method, bilirubin by Jendrassik and Grof method. The urea percentage was determined by modified Berthelot method and creatinine by alkaline picrate method in serum of mice using ENZOPAK/CHEMPAK reagent kits. **Results:** Compound conessine showed *in vitro* anti-plasmodial activity with its IC_{50} value 1.9 μg/ml and 1.3 μg/ml using schizont maturation and pLDH assay respectively. The compound showed cytotoxity IC_{50}= 14 μg/ml against L6 cells of rat skeletal muscle myoblast. The isolated compound from plant *H. antidysenterica* significantly reduced parasitaemia (at 10 mg/kg exhibited 88.95% parasite inhibition) in *P. berghei*-infected mice. Due to slightly toxic nature (cytotoxicity = 14), biochemical analysis (liver and kidney function test) of the serum from mice after administration of conessine were also observed. **Conclusion:** The present investigation demonstrates that the compound conessine exhibited substantial anti-malarial property. The isolated compound could be chemically modified to obtain a more potent chemical entity with improved characteristics against malaria.

141. **Singh SP**, Mohan L. Biological control of mosquitoes by insectivorous flycatcher birds. ***J Entomol Res* 2013; *37*(4): 359–64.**

ABSTRACT

Flycatcher is an insectivorous bird which primarily feeds on small insects and flies and plays an important role in biological control of insects including mosquitoes. The birds are highly motivated, efficient and cost effective pest controller, the feasibility of use of insectivorous flycatcher birds in mosquito control has been reviewed.

KEYWORDS: Insectivorous, flycatcher birds, mosquito, malaria and vector control.

142. **Singh RK**, **Haq S**, **Kumar G**, **Dhiman RC**. Bionomics and vectorial capacity of *Anopheles annularis* with special reference to India: A review. ***J Commun Dis* 2013; *45* (1&2): 1–16.**

ABSTRACT

Anopheles annularis is widely distributed mosquito species all over the country. *An. annularis* has been incriminated as a malaria vector in India, Sri Lanka, Bangladesh, Myanmar, Indonesia, Malaysia and China. In India, it has been reported to play an important role in malaria transmission as a secondary vector in certain parts of Assam, West Bengal and U.P. In Odisha and some neighbouring countries such as Sri Lanka, Nepal and Myanmar it has been recognised as a primary vector of malaria. This is a species complex of two sibling species A and B but the role of these sibling species in malaria transmission is not clearly known. *An. annularis* is resistant to DDT and dieldrin/HCH and susceptible to malathion and synthetic pyrethorides in most of the parts of India. In view of rapid change in ecological conditions, further studies are required on the bionomics of *An. annularis* and its role in malaria transmission in other parts of the country. Considering the importance of *An. annularis* as a malaria vector, the bionomics and its role in malaria transmission has been reviewed in this paper. In this communication, an attempt has been made to review its bionomics and its role as malaria vector. *An. annularis* is a competent vector of malaria, thus, due attention should be paid for its control under the vector control programmes specially in border states where it is playing a primary role in malaria transmission.

143. Cator LJ, **Thomas S**, Paaijmans KP, **Ravishankaran S**, **Justin JA**, Mathai MT, Read AF, Thomas MB, **Eapen A**. Characterizing microclimate in urban malaria transmission settings: A case study from Chennai, India. ***Malar J*** **2013; *12*: 84.**

ABSTRACT

Background: Environmental temperature is an important driver of malaria transmission dynamics. Both the parasite and vector are sensitive to mean ambient temperatures and daily temperature variation. To understand transmission ecology, therefore, it is important to determine the range of microclimatic temperatures experienced by malaria vectors in the field. **Methods**: A pilot study was conducted in the Indian city of Chennai to determine the temperature variation in urban microclimates and characterize the thermal ecology of the local transmission setting. Temperatures were measured in a range of probable indoor and outdoor resting habitats of*Anopheles stephensi* in two urban slum malaria sites. Mean temperatures and daily temperature fluctuations in local transmission sites were compared with standard temperature measures from the local weather station. The biological implications of the different temperatures were explored using temperature-dependent parasite development models to provide estimates of the extrinsic incubation period (EIP) of *Plasmodium vivax* and *Plasmodium falciparum*. **Results:** Mean daily temperatures within the urban transmission sites were generally warmer than those recorded at the local weather station. The main reason was that night-time temperatures were higher (and hence diurnal temperature ranges smaller) in the urban settings. Mean temperatures and temperature variation also differed between specific resting sites within the transmission environments. Most differences were of the order of 1-3°C but were sufficient to lead to important variation in predicted EIPs and hence, variation in estimates of transmission intensity. **Conclusions**: Standard estimates of environmental temperature derived from local weather stations do not necessarily provide realistic measures of temperatures within actual transmission environments. Even the small differences in mean temperatures or diurnal temperature ranges reported in this study can lead to large variations in key mosquito and/or parasite life history traits that determine transmission intensity. Greater effort should be directed at quantifying adult mosquito resting behaviour and determining the temperatures actually experienced by mosquitoes and parasites in local transmission environments.

In the absence of such highly resolved data, the approach used in the current study provides a framework for improved thermal characterization of transmission settings.

KEYWORDS: Temperature, Extrinsic incubation period, *Anopheles stephensi*, urban malaria.

144. **Srivastava P**, Ratha J, **Shah NK**, **Mishra N**, **Anvikar A**, **Sharma SK**, **Das MK**, **Valecha N**. A clinical and molecular study of artesunate+sulfadoxine-pyrimethamine in three districts of central and eastern India. ***Malar J*** **2013; *12*: 247.**

ABSTRACT

Background: Artesunate + sulphadoxine-pyrimethamine (AS + SP) is recommended throughout India as the first-line treatment for uncomplicated *falciparum* malaria. Due to the presence of several eco-epidemiological zones of malaria and variable drug pressure, it is necessary to evaluate the efficacy of this combination in different regions of India. The objective of this study was to use clinical and molecular methods to monitor the efficacy of AS + SP in three diverse sites. **Methods:** The study was undertaken in three high endemic sites of central and eastern India. Patients with uncomplicated *falciparum* malaria were enrolled and followed for 28 days. Molecular genotyping was conducted for merozoite surface protein (*msp1* and *msp2*) to differentiate between re-infection and recrudescence and for the *dhfr* and *dhps* genes to monitor antifolate drug resistance. **Results:** In all, 149 patients were enrolled at the three sites. The crude cure rates were 95.9%, 100%, and 100% in Ranchi, Keonjhar, and West Garo Hills respectively. PCR-corrected cure rates were 100% at all sites. In *dhfr*, 27% of isolates had triple mutations, while 46% isolates were double-mutants. The most prevalent mutation was S108N followed by C59R. 164 L mutation was observed in 43/126 (34%) isolates. In *dhps*, most (76%) of the isolates were wild-type. Only 2.5% (2/80) isolates showed double mutation. *dhfr-dhps* two locus mutation were observed in 16% (13/80) isolates. Parasite clearance time was not related with antifolate mutations. **Conclusions:** AS + SP combination therapy remained effective against *falciparum* malaria despite common mutations promoting resistance to antifolate drugs. Although the prevalence of double and triple mutations in *dhfr*was high, the prevalence of *dhfr-dhps* two locus mutations were low. Even isolates with *dhfr* triple and dhfr-dhps two locus mutations achieved adequate clinical and parasitological response.

KEYWORDS: artesunate+sulphadoxine-pyrimethamine, *Plasmodium falciparum*, dihydrofolate reductase, dihydropteroate synthetase, *falciparum* malaria.

145. **Singh N**, **Bharti PK**, **Singh MP**, Mishra S, **Shukla MM**, Sharma RK, **Singh RK**. Comparative evaluation of bivalent malaria rapid diagnostic tests versus traditional methods in field with special reference to heat stability testing in central India. ***PLoS ONE* 2013; *8*(3): e58080**.

ABSTRACT

Background: Malaria presents a diagnostic challenge in areas where both *Plasmodium falciparum* and *P.vivax* are co-endemic. Bivalent Rapid Diagnostic tests (RDTs) showed promise as diagnostic tools for *P.falciparum* and *P.vivax*. To assist national malaria control programme in the selection of RDTs, commercially available seven malaria RDTs were evaluated in terms of their performance with special reference to heat stability. **Methodology/Principal Findings:** This study was undertaken in four forested districts of central India (July, 2011– March, 2012). All RDTs were tested simultaneously in field along with microscopy as gold standard. These RDTs were stored in their original packing at 25°C before transport to the field or they were stored at 35°C and 45°C upto 100 days for testing the performance of RDTs at high temperature. In all 2841 patients with fever were screened for malaria of which 26% were positive for *P.falciparum*, and 17% for *P.vivax*. The highest sensitivity of any RDT for *P.falciparum* was 98% (95% CI; 95.9–98.8) and lowest sensitivity was 76% (95% CI; 71.7–79.6). For *P.vivax* highest and lowest sensitivity for any RDT was 80% (95% CI; 94.9 - 83.9) and 20% (95% CI; 15.6–24.5) respectively. Heat stability experiments showed that most RDTs for *P.falciparum* showed high sensitivity at 45°C upto 90 days. While for *P.vivax* only two RDTs maintained good sensitivity upto day 90 when compared with RDTs kept at room temperature. Agreement between observers was excellent for positive and negative readings for both *P.falciparum* and *P.vivax* (Kappa >0.6–0.9). **Conclusion:** This is first field evaluation of RDTs regarding their temperature stability. Although RDTs are useful as diagnostic tool for *P.falciparum* and *P.vivax* even at hightemperature, the quality of RDTs should be regulated and monitored more closely.

146. **Mehrunnisa A**, **Sharma A**, **Parasher H**, **Dhayal D**, **Singh OP**, **Nanda N**, **Adak T**. Comparative susceptibilities of species T and U of the *Anopheles fluviatilis* complex to *Plasmodium vinckei* petteri sporogony. ***J Med Entomol*** **2013; *50*(3): 594–7**.

ABSTRACT

Anopheles fluviatilis James is an important malaria vector in Indian subcontinent. *An. fluviatilis* exists as a complex of three sibling species, of which two species, T and U, have been colonized so far. Attempts were made to study the comparative susceptibility of species T and U of the *An. fluviatilis* complex to rodent malaria parasite *Plasmodium vinckei petteri* by using *Anopheles stephensi* Liston as calibrator for variable infectivity in different isolates. *An. stephensi*, which was used as control, became readily infected, with 60–65% mosquitoes carrying developing oocysts, whereas in species T and species U,≈50 and 63%, respectively, of mosquitoes carried oocyts. *An. fluviatilis* species T was found comparatively less susceptible to *P. v. petteri* sporogonic development compared with species U. Moreover, significantly lesser sporozoites rate (11%) was observed in species T compared with 31% in species U. Species T and species U are not considered as malaria vectors in India in the field. However, in the laboratory, both these species are able to support the malaria sporogony.

KEYWORDS: *Anopheles fluviatilis*, species complex, *Plasmodium vinckei petteri*, sporogony.

147. Sreenivasamurthy SK, Dey G, Ramu M, Kumar M, Gupta MK, **Mohanty AK**, Harsha HC, **Sharma P**, Kumar N, Pandey A, **Kumar A**, Prasad TS. A compendium of molecules involved in vector-pathogen interactions pertaining to malaria. ***Malar J*** **2013; *12*: 216.**

ABSTRACT

Malaria is a vector-borne disease causing extensive morbidity, debility and mortality. Development of resistance to drugs among parasites and to conventional insecticides among vector-mosquitoes necessitates innovative measures to combat this disease. Identification of molecules involved in the maintenance of complex developmental cycles of the parasites within the vector and the host can provide attractive targets to intervene in the disease transmission. In the last decade, several efforts have been made in identifying such molecules involved in mosquito-parasite interactions and, subsequently, validating their role in the development of parasites within

the vector. In this study, a list of mosquito proteins, which facilitate or inhibit the development of malaria parasites in the midgut, haemolymph and salivary glands of mosquitoes, is compiled. A total of 94 molecules have been reported and validated for their role in the development of malaria parasites inside the vector. This compendium of molecules will serve as a centralized resource to biomedical researchers investigating vector-pathogen interactions and malaria transmission.

KEYWORDS: knockdown, RNAi, gene silencing, *plasmodium*,*anopheles*, oocyst, sporozoite.

148. Tyagi RK, **Das MK**, Singh SS, Sharma YD. Discordance in drug resistance-associated mutation patterns in marker genes of *Plasmodium falciparum* and *Plasmodium knowlesi* during coinfections. ***J Antimicrob Chemother* 2013; *68*(5): 1081–8.**

ABSTRACT

Objectives: Human *Plasmodium knowlesi* infections have been reported from several South-East Asian countries, excluding India, but its drug susceptibility profile in mixed-infection cases remains unknown. **Methods:** The chloroquine resistance transporter (CRT) and dihydrofolate reductase (DHFR) genes of *P. knowlesi* and other *Plasmodium* species were sequenced from clinical isolates obtained from malaria patients living in the Andaman and Nicobar Islands, India. The merozoite surface protein-1 and 18S rRNA genes of *P. knowlesi* were also sequenced from these isolates. **Results:** Among 445 samples analysed, only 53 of them had *P. knowlesi*-specific gene sequences. While 3 of the 53 cases (5.66%) had *P. knowlesi*monoinfection, the rest were coinfected with *Plasmodium falciparum*(86.79%, *n*=46) or *Plasmodium vivax* (7.55%, *n*=4), but none with*Plasmodium malariae* or *Plasmodium ovale*. There was discordance in the drug resistance-associated mutations among the coinfecting *Plasmodium* species. This is because the *P. knowlesi* isolates contained wild-type sequences, while *P. falciparum* isolates had mutations in the CRT and DHFR marker genes associated with a higher level of chloroquine and antifolate drug resistance, respectively. The mutation pattern indicates that the same patient, having a mixed infection, may be harbouring the drug-susceptible *P. knowlesi* parasite and a highly drug-resistant *P. falciparum* parasite. **Conclusions:** A larger human population in South-East Asia may be at risk of *P. knowlesi* infection than reported so far. The different drug susceptibility genotypes of *P. knowlesi* from its coinfecting *Plasmodium*species in mixed infections adds a new dimension to the malaria control programme, requiring formulation of an appropriate drug policy.

KEYWORDS: *P. falciparum, P. knowlesi*, molecular markers, malaria zoonosis, epidemiology.

149. **Singh N**, **Chand SK**, **Bharti PK**, **Singh MP**, Chand G, **Mishra AK**, **Shukla MM**, Mahulia MM, Sharma RK. Dynamics of forest malaria transmission in Balaghat district, Madhya Pradesh, India. ***PLoS ONE* 2013; *8*(9): e73730.**

ABSTRACT

Background: An epidemiological and entomological study was carried out in Balaghat district, Madhya Pradesh, India to understand the dynamics of forest malaria transmission in a difficult and hard to reach area where indoor residual spray and insecticide treated nets were used for vector control. **Methods:** This community based cross-sectional study was undertaken from January 2010 to December 2012 in Baihar and Birsa Community Health Centres of district Balaghat for screening malaria cases. Entomological surveillance included indoor resting collections, pyrethrum spray catches and light trap catches. *Anophelines* were assayed by ELISA for detection of *Plasmodium* circumsporozoite protein. **Findings:** *Plasmodium falciparum* infection accounted for >80% of all infections. *P. vivax* 16.5%, *P. malariae* 0.75% and remaining were mixed infections of *P. falciparum, P. vivax* and *P. malariae*. More than, 30% infections were found in infants under 6 months of age. Overall, an increasing trend in malaria positivity was observed from 2010 to 2012 (chi-square for trend = 663.55; $P<0.0001$). Twenty five *Anopheles culicifacies* (sibling species C, D and E) were positive for circumsporozoite protein of *P. falciparum* (44%) and *P. vivax* (56%). Additionally, 2 *An. fluviatilis*, were found positive for *P. falciparum* and 1 for *P. vivax* (sibling species S and T). *An. fluviatilis* sibling species T was found as vector in forest villages for the first time in India. **Conclusion:** These results showed that the study villages are experiencing almost perennial malaria transmission inspite of indoor residual spray and insecticide treated nets. Therefore, there is a need for new indoor residual insecticides which has longer residual life or complete coverage of population with long lasting insecticide treated nets or both indoor residual spray and long lasting bed nets for effective vector control. There is a need to undertake a well designed case control study to evaluate the efficacy of these interventions.

150. **Haq S**, **Srivastava HC**. Efficacy of *Aphanius dispar* (Rüppell) an indigenous larvivorous fish for vector control in domestic tanks under the Sardar Sarovar Narmada project command area in District Kheda, Gujarat. ***J Vector Borne Dis*** **2013; 50(2): 137–40.**

151. **Bharti PK**, **Chand SK**, **Singh MP**, Mishra S, **Shukla MM**, Singh R, **Singh N**. Emergence of a new focus of *Plasmodium malariae* in forest villages of District Balaghat, central India: Implications for the diagnosis of malaria and its control. ***Trop Med Int Health*** **2013; 18(1): 12–7.**

ABSTRACT

Objective: During an epidemiological study (January–July 2012) on malaria in forest villages of Central India, *Plasmodium malariae*-like malaria parasites were observed in blood smears of fever cases. We aimed to confirm the presence of *P. malariae* using molecular tools *i.e*. species-specific nested polymerase chain reaction (PCR) and DNA sequencing. **Methods:** All fever cases or cases with history of fever in 25 villages of Balaghat district were screened for malaria parasite using bivalent rapid diagnostic test and microscopy after obtaining written informed consent. Nested PCR was employed on microscopically suspected*P. malariae* cases. DNA sequences in the target region for PCR diagnosis were analysed for all the suspected cases of *P. malariae*. **Results:** Among the 22 microscopy suspected *P. malariae* cases, nested PCR confirmed the identity of *P. malariae* in 19 cases. Among these 14 were mono *P. malariae* infections, three were mixed infection of *P. malariae* with *Plasmodium falciparum* and two were mixed infection of *P. malariae* with *Plasmodium vivax*. Clinically *P. malariae* subjects generally presented with fever and headache. However, the typical 3-day pattern of quantum malaria was not observed. The parasite density of *P. malariae* was significantly lower than that of *P. vivax*and *P. falciparum*. **Discussions:** *Plasmodium malariae* may have been in existence in forest villages of central India but escaped identification due to its close resemblance to *P. vivax*. The results re-affirm the importance of molecular methods of testing on routine basis for efficacious control strategies against malaria.

KEYWORDS: malaria, *Plasmodium malariae*, central India, malaria control.

152. **Shah NK**, Poole C, MacDonald PDM, **Srivastava B**, Schapira A, Juliano JJ, **Anvikar A**, Meshnick SR, **Valecha N**, **Mishra N**. Epidemiology of *Plasmodium falciparum* gametocytemia in India: Prevalence, agestructure, risk factors and the role of a predictive score for detection. ***Trop Med Int Health* 2013; *8*(7): 800–9.**

ABSTRACT

Objective: To characterise the epidemiology of *Plasmodium falciparum* gametocytemia and determine the prevalence, age structure and the viability of a predictive model for detection. **Methods:** We collected data from 21 therapeutic efficacy trials conducted in India during 2009–2010 and estimated the contribution of each age group to the reservoir of transmission. We built a predictive model for gametocytemia and calculated the diagnostic utility of different score cut-offs from our risk score. **Results:** Gametocytemia was present in 18% (248/1 335) of patients and decreased with age. Adults constituted 43%, school-age children 45% and under fives 12% of the reservoir for potential transmission. Our model **retained** age, sex, region and previous antimalarial drug intake as predictors of gametocytemia. The area under the receiver operator characteristic curve was 0.76 (95%CI:0.73,0.78), and a cut-off of 14 or more on a risk score ranging from 0 to 46 provided 91% (95%CI:88,95) sensitivity and 33% (95%CI:31,36) specificity for detecting gametocytemia. **Conclusions:** Gametocytemia was common in India and varied by region. Notably, adults contributed substantially to the reservoir for potential transmission. Predictive modelling to generate a clinical algorithm for detecting gametocytemia did not provide sufficient discrimination for targeting interventions.

KEYWORDS: malaria, *Plasmodium falciparum*, disease reservoirs, risk, algorithms, epidemiology, India.

153. Khan V, Zala DB, **Srivastava HC**. Entomological indicators during transmission season of dengue in Silvassa (India). ***J Parasit Dis* 2013; DOI 10.1007/s12639-013-0343-0.**

ABSTRACT

The entomological surveillance was conducted in urban, semi-urban/slum, industrial and residential areas during main transmission period from June to November 2012. In residential sites house index was 41.7–35.0, breteau index 71.7–136.7 and container index 11.6–20.2. During

transmission period all the values ware much higher than the threshold level. The causes of high values of entomological indicator appeared to be rapid industrialization, unawareness of the conditions or factors that can exacerbate mosquito breeding, water storage habits in community and un-implementation of health related legislation.

KEYWORDS: entomological indicators, dengue, *Aedes.*

154. **Mittal PK**, **Nanda N**, **Singh OP**, **Batra CP**, **Adak T**. Establishment of a focus on *Anopheles fluviatilis*, an important malaria vector near the National Thermal Power Corporation Project in Dadri CHC area in District Gautam Budh Nagar, Uttar Pradesh, India. ***J Vector Borne Dis* 2013; *50*(4): 307–10.**

KEYWORDS: *Anopheles fluviatilis*, establishment, Gautam Budh Nagar, malaria vector.

155. Rathi B, Singh AK, Kishan R, **Singh N**, Latha N, **Srinivasan S**, **Pandey KC**, Tiwari HK, Singh BK. Functionalized hydroxyethylamine based peptide nanostructures as potential inhibitors of falcipain-3, an essential proteases of *Plasmodium falciparum*. ***Bioorg Med Chem* 2013, *21*: 5503–9.**

ABSTRACT

Self-assembled peptide based nanostructures gained enough popularity due to their easy biocompatibility and numerous potential applications. An excellent model of self-assembly ofhydroxyethylamine based peptide nanostructures was synthesized and characterized by DLS and TEM. Spherical nano structures of I and III were observed with particle size ~50 and ~80nm, respectively. Further, I and III were screened against anti-malarial target, falcipain-3 (FP3), a crucial cysteine protease involved as a major hemoglobinase of *Plasmodium falciparum*. Interestingly, compound III completely inhibited the activity of FP3. The effective concentration (1.5μM) of III found to be more potent than I. This biochemical result was substantiated by molecular-docking studies indicating III to be best inhibitor of FP3. This is the first report showing that bis hydroxethylamine based peptide nanostructures could be very effective inhibitor of malarial cysteine proteases.

KEYWORDS: antimalarial chemotheraphy, BYZAHKRALXCGNO-XAZDILKDSA-N, Falcipain-3, hemoglobin hydrolysis, hydroxyethylamine, molecular self-assembly, QRJCFENHSFVGNL-MSOLQXFVSA-N, WSDASMLFNPNZOK-JFYQQWHZSA-N.

156. **Kumar N**, Pande V, **Bhatt RM**, **Shah NK**, **Mishra N**, **Srivastava B**, **Valecha N**, **Anvikar AR.** Genetic deletion of HRP2 and HRP3 in Indian *Plasmodium falciparum* population and false negative malaria rapid diagnostic test. ***Acta Trop*** **2013; *125*(1): 119–21.**

ABSTRACT

Genetic polymorphisms in diagnostic antigens are important factors responsible for variable performance of rapid diagnostic tests. Additionally, the failure of antigen expression due to gene deletion may also contribute to variable performance. We report Indian *Plasmodium falciparum* field isolates lacking both *Pfhrp2* and *Pfhrp3* genes leading to false negative results of rapid diagnostic tests. The study highlights need to determine the prevalence of *P. falciparum* isolates lacking these genes in larger field populations in India.

KEYWORDS: malaria, *Pfhrp*2, *Pfhrp*3, genetic deletion, rapid diagnostic tests, India.

157. **Awasthi G**, **Das A**. Genetics of chloroquineresistant malaria: A haplotypic view. ***Mem Inst Oswaldo Cruz*** **2013; *108*: 947–61.**

ABSTRACT

The development and rapid spread of chloroquine resistance (CQR) in *Plasmodium falciparum* have triggered the identification of several genetic target(s) in the *P. falciparum* genome. In particular, mutations in the *Pfcrt* gene, specifically, K76T and mutations in three other amino acids in the region adjoining K76 (residues 72, 74, 75 and 76), are considered to be highly related to CQR. These various mutations form several different haplotypes and *Pfcrt* gene polymorphisms and the global distribution of the different CQR-*Pfcrt* haplotypes in endemic and non-endemic regions of *P. falciparum* malaria have been the subject of extensive study. Despite the fact that the *Pfcrt* gene is considered to be the primary CQR gene in *P. falciparum* , several studies have suggested that this may not be the case. Furthermore, there is a poor correlation between the evolutionary implications of the *Pfcrt* haplotypes and the inferred migration of CQR *P. falciparum* based on CQR epidemiological surveillance data. The present paper aims to clarify the existing knowledge on the genetic basis of the different CQR-

*Pfcrt*haplotypes that are prevalent in worldwide populations based on the published literature and to analyse the data to generate hypotheses on the genetics and evolution of CQR malaria.

KEYWORDS: malaria, chloroquine, *Pfcrt* gene, haplotypes, evolution.

158. Carlton J, **Das A**, Escalante AA. Genomics, population genetics and evolutionary history of *Plasmodium vivax*. ***Adv Parasitol* 2013; *81*: 203–22**.

ABSTRACT

Plasmodium vivax is part of a highly diverse clade that includes several *Plasmodium* species found in nonhuman primates from Southeast Asia. The diversity of primate malarias in Asia is staggering; nevertheless, their origin was relatively recent in the evolution of *Plasmodium*. We discuss how humans acquired the lineage leading to *P. vivax* from a nonhuman primate determined by the complex geological processes that took place in Southeast Asia during the last few million years. We conclude that widespread population genomic investigations are needed in order to understand the demographic processes involved in the expansion of *P. vivax* in the human populations. India represents one of the few countries with widespread *vivax* malaria. Earlier studies have indicated high genetic polymorphism at antigenic loci and no evidence for geographic structuring. However, new studies using genetic markers in selectively neutral genetic regions indicate that Indian *P. vivax* presents complex evolutionary history but possesses features consistent with being part of the ancestral distribution range of this species. Such studies are possible due to the availability of the first *P. vivax* genome sequences. Next generation sequencing technologies are now paving the way for the sequencing of more *P. vivax* genomes that will dramatically increase our understanding of the unique biology of this species.

KEYWORDS: *Plasmodium vivax*, monkey malaria clade, comparative genomics, population genetics, evolution.

159. **Gupta P**, **Pande V**, **Eapen A**, **Singh V**. Genotyping of *MSP3β* gene in Indian *Plasmodium vivax*. ***J Vector Borne Dis* 2013; *50*(3): 197–201.**

ABSTRACT

Background & Objectives: The search for effective polymorphic markers in *Plasmodium vivax* is highly demanding to understand its transmission in a population. Due to the limited knowledge

existing for *P. vivax*, the search for polymorphic markers for population studies is ongoing. The *MSP* gene family of *Plasmodia* has been linked with immune evasion. To study the circulating parasite population *P. vivax* merozoite surface protein 3β (*PvMSP3β*) polymorphic marker was used to investigate the genetic diversity of *P. vivax* in natural infections. **Methods**: Polymorphism of *PvMSP3β* gene was determined in 46 *P. vivax* blood samples from six different regions of India by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) with *PstI*. **Results**: Two different parasite genotypes, *viz*. type-A and type-B were detected among 46 samples that were positive for PCR, based on the size of the amplification. RFLP analysis with *PstI* showed 22 allelic groups and 15.2% samples revealed mixed infections on analysis. **Conclusion**: *PvMSP3β* was found to be an effective molecular marker for *P. vivax* as it shows high diversity in India and multiple genotypes easily distinguishable without the need for sequencing.

KEYWORDS: genotyping, *MSP3β*, *Plasmodium vivax*, RFLP.

160. **Sharma A**, **Dhayal D**, **Singh OP**, **Adak T**, Bhatnagar RK. Gut microbes influence fitness and malaria transmission potential of Asian malaria vector *Anopheles stephensi*. ***Acta Trop* 2013; *128*(1): 41–7.**

ABSTRACT

The midgut of parasite transmitting vector, *Anopheles stephensi* is a physiologically dynamic ecological niche of resident microbes. The gut resident microbes of anisomorphic and physiologically variable male and female *A. stephensi* mosquitoes were different (Rani et al., 2009). To understand the possible interaction of gut microbes and mosquito host, we examined the contribution of the microbe community on the fitness of the adult mosquitoes and their ability to permit development of the malaria parasite. *A. stephensi* mosquitoes were fed with antibiotic to sterilize their gut to study longevity, blood meal digestion, egg laying and maturation capacity, and consequently ability to support malaria parasite development. The sterilization of gutimparted reduction in longevity by a median of 5 days in male and 2 days in female mosquitoes. Similarly, the sterilization also diminished the reproductive potential probably due to increased rate of the resorption of follicles in ovaries coupled with abated blood meal digestion in gut-sterilized females. Additionally, gut sterilization also led to increased susceptibility to oocyst development upon feeding on malaria infected blood. The susceptibility to malaria parasite introduced

upon gut sterilization of *A. stephensi* was restored completely upon re-colonization ofgut by native microbes. The information provided in the study provides insights into the role of the gut-resident microbial community in various life events of the mosquito that may be used to develop alternate malaria control strategies, such as paratransgenesis.

KEYWORDS: *anopheles*, fecundity, gut-biota, longevity, malaria transmittance.

161. **Shah NK**, **Tyagi P**, **Sharma SK**. The impact of artemisinin combination therapy and longlasting insecticidal nets on forest malaria incidence in tribal villages of India, 2006– 2011. ***PLoS ONE* 2013; *8*(2): e56740.**

ABSTRACT

Introduction: New tools for malaria control, artemisinin combination therapy (ACT) and long-lasting insecticidal nets (LLINs) were recently introduced across India. We estimated the impact of universal coverage of ACT and ACT plus LLINs in a setting of hyperendemic, forest malaria transmission. **Methods**: We reviewed data collected through active and passive case detection in a vaccine trial cohort of 2,204 tribal people residing in Sundargarh district, Odisha between 2006 and 2011. We compared measures of transmission at the village and individual level in 2006-2009 versus 2010-2011 after ACT (in all villages) and LLINs (in three villages) were implemented. **Results**: During 2006-2009 malaria incidence per village ranged from 156-512 per 1000 persons per year and slide prevalence ranged from 28-53%. Routine indoor residual spray did not prevent seasonal peaks of malaria. Post-intervention impact in 2010-2011 was dramatic with ranges of 14-71 per 1000 persons per year and 6-16% respectively. When adjusted for village, ACT alone decreased the incidence of malaria by 83% (IRR 0.17, 95%CI: 0.10, 0.27) and areas using ACT and LLINs decreased the incidence of malaria by 86% (IRR 0.14, 95%CI: 0.05, 0.38). After intervention, the age of malaria cases, their parasite density, and proportion with fever at the time of screening increased. **Conclusions**: ACT, and LLINs along with ACT, effectively reduced malaria incidence in a closely monitored population living in a forest ecotype. It is unclear whether LLINs were impactful when prompt and quality antimalarial treatment was available. In spite of universal coverage, substantial malaria burden remained.

162. **Rawat M**, **Vijay S**, Gupta Y, Tiwari PK, **Sharma A**. Imperfect duplicate insertions type of mutations in plasmepsin V modulates binding properties of PEXEL motifs of export proteins in Indian *Plasmodium vivax*. ***PLoS ONE* 2013; *8*(3): e60077.**

ABSTRACT

Introduction: Plasmepsin V (PM-V) have functionally conserved orthologues across the *Plasmodium genus* who's binding and antigenic processing at the PEXEL motifs for export about 200–300 essential proteins is important for the virulence and viability of the causative *Plasmodium*species. This study was undertaken to determine *P. vivax* plasmepsin V Ind (*Pv*PM-V-Ind) PEXEL motif export pathway for pathogenicity-related proteins/antigens export thereby altering*plasmodium* exportome during erythrocytic stages. **Method:** We identify and characterize *Plasmodium vivax* plasmepsin-V-Ind (mutant) gene by cloning, sequence analysis, in *silico* bioinformatic protocols and structural modeling predictions based on docking studies on binding capacity with PEXEL motifs processing in terms of binding and accessibility of export proteins. **Results:** Cloning and sequence analysis for genetic diversity demonstrates *Pv*PM-V-Ind (mutant) gene is highly conserved among all isolates from different geographical regions of India. Imperfect duplicate insertion types of mutations (SVSE from 246–249 AA and SLSE from 266–269 AA) were identified among all Indian isolates in comparison to *P.vivax* Sal-1 (*Pv*PM-V-Sal 1) isolate. *In silico* bioinformatics interaction studies of PEXEL peptide and active enzyme reveal that *Pv*PM-V-Ind (mutant) is only active in endoplasmic reticulum lumen and membrane embedding is essential for activation of plasmepsin V. Structural modeling predictions based on docking studies with PEXEL motif show significant variation in substrate protein binding of these imperfect mutations with data mined PEXEL sequences. The predicted variation in the docking score and interacting amino acids of *Pv*PM-V-Ind (mutant) proteins with PEXEL and lopinavir suggests a modulation in the activity of *Pv*PM-V in terms of binding and accessibility at these sites. **Conclusion/Significance:** Our functional modeled validation of *Pv*PM-V-Ind (mutant) imperfect duplicate insertions with data mined PEXEL sequences leading to altered binding and substrate accessibility of the enzyme makes it a plausible target to investigate export mechanisms for *in silico* virtual screening and novel pharmacophore designing.

163. **Dua VK**, Kumar A, **Pandey AC**, **Kumar S**. Insecticidal and genotoxic activity of *Psoralea corylifolia* Linn. (Fabaceae) against *Culex quinquefasciatus* Say, 1823. ***Parasit Vectors* 2013; *6*: 30.**

ABSTRACT

Background: Indiscriminate use of synthetic insecticides to eradicate mosquitoes has caused physiological resistance. Plants provide a reservoir of biochemical compounds; among these compounds some have inhibitory effect on mosquitoes. In the present study the larvicidal, adulticidal and genotoxic activity of essential oil of *Psoralea corylifolia* Linn. against *Culex quinquefasciatus* Say was explored. **Methods:** Essential oil was isolated from the seeds of *P. corylifolia* Linn. Larvicidal and adulticidal bioassay of *Cx. quinquefasciatus* was carried out by WHO method. Genotoxic activity of samples was determined by comet assay. Identification of different compounds was carried out by gas chromatography- mass spectrometry analysis. **Results:** LC_{50} and LC_{90} values of essential oil were 63.38±6.30 and 99.02±16.63 ppm, respectively against *Cx. quinquefasciatus* larvae. The LD_{50} and LD_{90} values were 0.057±0.007 and 0.109±0.014 mg/cm^2 respectively against adult *Cx. quinquefasciatus*,. Genotoxicity of adults was determined at 0.034 and 0.069 mg/cm^2. The mean comet tail length was 6.2548±0.754 μm and 8.47±0.931 μm and the respective DNA damage was significant *i.e.* 6.713% and 8.864% in comparison to controls. GCMS analysis of essential oil revealed 20 compounds. The major eight compounds were caryophyllene oxide (40.79%), phenol,4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl) (20.78%), caryophyllene (17.84%), α-humulene (2.15%), (+)- aromadendrene (1.57%), naphthalene, 1,2,3,4-tetra hydro-1,6-dimethyle-4-(1-methyl)-, (1S-cis) (1.53%), trans-caryophyllene (0.75%), and methyl hexadecanoate (0.67%). **Conclusion:** Essential oil obtained from the seeds of *P. corylifolia* showed potent toxicity against larvae and adult *Cx. quinquefasciatus*. The present work revealed that the essential oil of *P. corylifolia* could be used as environmentally sound larvicidal and adulticidal agent for mosquito control.

KEYWORDS: larvicidal activity, adulticidal activity, genotoxicity, dna damage, essential oil, *Psoralea corylifolia, Culex quinquefasciatus,* GCMS.

164. **Singh RK**, **Haq S**, **Kumar G**, **Mittal PK**, **Dhiman RC**. Insecticide-susceptibility status of dengue vectors *Aedes aegypti* and *Aedes albopictus* in India: A review. ***Dengue Bull* 2013; *37*: 177–91**

ABSTRACT

In India, *Aedes aegypti* is widely distributed and plays a key role in dengue transmission, as a principal vector. In addition, *Aedes albopictus*, a feral species that breeds in outdoor premises such as tree holes and artificial containers, is surmised as a dengue vector in various areas. This paper reviews studies carried out in India in which insecticides were tested against *Aedes aegypti* and *Aedes albopictus* and where insecticide resistance was reported. Public databases were also searched, using relevant keywords. The review of literature on susceptibility status revealed widespread resistance against dichlorodiphenyltrichloroethane (DDT) in adult as well as immature stages of both *Aedes aegypti* and *Aedes albopictus* mosquitoes. Adult *Aedes aegypti* and *Aedes albopictus* have been found to be susceptible to malathion (organophosphate) and synthetic pyrethroids. There are some reports of tolerance and development of resistance against malathion in the adult as well as larval stages of *Aedes* mosquito. In general, the aquatic stages of *Aedes aegypti* are still susceptible to conventional larvicides, namely temephos, *Bacillus thuringiensis israelensis* (Bti), etc., which are commonly used for larval control in the country. The immature stages of *Aedes* mosquitoes have shown a tendency to develop induced resistance to temephos under laboratory conditions. In epidemic conditions, spraying with an appropriate insecticide, to which both *Aedes aegypti* and *Aedes albopictus* mosquitoes are fully susceptible, may be undertaken for rapid control.

KEYWORDS: dengue vector, India, insecticide susceptibility, review.

165. **Mishra N**, **Gupta R**, **Singh S**, **Rana R**, **Shahi B**, **Das MK**, **Anvikar AR**, **Valecha N**. Insights following change in drug policy: A descriptive study for antimalarial prescription practices in children of public sector health facilities in Jharkhand state of India. ***J Vector Borne Dis* 2013; *50*(4): 271–7.**

ABSTRACT

Background & Objectives: Widespread resistance to chloroquine was the mainstay to implement artemisininbased combination therapy (ACT) in the year 2007 in few malaria endemic states in India including Jharkhand as the first line of treatment for uncomplicated *Plasmodium falciparum* malaria. This study was conducted in Jharkhand state of the country just after the implementation of ACT to assess the prevailing antimalarial drug prescribing practices, availability of antimalarial drugs and the acceptability of the new policy by the health professionals for the treatment of uncomplicated *P. falciparum* malaria patients particularly in children ≤15 yr of age. **Methods**: This is a cross-sectional study in children aged ≤15 yr with malaria or to whom antimalarial drug was prescribed. Main outcome measure was prescription of recommended ACT in children aged ≤15 yr with malaria in the selected areas of Jharkhand. **Results**: In the year 2008, artemisinin-based combination therapy (ACT) was implemented in 12 districts of the studied state; however, the availability of ACT was confirmed only in five districts. Antimalarial prescription was prevalent amongst the undiagnosed (8.4%), malaria negative (64.3%) and unknown blood test result (1.2%) suggesting the prevalence of irrational treatment practices. ACT prescription was very low with only 3.2% of confirmed *falciparum* malaria patients receiving it while others received either non-artesunate (NA) treatment (88.1%) including chloroquine (CQ) alone, CQ + Primaquine (PQ)/other drugs, sulphadoxine-pyrimethamine (SP) alone, SP + other drugs or artemisinin monotherapy (AM) treatment (6.3%). Still others were given nonantimalarial treatment (NM) in both malaria positive (0.3%) and malaria negative (2.1%) cases. **Interpretation & conclusion**: Despite the change in drug policy in the studied state the availability and implementation of ACT was a major concern. Nevertheless, the non-availability of blister packs for children aged ≤15 yr was the main hindrance in the implementation of the recommended antimalarial. Availability, training and participation of health professionals in decision-making are the key elements to improve adherence to new treatment guidelines. This study provided evidence for the requirement of age-specific blister packs in the country and the national programme has introduced age-specific blister packs in the country in 2010. This baseline information will be useful to monitor the progress in ACT implementation in the country.

KEYWORDS: ACT, antimalarial prescription practices, implementation of drug policy, India.

166. **Prajapati SK**, **Singh OP**. Insights into the invasion biology of *Plasmodium vivax*. ***Front Cell Infect Microbiol*** **2013; *3*: 8.**

167. Baeza A, Bouma M J, **Dhiman RC**, Baskerville EB, Ceccoto P, **Yadav RS**, Pascual M. Longlasting transition toward sustainable elimination of desert malaria under irrigation development. ***Proc Nat Acad Sci USA* 2013; *110*(37): 15157–62.**

ABSTRACT

In arid areas, people living in the proximity of irrigation infrastructure are potentially exposed to a higher risk of malaria due to changes in ecohydrological conditions that lead to increased vector abundance. However, irrigation provides a pathway to economic prosperity that over longer time scales is expected to counteract these negative effects. A better understanding of this transition between increased malaria risk and regional elimination, in particular whether it is slow or abrupt, is relevant to sustainable development and disease management. By relying on space as a surrogate for stages of time, we investigate this transition in a semidesert region of India where a megairrigation project is underway and expected to cover more than 1,900 million hectares and benefit around 1 million farmers. Based on spatio-temporal epidemiological cases of *Plasmodium vivax* malaria and land-use irrigation from remote sensing sources, we show that this transition is characterized by an enhanced risk in areas adjacent to the trunk of the irrigation network, despite a forceful and costly insecticide-based control. Moreover, this transition between climate-driven epidemics and sustained low risk has already lasted a decade. Given the magnitude of these projects, these results suggest that increased health costs have to be planned for over a long time horizon. They further highlight the need to integrate assessments of both health and environmental impacts to guide adaptive mitigation strategies. Our results should help to define and track these transitions in other arid parts of the world subjected to similar tradeoffs.

KEYWORDS: agricultural development, environmental health, epidemic malaria, irrigation gradient, vector-borne diseases.

168. **Pandey KC**. Macromolecular inhibitors of malarial cysteine proteases: An invited review. ***J Biomed Sci Engg* 2013; *6*: 885–95.**

ABSTRACT

There are evidences indicating that cysteine proteases play an essential role in malaria parasites; therefore, an obvious area of investigation is the inhibition of these enzymes to treat malaria. Small cysteine protease inhibitors of malaria are well studied, but macromolecular nature of inhibitor is a new field to explore. In malarial cysteine proteases, there are macromolecular endogenous inhibitors playing important roles in regulation of the cysteine protease activity of parasite and host. Recent studies suggested that there are known and characterized endogenous inhibitors like falstatin present in *P. falciparum*, PbICP (inhibitor of cysteine protease in *P. berghei*), PyICP (inhibitor of cysteine protease in *P. yoelli*), and other macromolecular inhibitors which are the prodomain of enzyme itself regulating the activity of the mature enzyme. All the known macromolecular endogenous inhibitors are using specific loop-like structure to interact with malarial cysteine proteases. The majority of macromolecular inhibitors are competitive in nature, and block access to the active site of their target protease, but do not bind in a strictly substrate-like manner. They rather interact with the protease subsites and catalytic residues in a non-catalytically competent manner. In future, designing inhibitors based on these protein-protein interactions will be a new approach in the field of malaria. Since macromolecular inhibitors can gain potency through the burial of a large surface area and specificity through contacts with secondary binding sites critical for inhibition, and could be less prone to drug resistant mutation.

KEYWORDS: malaria, cysteine protease, hemoglobinase, macromolecular inhibitor, protein-protein interaction.

169. Cash BA, Rodo X, Ballester J, Bouma MJ, Baeza A, **Dhiman RC**, Pascual M. Malaria epidemics and the influence of the tropical South Atlantic on the Indian monsoon. ***Nature Climate Change* 2013; *3*: 502–7.**

ABSTRACT

The existence of predictability in the climate system beyond the relatively short timescales of synoptic weather1, 2 has provided significant impetus to investigate climate variability and its consequences for society. In particular, relationships between the relatively slow changes in sea surface temperature (SST) and climate variability at widely removed points across the globe provide a basis for statistical and dynamical efforts to predict numerous phenomena, from rainfall to disease incidence, at seasonal to decadal timescales. We describe here a remote influence,

identified through observational analysis and supported through numerical experiments with a coupled atmosphere–ocean model, of the tropical South Atlantic (TSA) on both monsoon rainfall and malaria epidemics in arid northwest India. Moreover, SST in the TSA is shown to provide the basis for an early warning of anomalous hydrological conditions conducive to malaria epidemics four months later, therefore at longer lead times than those afforded by rainfall. We find that the TSA is not only significant as a modulator of the relationship between the monsoon and the El Niño/Southern Oscillation, as has been suggested by previous work3, 4, but for certain regions and temporal lags is in fact a dominant driver of rainfall variability and hence malaria outbreaks.

170. Nagaraj VA, Sundaram B, Varadarajan NM, **Subramani PA**, **Kalappa DM**, **Ghosh SK**, Padmanaban G. Malaria parasite-synthesized heme is essential in the mosquito and liver stages and complements host heme in the blood stages of infection. ***PLoS Pathogen*** **2013; *9*(8): e1003522.**

ABSTRACT

Heme metabolism is central to malaria parasite biology. The parasite acquires heme from host hemoglobin in the intraerythrocytic stages and stores it as hemozoin to prevent free heme toxicity. The parasite can also synthesize heme *de novo*, and all the enzymes in the pathway are characterized. To study the role of the dual heme sources in malaria parasite growth and development, we knocked out the first enzyme, δ-aminolevulinate synthase (ALAS), and the last enzyme, ferrochelatase (FC), in the heme-biosynthetic pathway of *Plasmodium berghei*(*Pb*). The wild-type and knockout (KO) parasites had similar intraerythrocytic growth patterns in mice. We carried out *in vitro* radiolabeling of heme in *Pb*-infected mouse reticulocytes and*Plasmodium falciparum*-infected human RBCs using [4-^{14}C] aminolevulinic acid (ALA). We found that the parasites incorporated both host hemoglobin-heme and parasite-synthesized heme into hemozoin and mitochondrial cytochromes. The similar fates of the two heme sources suggest that they may serve as backup mechanisms to provide heme in the intraerythrocytic stages. Nevertheless, the *de novo* pathway is absolutely essential for parasite development in the mosquito and liver stages. *Pb*KO parasites formed drastically reduced oocysts and did not form sporozoites in the salivary glands. Oocyst production in *Pb*ALASKO parasites recovered when mosquitoes received an ALA supplement. *Pb*ALASKO sporozoites could infect mice only when the mice received an ALA supplement. Our results indicate the potential for new therapeutic interventions targeting the heme-biosynthetic pathway in the parasite during the mosquito and liver stages.

171. **Thakur RS**, Tousif S, **Awasthi V**, **Sanyal A**, **Atul PK**, **Punia P**, **Das J**. Mesenchymal stem cells play an important role in host protective immune responses against malaria by modulating regulatory T-cells. ***Eur J Immunol* 2013; *43*: 2070–7.**

ABSTRACT

Plasmodium spp. parasites, the causative agents of malaria, survive and replicate in human hosts by modulating host protective immune responses. In a rodent model, malaria manifests as a severe splenomegaly, with infiltration of cells and lympho-proliferation as major contributing factors of the immunopathology. However, the cellular contents and the functions of these cells have not been well studied. Here, we report that *Plasmodium berghei* infection of mice leads to massive recruitment of mesenchymal stem cells (MSCs) in secondary lymphoid organs. Infusion of these cells into naïve mice was able to confer host resistance against malaria. Furthermore, MSCs augmented interleukin (IL)-12 production but suppressed IL-10 production in recipient animals. In addition, we observed dramatic reductions of regulatory T (Treg) cells in animals that received MSCs. Taken together, our findings have identified recruitment of MSCs as a novel host protective mechanism adopted by the host to combat malaria by modulating Treg-cell responses.

KEYWORDS: hemozoin, malaria, mesenchymal stem cell, regulatory T cell.

172. **Mallick PK**, Sutton PL, Singh R, **Singh OP**, **Dash AP**, Singh AK, Carlton JM, Bhasin VK. Microsatellite analysis of chloroquine resistance associated alleles and neutral loci reveal genetic structure of Indian *Plasmodium falciparum*. ***Infect Genet Evol* 2013; *19*: 164– 75.**

ABSTRACT

Efforts to control malignant malaria caused by *Plasmodium falciparum* are hampered by the parasite's acquisition of resistance to antimalarial drugs, *e.g.*, chloroquine. This necessitates evaluating the spread of chloroquine resistance in any malaria-endemic area. India displays highly variable malaria epidemiology and also shares porous international borders with malaria-endemic Southeast Asian countries having multi-drug resistant malaria. Malaria epidemiology in India is believed to be affected by two major factors: high genetic diversity and evolving drugresistance in *P. falciparum*. How transmission intensity of malaria can influence the genetic

structure of chloroquine-resistant *P. falciparum* population in India is unknown. Here, geneticdiversity within and among *P. falciparum* populations is analyzed with respect to their prevalence and chloroquine resistance observed in 13 different locations in India. Microsatellites developed for *P. falciparum*, including three putatively neutral and seven microsatellites thought to be under a hitchhiking effect due to chloroquine selection were used. Genetic hitchhiking is observed in five of seven microsatellites flanking the gene responsible for chloroquine resistance. Geneticadmixture analysis and F-statistics detected genetically distinct groups in accordance with transmission intensity of different locations and the probable use of chloroquine. A large geneticbreak between the chloroquine-resistant parasite of the Northeast-East-Island group and Southwest group (F_{ST}=0.253, P<0.001) suggests a long period of isolation or a possibility of different origin between them. A pattern of significant isolation by distance was observed in low transmission areas (r=0.49, P=0.003, N=83, Mantel test). An unanticipated pattern of spread of hitchhiking suggests genetic structure for Indian *P. falciparum* population. Overall, the study suggests that transmission intensity can be an efficient driver for genetic differentiation at bothneutral and adaptive loci across India.

KEYWORDS: chloroquine resistance, gene-flow, hitchhiking, India, *Plasmodium falciparum*, transmission area.

173. Nizamuddin Mohammad, Kogan F, **Dhiman RC**, Guo Wei, Roytman L. Modelling and forecasting malaria in Tripura, India using NOAA/AVHRR-based vegetation health indices. ***Int J Remote Sens App*** **2013; *3*(3): 108–16.**

ABSTRACT

Improved forecasting, prevention and control of epidemics are the key technical elements for malaria eradication program. The objective is to use NOAA/AVHRR environmental satellite data to produce weather seasonal forecasts for using as a proxy for predicting malaria epidemics in Tripura state, India which has one of the highest endemic of malaria cases in the country. An algorithm has been reported that uses Vegetation Health (VH) Indices (Vegetation Condition Index (VCI) and Temperature Condition Index (TCI)) computed from Advance Very High Resolution Radiometer (AVHRR) data flown on NOAA afternoon polar orbiting satellite. A significant relationship between satellite data and annual malaria incidences is found at least three months

before the major malaria transmission period. Principal component regression (PCR) method was used to develop a model to predict malaria as a function of the TCI. The simulated results were compared with observed malaria statistics showing that the error of estimations of malaria is insignificant. Optical remote sensing therefore is a valuable tool to estimate malaria well in advance so that preventive measures can be taken.

KEYWORDS: AVHRR, VCI, TCI, malaria, principal component regression.

174. **Singh SP**, Mohan L. Studies on Morphological variations of spiracles in three vector species of mosquitoes. ***J Entomol Res* 2013; *37*(2): 177– 81.**

ABSTRACT

Laboratory reared and wild populations of *Anopheles stephensi*, vector of malaria; *Culex quinquefasciatus*, vector of filaria and *Aedes aegypti*, vectors of dengue, were subjected to study the spiracular indices. The average length of anterior spiracle of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* ranged from 0.1 to 0.12, 0.11 to 0.14 and 0.14 to 0.15 mm respectively. Similarly the average length of posterior spiracle ranged from 0.1 to 0.12, 0.13 to 0.15 and 0.14 to 0.15 mm respectively. The average of spiracular indices of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* ranged from 7.07 to 8.13, 8.05 to 9.05 and 9.02 to 10.01 mm respectively. In wild populations, the average length of anterior spiracle of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* ranged from 0.07 to 0.11, 0.12 to 0.13 and 0.14 to 0.16 mm respectively. The average length of posterior spiracle ranged from 0.08 to 0.11, 0.13 to 0.14 and 0.14 to 0.17 mm respectively. The average of spiracular indices of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* ranged from 6.9 to 7.10, 8.05 to 9.6 and 11.01 to 11.7 mm respectively. These measures provide structurally differentiation of mosquito vectors.

KEYWORDS: mosquito vector, thoracic spiracle, spiracular indices, malaria.

175. **Singh SP**, **Mittal PK**. Mosquito repellent and oviposition deterrent activities of *Solanum nigrum* seed extract against mosquito vectors. ***Online Int Interdiscip Res J* 2013; *3*(6).**

ABSTRACT

This study was carried out to evaluate mosquito repellent and oviposition deterrent activities of *Solanum nigrum* against malaria vector *Anopheles stephensi*. Hexane extract of the seeds of *Solanum nigrum* was used for repellent and oviposition deterrent activity against mosquito vector *Anopheles stephensi* Liston (Diptera: Culicidae) in laboratory bio-assays. Percent protection obtained against *An. stephensi* was 100% in 0 hours and 81% after 6 hours at the 10% concentration of the extract as compared to 100% after 6 hours at 2.5% DEET solution. The concentrations of the hexane extract of the seeds of *Solanum nigrum* ranging between 0.03125% and 0.5% showed 27 to 99.5% oviposition deterrence in treated bowls as compared to untreated control. These observations show that the *Solanum nigrum* seed extract is an effective personal protection measure and oviposition deterrent against mosquito vectors.

KEYWORD: *Solanum nigrum*, repellency, oviposition deterrent, *Anopheles stephensi*.

176. **Khan N**, Pande V, **Das A**. NAT2 sequence polymorphisms and acetylation profiles in Indians. ***Pharmacogenomics* 2013; *14*(3): 289–303.**

ABSTRACT

Background: NAT2, a broad-spectrum drug-metabolizing gene, is of high pharmacogenetic interest. Based on seven different mutations in the NAT2 gene, an individual can either be categorized as a slow or fast acetylator. **Materials & methods:** In order to characterize acetylation profiles of Indians, where data are poorly available, we sequenced the 873 bp NAT2 coding region in 250 Indians, covering the whole of India including three tribes. **Results:** Altogether, 35 NAT2 alleles forming two acetylator phenotypes (distributed almost in equal proportion in India) were found; while the alleles determining slow acetylators were highly differentiated, the fast acetylator alleles were less in number but highly frequent. **Conclusion:** Interestingly, distribution of two different acetylation phenotypes correlated well with historical dietary pattern in India. The neighbor-joining phylogenetic tree based on NAT2 gene polymorphisms in worldwide humans revealed genetic affinities among populations with similar acetylation phenotypes, which also placed Indians and Africans together in a single cluster.

KEYWORDS: acetylation phenotypes, drug metabolization, India, N-acetyltransferase 2, NAT2, pharmacogenetics.

177. **Prajapati SK**, **Joshi H**, Carlton JM, Rizvi MA. *Neutral polymorphisms in putative housekeeping genes and tandem repeats unravels the population genetics and evolutionary history of Plasmodium vivax in India*. ***PLoS Negl Trop Dis*** **2013; *7*(9): e2425.**

ABSTRACT

The evolutionary history and age of *Plasmodium vivax* has been inferred as both recent and ancient by several studies, mainly using mitochondrial genome diversity. Here we address the age of *P. vivax* on the Indian subcontinent using selectively neutral housekeeping genes and tandem repeat loci. Analysis of ten housekeeping genes revealed a substantial number of SNPs (n=75) from 100 *P. vivax* isolates collected from five geographical regions of India. Neutrality tests showed a majority of the housekeeping genes were selectively neutral, confirming the suitability of housekeeping genes for inferring the evolutionary history of *P. vivax*. In addition, a genetic differentiation test using housekeeping gene polymorphism data showed a lack of geographical structuring between the five regions of India. The coalescence analysis of the time to the most recent common ancestor estimate yielded an ancient TMRCA (232,228 to 303,030 years) and long-term population history (79,235 to 104,008) of extant *P. vivax* on the Indian subcontinent. Analysis of 18 tandem repeat loci polymorphisms showed substantial allelic diversity and heterozygosity per locus, and analysis of potential bottlenecks revealed the signature of a stable *P. vivax* population, further corroborating our ancient age estimates. For the first time we report a comparable evolutionary history of *P. vivax* inferred by nuclear genetic markers (putative housekeeping genes) to that inferred from mitochondrial genome diversity.

178. **Shah NK**, Schapira A, Juliano JJ, **Srivastava B**, MacDonald PDM, Poole C, **Anvikar A**, Meshnick SR, **Valecha N**, **Mishra N**. Nonrandomized controlled trial of artesunate plus sulfadoxine-pyrimethamine with or without primaquine for preventing posttreatment circulation of *Plasmodium falciparum* gametocytes. ***Antimicrob Agents Chemother*** **2013; *57*(7): 2948–54.**

ABSTRACT

Artemisinin combination therapies eliminate immature *Plasmodium falciparum*gametocytes but not mature gametocytes, which may persist for up to 1 month posttreatment. A single dose of primaquine, which is inexpensive and effective against mature gametocytes, could be added to

further reduce the potential for posttreatment parasite transmission. Currently, we have few data regarding the effectiveness or safety of doing so. We collected data from 21 therapeutic efficacy trials of the National Antimalarial Drug Resistance Monitoring System of India conducted during 2009 to 2010, wherein 9 sites used single-dose primaquine (0.75 mg/kg of body weight) administered on day 2 along with artesunate plus sulfadoxine-pyrimethamine (AS+SP) while 12 did not. We estimated the effect of primaquine on posttreatment gametocyte clearance and the total number of gametocyte-weeks as determined by microscopy. We compared the median area under the curve for gametocyte density and reported adverse events. One thousand three hundred thirty-five patients completed the antimalarial drug treatment. Adjusting for region, primaquine increased the rate of gametocyte clearance (hazard ratio, 1.9; 95% confidence interval [CI], 1.1 to 3.3), prevented 45% (95% CI, 19 to 62) of posttreatment gametocyte-weeks, and decreased the area under the gametocyte density curve over the 28-day follow-up compared to AS+SP alone (*P* value = 0.01). The results were robust to other adjustment sets, and the estimated effect of primaquine increased during sensitivity analysis on the measurement of exposure time. No serious adverse events were detected. In conclusion, the addition of primaquine to AS+SP was effective in reducing the posttreatment presence of *P. falciparum* gametocytes. Primaquine was well tolerated and could be administered along with an artemisinin combination therapy as the first-line therapy.

179. **Nanda N**, **Singh OP**, **Dua VK**, **Pandey AC**, **Nagpal BN**, **Adak T**, **Dash AP**, **Subbarao SK**. Population cytogenetic and molecular evidence for existence of a new species in *Anopheles fluviatilis* complex (Diptera: Culicidae). ***Infect Genet Evol*** **2013; *13*: 218– 23.**

ABSTRACT

Anopheles fluviatilis James, an important malaria vector in the Oriental region has been established as a complex of at least three cryptic species which vary in their biological characteristics and malaria transmission potential. The sibling species S, T and U of *Fluviatilis* Complex can be identified by examination of species-specific fixed inversions in the polytene chromosomes and can also be differentiated by an allele-specific PCR assay based on differences in the D3 region of 28S ribosomal DNA (rDNA) of these species. Here we report anew *An. fluviatilis* population from villages under Laksar Community Health Centre, District Haridwar (Uttarakhand state), India which differs from the three sibling species of *Fluviatilis* Complex by two fixed paracentric

inversions, s(1) and S in polytene chromosome arms 2 and 3 respectively. Longitudinal study carried out in study villages showed that the new cytotype was sympatric with species T and U in all the collections and no inversion heterozygotes were observed between them. Thus presence of two fixed paracentric inversions in polytene chromosomes with total absence of inversion heterozygotes demonstrates reproductive isolation which unequivocally establishes this cytological variant as a new species, provisionally designated as species V in the *Fluviatilis* Complex. Analysis of DNA sequences of D3 domain of 28S rDNA and ITS 2 region has also shown that species V is distinctly different from species S, T and U. With the discovery of new species in the *Fluviatilis* Complex, in-depth studies are required to know its distribution pattern and biological characteristics and to ascertain its role in malaria transmission.

KEYWORDS: *Anopheles fluviatilis*, sibling species, polytene chromosomes, paracentric inversions, 28S rDNA, ITS2 region.

180. Roy M, Bouma MJ, Ionides EL, **Dhiman RC**, Pascual M. The potential elimination of *Plasmodium vivax* malaria by relapse treatment: Insights from a transmission model and surveillance data from NW India. ***PLoS Negl Trop Dis* 2013; *7*(1): e1979.**

ABSTRACT

Background: With over a hundred million annual infections and rising morbidity and mortality, *Plasmodium vivax* malaria remains largely a neglected disease. In particular, the dependence of this malaria species on relapses and the potential significance of the dormant stage as a therapeutic target, are poorly understood. **Methodology/Principal Findings**: To quantify relapse parameters and assess the population-wide consequences of anti-relapse treatment, we formulated a transmission model for *P. vivax* suitable for parameter inference with a recently developed statistical method based on routine surveillance data. A low-endemic region in NW India, whose strong seasonality demarcates the transmission season, provides an opportunity to apply this modeling approach. Our model gives maximum likelihood estimates of 7.1 months for the mean latency and 31% for the relapse rate, in close agreement with regression estimates and clinical evaluation studies in the area. With a baseline of prevailing treatment practices, the model predicts that an effective anti-relapse treatment of 65% of those infected would result in elimination within a decade, and that periodic mass treatment would dramatically reduce the burden of the disease in a few years. **Conclusion/Significance**: The striking dependence of *P. vivax* on relapses for

survival reinforces the urgency to develop more effective anti-relapse treatments to replace Primaquine (PQ), the only available drug for the last fifty years. Our methods can provide alternative and simple means to estimate latency times and relapse frequency using routine epidemiological data, and to evaluate the population-wide impact of relapse treatment in areas similar to our study area.

181. Srivastava DK, **Dev V**, Bhattacharya S. Predatory potential of indigenous and exotic larvivorous fish for control of mosquito breeding. ***J App Biosci* 2013; *39*(2): 127–30.**

182. **Singh R**, **Savargaonkar D**, **Bhatt R**, **Valecha N**. Rapid detection of *Plasmodium vivax* in saliva and blood using loop mediated isothermal amplification (LAMP) assay. ***J Infect* 2013; *67*(3): 245–7.**

183. **Mallick PK**, **Singh R**, **Singh OP**, Singh AK, Bhasin VK, **Valecha N**. Reduced heterozygosity at intragenic and flanking microsatellites of *pfcrt* gene establishes natural selection based molecular evolution of chloroquine-resistant *Plasmodium falciparum* in India. ***Infect Genet Evol* 2013; *20*: 407–12.**

ABSTRACT

The positive selection of a nucleotide substitution in exon 2 of *Plasmodium falciparum chloroquine resistance transporter* (*pfcrt*) gene (mutation responsible for chloroquine resistance) causes a reduction in variation of neutral loci close to the gene. This reduction in allelic diversity around flanking regions of *pfcrt* gene was reported in worldwide chloroquine resistant isolates and referred as selective sweep. In *Plasmodium falciparum* isolates of India, the selective sweep in flanking loci of *pfcrt* gene is well established, however, high allelic diversity observed in intragenic microsatellites of *pfcrt* gene implied an ongoing genetic recombination. To understand, if molecular evolution of chloroquine-resistant *P. falciparum* isolates in India follow a selective sweep model, we analyzed genetic diversity at both seven intragenic and seven flanking microsatellites of *pfcrt* (−24 to +106 kb) gene in chloroquine sensitive and resistant parasites originating from high and low transmission areas. We observed low expected heterozygosity at all loci of resistant *pfcrt*-haplotypes (H_e = 0–0.77) compared to the wild-type (H_e = 0.38–0.96). Resistant SVMNT from high transmission areas showed significantly higher mean H_e (P = 0.03, *t*-

test) at both intragenic and *pfcrt*-flanking loci (−24 to +22 kb) in comparison to low transmission areas. Our observation of reduction in variation at both intragenic and flanking loci of mutant *pfcrt* gene confirmed the selective sweep model of natural selection in chloroquine resistant *P. falciparum* isolates in India.

KEYWORDS: chloroquine resistance, *pfcrt* gene; microsatellite, heterozygosity, *plasmodium*.

184. **Prajapati SK**, **Singh OP**. Remodeling of human red cells infected with *Plasmodium falciparum* and the impact of PHIST proteins. ***Blood Cells Mol Dis*** **2013; *51*(3): 195–202.**

ABSTRACT

In an infected erythrocyte (iRBC), renovation and decoration are crucial for malarial parasite survival, pathogenesis and reproduction. Host cell remodeling is mediated by an array of diverse parasite-encoded export proteins that traffic within iRBC. These remodeling proteins extensively modify the membrane and cytoskeleton of iRBC and help in formation of parasite-induced novel organelles such as 'Maurer's Cleft (MC), tubulovesicular network (TVN) and parasitophorous vacuole membrane (PVM) inside the iRBC. The genome sequence of *Plasmodium falciparum* shows expansion of export proteins, which suggests a complex requirement of these export proteins for specific pathogenesis and erythrocyte remodeling. *Plasmodium helical intersperse sub-telomeric (PHIST)* is a family of seventy-two small export proteins and many of its recently discovered functional characteristics suggest an intriguing putative role in modification of an iRBC. This review highlights the recent advances in parasite genomics, proteomics, and cell biology studies unraveling the host cell modification; providing a speculation on the impact of PHIST proteins in modification of the iRBC.

KEYWORDS: *Plasmodium falciparum*, host cell remodeling, remodeling proteins, phist proteins, erythrocyte.

185. Kant R, **Haq S**, **Srivastava HC**, **Sharma VP**. Review of the bioenvironmental methods for malaria control with special reference to the use of larvivorous fishes and composite fish culture in central Gujarat, India. ***J Vector Borne Dis*** **2013; *50*: 1 –12.**

ABSTRACT

Mosquito control with the use of insecticides is faced with the challenges of insecticide resistance in disease vectors, community refusal, their high cost, operational difficulties, and environmental concern. In view of this, integrated vector control strategies with the use of larvivorous fishes such as Guppy (*Poecilia reticulata*) and Gambusia (*G. affinis*) as biological control agents were used in controlling mosquito breeding in different types of breeding places such as intradomestic containers, various types of wells, rice-fields, pools, ponds and elsewhere in malaria prone rural areas of central Gujarat. Attempts were also made to demonstrate composite fish culture in unused abandoned village ponds by culturing Guppy along with the food fishes such as Rohu (*Labeo rohita*), Catla (*Catla catla*) and Mrigal (*Cirrhinus mrigala*). Income generated from these ponds through sale of fishes was utilized for mosquito control and village development. The technology was later adopted by the villagers themselves and food fish culture was practised in 23 ponds which generated an income of `1,02,50,992 between 1985 and 2008. The number of villages increased from 13 to 23 in 2008 and there was also gradual increase of income from ` 3,66,245 in 1985–90 to ` 55,06,127 in 2002–08 block. It is concluded that larvivorous fishes can be useful tool in controlling mosquito breeding in certain situations and their use along with composite fish culture may also generate income to make the programme self-sustainable.

KEYWORDS: composite fish culture, larvivorous fishes, malaria, mosquito larval control.

186. **Valecha N**, **Srivastava B**, Dubhashi NG, Rao BH, **Kumar A**, **Ghosh SK**, **Singh JP**, Kiechel JR, Sharma B, Jullien V, **Dash AP**, Taylor WR, **Anvikar AR**. Safety, efficacy and population pharmacokinetics of fixed-dose combination of artesunate-mefloquine in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in India. *J **Vector Borne Dis*** **2013; *50*(4): 258–64.**

ABSTRACT

Background & Objectives: India has switched over to artemisinin-based combination therapy (ACT) for the treatment of acute uncomplicated *Plasmodium falciparum* malaria and the ACT used in the national programme is artesunate + sulphadoxine-pyrimethamine. Since the efficacy of ACT is dependent also on the partner drug, there is a need to evaluate and deploy multiple ACTs. **Methods**: This multicentre, single-arm, open-label clinical trial was carried out to assess the efficacy, safety and population pharmacokinetics of a fixed dose combination (FDC) artesunate mefloquine (ASMQ) in *P. falciparum* infected, Indian adults at Panjim, Goa, and Mangalore,

Karnataka between December 2007 and November 2008. **Results**: A total of 77 patients (males 74) were screened and enrolled: 42 at Goa and 35 at Mangalore with a median age of 25 yr (range 18–55 yr). One patient failed in treatment on D53, a PCR proven new infection, seven developed recurrent *vivax* parasitaemia and 11 did not have a parasitological endpoint. By per protocol analysis, the D63 cure rate was 58/59 (98.3; 95% C.I. 90.9–99.9%), and 58/58, with PCR correction. ASMQ was welltolerated and no serious adverse events were reported. **Interpretation & Conclusion:** The study showed that the ASMQ FDC was efficacious and well-tolerated for the treatment of acute, uncomplicated *P. falciparum* malaria in highly endemic, chloroquine resistant areas of Goa and Mangalore. It is a viable option for India.

KEYWORDS: artesunate, India, malaria, mefloquine, *P. falciparum*, pharmacokinetics.

187. **Singh RK**, **Haq S**, **Dhiman RC**. Studies on knowledge, attitude and practices in malaria endemic tribal areas of Bihar and Jharkhand, India. ***J Trop Dis*** **2013; *1*(3): 110.**

ABSTRACT

Knowledge, attitude and practices (KAP) in tribal and rural population of four malaria endemic districts in Bihar and Jharkhand states in respect of malaria were studied. The results of this study showed that most of the respondents (92.5%) were known to malaria and aware of the common symptoms of malaria (82.4%) like fever, shivering and cold etc. However, considerable (28.4%) number was not aware that malaria is caused by mosquito bite. Knowledge about resting sites of malaria vectors was good as 48.8% respondents reported cattle sheds, 32.4% respondents reported human dwellings and 15% damp dark places. Most of the respondents were not aware of mosquito breeding associated with clean water bodies. The attitude of respondents towards vector control programme was very poor as 67.8% respondents lost faith in DDT spraying because of its ineffectiveness in controlling the mosquito nuisance. More than two third of respondents were regular user of treated bed nets and it was considered the best option for protection from malaria and mosquito bite. Proper health education is warranted to increase community knowledge and awareness in local language at the individual and community level to promote malaria prevention, to enhance the IRS coverage and use of bed nets for successful malaria control. Insecticide spray may be more acceptable to the communities, if appropriate and more effective insecticide is being used.

KEYWORDS: malaria, vector mosquito, IRS, bed nets, KAP.

188. **Bali P**, **Pradhan S**, **Sharma D**, **Adak T**. Toll like receptor 2 and 4 polymorphisms in malaria endemic populations of India. ***Hum Immunol* 2013; *74*(2): 223–9.**

ABSTRACT

Toll like receptors (TLRs) play a pivotal role in recognizing the invading malaria parasite *Plasmodium*, thus genetic makeup of the exposed population can be of utmost importance for its predisposition to malaria. In this study 264 malaria patients from seven different eco epidemiological regions of India were genotyped for TLR2 and TLR4 polymorphisms using DNA sequencing methods. No variation was observed at residue positions 677 and 753 in TLR2 whereas residue positions 299 and 399 in TLR4 were highly polymorphic. The GC haplotype (Asp299Gly/Thr399Thr) was observed at the highest frequency in populations of East Singhbhum, Vizianagaram and North Goa and absent in Kolkata, Dakshin Kannada and Nicobar district. All polymorphisms were in Hardy Weinberg equilibrium. Populations of Kolkata, Nicobar district, Sundergarh and Dakshin Kannada were observed to be closely related. TLR2 polymorphism was absent in the Indian population and an overall heterogeneous pattern of TLR4 polymorphism can be attributed to genetic drift. However it can be inferred that GC haplotype is under the process of natural selection in the Indian population and one of the factors contributing to its selection could be predominance of *Plasmodium falciparum* in these regions.

189. **Yadav S**, **Singh SP**, **Mittal PK**. Toxicity of *Thevetia peruviana* (yellow oleander) against larvae of *Anopheles stephensi* and *Aedes aegypti* vectors of malaria and dengue. ***J Entomol Zool Stud* 2013; *1*(6): 85–7.**

ABSTRACT

The secondary metabolites (terpenes, acetogenins and alkaloids produced by the plants have been explored for their utility in mosquito control as it has been already proven that some of the plant compounds are toxic to the target organism but harmless to mankind. Basic research for the synthesis of new pesticides is need of the hour. This study was conducted using secondary metabolites of plant extract of *Thevetia peruviana* to study its larvicidal properties against the

larvae of Malaria and Dengue vectors. The toxicity of the leaf extract of *Thevetia peruviana* was evaluated against the larvae of *Anopheles stephensi,* and *Aedes aegypti* mosquitoes. Mean LC50 values of the petroleum ether, chloroform, acetone and methanol extracts obtained from leaves of *Thevetia peruviana* against the larvae of *An.stephensi* and *Aedes aegypti* mosquitoes after 24 hours were determined as 0.045, >0.05, 0.026, 0041 and 0.038, >0.05, 0.021 and 0.036%, respectively. However delayed impact after 3 days with chloroform extract and indicated that the larvicidal action is probably due to the insect growth inhibition.

KEYWORDS: *Thevetia peruviana,* toxicity, malaria larvae, *Anopheles stephensi, Aedes aegypti*.

190. Gokhale K, Patil DP, Dhotre DP, **Dixit R**, Mendki MJ, Patole MS, Shouche YS. Transcriptome analysis of *Anopheles stephensi* embryo using expressed sequence tags. ***J Biosci* 2013; *38*(2): 301–9.**

ABSTRACT

Germ band retraction (GBR) stage is one of the important stages during insect development. It is associated with an extensive epithelial morphogenesis and may also be pivotal in generation of morphological diversity in insects. Despite its importance, only a handful of studies report the transcriptome repertoire of this stage in insects. Here, we report generation, annotation and analysis of ESTs from the embryonic stage (16-22 h post fertilization) of laboratoryreared *Anopheles stephensi* mosquitoes. A total of 1002 contigs were obtained upon clustering of 1140 high-quality ESTs, which demonstrates an astonishingly low transcript redundancy (12.1 percent). Putative functions were assigned only to 213 contigs (21 percent), comprising mainly of transcripts encoding protein synthesis machinery. Approximately 78 percent of the transcripts remain uncharacterized, illustrating a lack of sequence information about the genes expressed in the embryonic stages of mosquitoes. This study highlights several novel transcripts, which apart from insect development, may significantly contribute to the essential biological complexity underlying insect viability in adverse environments. Nonetheless, the generated sequence information from this work provides a comprehensive resource for genome annotation, microarray development, phylogenetic analysis and other molecular biology applications in entomology.

191. **Singh SP**, Mohan L. Variations in the ommatidia and compound eyes of three species of mosquito vectors. ***J Entomol Zool Stud*** **2013; *1*(5): 15–20.**

ABSTRACT

The compound eye is one of the most important organs of the insects, which is made up of compact individual eye elements known as ommatidia. Each ommatidium is externally visible as a facet. The number of ommatidia was found to be different in numbers in compound eyes of different mosquitoes vector species (*Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*), which was manually counted by corneal spreads. Laboratory- reared mosquitoes were used in this study. The mean number of ommatidia was different in numbers among adults (610-900) and pupae (455-896) in three different mosquito species examined *Anopheles stephensi*, *Culex quinquefasciatus* and *Aeedes aegypti* respectively. Variability was observed in number of ommatidia in aquatic and adult stages within the species and among three different mosquito species. The ommatidia in compound eye of the mosquito vectors were found to be hexagonal shape and regular in size. It was observed that the *An. stephensi* male and female have dichoptic eye whereas *Cx. quinquefasciatus* and *Ae. aegypti* male and female have holoptic eye.

KEYWORDS: mosquito vectors, compound eye, ommatidia, malaria.

Titles: A-Z

2014

192. Masur U, **Kumar H**, **Kumar A**. Anti-larval effects of leaf and callus extract of *Dysoxylum binectariferum* against urban malaria vector, *Anopheles stephensi*. ***J Nat Prod* 2014; *7*: 147-54.**

ABSTRACT

Dysoxylum binectariferum Hook is a large tree endemic to Western Ghats of India and shows incredibly high yields of *rohitukine*. Methanolic extracts of leaves of this plant were tested against 3rd& 4th instar larvae of *Anopheles stephensi*, a malaria vector in urban areas of India. Attempts were also made in the present investigation to tissue culture the leaf and compare the efficacy of callus extract with that of the leaf extract against the larvae of *An. stephensi*. The dose mortality data were subjected to log probit regression analysis to determine median lethal concentrations, LC50 and LC90 after exposure for 24h. Accordingly, 97.5% mortality was observed at 18000 ppm concentration of leaf extract, with LC50 13465 (95% CI: 12845-14182ppm) and LC9018009 (95% CI: 16653-20368) ppm respectively. The callus extract showed 98.75% mortality at 2000 ppm with LC50 & LC90 values 907 (95% CI: 726-1133) ppm and 1961 (95% CI: 1478-3522) ppm respectively. Results revealed that both leaf and callus extract have activity against *Anopheles*

stephensi larvae but compared to the leaf, callus appears more efficient. They could be potential source of herbal-based insecticide for control of disease vectors.

KEYWORDS: *D. binectariferum*, *An. Stephensi*, larvicidal activity, leaf extract, callus extract.

193. **Anvikar AR**, Arora U, Sonal GS, **Mishra N**, **Shahi B**, **Savargaonkar D**, **Kumar N**, **Shah NK**, **Valecha N**. Antimalarial drug policy in India: Past, present & future. ***Indian J Med Res* 2014; *139* (2): 205-215.**

ABSTRACT

The use of antimalarial drugs in India has evolved since the introduction of quinine in the 17th century. Since the formal establishment of a malaria control programme in 1953, shortly after independence, treatments provided by the public sector ranged from chloroquine, the mainstay drug for many decades, to the newer, recently introduced artemisinin based combination therapy. The complexity of considerations in antimalarial treatment led to the formulation of a National Antimalarial Drug Policy to guide procurement as well as communicate best practices to both public and private healthcare providers. Challenges addressed in the policy include the use of presumptive treatment, the introduction of alternate treatments for drug-resistant malaria, the duration of primaquine therapy to prevent relapses of *vivax* malaria, the treatment of malaria in pregnancy, and the choice of drugs for chemoprophylaxis. While data on antimalarial drug resistance and both public and private sector treatment practices have been recently reviewed, the policy process of setting national standards has not. In this perspective on antimalarial drug policy, this review highlights its relevant history, analyzes the current policy, and examines future directions.

KEYWORDS antimalarial, drug, India, malaria, treatment, policy.

194. Wilson AL, **Dhiman RC**, Kitron U, Scott TW, van den Berg H, Lindsay SW. Benefit of insecticide-treated nets, curtains and screening on vector borne diseases, excluding malaria: a systematic review and meta-analysis. ***PLoS Negl Trop Dis* 2014 Oct 9; *8*(10):e3228.**

ABSTRACT

Introduction: Insecticide-treated nets (ITNs) are one of the main interventions used for malaria control. However, these nets may also be effective against other vector borne diseases (VBDs). We conducted a systematic review and meta-analysis to estimate the efficacy of ITNs, insecticide-treated curtains (ITCs) and insecticide-treated house screening (ITS) against Chagas disease, cutaneous and visceral leishmaniasis, dengue, human African trypanosomiasis, Japanese encephalitis, lymphatic filariasis and onchocerciasis. **Methods:** MEDLINE, EMBASE, LILACS and Tropical Disease Bulletin databases were searched using intervention, vector- and disease-specific search terms. Cluster or individually randomised controlled trials, non-randomised trials with pre- and post-intervention data and rotational design studies were included. Analysis assessed the efficacy of ITNs, ITCs or ITS versus no intervention. Meta-analysis of clinical data was performed and percentage reduction in vector density calculated. **Results:** Twenty-one studies were identified which met the inclusion criteria. Meta-analysis of clinical data could only be performed for four cutaneous leishmaniasis studies which together showed a protective efficacy of ITNs of 77% (95%CI: 39%–91%). Studies of ITC and ITS against cutaneous leishmaniasis also reported significant reductions in disease incidence. Single studies reported a high protective efficacy of ITS against dengue and ITNs against Japanese encephalitis. No studies of Chagas disease, human African trypanosomiasis or onchocerciasis were identified. **Conclusion:** There are likely to be considerable collateral benefits of ITN roll out on cutaneous leishmaniasis where this disease is co-endemic with malaria. Due to the low number of studies identified, issues with reporting of entomological outcomes, and few studies reporting clinical outcomes, it is difficult to make strong conclusions on the effect of ITNs, ITCs or ITS on other VBDs and therefore further studies be conducted. Nonetheless, it is clear that insecticide-treated materials such as ITNs have the potential to reduce pathogen transmission and morbidity from VBDs where vectors enter houses.

195. **Singh RK**, **Haq S**, **Kumar G**, **Mittal PK**, **Dhiman RC**. Bionomics and vector potential of *Anopheles subpictus* as a malaria vector in India: An overview. ***Int J Mosq Res*** **2014, *1*(1); 29-37.**

ABSTRACT

Anopheles subpictus has been recognised as an important vector of malaria in Sri Lanka and some other countries like Malaysia and Maldives. It has been found to play an important role in malaria

transmission as a secondary vector in certain parts of Odisha and coastal areas of south India. *An. subpictus* is a widely distributed mosquito species that breeds in a variety of fresh as well as saline water habitats. The species is a complex of four sibling species provisionally designated as: sibling species A, B, C and D, but the role of these sibling species in malaria transmission is not clearly known. As there is limited research work available on this species in India, it was thought prudent to review the bionomics and the role of *An. subpictus* in malaria transmission in Indian context. Further studies are required on the bionomics of *An. subpictus* and its role in malaria transmission in other parts of the country under the influence of changing ecological conditions.

KEYWORDS: *Anopheles subpictus*, distribution, India; malaria, species complex, vector.

196. Jain S, Rana V, Shrinet J, **Sharma A**, **Tridibes Adak**, **Sunil S**, Bhatnagar RK. Blood feeding and *Plasmodium* infection alters the miRNome of *Anopheles stephensi*. ***PLoS One* 2014 May 27; *9*(5): e98402.**

ABSTRACT

Blood feeding is an integral process required for physiological functions and propagation of the malaria vector *Anopheles*. During blood feeding, presence of the malaria parasite, *Plasmodium*in the blood induces several host effector molecules including microRNAs which play important roles in the development and maturation of the parasite within the mosquito. The present study was undertaken to elucidate the dynamic expression of miRNAs during gonotrophic cycle and parasite development in *Anopheles stephensi*. Using next generation sequencing technology, we identified 126 miRNAs of which 17 were novel miRNAs. The miRNAs were further validated by northern hybridization and cloning. Blood feeding and parasitized blood feeding in the mosquitoes revealed regulation of 13 and 16 miRNAs respectively. Expression profiling of these miRNAs revealed that significant miRNAs were down-regulated upon parasitized blood feeding with a repertoire of miRNAs showing stage specific up-regulation. Expression profiles of significantly modulated miRNAs were further validated by real time PCR. Target prediction of regulated miRNAs revealed overlapping targeting by different miRNAs. These targets included several metabolic pathways including metabolic, redox homeostasis and protein processing machinery components. Our analysis revealed tight regulation of specific miRNAs post blood feeding and parasite infection in *An. stephensi*. Such regulated expression suggests possible role of these miRNAs during gonotrophic cycle in mosquito. Another set of miRNAs were also significantly regulated at 42 h

and 5 days post infection indicating parasite stage-specific role of host miRNAs. This study will result in better understanding of the role of miRNAs during gonotrophic cycle and parasite development in mosquito and can probably facilitate in devising novel malaria control strategies at vector level.

197. **Shalini** S, **Chaudhuri S**, Sutton PL, **Mishra N**, Srivastava N, David JK, **Ravindran KJ**, Carlton JM, **Eapen A**. Chloroquine efficacy studies confirm drug susceptibility of *Plasmodium vivax* in Chennai, India. ***Malar J* 2014 Mar 31; *13*: 129.**

ABSTRACT

Background: Assessing the *Plasmodium vivax* burden in India is complicated by the potential threat of an emerging chloroquine (CQ) resistant parasite population from neighbouring countries in Southeast Asia. Chennai, the capital of Tamil Nadu and an urban setting for *P. vivax* in southern India, was selected as a sentinel site for investigating CQ efficacy and sensitivity in *vivax* malaria. **Methods:** CQ efficacy was evaluated with a 28-day *in vivo* therapeutic study, while CQ sensitivity was measured with an *in vitro* drug susceptibility assay. In both studies, isolates also underwent molecular genotyping to investigate correlations between parasite diversity and drug susceptibility to CQ. Molecular genotyping included sequencing a 604 base pair (bp) fragment of the *P. vivax multidrug resistant gene-1* (*Pvmdr1*) for single nucleotide polymorphisms (SNPs) and also the amplification of eight microsatellite (MS) loci located across the genome on eight different chromosomes. **Results:** In the 28-day *in vivo* study (*N*=125), all subjects were aparasitaemic by Day 14. Passive case surveillance continuing beyond Day 28 in 22 subjects exposed 17 recurrent infections, which ranged from 44 to 148 days post-enrollment. *Pvmdr1* sequencing of these recurrent infections revealed that 93.3% had identical mutant haplotypes (958M/Y976/1076L) to their baseline Day 0 infection. MS genotyping further revealed that nine infection pairs were related with ≥75% haplotype similarity (same allele at six or more loci). To test the impact of this mutation on CQ efficacy, an *in vitro* drug assay (*N*=68) was performed. No correlation between IC_{50} values and the percentage of ring-stage parasites prior to culture was observed (r_{sadj}: -0.00063, p = 0.3307) and the distribution of alleles among the *Pvmdr1* SNPs and MS haplotypes showed no significant associations with IC_{50} values. **Conclusions:** *Plasmodium vivax* was found to

be susceptible to CQ drug treatment in both the *in vivo* therapeutic drug study and the *in vitro* drug assay. Though the mutant 1076L of *Pvmdr1* was found in a majority of isolates tested, this single mutation did not associate with CQ resistance. MS haplotypes revealed strong heterogeneity in this population, indicating a low probability of reinfection with highly related haplotypes.

KEYWORDS: *Plasmodium vivax,* Chloroquine, *In vitro, In vivo* Genetic diversity, Chennai.

198. **Singh R**, **Singh DP**, Gupta R, **Savargaonkar D**, **Singh OP**, **Nanda N**, **Bhatt RM**, **Valecha N**. Comparison of three PCR-based assays for the non-invasive diagnosis of malaria: detection of *Plasmodium* parasites in blood and saliva. ***Eur J Clin Microbiol Infect Dis*** **2014 Sep; *33*(9):1631-9.**

ABSTRACT

The conventional molecular diagnosis of malaria uses 18S rRNA-based PCR assay employing blood samples. This assay presents limitation in terms of long turnaround time and increased chances of false-positive results. Here, we evaluated one-step singleplex or multiplex PCR assay based on high copy species-specific consensus repeat sequences (CRS) along with standard 18S rRNA nested PCR (18S n-PCR) assay to detect *P. falciparum* and *P. vivax*infection using blood and saliva samples from Indian febrile patients. Out of 327 patients, 187 were found to be positive for malaria parasites by microscopic examination of peripheral blood smears. Among these 130 were *P. vivax* and 57 were *P. falciparum* cases. The18S n-PCR assay and CRS PCR assay identified 186 out of 187 cases (99.4 %). Multiplex CRS PCR assay detected *Plasmodium*in 176 out of 187 cases (94.1 %). Both singleplex and multiplex CRS PCR assay identified 6 mixed infection cases, while 18S n-PCR assay detected 10 mixed infection cases of *P. vivax* and *P. falciparum*, which were not recognized by microscopy. Non-invasive *Plasmodium* detection rate with DNA derived from saliva samples was highest for 18S n-PCR (87.36 %), followed by singleplex CRS (81 %) and multiplex CRS PCR assay (70.5 %). Specificity for *P. vivax* and *P. falciparum* detection for all assays was 98.48 % and 100 % respectively. Detection rate for *P. vivax* in saliva correlated with parasite density for CRS target-based assays. The species-specific CRS PCR, either as a singleplex or multiplex assay, can have an impact on diagnosis and epidemiological studies in malaria.

199. **Sundararaj S**, Saxena AK, Sharma R, **Vashisht K**, **Sharma S**, **Anvikar A**, **Dixit R**, Rosenthal PJ, **Pandey KC**. Cross-Talk between malarial cysteine proteases and falstatin: The BC loop as a hot-spot target. ***PLoS ONE* 2014; *9*(4): e93008.**

ABSTRACT

Cysteine proteases play a crucial role in the development of the human malaria parasites*Plasmodium falciparum* and *Plasmodium vivax*. Our earlier studies demonstrated that these enzymes are equipped with specific domains for defined functions and further suggested the mechanism of activation of cysteine proteases. The activities of these proteases are regulated by a new class of endogenous inhibitors of cysteine proteases (ICPs). Structural studies of the ICPs of *Trypanosoma cruzi* (chagasin) and *Plasmodium berghei* (PbICP) indicated that three loops (termed BC, DE, and FG) are crucial for binding to target proteases. Falstatin, an ICP of *P. falciparum*, appears to play a crucial role in invasion of erythrocytes and hepatocytes. However, the mechanism of inhibition of cysteine proteases by falstatin has not been established. Our study suggests that falstatin is the first known ICP to function as a multimeric protein. Using site-directed mutagenesis, hemoglobin hydrolysis assays and peptide inhibition studies, we demonstrate that the BC loop, but not the DE or FG loops, inhibits cysteine proteases of *P. falciparum* and *P. vivax* via hydrogen bonds. These results suggest that the BC loop of falstatin acts as a hot-spot target for inhibiting malarial cysteine proteases. This finding suggests new strategies for the development of anti-malarial agents based on protease-inhibitor interactions.

200. **Mishra N**, **Kaitholia K**, **Srivastava B**, **Shah NK**, **Narayan JP**, **Dev V**, **Phookan S, Anvikar AR, Rama R, Bharti RS,** Sonal GS, Dharival AC**, Valecha N.** Declining efficacy of artesunate plus sulphadoxine-pyrimethamine in northeastern India. ***Malar J* 2014; *13*: 284.**

ABSTRACT

Background: Anti-malarial drug resistance in *Plasmodium falciparum* in India has historically travelled from northeast India along the Myanmar border. The treatment policy for *P. falciparum* in the region was, therefore, changed from chloroquine to artesunate (AS) plus sulphadoxine-pyrimethamine (SP) in selected areas in 2005 and in 2008 it became the first-line treatment. Recognizing that resistance to the partner drug can limit the useful life of this

combination therapy, routine *in vivo* and molecular monitoring of anti-malarial drug efficacy through sentinel sites was initiated in 2009. **Methods:** Between May and October 2012, 190 subjects with acute uncomplicated *falciparum* malaria were enrolled in therapeutic efficacy studies in the states of Arunachal Pradesh, Tripura, and Mizoram. Clinical and parasitological assessments were conducted over 42 days of follow-up. Multivariate analysis was used to determine risk factors associated with treatment failure. Genotyping was done to distinguish re-infection from recrudescence as well as to determine the prevalence of molecular markers of antifolate resistance among isolates. **Results:** A total of 169 patients completed 42 days of follow-up at three sites. The crude and PCR-corrected Kaplan-Meier survival estimates of AS + SP were 60.8% (95% CI: 48.0-71.4) and 76.6% (95% CI: 64.1-85.2) in Gomati, Tripura; 74.6% (95% CI: 62.0-83.6) and 81.7% (95% CI: 69.4-89.5) in Lunglei, Mizoram; and, 59.5% (95% CI: 42.0-73.2) and 82.3% (95% CI: 64.6-91.6) in Changlang, Arunachal Pradesh. Most patients with *P. falciparum* cleared parasitaemia within 24 hours of treatment, but eight, including three patients who failed treatment, remained parasitaemic on day 3. Risk factors associated with treatment failure included age < five years, fever at the time of enrolment and AS under dosing. No adverse events were reported. Presence of *dhfr*plus *dhps* quintuple mutation was observed predominantly in treatment failure samples. **Conclusion:** AS + SP treatment failure was widespread in northeast India and exceeded the threshold for changing drug policy. Based on these results, in January 2013 the expert committee of the National Vector Borne Disease Control Programme formulated the first subnational drug policy for India and selected artemether plus lumefantrine as the new first-line treatment in the northeast. Continued monitoring of anti-malarial drug efficacy is essential for effective malaria control.

KEYWORDS: *Plasmodium falciparum*, Artesunate + sulphadoxine-pyrimethamine (AS + SP), Artemisinin combination therapy (ACT), *Dihydrofolate reductase*(*dhfr*), *Dihyropteroate synthase* (*dhps*).

201. **Sharma P**, **Sharma S**, Mishra A, **Thomas T**, **Tanwee D De** , **Verma S**, **Vandana K**, **Rohilla SL**, **Singh N**, **Pandey KC**, **Dixit R**. Deep sequencing revealed “Plant like transcripts” in mosquito *A. culicifacies*: an evolutionary puzzle. ***BioRxiv* 2014*;* doi: 10.1101/010009.**

ABSTRACT

As adult female mosquito's salivary gland facilitate blood meal uptake and pathogen transmission *e.g*. *Plasmodium,* virus etc., a plethora of research has been focused to understand the mosquito-vertebrate-pathogen interactions. Despite the fact that mosquito spends longer time over nectar sugar source, the fundamental question "how adult female salivary gland manages molecular and functional relationship during sugar vs. blood meal uptake" remains unanswered. Currently, we are trying to understand these molecular relationships under dual feeding conditions in the salivary glands of the mosquito *Anopheles culicifacies*. During functional annotation of salivary transcriptome database, unexpectedly we discovered a cluster of salivary transcripts encoding plant like proteins. Our multiple experimental validations confirmed that Plant like transcripts (PLTs) are of mosquito origin and may encode functional proteins. A comprehensive molecular analysis of the PLTs and ongoing metagenomic analysis of salivary microbiome provide first evidence that how mosquito may have been benefited from its association with plant host and microbes. Future understanding of the underlying mechanism of the feeding associated molecular responses may provide new opportunity to control vector borne diseases.

202. **Dev V**, Khound K, **Tewari GG**. Dengue vectors in urban and suburban Assam, India: entomological observations. ***WHO South-East Asia J Public Health.*** **2014; *3*(1): 51–9.**

ABSTRACT

Background: Dengue is rapidly becoming established in north-east India and spreading, on account of rapid urbanization and population movement, with reported morbidity and attributable death cases. This study aims to determine the seasonal abundance of *Aedes* (*Stegomyia*) albopictus and *Aedes* (*Stegomyia*) aegypti in Guwahati metropolis and suburban settlements; to characterize the breeding resources for these mosquitoes; and to ascertain the status of their susceptibility to adulticides and larvicides. **Methods**: Mosquito larval surveys were carried out in different localities in both Guwahati city and adjoining suburbs from January to December 2013, to determine the seasonal abundance of disease vectors and their breeding preferences. The insecticide susceptibility status of mosquito adults and larval populations of both *Aedes aegypti* and *Aedes albopictus* was ascertained, using World Health Organization standard diagnostic concentrations and test procedures. **Results**: The study revealed that both *Aedes aegypti* and *Aedes albopictus* are widely abundant in Guwahati city and suburbs, and breeding in a wide variety of resources. *Aedes albopictus*, however, was the predominant mosquito species in suburbs, breeding

preferentially in flower vases, cut-bamboo stumps and leaf axils. *Aedes aegypti* was the most common in the city, breeding predominantly in discarded tyres, cement tanks and used battery boxes. Both *Aedes aegypti* and *Aedes albopictus* were resistant to dichlorodiphenyltrichloroethane (DDT; 4%), but susceptible to malathion (5%), and exhibited a varied response to pyrethroids. However, larval populations of both these mosquito species were susceptible to larvicides, including malathion (1.0 mg/L), temephos (0.02 mg/L) and fenthion (0.05 mg/L), at much lower dosages than diagnostic concentrations. **Conclusion**: Given the seasonal abundance and case incidence in city areas, it is highly probable that *Aedes aegypti* is the predominant mosquito vector transmitting dengue virus. The study results have direct relevance for the state dengue-control programme, for targeting interventions and averting outbreaks and spread of disease.

KEYWORDS: *Aedes aegypti*, *Aedes albopictus*, Assam, dengue, insecticide susceptibility status, mosquito breeding habitats, north-east India, seasonal abundance.

203. **Chauhan K**, Pande V, **Das A**. DNA sequence polymorphisms of the *pfmdr1* gene and association of mutations with the *pfcrt* gene in Indian *Plasmodium falciparum* isolates. ***Infect Genet Evol.* 2014 Aug; *26*:213-22.**

ABSTRACT

Mutations in the *Plasmodium falciparum* multidrug resistance (*pfmdr1*) gene are known to provide compensatory fitness benefits to the chloroquine (CQ)-resistant malaria parasites and are often associated with specific mutations in the *P. falciparum* CQ resistant transporter (*pfcrt*) gene. Prevalence of the specific mutations in these two genes across different malaria endemic regions was mostly studies. However, reports on mutations in the *pfmdr1* gene and their geneticassociations with mutations in the *pfcrt* gene in Indian *P. falciparum* field isolates are scarce. We have sequenced a 560 bp region of *pfmdr1* coding sequence in 64 *P. falciparum* isolates collected from different malaria endemic populations in India. Twenty out of these 64 isolates were laboratory cultured with known in vitro CQ sensitiveness (10 sensitive and 10 resistant). Three low frequency mutations (two non-synonymous and one synonymous) in the *pfmdr1* gene were segregating in Indian isolates in addition to the predominant Y_{86} and Y_{184} ones, with high haplotype and nucleotide diversity in the field isolates in comparison to the cultured ones. No statistically significant genetic association between the mutations in the *pfmdr1* and *pfcrt*

gene could be detected; almost all observed associations were intragenic in nature. The results on thegenetic diversity of the *pfmdr1* gene were discussed in term of evolutionary perspectives in Indian *P. falciparum*, with possible future potential of gaining further insights on this gene in view of evolving malaria parasites resistant to artemisinin partner drugs.

KEYWORDS: India, *pfcrt*, *Plasmodium falciparum*, Single Nucleotide Polymorphisms (SNPs), *pfmdr1*.

204. **Mbenda HG**, **Awasthi G**, Singh PK, Gouado I, **Das A**. Does malaria epidemiology project Cameroon as 'Africa in miniature. ***J Biosci* 2014 Sep; *39*(4):727-38.**

ABSTRACT

Cameroon, a west-central African country with a ~ 20 million population, is commonly regarded as 'Africa in miniature' due to the extensive biological and cultural diversities of whole Africa being present in a single-country setting. This country is inhabited by ancestral human lineages in unique eco-climatic conditions and diverse topography. Over 90 percent Cameroonians are at risk of malaria infection, and ~ 41 percent have at least one episode of malaria each year. Historically, the rate of malaria infection in Cameroon has fluctuated over the years; the number of cases was about 2 million in 2010 and 2011. The Cameroonian malaria control programme faces an uphill task due to high prevalence of multidrug-resistant parasites and insecticide-resistant malariavectors. Above all, continued human migration from the rural to urban areas as well as population exchange with adjoining countries, high rate of ecological instabilities caused by deforestation, poor housing, lack of proper sanitation and drainage system might have resulted in the recent increase in incidences of malaria and other vector-borne diseases in Cameroon. The available data on eco-environmental variability and intricate malaria epidemiology in Cameroon reflect the situation in the whole of Africa, and warrant the need for in-depth study by using modern surveillance tools for meaningful basic understanding of the malaria triangle (host-parasite-vector-environment).

205. **Sharma SK**, **Tyagi PK**, **Upadhyay AK**, **Haque MA**, Agrawal OP. Efficacy, human safety and collateral benefits of alphacypermethrin treated long-lasting insecticidal net (Interceptor(R) in a

hyperendemic tribal area of Orissa, India. ***J Trop Dis*** **2014; *2*(3): 135. doi:10.4172/2329-891X.1000135.**

ABSTRACT

Interceptor net® is a long-lasting insecticidal net (LN) made of multifilament polyester fabric in which the insecticide alphacypermethrin is incorporated directly into the polymers at a dose of 200 mg/m2. This paper presents the results of an efficacy trial on Interceptor nets on malaria transmission in an area under the influence of pyrethroid susceptible vector species *Anopheles culicifacies* and *A. fluviatilis* in Sundargarh District, Orissa, India. There was a reduction of 57-76% in malaria incidence in Interceptor net area as compared to the control areas. Cross-sectional point prevalence surveys showed a reduction of 73.1% and 40% in malaria prevalence in Interceptor net and untreated net users respectively, whereas there was an increase of 17% in no net villages. The net usage rate in the study population was between 80-98% during different months. With respect to the adverse effects of the insecticide, people reported skin irritations but transitory in nature hence did not pose any danger. Interceptor nets also provided collateral benefits in terms of relief from other household pests such as head lice, bed bugs, cockroaches, ants and houseflies.

KEYWORDS: long-lasting insecticidal net, alphacypermethrin, interceptor net, human safety, efficacy trial, Orissa, India.

206. **Dhiman RC**. Emerging vector-borne zoonoses: eco-epidemiology and public health implications in India. ***Front Public Health*****. 2014 Sep 30; *2*:168.**

ABSTRACT

The diseases originating from animals or associated with man and animals are remerging and have resulted in considerable morbidity and mortality. The present review highlights the re-emergence of emerging mainly zoonotic diseases like chikungunya, scrub typhus, and extension of spatial distribution of cutaneous leishmaniasis from western Rajasthan to Himachal Pradesh, Kerala, and Haryana states; West Nile virus to Assam, and non-endemic areas of Japanese encephalitis (JE) like Maharashtra and JE to Delhi; Crimean-Congo hemorrhagic fever making inroads in Ahmedabad; and reporting fifth parasite of human malaria with possibility of zoonosis have been

highlighted, which necessitates further studies for prevention and control. Emphasis has been given on understanding the ecology of reservoir hosts of pathogen, micro niche of vector species, climatic, socioeconomic risk factors, etc. Development of facilities for diagnosis of virus from insects, reservoirs, and human beings (like BSL4, which has been established in NIV, Pune), awareness about symptoms of new emerging viral and other zoonotic diseases, differential diagnosis, risk factors (climatic, ecological, and socioeconomic) and mapping of disease-specific vulnerable areas, and mathematical modeling for projecting epidemiological scenario is needed for preparedness of public health institutes. It is high time to understand the ecological link of zoonotic or anthroponotic diseases for updated risk maps and epidemiological knowledge for effective preventive and control measures. The public health stakeholders in India as well as in Southeast Asia should emphasize on understanding the eco-epidemiology of the discussed zoonotic diseases for taking preventive actions.

KEYWORDS: India, *P. knowlesi*, public health, chikungunya virus, cutaneous leishmaniasis, scrub typhus, vector-borne disease, zoonotic diseases.

207. **Singh RK**, **Mittal PK**, **Dhiman RC**. Evaluation of mosquito larvicidal efficacy of leaf extract of a cactus plant, *Agave sisalana* (Family- Agavaceae). ***J Entomol Zool stud*** **2014; *2*(1); 83-6.**

ABSTRACT

The leaf extract of *Agave sisalana* was tested as a larvicide against three vector mosquito species *viz., Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. Preliminary results showed that the 2% dilution of the leaf extract produced 100% mortality of IIIrd instar larvae of *An*. stephensi and 1% dilution produced 100% mortality in case of *Cx. quinquefasciatus* and *Ae. aegypti*. For further bioassays, the LC50 value of dried crude, methanol and petroleum ether extracts of *Ag. sisalana* leaves were determined against IIIrd/IVth instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* and these values were 75, 86 & 76 ppm; 36, 82 & 220 ppm and 27, 51 & 31 ppm, respectively. The present study revealed that *Ag. sisalana* leaf extract possess larvicidal activity against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* and can be exploited for their control under integrated approach of vector control.

KEYWORDS: mosquito larvicide, *Agave sisalana*, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*.

208. **Gupta P**, Pande V, **Das A**, **Singh V**. Genetic Polymorphisms in *VIR* Genes among Indian *Plasmodium vivax* Populations. ***Korean J Parasitol* 2014; *52*(5): 557-64.**

ABSTRACT

The *vir* genes are antigenic genes and are considered to be possible vaccine targets. Since India is highly endemic to *Plasmodium vivax*, we sequenced 5 different *vir* genes and investigated DNA sequence variations in 93 single-clonal *P. vivax* isolates. High variability was observed in all the 5 *vir* genes; the *vir* 1/9 gene was highly diverged across Indian populations. The patterns of genetic diversity do not follow geographical locations, as geographically distant populations were found to be genetically similar. The results in general present complex genetic diversity patterns in India, requiring further in-depth population genetic and functional studies.

KEYWORDS: *Plasmodium vivax*, vir gene, DNA sequence polymorphism, India.

209. **Gupta P**, **Singh R**, Khan H, Raza A, **Yadavendu V**, **Bhatt RM**, **Singh V**. Genetic Profiling of the *Plasmodium falciparum* population using antigenic molecular markers. ***Scientific World J* 2014; 2014. doi: 140867.**

ABSTRACT

About 50% of malaria infections in India are attributed to *Plasmodium falciparum* but relatively little is known about the genetic structure of the parasite populations. The molecular genotyping of the parasite populations by merozoite surface protein (msp1 and msp2) and glutamate-rich protein (glurp) genes identifies the existing parasite population in the regions which help in understanding the molecular mechanisms involved in the parasite's drive for survival. This study reveals the genetic profile of the parasite population in selected regions across the country with varying degree of endemicity among them. We also report the prevalence of *Pfcrt* mutations in this parasite population to evaluate the pattern of drug resistance development in them.

210. Price RN, Von SL, **Valecha N**, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax*: a systematic review and meta-analysis. ***Lancet Infect Dis* 2014 Oct; *14*(10): 982-91.**

ABSTRACT

Background: Chloroquine is the first-line treatment for *Plasmodium vivax* malaria in most endemic countries, but resistance is increasing. Monitoring of antimalarial efficacy is essential, but in *P vivax* infections the assessment of treatment efficacy is confounded by relapse from the dormant liver stages. We systematically reviewed *P vivax* malaria treatment efficacy studies to establish the global extent of chloroquine resistance. **Methods:** We searched Medline, Web of Science, Embase, and the Cochrane Database of Systematic Reviews to identify studies published in English between Jan 1, 1960, and April 30, 2014, which investigated antimalarial treatment efficacy in *P vivax* malaria. We excluded studies that did not include supervised schizonticidal treatment without primaquine. We determined rates of chloroquine resistance according to *P vivax* malaria recurrence rates by day 28 whole-blood chloroquine concentrations at the time of recurrence and study enrolment criteria. **Findings:** We identified 129 eligible clinical trials involving 21694 patients at 179 study sites and 26 case reports describing 54 patients. Chloroquine resistance was present in 58 (53%) of 113 assessable study sites, spread across most countries that are endemic for *P vivax*. Clearance of parasitaemia assessed by microscopy in 95% of patients by day 2, or all patients by day 3, was 100% predictive of chloroquine sensitivity. **Interpretation:** Heterogeneity of study design and analysis has confounded global surveillance of chloroquine-resistant *P vivax*, which is now present across most countries endemic for *P vivax*. Improved methods for monitoring of drug resistance are needed to inform antimalarial policy in these regions.

211. **Anushrita**, **Nagpal BN**, **Srivastava A**, **Saxena R**, Kapoor N, **Chand SK**, **Gupta SK**, **Dash AP**, **Dua VK**, **Valecha N**. Health Impact Assessment – a retrospective study for prospective approach in Madhya Pradesh, India. ***Austin J Infect Dis* 2014; *1*(3): 7.**

ABSTRACT

Dams and Irrigation channels have long been a topic of serious debate, principally because of their repercussions over human health despite their many promising benefits on stakeholders. Construction activities continue for many years which further change the micro climatic condition of surrounding areas. This rapid change of climatic condition and vector dynamics bring a lot of challenges to control strategies of respective authorities. Indira Sagar (ISP) and Omkareshwar

(OSP) dams are two major dam projects in Madhya Pradesh (MP), India and have created many changes at a very large scale in various domains of health. Health Impact Assessment (HIA) is the tool to bring all such complexities at a common platform and to provide decision-makers a set of evidence-based recommendations about the proposal. In view of this, Narmada Valley Development Authority (NVDA) of MP state provided a project along with funding to National Institute of Malaria Research (NIMR), Delhi to conduct a retrospective HIA study of sentinel villages, rehabilitation and resettlement (RR) colonies along with temporary labor settlements of ISP and OSP dam project area starting from 2004 onwards (2004-2013) with especial focus on malaria along with other VBDs. Entomological and epidemiological surveys were conducted thrice a year in three seasons namely pre-monsoon, monsoon and post-monsoon seasons. Baseline surveys were done during 2004-05 to analyze the situation. After 2005, various situation specific mitigation measures were suggested to the state health authorities, NVDA and NHDC based on Integrated Vector Management (IVM) approach and were implemented simultaneously to bring down the disease incidence and the impact was studied during subsequent years. During the initial years man hour density of *An culicifacies* (major rural vector) for the selected villages was observed to be very high (2-278). After intervention it was observed to be as low as (0-9). Density of other vector species *viz Culex quinquefasciatus* and *Aedes aegypti* could also be reduced to zero in later phases of studies. The impact of interventions for this study could be seen on the number of malaria cases in study areas. The malaria cases were also high in 2004 (299) which reduced to 2 cases due to stakeholder's participation and early detection and prompt treatment. The current study brings for the first time a retrospective health impact assessment of a dam impoundment with respect to the mosquito borne diseases especially malaria.

KEYWORDS: health impact assessment, malaria, dams, irrigation, canals.

212. **Prajapati SK**, **Singh OP**. Identification of a *vir-orthologous* immune evasion gene family from primate malaria parasites. ***Parasitology*** **2014 Apr; *141*(5): 641-5.**

ABSTRACT

The immune evasion gene family of malaria parasites encodes variant surface proteins that are expressed at the surface of infected erythrocytes and help the parasite in evading the host immune response by means of antigenic variation. The identification of *Plasmodium vivax vir* orthologous

immune evasion gene family from primate malaria parasites would provide new insight into the evolution of virulence and pathogenesis. Three *vir* subfamilies *viz. vir-B, vir-D* and *vir-G* were successfully PCR amplified from primate malaria parasites, cloned and sequenced. DNA sequence analysis confirmed orthologues of *vir-D* subfamily in *Plasmodium cynomolgi, Plasmodium simium, Plasmodium simiovale* and *Plasmodium fieldi*. The identified *vir-D* orthologues are 1–9 distinct members of the immune evasion gene family which have 68–83% sequence identity with *vir-D* and 71·2–98·5% sequence identity within the members identified from primate malaria parasites. The absence of other *vir* subfamilies among primate malaria parasites reflects the limitations in the experimental approach. This study clearly identified the presence of *vir-D* like sequences in four species of *Plasmodium* infecting primates that would be useful in understanding the evolution of virulence in malaria parasites.

KEYWORDS: primate malaria parasites, immune evasion gene family, antigenic variation, virulence, pathogenesis.

213. **Saxena R**, **Das MK**, **Nagpal BN**, **Srivastava A**, **Gupta SK**, **Kumar A**, **Tomar AS**, Sinha AT, Vidyotma R, Jeyaseelan AT, Baraik VK, **Singh VP**. Identification of malaria risk factors for control by focused interventions in Ranchi district, Jharkhand, India. ***J Vector Borne Dis 2014; 51(4):276-81*.**

ABSTRACT

Background & Objectives: Ranchi, the capital of Jharkhand state is endemic for malaria, particularly the Bundu Primary Health Centre (PHC) is the worst affected. Therefore, a study was initiated during 2009 using remote sensing (RS) and geographical information system (GIS) to identify risk factors responsible for high endemicity in this PHC. **Methods**: Bundu and Angara in Ranchi district were identified as high and low malaria endemic PHCs based on epidemiological data of three years (2007–09). The habitation, streams, other water body, landform, PHC and village boundary thematic maps were prepared using IRS-P6/LISS III-IV imageries and macro level breeding sites were identified. Digital elevation model (DEM) of the PHCs was generated using Cartosat Stereo Pair images and from DEM, slope map was derived to calculate flat area. From slope, aspect map was derived to indicate direction of water flow. Length of perennial streams, area under rocky terrain and buffer zones of 250, 500 and 750 m were constructed around streams. High resolution remote sensing imageries were used to identify micro level breeding sites.

Based on macro-micro breeding sites, six villages from each PHC were selected randomly having combination of different parameters representing all ecotypes. Entomological data were collected during 2010–11 in pre- and post-monsoon seasons following standard techniques and analyzed statistically. Differential analysis was attempted to comprehend socioeconomic and other determinants associated with malaria transmission. **Results**: The study identified eight risk factors responsible for higher malaria endemicity in Bundu in comparison to Angara PHC based on ecological, entomological, socioeconomic and other local parameters. **Conclusion**: Focused interventions in integrated vector management (IVM) mode are required to be carried out in the district for better management and control of disease.

KEYWORDS: DEM, GIS, malaria risk factors, remote sensing, slope, stone quarries.

214. **Saxena R**, **Nagpal BN**, **Singh VP**, **Srivastava A**, **Dev V**, **Sharma MC**, **Gupta HP**, **Tomar AS**, Sharma S, **Gupta SK**. Impact of deforestation on known malaria vectors in Sonitpur district of Assam, India. ***J Vector Borne Dis*** **2014; *51*(3): 211-5.**

ABSTRACT

Background & Objectives: An alarming rate of deforestation has been reported from Sonitpur district of Assam, India therefore, a study was initiated during 2009 using remote sensing (RS) to assess deforested areas in the district and to study the impact on malaria vectors in order to formulate appropriate control strategy. **Methods**: RS imageries of 2000 and 2009 were used to assess deforested areas in the selected district. Entomological data were collected in four surveys during 2009–2011. The data were analyzed statistically using test of single proportions ($\chi 2$) and pair-wise comparison. Vector incrimination was done using enzyme-linked immunosorbent assay (ELISA) and entomological inoculation rate (EIR) was calculated to estimate transmission intensity. **Results**: The deforested areas were identified in north-western parts of Sonitpur district falling in Dhekiajuli Primary Health Centre (PHC). The forest cover of the PHC decreased >50% during 2000–2009. Five species of *anopheline* vectors were collected. *Anopheles minimus* sensu lato (s.l.) was collected least abundantly while *An. culicifacies* s.l. prevailed most abundantly and significant difference was observed between proportions of the collected vector species. Pair-wise comparison between *An. culicifacies* s.l. and *An. minimus* s.l. was also found statistically significant indicating that *An. culicifacies* s.l. is establishing its population in deforested areas. *An.*

culicifacies s.l. was found ELISA positive and EIR was measured as 4.8 during transmission season. **Conclusion**: *An. culicifacies* s.l. replaced *An. minimus* s.l., the vector of malaria in northeast India and was found ELISA positive, therefore could have possible role in malaria transmission in the deforested areas of the district.

KEYWORDS: deforestation, entomological inoculation rate, GPS, remote sensing, vector incrimination.

215. **Dua VK**, **Srivastava A**, **Pandey AC**, **Gupta NC**. Impact of Integrated vector control strategy on malaria incidence and disability adjusted life years (Daly) at Bharat Heavy Electricals Limited, Hardwar, India-Results of 25 years study. ***Austin J Infect Dis* 2014; *1*(2):1-7.**

ABSTRACT

A project on Integrated Vector Control strategy on malaria was launched at Bharat Heavy Electricals Limited, Hardwar, and Uttarakhand, India in 1986. Major components of intervention strategy were source reduction, improving drainage, application of Expanded Polysterene Beads (EPS), biological control, use of larvivorous fishes *Poecilia reticulata* and *Gambusia affinis*, bacterial larvicides Bactoculicide *(Bacillus thuringiensis israelensis* and Spherix (*B. sphaericus*), limited use of fogging with malathion for the control of *Culex* mosquitoes, health education and community participation, prompt case detection, proper treatment and follow up of all malaria positive cases. Impact of Integrated Vector Control strategy on malaria incidence with time and estimation of diseases burden in term of Disability Adjusted Life Years (DALY) are presented in this paper.Implementation of Integrated Vector Control strategy on malaria incidence, at BHEL, Hardwar drastically reduced malaria cases from 2733 in 1986 to 593 in 1987 and constantly decreased up to 1994 with total cases of 96 which implied that there is no indigenous transmission in BHEL area. A slight increase of malaria cases was recorded from 1995 to 1997 with the total cases of 245, 286 and 238 respectively and then decreased constantly till 2011with an overall reduction above 95%. Highest DALY lost was estimated for the age category ≥ 14 in 1986, which showed a sharp decline over the years. However a general decline in DALY was observed in all age groups. Integrated Vector Control strategy on malaria incidence in industries complex at BHEL Hardwar with limited use of insecticide is highly effective, sustainable in long run, economical and resulted drastic reduction in DALY besides major reduction in malaria cases.

KEYWORDS: integrated vector control strategy on malaria, disability adjusted life year (Daly), malaria incidence.

216. Venkatesan R, **Ravindran J**, **Eapen A**, William J. Insecticidal and growth regulating activity of crude extracts of *Cassia occidentalis* L. (Caesalpinaceae) against the urban malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). ***Asian Pac J Trop Dis*** **2014; *4*(2): S578-82.**

ABSTRACT

Objective: To investigate insecticidal and growth regulating activity of crude leaf extracts of *Cassia occidentalis* L. (Caesalpinaceae) against the urban malaria vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). **Methods:** Larvicidal activity was studied against third instar larvae for 24 h at concentrations of 62.5, 125.0, 250.0, 500.0, 1000. 0, 2 000.0, 4 000.0 and 8 000.0 mg/L. The effect on development and growth of immature mosquitoes was studied at concentrations of 125.0, 250.0 and 500.0 mg/L and was assessed by growth index. Adulticidal activity on topical application was studied on test dosages of 0.01, 0.05, 0.10, 0.25 and 0.50 μg per newly emerged unfed adult female mosquito. **Results:** Larvicidal activity was poor, not proportional to concentration and LC50 values were above the acceptable dose of 10 mg/L. Potential growth regulating activity was observed and the growth index was 277.5, 111.0 and 27.5 times less than control, respectively. Average larval pupal transformation was 5.8, 4.3 and 96.0 times greater than pupal adult transformation in hexane, 5.0, 4.3 and 48.4 in ethyl acetate, and 4.8, 4.2 and 10.2 in methanol extract, respectively. Adulticidal activity was the highest in ethyl acetate, followed by hexane and methanol extract with LD50 values of 0.23, 0.32 and 0.64 μg/female mosquito, respectively. **Conclusions:** The crude leaf extracts of Cassia occidentalis studied showed poor larvicidal, potential growth regulating and a moderate level of adulticidal activity.

KEYWORDS: cassia occidentalis, leaf extracts, larvicidal, growth regulating, adulticidal, *Anopheles stephensi*.

217. **Singh RK**, **MittaL PK**, **Kumar G**, **Dhiman RC**. Insecticide susceptibility status of *Aedes aegypti* and *Anopheles stephensi* larvae against temephos in Delhi. ***Int J Mosq Res*** **2014, *1*(3); 69-73.**

ABSTRACT

Temephos is used as a larvicide in urban areas in India to control the population of mosquito vectors *viz*. *Anopheles stephensi* and *Aedes aegypti*. The susceptibility status of *Ae. aegypti* and *An. stephensi* to temophos in various zones of Municipal Corporation of Delhi was evaluated using the WHO method for determining larval susceptibility test kit. Results revealed that the larval mortality of *Ae. aegypti* collected from different localities ranged between 64.88% to 98.22%. The highest mortality was recorded from Sangam Vihar (98.22%) and lowest was recorded from Majnu ka tila (64.88%). *Ae. aegypti* larvae collected from Sangam Vihar locality was found fully susceptible to temephos, from two localities *viz*. Uttam Nagar and Pitampura of study area were tolerant to temephos, and from five localities *viz*. Majnu ka tila, Shastri Park, Mayur Vihar II, Tilak Bridge and Nagal Dewat showed development of resistance against temephos at diagnostic concentrations. However, larval populations of *An. stephensi* were fully susceptible to temephos in all the localities. The present study indicates the possible development of resistance against temephos in the larvae of *Ae. aegypti* in some areas in Delhi.

KEYWORDS: *Anopheles stephensi*, *Aedes aegypti*, insecticide susceptibility status and temephos.

218. **Singh RK**, **Kumar G**, **Mittal PK**. Insecticide Susceptibility status of malaria vectors in India: A review. ***Int Jour Mosq Res*** **2014, *1*(1): 5-9.**

ABSTRACT

Malaria is a disease caused by the biting of the *Anopheline* mosquito vectors. Vector control is the major component of the strategy for malaria control which aims to prevent parasite transmission through interventions targeting adult malaria vectors. For this, chemical, biological and mechanical methods are applied. In chemical approach of controlling malarial mosquitoes, insecticides have been used extensively for larviciding, indoor residual spraying and impregnation of bed nets in the last few decades. As a result of this, vector resistance to these insecticides have been recorded in various parts of the country and mosquitoes have developed wide spread resistance to some of these insecticides. There is need for countrywide and regular surveys for monitoring the insecticide susceptibility status of major vectors and assessing their implications on vector control activities. In India, most of the studies revealed that resistance against DDT is prevalent in most of the

malaria vector species. Bye and large, *An. culicifacies* and *An. stephensi* are resistant to malathion also and resistance against synthetic pyrethroid is developing. Moreover, *An. fluviatilis*, *An. minimus* and *An. annularis* are susceptible to malathion and deltamethrin. As the chemical molecules available for the role of insecticide are very few and invention of new molecules takes time, this is the need of time that increasing trend of resistance status of mosquitoes against the insecticides used in the vector control programme have to be minimized. There are only a few reports on the susceptibility status of the mosquitoes against various insecticides and thus more emphasis on these studies should be given. Again the appropriate use of the insecticides like rationale use with rotation of insecticides and insecticide combinations can be an effective strategy to combat this insecticide resistance.

KEYWORDS: malaria vector, insecticide susceptibility, review, India.

219. Kanchan K, Jha P, Pati SS, Mohanty S, Mishra SK, **Sharma SK**, Awasthi S, Venkatesh V, Habib S. Interferon-γ (*IFNG*) microsatellite repeat and single nucleotide polymorphism haplotypes of IFN-α receptor (*IFNAR1*) associated with enhasced malaria susceptibility in Indian populations. ***Infect Genet Evol* 2014; *29*: 6-14.**

ABSTRACT

Pro-inflammatory cytokines IFNγ and IFNα function through their cellular receptors IFNγR1 and IFNαR1, respectively to mediate immune processes during malaria infection. A total of 21 SNPs, 2 ins/del polymorphisms and a microsatellite repeat, selected on the basis of their reported association with infectious diseases including malaria in world populations, were analysed for association with *Plasmodium falciparum* malaria susceptibility in a case-control study with adult patients and ethnically-matched controls drawn from a disease meso- to hyperendemic and a nonendemic region of India. Among the five *IFNG* SNPs tested, an intron 3 and a 3'UTR SNP associated with disease in the endemic region. In addition, large $(CA)_n$ repeats of *IFNG* intron 1 associated with protection from severe malaria in the endemic region (severe vs. control, odds ratio=0.21, 95% CI=0.08-0.52, $P=1.3 \times 10^{(-4)}$). The TA11CAG haplotype (rs2069705 T/C, rs2430561 A/T, rs3138557 (CA)n, rs2069718 T/C, rs2069727 A/G, rs2069728 G/A) carrying a short CA_{11} repeat also exhibited very strong association with severe malaria, particularly in the endemic region (severe vs. control, OR=14.56, 95% CI=3.39-85.81, $P=3 \times 10^{(-5)}$). One SNP each from the *IFNA8* and *IFNA17* of *IFNA* gene cluster had a protective effect in the non-endemic

region but not in the endemic region. A promoter and an intron 2 SNP of *IFNAR1* were risk factors for disease and the *IFNAR1* haplotype GCCAGG (rs2843710 C/G, rs2850015 C/T, +6993 C/T, rs2243594 A/G, rs1012335 G/C, rs2257167 G/C) carrying both the risk alleles strikingly associated with disease manifestation in the endemic region (severe vs. control, OR=27.14, 95% CI=3.12-1254, $P=2 \times 10^{(-5)}$; non-severe vs. control, OR=61.87, 95% CI=10.08-2521, $P=1 \times 10^{(-8)}$). The data indicates dissimilar contribution of cytokine and cytokine receptor variants to disease in populations residing in areas of differential malaria endemicity.

KEYWORDS: cytokine, haplotypes, malaria susceptibility, polymorphisms, receptor.

220. Sukhthankar JH, **Kumar H**, Godhinno MHS, **Kumar A**. Larvicidal activity of methanolic leaf extracts of plant *Chromolaena odorata* L. (Asteraceae) against some vector mosquitoes in India. ***International J Mosq Res 2014. 1*(3): 33-8.**

ABSTRACT

Mosquitoes transmit malaria, filariasis, dengue, chikungunya, *etc*. Repeated use of insecticides for mosquito control has caused development of resistance, adverse effects on non-target organisms and serious environmental concerns. Hence alternative control measures are being explored inter alia plant based insecticides. We carried out larvicidal bioassays with methanolic extract of leaves of *Chromolaena odorata* (family Asteraceae) against late instar larvae of disease vectors *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The highest mortality was observed in *Cx. quinquefasciatus* [LC50 = 43 ppm, (95% CI: 34 - 48 ppm); LC90 = 110 ppm (CI: 94 - 135 ppm)] followed by *Ae. aegypti* [LC50 = 138 ppm, (CI: 121 - 157 ppm); LC90 = 463 ppm (CI: 386 - 584 ppm)] and *An. stephensi* [LC50 = 1613 ppm (CI: 1364 - 1890 ppm); LC90 = 8306 ppm (CI: 6598 - 11076 ppm)]. Being larvicidal, leaf extracts of *Chromolaena odorata* could be explored further.

KEYWORDS: *Chromolaena odorata*, leaf extract, *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes aegypti*, larvicidal activity.

221. **Kar NP**, **Kumar A**, **Singh OP**, Carlton JM, **Nanda N**. A review of Malaria transmission dynamics in forest ecosystems. ***Parasit Vectors 2014; 7*(1): 265-76**.

ABSTRACT

Malaria continues to be a major health problem in more than 100 endemic countries located primarily in tropical and sub-tropical regions around the world. Malaria transmission is a dynamic process and involves many interlinked factors, from uncontrollable natural environmental conditions to man-made disturbances to nature. Almost half of the population at risk of malaria lives in forest areas. Forests are hot beds of malaria transmission as they provide conditions such as vegetation cover, temperature, rainfall and humidity conditions that are conducive to distribution and survival of malaria vectors. Forests often lack infrastructure and harbor tribes with distinct genetic traits, socio-cultural beliefs and practices that greatly influence malaria transmission dynamics. Here we summarize the various topographical, entomological, parasitological, human ecological and socio-economic factors, which are crucial and shape malaria transmission in forested areas. An in-depth understanding and synthesis of the intricate relationship of these parameters in achieving better malaria control in various types of forest ecosystems is emphasized.

KEYWORDS: forest malaria, transmission dynamics, deforestation, vector behavior, socio-economic factors, tribal communities.

222. **Vijay S**, **Rawat M**, **Sharma A**. Mass spectrometry based proteomic analysis of salivary glands of urban malaria vector *Anopheles stephensi*. ***Biomed Res Int*** **2014; doi: 686319**.

ABSTRACT

Salivary gland proteins of *Anopheles* mosquitoes offer attractive targets to understand interactions with sporozoites, blood feeding behavior, homeostasis, and immunological evaluation of malaria vectors and parasite interactions. To date limited studies have been carried out to elucidate salivary proteins of *An. stephensi* salivary glands. The aim of the present study was to provide detailed analytical attributives of functional salivary gland proteins of urban malaria vector *An. stephensi*. A proteomic approach combining one-dimensional electrophoresis (1DE), ion trap liquid chromatography mass spectrometry (LC/MS/MS), and computational bioinformatic analysis was adopted to provide the first direct insight into identification and functional characterization of known salivary proteins and novel salivary proteins of *An. stephensi*. Computational studies by online servers, namely, MASCOT and OMSSA algorithms, identified a total of 36 known salivary

proteins and 123 novel proteins analysed by LC/MS/MS. This first report describes a baseline proteomic catalogue of 159 salivary proteins belonging to various categories of signal transduction, regulation of blood coagulation cascade, and various immune and energy pathways of *An. stephensi* sialotranscriptome by mass spectrometry. Our results may serve as basis to provide a putative functional role of proteins in concept of blood feeding, biting behavior, and other aspects of vector-parasite host interactions for parasite development in anopheline mosquitoes.

223. **Tyagi S**, Pande V, **Das A**. Mitochondrial genome sequence diversity of Indian *Plasmodium falciparum* isolates. ***Mem Inst Oswaldo Cruz* 2014 Jul; *109*(4): 494-8.**

ABSTRACT

We have analysed the whole mitochondrial (*mt*) genome sequences (each ~6 kilo nucleotide base pairs in length) of four field isolates of the malaria parasite *Plasmodium falciparum* collected from different locations in India. Comparative genomic analyses of *mt* genome sequences revealed three novel India-specific single nucleotide polymorphisms. In general, high *mt* genome diversity was found in Indian *P. falciparum*, at a level comparable to African isolates. A population phylogenetic tree placed the presently sequenced Indian *P. falciparum* with the global isolates, while a previously sequenced Indian isolate was an outlier. Although this preliminary study is limited to a few numbers of isolates, the data have provided fundamental evidence of the *mt* genome diversity and evolutionary relationships of Indian *P. falciparum*with that of global isolates.

KEYWORDS: malaria, *Plasmodium falciparum*, mitochondrial genome, evolution, India.

224. **Srivastava H**, Huong NT, Arunyawat U, **Das A**. Molecular population genetics of the NADPH cytochrome P450 reductase (CPR) gene in *Anopheles* minimus. ***Genetica* 2014 Aug; *142*(4): 295-315.**

ABSTRACT

Development of insecticide resistance (IR) in mosquito vectors is a primary huddle to malaria control program. Since IR has genetic basis, and genes constantly evolve with response to environment for adaptation to organisms, it is important to know evolutionary pattern of genes

conferring IR in malaria vectors. The mosquito *Anopheles minimus* is a major malaria vector of the Southeast (SE) Asia and India and is susceptible to all insecticides, and thus of interest to know if natural selection has shaped variations in the gene conferring IR. If not, the DNA fragment of such a gene could be used to infer population structure and demography of this species of malaria vector. We have therefore sequenced a *569 bp DNA segment of the NADPH cytochrome P450 reductase (CPR) gene (widely known to confer IR) in 123 individuals of *An. minimus* collected in 10 different locations (eight Indian, one Thai and one Vietnamese). Two Indian population samples were completely mono-morphic in the CPR gene. In general, low genetic diversity was found with no evidence of natural selection in this gene. The data were therefore analyzed to infer population structure and demography of this species. The 10 populations could be genetically differentiated into four different groups; the samples from Thailand and Vietnam contained high nucleotide diversity. All the 10 populations conform to demographic equilibrium model with signature of past population expansion in four populations. The results in general indicate that the *An. minimus* mosquitoes sampled in the two SE Asian localities contain several genetic characteristics of being parts of the ancestral population.

KEYWORDS: malaria, *Anopheles minimus*, insecticide resistance, NADPH cytochrome P450 reductase (CPR) gene, molecular population genetics.

225. **Dixit J**, Arunyawat U, Huong NT, **Das A**. Multilocus nuclear DNA markers reveal population structure and demography of *Anopheles minimus*. ***Mol Ecol.* 2014 Nov; *23*(22): 5599-618.**

ABSTRACT

Utilization of multiple putatively neutral DNA markers for inferring evolutionary history of species population is considered to be the most robust approach. Molecular population genetic studies have been conducted in many species of *Anopheles* genus, but studies based on single nucleotide polymorphism (SNP) data are still very scarce. *Anopheles minimus* is one of the principal malaria vectors of Southeast (SE) Asia including the Northeastern (NE) India. Although population genetic studies with mitochondrial genetic variation data have been utilized to infer phylogeography of the SE Asian populations of this species, limited information on the population structure and demography of Indian *An. minimus* is available. We herewith have developed multilocus nuclear genetic approach with SNP markers located in X chromosome of *An. minimus*

in eight Indian and two SE Asian population samples (121 individual mosquitoes in total) to infer population history and test several hypotheses on the phylogeography of this species. While the Thai population sample of *An. minimus* presented the highest nucleotide diversity, majority of the Indian samples were also fairly diverse. In general, *An. minimus* populations were moderately substructured in the distribution range covering SE Asia and NE India, largely falling under three distinct genetic clusters. Moreover, demographic expansion events could be detected in the majority of the presently studied populations of *An. minimus*. Additional DNA sequencing of the mitochondrial COII region in a subset of the samples (40 individual mosquitoes) corroborated the existing hypothesis of Indian *An. minimus* falling under the earlier reported mitochondrial lineage B.

KEYWORDS: *Anopheles minimus*, demography, India, population structure, single nucleotide polymorphisms.

226. **Tyagi S**, Pande V, **Das A**. New insights into the evolutionary history of *Plasmodium falciparum* from mitochondrial genome sequence analyses of Indian isolates. ***Mol Ecol* 2014, Jun; *23*(12): 2975-87.**

ABSTRACT

Estimating genetic diversity and inferring the evolutionary history of *Plasmodium falciparum* could be helpful in understanding origin and spread of virulent and drug-resistant forms of the malaria pathogen and therefore contribute to malaria control programme. Genetic diversity of the whole mitochondrial (mt) genome of *P. falciparum* sampled across the major distribution ranges had been reported, but no Indian *P. falciparum* isolate had been analysed so far, even though India is highly endemic to *P. falciparum* malaria. We have sequenced the whole mt genome of 44 Indian field isolates and utilized published data set of 96 genome sequences to present global genetic diversity and to revisit the evolutionary history of *P. falciparum*. Indian *P. falciparum* presents high genetic diversity with several characteristics of ancestral populations and shares many of the genetic features with African and to some extent Papua New Guinean (PNG) isolates. Similar to African isolates, Indian *P. falciparum* populations have maintained high effective population size and undergone rapid expansion in the past with oldest time to the most recent common ancestor (TMRCA). Interestingly, one of the four single nucleotide polymorphisms (SNPs) that

differentiates *P. falciparum* from *P. falciparum*-like isolates (infecting non-human primates in Africa) was found to be segregating in five Indian *P. falciparum* isolates. This SNP was in tight linkage with other two novel SNPs that were found exclusively in these five Indian isolates. The results on the mt genome sequence analyses of Indian isolates on the whole add to the current understanding on the evolutionary history of *P. falciparum*.

KEYWORDS: evolutionary history, India, mitochondrial genome, *Plasmodium falciparum*, single nucleotide polymorphisms.

227. **Raghavendra K**, **Barik TK**, **Sharma SK**, **Das MK**, **Dua VK**, **Pandey A**, **Ojha VP**, **Tiwari SN**, **Ghosh SK**, **Dash AP**. A note on the insecticide susceptibility status of principal malaria vector *Anopheles culicifacies* in four states of India**.** ***J Vector Borne Dis*** **2014, Sep; *51*(3): 230-4.**

ABSTRACT

Background & Objectives: The major malaria vector, *Anopheles culicifacies* Giles is reported to contribute ~ 65% of the malaria cases in India. This species developed resistance to DDT and later to HCH, malathion and also to pyrethroids in some states due to their use in the national malaria control programme. In the present study, insecticide susceptibility of this species was monitored in four states of India. **Methods**: To determine insecticide susceptibility status of the major malaria vector *An. culicifacies*, adult mosquitoes were collected from different localities of 32 tribal districts in the states of Andhra Pradesh, Odisha, Jharkhand and West Bengal during October/November 2009–10. Mosquitoes were collected from stratified ecotypes comprising a group of districts in West Bengal and individual districts in three other states. Mosquitoes were exposed to papers treated with WHO diagnostic dose: 4% DDT, 5% malathion and 0.05% deltamethrin following the WHO tube method. **Results**: Results provided the susceptibility status of *An. culicifacies* to different insecticides used in the public health programme in 32 districts in four states. *An. culicifacies* was found resistant to DDT (mortality range 0–36%) in all the 32 districts; to malathion it was resistant in 14 districts, verification required in 10 districts and susceptible in eight districts (mortality range 32.2–100%). It was resistant to deltamethrin in four districts, verification required in 11 districts and susceptible in 17 districts (mortality range 43.3–100%). **Interpretation & conclusion**: Development of widespread resistance to insecticides used in public health sprays for vector control including to pyrethroids in *An. culicifacies* in the

surveyed districts is of great concern for the malaria control programme as the major interventions for vector control are heavily reliant on chemical insecticides, mainly synthetic pyrethroids used both for indoor residual spraying and for long-lasting insecticidal nets. Thus, there is a need to periodically monitor and update the susceptibility status of malaria vector(s) to suggest alternative vector control strategies for effective disease management.

KEYWORDS: *Anopheles culicifacies*, India, insecticide resistance, vector control.

228. **Ngassa Mbenda HG**, **Das A**. Occurrence of multiple chloroquine-resistant *Pfcrt* haplotypes and emergence of the S(agt)VMNT type in Cameroonian *Plasmodium falciparum*. ***J Antimicrob Chemother* 2014 Feb; *69*(2): 400-3.**

ABSTRACT

Objectives: The main objective of this study was to unravel the distribution of different *Pfcrt* genotypes in the central, littoral, eastern and southern regions of Cameroon and also in locations bordering Gabon and Equatorial Guinea. This is because (i) the chloroquine-resistant malaria parasite*Plasmodium falciparum* shows a wide occurrence in Cameroon, (ii) mutations in the 72nd to 76th amino acid positions of the *Pfcrt* gene are known to confer resistance to chloroquine, and (iii) only a single chloroquine-resistant haplotype ($C_{72}V_{73}I_{74}E_{75}T_{76}$) has so far been reported in Cameroon. **Methods:** We followed a molecular approach with DNA sequencing of the second exon of the *Pfcrt* gene to identify single nucleotide polymorphisms in 180 *P. falciparum* field isolates sampled in five different locations in Cameroon. **Results:** The chloroquine-resistant *Pfcrt* CVIET haplotype was most abundant, followed by the wild-type CVMNK haplotype. Five hitherto unreported chloroquine-resistant *Pfcrt* haplotypes were detected for the first time in Cameroonian *P. falciparum*, including the surprise appearance of the $S_{(agt)}$VMNT haplotype. **Conclusions:** The high observed haplotype diversity of the chloroquine-resistant *Pfcrt* gene and the appearance of the $S_{(agt)}$VMNT haplotype are daunting and can be attributed to drug pressure and/or the misuse of chloroquine and/or amodiaquine in Cameroon.

KEYWORDS: chloroquine resistance, *P. falciparum*, malaria, Cameroon.

229. Gupta P, **Anvikar AR**, **Valecha N**, Gupta YK. Pharmacovigilance practices for better healthcare delivery: knowledge and attitude study in the national malaria control programme of India. ***Malar Res Treat*** **2014; 2014: 837427.**

ABSTRACT

Background: With large scale rollout of artemisinin based therapy in the National Malaria Control Programme of India, a risk management plan is needed. This depends on adverse drug reaction (ADR) reporting by the healthcare professionals (HCPs). For the programme to be successful, an understanding of the mindset of HCPs is critical. Hence, the present study was designed to assess and compare the ADR reporting beliefs of HCPs involved in the National Malaria Control Programme of India. **Methods:** A cross–sectional survey was conducted amongst the HCPs who manage malaria up to the district level in India. A 5-point Likert scale-based questionnaire was developed as a study tool. **Results**: A total of 154 HCPs participated in the study (age: 42.4 ± 10.1 years with 33.8% being females). About 61% felt that only medically qualified HCPs are responsible for ADR reporting. Likeliness to report in future was mentioned by 45% HCPs. The knowledge score was relatively lower for life science graduates (P = 0.09). Knowledge correlated positively with attitude (r 2 = 0.114; P < 0.0001). **Conclusion**: Based on the caveats identified, a specific and targeted in-service education with hands-on training on ADR monitoring and reporting needs to be designed to boost real time pharmacovigilance in India.

230. Keluskar P, **Singh V**, **Gupta P**, Ingle S. *Plasmodium falciparum* and *Plasmodium vivax* specific lactate dehydrogenase: genetic polymorphism study from Indian isolates. ***Infect Genet Evol*** **2014 Aug; *26*: 313-22.**

ABSTRACT

Control and eradication of malaria is hindered by the acquisition of drug resistance by*Plasmodium* species. This has necessitated a persistent search for novel drugs and more efficient targets. *Plasmodium* species specific lactate dehydrogenase is one of the potential therapeutic and diagnostic targets, because of its indispensable role in endoerythrocytic stage of the parasite. A target molecule that is highly conserved in the parasite population can be more effectively used in diagnostics and therapeutics, hence, in the present study polymorphism in *Pf*LDH

(*Plasmodiumfalciparum* specific LDH) and *Pv*LDH (*Plasmodiumvivax* specific LDH) genes was analyzed using PCR-single strand confirmation polymorphism (PCR-SSCP) and sequencing. Forty-six *P. falciparum* and thirty-five *P. vivax* samples were screened from different states of India. Our findings have revealed presence of a single ***Pf***LDH genotype and six *Pv*LDH genotypes among the studied samples. Interestingly, along with synonymous substitutions, nonsynonymous substitutions were reported to be present for the first time in the *Pv*LDH genotypes. Further, through amino acid sequence alignment and homology modeling studies we observed that the catalytic residues were conserved in all *Pv*LDH genotypes and the nonsynonymous substitutions have not altered the enzyme structure significantly. Evolutionary genetics studies have confirmed that *Pf*LDH and *Pv*LDH loci are under strong purifying selection. Phylogenetic analysis of the pLDH gene sequences revealed that *P. falciparum* compared to *P. vivax*, has recent origin. The study therefore supports *Pf*LDH and *Pv*LDH as suitable therapeutic and diagnostic targets as well as phylogenetic markers to understand the genealogy of malaria species.

KEYWORDS: *Plasmodium*, lactate dehydrogenase, genetic polymorphism, malaria therapeuticdiagnostic target.

231. **Savargaonkar D**, **Shah N**, **Das MK**, **Srivastava B**, **Valecha N**. *Plasmodium malariae* infection: A case of missed diagnosis. ***J Vector Borne Dis* 2014; *51*(2): 149–51**.

232. Tanwar OP. Rikta S, Marella A, Alam Md. M, Akhter M, **Dua VK**. Prediction and comparison of drug likeliness properties of primaquine and its structural analogues using *In-Silico* ADME and Toxicity Prediction Tools. ***J Adv Bioinform App Res* 2014; *5*(3): 172-82.**

ABSTRACT

Five primaquine (PQ) analogues have been isolated by peroxy disulfate oxidation and were tested for antimalarial activity against *Plasmodium yoelli* infected mice. To develop them as promising antimalarial agents in-silico druglikeness studies were carried out. The analogue 6-Methoxy-5,8-di-(4-amino-1-methylbutyl-amino)-quinoline [P1] was found to be promising antimalarial agent as it was found nontoxic in in-silico studies and obeys all druglikeness rules. The drug likeliness properties of primaquine analogues were determined using various in-silico tools like ADMETPredictor™, QikProp, Molinspiration and Osiris drug like property calculators. Each in-

silico study supported the drugability of analogue P1. Result of in-silico studies shows that analogue P1 is safer and can be a drug of choice for radical cure of malaria infection.

KEYWORDS: malaria, antimalarial, primaquine (pq), qikprop, *Plasmodium yoelli,* and drug likeness.

233. **Singh RK**, **Mittal PK**, **Kumar G**, **Dhiman RC**. Prevalence of *Aedes* mosquitoes in various localities of Delhi during dengue transmission season. ***Entomol Appl Sci Lett* 2014; *1* (4): 16-21.**

ABSTRACT

Entomological investigations were carried out in various localities of different Municipal Zones of Delhi, India, from July to November 2012 with a view to study the prevalence, distribution and stratification of areas for *Aedes* species and to identify high risk areas in the town prone to dengue/ DHF outbreak. A total of 1050 houses including some outdoor habitats were searched for *Aedes* breeding. Of the total houses surveyed, *Aedes* breeding could be detected in domestic containers/drums, water storage tanks in 276 houses. In all, a total of 8972 water containers were searched, out of which 1893 were found positive for *Aedes* breeding. The overall house index (HI), container index (CI), breteau index (BI), and pupal index (PI) were 26.28, 21.09, 180.28 and 67.33 respectively. *Aedes aegypti* was the predominant species in intra-domestic and peri-domestic containers, while *Aedes albopictus* and *Aedes vittatus* larvae were also observed in outdoor habitats. *Aedes aegypti* breeding was detected in all the localities, where dengue cases were recorded during the past 3 years. Based on the various indices, *Aedes aegypti* population was most prevalent in Sangam Vihar locality in MCD south Zone, irrespective to the number of dengue cases.

KEYWORDS: dengue fever, *Aedes* breeding, house index, container index, breteau index, pupal index, Delhi.

234. **Prajapati SK**, Culleton R, **Singh OP**. Protein trafficking in *Plasmodium falciparum*-infected red cells and impact of the expansion of exported protein families. ***Parasitology* 2014 July 30; *141*(12): 1533-43.**

ABSTRACT

Erythrocytes are extensively remodelled by the malaria parasite following invasion of the cell. *Plasmodium falciparum* encodes numerous virulence-associated and host-cell remodelling proteins that are trafficked to the cytoplasm, the cell membrane and the surface of the infected erythrocyte. The export of soluble proteins relies on a sequence directing entry into the secretory pathways in addition to an export signal. The export signal consisting of five amino acids is termed the *Plasmodium* export element (PEXEL) or the vacuole transport signal (VTS). Genome mining studies have revealed that PEXEL/VTS carrying protein families have expanded dramatically in *P. falciparum*compared with other malaria parasite species, possibly due to lineage-specific expansion linked to the unique requirements of *P. falciparum* for host-cell remodelling. The functional characterization of such genes and gene families may reveal potential drug targets that could inhibit protein trafficking in infected erythrocytes. This review highlights some of the recent advances and key knowledge gaps in protein trafficking pathways in *P. falciparum*-infected red cells and speculates on the impact of exported gene families in the trafficking pathway.

KEYWORDS: *Plasmodium falciparum*, erythrocyte remodeling, protein trafficking, exported proteins, gene family expansion,

235. Sundaram B, Varadarajan NM, **Pradeep AS**, **Ghosh SK**, Nagaraj VA. "Purification of a recombinant histidine-tagged lactate dehydrogenase from the malaria parasite, *Plasmodium vivax*, and characterization of its properties". ***Biotechnol Lett*** **2014 Dec; *36*(12): 2473-80.**

ABSTRACT

Lactate dehydrogenase (LDH) of the malaria parasite, *Plasmodium vivax* (*Pv*), serves as a drug target and immunodiagnostic marker. The LDH cDNA generated from total RNA of a clinical isolate of the parasite was cloned into pRSETA plasmid. Recombinant his-tagged *Pv*LDH was over-expressed in *E. coli*Rosetta2DE3pLysS and purified using Ni^{2+}-NTA resin giving a yield of 25–30 mg/litre bacterial culture. The recombinant protein was enzymatically active and its catalytic efficiency for pyruvate was $5.4 \times 10^8\ min^{-1}\ M^{-1}$, 14.5 fold higher than a low yield preparation reported earlier to obtain *Pv*LDH crystal structure. The enzyme activity was inhibited by gossypol and sodium oxamate. The recombinant *Pv*LDH was reactive in lateral flow immunochromatographic assays detecting pan- and *vivax*-specific LDH. The soluble

recombinant *Pv*LDH purified using heterologous expression system can facilitate the generation of *vivax* LDH-specific monoclonals and the screening of chemical compound libraries for*Pv*LDH inhibitors.

KEYWORDS: Enzyme assay, immunochromatographic assay, Lactate dehydrogenase, malaria *Plasmodium vivax*, recombinant enzyme.

236. **Singh V**, **Gupta P**, Pande V. Revisiting the multigene families: *Plasmodium var* and *vir* genes. ***J Vector Borne Dis*** **2014 Jun; *51*(2):75-81.**

ABSTRACT

Malaria is an infectious disease that is widespread in tropical and subtropical regions. The malaria parasite is able to skip the host immunity and thus maintains not only persistent but also repeated infections. There are a number of multigene families in *Plasmodium* that code for the variant antigens and are targets for protective immunity. In this article, we summarize the virulence genes of *P. falciparum* (var genes) and *P. vivax* (vir genes) which play key roles in disease pathogenesis by evading elimination by the host immune system. These genes occurring within the parasite population are mostly present in the subtelomeric regions of the chromosome.

KEYWORDS: multigene family, *plasmodium*, *var* and *vir* genes.

237. **Punita S**, **Swati S**, Maurya RK, **Das De T**, **Thomas T**, **Lata S**, **Singh N**, **Pandey KC**, **Valecha N**, **Dixit R.** Salivary glands harbor more diverse microbial communities than gut in *Anopheles culicifacies*. ***Parasit Vectors*** **2014, May 20; *7*(1): 235.**

ABSTRACT

Background: In recent years, it has been well documented that gut flora not only influence mosquito physiology, but also significantly alter vector competency. Although, salivary gland and gut constitute key partners of the digestive system, it is still believed that salivary glands may harbor less flora than gut (Parasit Vectors 6: 146, 2013). **Methods:** Using a metagenomic approach, we have identified for the first time the diverse microbial community associated with these two physiologically different tissues of the digestive system in the mosquito *Anopheles culicifacies*. **Results:** A total of 17 different phyla could be assigned to the whole metagenomic

dataset, predominated by the phylum Proteobacteria, Firmicutes, Bacteriodetes, Tenericutes and Actinomycetes. Common bacteria included the members of *Enhydrobacter*, *Agromonas*, *Serratia*, *Ralsonia*,*Lactobacillus*, *Pseudomonas*, *Streptococcus*, *Rubrobacter*, *Anaerococcus*,*Methylobacterium*, *Turicibacter*, *Elizabethkingia* etc. in both the tissues representing 'core microbiota' of the mosquito digestive system. Salivary associated unique bacterial community included the members of *Chloriflexi*, *Chlorobi*, *Cyanobacteria*, *Nitrospira*, *TM7*, *Armatimonadetes*,*Planctomycetes*, *Fibrobacteres* etc.
Conclusion: We find that the salivary gland microbial community structure is more diverse than gut of the mosquito, probably due to differential feeding associated engagements such as food acquisition, ingestion and digestion processes.

238. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chour CM, Nguon C, Sovannaroth S, Pukrittayakamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thenh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CL, Tshefu AK, **Mishra N**, **Valecha N**, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashrahel M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samed R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J , Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunaso lC, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. ***N Eng J Med*** **2014; *371*: 411-23**.

239. **Elamathi N**, **Barik TK**, **Verma V**, **Velamuri PS**, **Bhatt RM**, **Sharma SK**, **Raghavendra K**. Standardization of a bottle assay--an indigenous method for laboratory and field monitoring of insecticide resistance and comparison with WHO adult susceptibility test. ***Parasitol Res*** **2014; *113*(10): 3859-66.**

ABSTRACT

The WHO adult susceptibility test is in use for insecticide resistance monitoring. Presently, materials are being imported from the Universiti Sains Malaysia, Malaysia and sometimes it is cost prohibitive. As an alternative, we present here a method of bottle bioassay using indigenous material. Different aspects related to the assay were studied and validated in the field. Bottle assay was standardized in the laboratory by using locally sourced material and laboratorymaintained insecticide-susceptible *Anopheles stephensi* and *Aedes aegypti* strains against technical grade deltamethrin and cyfluthrin insecticides dissolved in ethanol in a range of different concentrations. The frequency of use of the deltamethrin-coated bottles and shelf-life were determined. Discriminating dose for deltamethrin and cyfluthrin was 10 µg against *An. stephensi* and 2 µg against *Ae. aegypti* females. Insecticide-coated bottles stored at 25 to 35 °C can be used for three exposures within 7 days of coating. The study carried out in the laboratory was validated on wild caught *An. culicifacies* in the states of Odisha and Chhattisgarh against deltamethrin-coated bottles in comparison to WHO adult susceptibility test. Results of the study indicated that deltamethrin-coated bottles were effective up to three exposures within 7 days of coating for field population and 100 % mortality was recorded within 35 min as observed in laboratory studies for field collected susceptible population. Also in the WHO adult susceptibility test, 100 % knock-down within 35 min and 100 % mortality after 24 h holding period were observed in susceptible population, while in it was 50 % knock-down in 1 h and 64 % mortality after 24 h holding period for resistant population (50 % mortality in bottle assay in 60 min). The bottle assay can be used as an alternative to the WHO adult susceptibility test both in the laboratory and field for monitoring insecticide resistance in mosquito vectors using locally sourced material.

KEYWORDS: bottle assay, insecticide resistance, mosquitoes, shelf-life, usability period, deltamethrin.

240. **Thomas T**, **Tanwee D De**, **Sharma P**, **Verma S, Rohilla S**, **Pandey KC**, **Dixit R**. Structural and functional prediction analysis of mosquito Ninjurin protein: Implication in the Innate Immune Responses in *Anopheles stephensi*. ***Int J Mosq Res*** **2014 Dec; *1*(4): 60-5.**

ABSTRACT

Ninjurin, identified as a two-pass transmembrane protein is induced upon nerve injury in vertebrates. Recent studies demonstrate that ninjurins are cell adhesion molecules, capable of

regulating many cellular functions *viz*. embryogenesis, injury, inflammation, signals etc. However, their structural and functional properties controlling these cellular responses, especially innate immune responses have not been investigated in detail. Through comprehensive molecular and functional genomics approach, here we characterize and predict the possible role of mosquito ninjurin (AsNinj) in hemocyte mediated cellular immune response. Molecular modeling analyses provide crucial information about the key residues of ninjurin proteins, for further in vitro and in vivo functional analysis.

KEYWORDS: mosquito, ninjurin, malaria, hemocyte, innate immunity.

241. Sudheer Ch, Kumar D, Sohani SK, Malik A, Chahar BR, **Dhiman RC**. A support Vector Machine-FireFly Algorithm based forecasting model to determine malaria transmission. ***Neurocomputing*** **2014; *129*: 279–88.**

ABSTRACT

Accurate and reliable forecasts of malarial incidences are necessary for the health authorities to ensure the appropriate action for the control of the outbreak. In this study, a novel method based on coupling the Firefly Algorithm (FFA) and Support Vector Machines (SVM) has been proposed to forecast the malaria incidences. The performance of SVM models depends upon the appropriate choice of SVM parameters. In this study FFA has been employed for determining the parameters of SVM. The proposed SVM-FFA model has been adopted in predicting the malarial incidences in Jodhpur and Bikaner area where the malaria transmission is unstable. Monthly averages of rainfall, temperature, relative humidity and malarial incidences have been considered as input variables. Time series of monthly notifications of malaria cases has been obtained from primary health centers and from other local health facilities for a period of January 1998 to December 2002 in the region of Bikaner and from January 1998 to December 2000 in Jodhpur region. Further, the rainfall, relative humidity and temperature data have been obtained from meteorological records. The performance of the proposed SVM-FFA model has been compared with Artificial Neural Networks (ANN), Auto-Regressive Moving Average method and also with Support Vector Machine. The results indicate that the proposed SVM-FFA model provides more accurate forecasts compared to the other traditional techniques. Further, it has been recommended to carry out

additional strides to explore the utility and efficacy of SVM-FFA model. Thus SVM-FFA can be an alternate tool to facilitate the control of vector borne diseases like malaria.

KEYWORDS: SVM, malarial incidences, forecasting, time series, FFA.

242. **Singh N**, **Shukla MM**, Chand G, Barde PV, **Singh MP**. Vector Borne Diseases in Central India with special reference to Malaria, Filaria, Dengue and Chikungunya. ***WHO South-East Asia J Public Health*** **2014; *3*(1): 28-35.**

ABSTRACT

Background: Vector-borne diseases (VBDs) caused by parasites and viruses are a major cause of morbidity and mortality in Madhya Pradesh (MP), central India. These diseases are malaria, lymphatic filariasis, dengue and chikungunya. Epidemiological information is lacking on different VBDs that are commonly prevalent in rural-tribal areas of MP, except on malaria. **Methods**: The studies were carried out at the request of Government of Madhya Pradesh, in three locations where many VBDs are endemic. Data on malaria/filaria prevalence were collected by repeatedly undertaking cross-sectional parasitological surveys in the same areas for 3 years. For dengue and chikungunya, suspected cases were referred to the research centre. **Results**: Monitoring of results revealed that all the diseases are commonly prevalent in the region, and show year-to-year variation. Malaria slide positivity (the number of malaria parasitaemic cases, divided by the total number of blood smears made) was 18.7% (190/1018), 16.4% (372/2266) and 20.4% (104/509) respectively in the years 2011, 2012 and 2013. There was a strong age pattern in both *Plasmodium vivax* and *P. falciparum*. The slide *vivax* rate was highest among infants, at 5% (odds ratio [OR] = 3.8; 95% confidence interval [CI] = 1.5 to 9.4; $P< 0.01$). The prevalence of dengue was 48% (dengue viruses 1 and 4 – DENV-1 and DENV-4), 59% (DENV-1) and 34% (DENV-3) respectively, in the years 2011, 2012 and 2013 among referred samples, while for chikungunya very few samples were found to be positive. **Conclusion**: Despite recent advances in potential vaccines and new therapeutic schemes, the control of VBDs remains difficult. Therefore, interruption of transmission still relies on vector-control measures. A coordinated, consistent,

integrated vector-management approach is needed to control malaria, filaria, dengue and chikungunya.

KEYWORDS: Chikungunya, dengue, filaria, malaria, Madhya Pradesh, vectorborne diseases.

243. **Tyagi S**, Pande V, **Das A**. Whole mitochondria genome sequence of an Indian *Plasmodium falciparum* isolate. ***Korean J Parasitol* 2014; *52*(1): 99-103.**

ABSTRACT

Mitochondrial genome sequence of malaria parasites has served as a potential marker for inferring evolutionary history of the *Plasmodium* genus. In *Plasmodium falciparum*, the mitochondrial genome sequences from around the globe have provided important evolutionary understanding, but no Indian sequence has yet been utilized. We have sequenced the whole mitochondrial genome of a single *P. falciparum* field isolate from India using novel primers and compared with the 3D7 reference sequence and 1 previously reported Indian sequence. While the 2 Indian sequences were highly divergent from each other, the presently sequenced isolate was highly similar to the reference 3D7 strain.

KEYWORDS: *Plasmodium falciparum*, malaria, whole genome sequencing, mitochondrial genome, India.

Titles: A-Z

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244. **Uragayala S**, **Verma V**, Natarajan E, **Velamuri PS**, **Kamaraju R**. Adulticidal & larvicidal efficacy of three neonicotinoids against insecticide susceptible & resistant mosquito strains. ***Indian J Med Res*** **2015 Dec; *142* (7): 64-70.**

ABSTRACT

Background & Objectives: Due to ever growing insecticide resistance in mosquitoes to commonly used insecticides in many parts of the globe, there is always a need for introduction of new insecticides for the control of resistant vector mosquitoes. In this study, larvicidal and adulticidal efficacies of three neonicotinoids (imidacloprid, thiacloprid and thiamethoxam) were tested against resistant and susceptible populations of *Anopheles stephensi* Liston 1901, *Aedes (Stegomyia) aegypti Linnaeus,* and *Culex quinquefasciatus* Say (Diptera: Culicidae). **Methods**: Laboratory-reared mosquito species were used. Insecticide susceptibility tests were done using standard WHO procedures and using diagnostic dosages of insecticide test papers and larvicides. Adulticidal efficacy of candidate insecticides was assessed using topical application method and

larval bioassays were conducted using standard WHO procedure. **Results**: The results of topical application on 3-5 day old female mosquitoes indicated that resistant strain of *An. stephensi* registered lower LC50values than the susceptible strain. Among the three insecticides tested, thiacloprid was found more effective than the other two insecticides. *Culex quinquefasciatus* registered lowest LC50 for imidacloprid than the other two mosquito species tested. In larval bioassays, the LC50 values registered for imidacloprid were in the order of *Cx. quinquefasciatus* <*An. stephensi* (SS) <*An. stephensi*(RR) <*Ae. aegypti*. In case of thiacloprid, the order of efficacy (LC50) was *Cx. quinquefasciatus* <*An. stephensi* (SS) <*An. stephensi* (RR), whereas in case of thiamethoxam, the larvicidal efficacy was in the order of *An. stephensi* (RR) <*An. stephensi* (SS) <*Cx. quinquefasciatus*. **Interpretation & Conclusions**: The present study indicated that insecticide resistant strains of mosquito species tested showed more susceptibility to the three neonicotinoids tested, and the possibility of using neonicotinoids for the control of resistant mosquitoes should be explored.

KEYWORDS: *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, imidacloprid, resistance, thiacloprid, thiamethoxam.

245. **Vijay S**, **Rawal R**, **Kadian K**, **Raghavendra K**, **Sharma A**. Annotated differentially expressed salivary proteins of susceptible and insecticide-resistant mosquitoes of *Anopheles stephensi*. ***PLoS One*** **2015; *10*(3): e0119666.**

ABSTRACT

Vector control is one of the major global strategies for control of malaria. However, the major obstacle for vector control is the development of multiple resistances to organochlorine, organophosphorus insecticides and pyrethroids that are currently being used in public health for spraying and in bednets. Salivary glands of vectors are the first target organ for human-vector contact during biting and parasite-vector contact prior to parasite development in the mosquito midguts. The salivary glands secrete anti-haemostatic, anti-inflammatory biologically active molecules to facilitate blood feeding from the host and also inadvertently inject malaria parasites into the vertebrate host. The *Anopheles stephensi* mosquito, an urban vector of malaria to both human and rodent species has been identified as a reference laboratory model to study mosquito—parasite interactions. In this study, we adopted a conventional proteomic approach of 2D-

electrophoresis coupled with MALDI-TOF mass spectrometry and bioinformatics to identify putative differentially expressed annotated functional salivary proteins between *An. stephensi* susceptible and multiresistant strains with same genetic background. Our results show 2D gel profile and MALDI-TOF comparisons that identified 31 differentially expressed putative modulated proteins in deltamethrin/DDT resistant strains of *An. stephensi*. Among these 15 proteins were found to be upregulated and 16 proteins were downregulated. Our studies interpret that *An. stephensi* (multiresistant) caused an upregulated expression of proteins and enzymes like cytochrome 450, short chain dehyrdogenase reductase, phosphodiesterase etc that may have an impact in insecticide resistance and xenobiotic detoxification. Our study elucidates a proteomic response of salivary glands differentially regulated proteins in response to insecticide resistance development which include structural, redox and regulatory enzymes of several pathways. These identified proteins may play a role in regulating mosquito biting behavior patterns and may have implications in the development of malaria parasites in resistant mosquitoes during parasite transmission.

246. Nagraj VA, Mukhi D, Satishkumar V, **Pradeep AS**, **Ghosh SK**, Pandey RR, Shetty MC, Padmanaban G**.** Asparagine requirement in *Plasmodium berghei* as a target to prevent malaria transmission and liver infections. ***Nat Comm* 2015; *6*: 8775. doi: 10.1038/ncomms9775 2015.**

ABSTRACT

The proteins of *Plasmodium*, the malaria parasite, are strikingly rich in asparagine. *Plasmodium* depends primarily on host haemoglobin degradation for amino acids and has a rudimentary pathway for amino acid biosynthesis, but retains a gene encoding asparagine synthetase (AS). Here we show that deletion of AS in *Plasmodium berghei* (*Pb*) delays the asexual- and liver-stage development with substantial reduction in the formation of ookinetes, oocysts and sporozoites in mosquitoes. In the absence of asparagine synthesis, extracellular asparagine supports suboptimal survival of *Pb*AS knockout (KO) parasites. Depletion of blood asparagine levels by treating *Pb*ASKO-infected mice with asparaginase completely prevents the development of liver stages, exflagellation of male gametocytes and the subsequent formation of sexual stages. *In vivo*supplementation of asparagine in mice restores the exflagellation of*Pb*ASKO

parasites. Thus, the parasite life cycle has an absolute requirement for asparagine, which we propose could be targeted to prevent malaria transmission and liver infections.

247. Gupta H, Jain A, Saadi AV, Vasudevan TG, Hande MH, D'Souza SC, **Ghosh SK**, Umakanth S, Satyamoorthy K. Categorical complexities of *Plasmodium falciparum* malaria in individuals is associated with genetic variations in *ADORA2A* and *GRK5* genes. ***Infect Genet Evol.*** **2015; *34*: 188-99.**

ABSTRACT

In the erythrocytes, malaria parasite entry and infection is mediated through complex membrane sorting and signaling processes. We investigated the effects of single-locus and multilocus interactions to test the hypothesis that the members of the GPCR family genes, adenosine A2a receptor (*ADORA2A*) and G-protein coupled receptor kinase5 (*GRK5*), may contribute to the pathogenesis of malaria caused by *Plasmodium falciparum* (*Pf*) independently or through complex interactions. In a case-control study of adults, individuals affected by *Pf* malaria (complicated *n*=168; uncomplicated *n*=282) and healthy controls (*n*=450) were tested for their association to four known SNPs in *GRK5* (rs2230345, rs2275036, rs4752307 and rs11198918) and two in *ADORA2A* (rs9624472 and rs5751876) genes with malaria susceptibility, using techniques of polymerase chain reaction-restriction fragment length polymorphisms and direct DNA sequencing. Single-locus analysis showed significant association of 2 SNPs; rs5751876 (OR=3.2(2.0-5.2); *p*=0.0006) of *ADORA2A* and rs2230345 (OR=0.3(0.2-0.5); *p*=0.0006) of *GRK5* with malaria. The mean of the serum creatinine levels were significantly higher in patients with variant GG (*p*=0.006) of rs9624472 in *ADORA2A* gene compared to AA and AG genotypes in complicated *Pf* malaria cases, with the G allele also showing increased risk for malaria(OR=1.3(1.1-1.6); *p*=0.017). Analyses of predicted haplotypes of the two *ADORA2A* and the four *GRK5* SNPs have identified the haplotypes that conferred risk as well as resistance to malariawith statistical significance. Molecular docking analysis of evolutionary rs2230345 SNP indicated a stable activity of *GRK5* for the mutant allele compared to the wild type. Further, generalized multifactor dimensionality reduction to test the contribution of individual effects of the six polymorphisms and higher-order interactions to risk of symptoms/clinical complications ofmalaria suggested a best

six-locus model showing statistical significance. The study provides evidence for the role of *ADORA2A* and *GRK5* that might influence the etiology of malariainfection.

KEYWORDS: *ADORA2A*, association, *GRK5*, gene interaction, malaria, SNPs.

248. **Mann R**, **Sharma S**, **Mishra N**, **Valecha N**, **Anvikar AR**. Comparative assessment of genomic DNA extraction processes for *Plasmodium*: Identifying the appropriate method. ***J Vector Borne Dis*** **2015 Dec; *52*(4): 273-80.**

ABSTRACT

Plasmodium DNA, in addition to being used for molecular diagnosis of malaria, find utility in monitoring patient responses to antimalarial drugs, drug resistance studies, genotyping and sequencing purposes. Over the years, numerous protocols have been proposed for extracting *Plasmodium* DNA from a variety of sources. Given that DNA isolation is fundamental to successful molecular studies, here we review the most commonly used methods for *Plasmodium* genomic DNA isolation, emphasizing their pros and cons. A comparison of these existing methods has been made, to evaluate their appropriateness for use in different applications and identify the method suitable for a particular laboratory based study. Selection of a suitable and accessible DNA extraction method for *Plasmodium* requires consideration of many factors, the most important being sensitivity, cost-effectiveness and, purity and stability of isolated DNA. Need of the hour is to accentuate on the development of a method that upholds well on all these parameters.

KEYWORDS: DNA isolation, malaria, molecular tools, *plasmodium*, protocols.

249. **Kumar V**, **Nagpal BN**, Pande V, **Srivastava A**, **Gupta SK**, **Anushrita**, **Singh VP**, **Singh H**, **Saxena R**, Tuli NR, Yadav NK, Paul R, **Valecha N**, Telle O. Comparison of *Aedes aegypti* breeding in localities of different socio-economic groups of Delhi, India. ***Int J Mosq Res*** **2015; *2*(3) Part B: 83–8.**

ABSTRACT

An entomological survey was carried out in different socio-economic groups of selected localities in Delhi during June, 2013 to May, 2014 with a view to study the prevalence and distribution of *Aedes aegypti* mosquito for appropriate interventions. A door-to-door entomological survey was

carried out to find out the *Aedes* breeding in all types of water filled containers present in and around houses and their premises and immature stages of *Aedes* mosquitoes were collected. In larval survey, different indices were used to record *Aedes aegypti* density level. In all the localities surveyed during transmission season, solid waste was observed to be most preferred breeding site whereas overhead tanks (OHTs) and curing tanks were found to be the most preferred breeding containers during non-transmission season. Plastic containers (29%) in low income group (LIG); solid waste (27%) and plastic containers (26%) in Medium Income Group (MIG); and solid waste (27%) and curing tanks (21%) in High Income Group (HIG) were the most preferred breeding containers for the breeding of *Aedes aegypti*. The house index was higher in the months of August and September in LIG, June- July in MIG and June in HIG households. The BI in MIG households was below critical level (*i.e*. 20) while it was observed to be higher in HIG & LIG households during the month of September *i.e* 22.45 & 25.22 respectively. The CI was observed to be higher in all the three types of colonies which were 8.35, 7.5 & 13.49 in HIG, MIG & LIG respectively, in the month of September. Containers found in Low income group (LIG) is contributing more to the *Aedes aegypti* breeding than MIG and HIG localities. The over head tanks and curing tanks are the preferred breeding sites in non transmission season whereas solid waste and plastic containers are amongst preferred breeding sites during transmission season. The study concluded that the targeted intervention including sustained vector surveillance could help in controlling the sudden upsurge of dengue in a densely populated city like Delhi.

KEYWORDS: dengue, *Aedes aegypti*, larvae, indices, surveillance.

250. **Srivastava B**, **Anvikar AR**, **Ghosh SK**, **Mishra N**, **Kumar N**, Houri-Yafin A, Pollak JJ, Salpeter SJ, **Valecha N**. Computer-vision-based technology for fast, accurate and cost effective diagnosis of malaria. ***Malar J*** **2015 Dec 30; *14*(1): 526.**

ABSTRACT

Background: Microscopy has long been considered to be the gold standard for diagnosis of malaria despite the introduction of newer assays. However, it has many challenges like requirement of trained microscopists and logistic issues. A vision based device that can diagnose malaria, provide speciation and estimate parasitaemia was evaluated. **Methods:** The device was evaluated using samples from 431 consented patients, 361 of which were initially screened by

RDT and microscopy and later analysed by PCR. It was a prospective, non-randomized, blinded trial. Quantification of parasitaemia was performed by two experienced technicians. Samples were subjected to diagnosis by Sight Dx digital imaging scanning. **Results:** The sensitivity and specificity of the SightDx P1 device for analysed samples were found to be 97.05 and 96.33 %, respectively, when compared to PCR. When compared to microscopy, sensitivity and specificity were found to be 94.4 and 95.6 %, respectively. The device was able to speciate 73.3 % of the PCR *Plasmodium falciparum* positive samples and 91.4 % of PCR *Plasmodium vivax* positive samples. **Conclusion:** The ability of the device to detect parasitaemia as compared with microscopy was within 50 % in 71.3 % of cases and demonstrated a correlation coefficient of 0.89.
KEYWORDS: malaria, diagnosis, PCR, device, sensitivity, specificity.

251. **Nagpal BN**, **Ghosh SK**, **Eapen A**, **Srivastava A**, **Sharma MC**, **Singh VP**, Parashar BD, Prakash Shri, Mendki MJ, Tikar SN, **Saxena R**, **Gupta S**, **Tiwari SN**, **Ojha VP**, **Ravindran KJ**, Ganesan K, Rao AN, Sharma RS, Tuli NR, Yadav NK, Vijayaraghavan R, **Dua VK**, **Dash AP**, Kaushik MP, Joshi PL, **Valecha N**. Control of *Aedes aegypti* and *Ae. albopictus*, the vectors of dengue and chikungunya, by using pheromone C21 with an insect growth regulator: Results of multicentric trials from 2007-12 in India. ***J Vector Borne Dis*** **2015; *52*: 224-31.**

ABSTRACT

Background & Objectives: *Aedes* mosquito control has gained much importance nowadays in view of rise in number of reported cases of dengue and chikungunya in India and other countries. In the present study, C21 attracticide (containing a pheromone and an insect growth regulator—IGR, developed by Defence Research and Development Establishment (DRDE), Gwalior, India was tested for its feasibility for surveillance and control of *Aedes* mosquito in a multicentric mode from October 2007 to June 2012 in urban (Delhi, and Bengaluru district, Karnataka) and suburban (Alappuzha district, Kerala) settings of the country in three phases. **Methods**: Across the randomly selected households in each study area, two to four containers treated with attracticide (experimental) and untreated (control) were placed and monitored by trained surveillance workers on weekly/ fortnightly basis for determining the presence of eggs, larvae and pupae. Container positivity, percent larvae, egg and pupae collected were determined during different phases and analyzed statistically using SPSS 18.0. **Results**: Container positivity was found statistically

significant at Bengaluru and Alappuzha, Kerala while in Delhi, it was found non-significant. Eggs collected from experimental containers were significantly higher in comparison to control at all the locations except Delhi. Also larvae collected from control containers were significantly higher at all the locations except Bengaluru. Pupae collected from control containers remained significantly higher at all the locations as no pupal formation was recorded from experimental containers. **Interpretation & Conclusion**: The use of C21 attracticide hampered pupal formation, thus inhibiting adult population in the study areas. The study established that C21 attracticide was efficacious in the field conditions and has potential for use in surveillance and management of dengue and chikungunya mosquitoes.

KEYWORDS: attracticide, C21, chikungunya, dengue, surveillance.

252. **Sharma P**, **Das De T**, **Sharma S**, Mishra AK, **Thomas T**, **Verma S**, **Kumari V**, **Lata S**, **Singh N**, **Valecha N**, **Pandey KC**, **Dixit R**. Deep sequencing revealed molecular signature of horizontal gene transfer of plant like transcripts in mosquito *Anopheles culicifacies*: an evolutionary puzzle. ***F1000Research*** **2015 Dec 30; *4*: 1523.**

ABSTRACT

In prokaryotes, horizontal gene transfer (HGT) has been regarded as an important evolutionarydrive to acquire and retain beneficial genes for their survival in diverse ecologies. However, in eukaryotes, the functional role of HGTs remains questionable, although current genomic tools are providing increased evidence of acquisition of novel traits within non-mating metazoan species. Here, we provide another transcriptomic evidence for the acquisition of massive plant genes in the mosquito, *Anopheles culicifacies*. Our multiple experimental validations including genomic PCR, RT-PCR, real-time PCR, immuno-blotting and immuno-florescence microscopy, confirmed that plant like transcripts (PLTs) are of mosquito origin and may encode functional proteins. A comprehensive molecular analysis of the PLTs and ongoing metagenomic analysis of salivary microbiome provide initial clues that mosquitoes may have survival benefits through the acquisition of nuclear as well as chloroplast encoded plant genes. Our findings of PLTs further support the similar questionable observation of HGTs in other higher organisms, which is still a controversial and debatable issue in the community of evolutionists. We believe

future understanding of the underlying mechanism of the feeding associated molecular responses may shed new insights in the functional role of PLTs in the mosquito.

KEYWORDS: mosquito, plant like transcript, salivary gland, feeding, malaria, microbial flora.

253. Kleinschmidt I, Mnzava AP, Kafy HT, Mbogo C, Bashir AI, Bigoga J, Adechoubou A, **Raghavendra K**, Knox TB, Malik EM, Nkuni ZJ, Bayoh N, Ochomo E, Fondjo E, Kouambeng C, Awono-Ambene HP, Etang J, Akogbeto M, **Bhatt R**, **Swain DK**, Kinyari T, Njagi K, Muthami L, Subramaniam K, Bradley J, West P, Massougbodji A, Okê-Sopoh M, Hounto A, Elmardi K, **Valecha N**, Kamau L, Mathenge E, Donnelly MJ. Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi-country investigation. ***Malar J*** **2015, Jul 22; *14*:282. doi: 10.1186/s12936-015-0782-4.**

ABSTRACT

Background: Progress in reducing the malaria disease burden through the substantial scale up of insecticide-based vector control in recent years could be reversed by the widespread emergence of insecticide resistance. The impact of insecticide resistance on the protective effectiveness of insecticide-treated nets (ITN) and indoor residual spraying (IRS) is not known. A multi-country study was undertaken in Sudan, Kenya, India, Cameroon and Benin to quantify the potential loss of epidemiological effectiveness of ITNs and IRS due to decreased susceptibility of malaria vectors to insecticides. The design of the study is described in this paper. **Methods:** Malaria disease incidence rates by active case detection in cohorts of children, and indicators of insecticide resistance in local vectors were monitored in each of approximately 300 separate locations (clusters) with high coverage of malaria vector control over multiple malaria seasons. Phenotypic and genotypic resistance was assessed annually. In two countries, Sudan and India, clusters were randomly assigned to receive universal coverage of ITNs only, or universal coverage of ITNs combined with high coverage of IRS. Association between malaria incidence and insecticide resistance, and protective effectiveness of vector control methods and insecticide resistance were estimated, respectively. **Results:** Cohorts have been set up in all five countries, and phenotypic resistance data have been collected in all clusters. In Sudan, Kenya, Cameroon and Benin data

collection is due to be completed in 2015. In India data collection will be completed in 2016. **Discussion:** The paper discusses challenges faced in the design and execution of the study, the analysis plan, the strengths and weaknesses, and the possible alternatives to the chosen study design.

254. Singh AK, Rajendran V, **Pant A**, Ghosh PC, **Singh N**, Latha N, Garg S, **Pandey KC**, Singh BK, Rathi B. Design, synthesis and biological evaluation of functionalized phthalimides: a new class of antimalarials and inhibitors of falcipain-2, a major hemoglobinase of malaria parasite. ***Bioorg Med Chem* 2015; *23*(8): 1817-27.**

ABSTRACT

Phthalimides functionalized with cyclic amines were synthesized, characterized and screened for their in vitro antimalarial efficacy against *Plasmodium falciparum* (*Pf3D7*). Of all the listedphthalimides evaluated, 14 and 24 were identified as potent antimalarial agents as advocated by assessment of their ability to inhibit [(3)H] hypoxanthine incorporation in the nucleic acid of parasites. In addition, phthalimides 14 and 24 were incubated for 60 and 90h and an enhanced antimalarial effect was noticed with increase in time to great extent. A reduction in IC50 values was observed with increase in exposure time of the parasite to the compounds. A symmetric phthalimide, 24 possessing piperazine as linker unit was identified as the most potent antimalarial agent with IC50 values of 5.97±0.78, 2.0±1.09 and 1.1±0.75μM on incubation period of 42, 60 and 90h, respectively. The abnormal morphologies such as delay in developmental stages, growth arrest and condensed nuclei of parasite were observed with the aid of microscopic studies upon exposure with 14 and 24. The evaluation of 14 and 24 against chloroquine resistant strain, (*Pf7GB*) of *P. falciparum* afforded IC50 values, 13.29±1.20 and 7.21±0.98μM, respectively. The combination of 24 with artemisinin (ART) showed enhanced killing of parasite against *Pf3D7*. Further, all phthalimides were evaluated for their activity against falcipain-2 (FP2), a majorhemoglobinase of malarial parasite. The enzymatic assay afforded 6 as most active member against FP2. To the best of our knowledge this is the initial study represents phthalimide protected amino acids functionalized with cyclic amines as potent antimalarial agents.

KEYWORDS: docking, Falcipain-2, phthalimides, piperazine, *Plasmodium falciparum*.

255. **Vikram K**, **Nagpal BN**, Pande V, **Srivastava A**, **Saxena R**, **Singh H**, **Anushrita**, **Gupta SK**, Tuli NR, Yadav NK, Olivier T, Richard P, **Valecha N**. Detection of dengue virus in individual *Aedes aegypti* mosquitoes in Delhi, India. ***J Vector Borne Dis*** **2015; *52*(2): 129-33.**

ABSTRACT

Background & Objectives: Delhi, the capital city of India, has so far witnessed several outbreaks of dengue fever since 1967 (last one reported in 2013). Improved virological and entomological surveillance are the only tools that can help in prevention of dengue as well as in the development of dengue control programmes. The aim of the study was to conduct a prospective field study to detect dengue virus in adult *Aedes aegypti* mosquitoes collected from various localities represented by different socioeconomic groups in Delhi. **Methods**: The study areas were selected and categorized into high, medium and low income groups on the basis of socioeconomical characteristics of the resident population, where dengue cases were reported during the past three years by MCD. Dengue viral infection was detected in the head squash of each adult mosquito by immunofluorescent assay (IFA) employing monoclonal antibodies against dengue virus (DENV). A total of 2408 females and 1206 males of *Ae. aegypti* were collected and tested by IFA. **Results**: Out of 2408 *Ae. aegypti* females, 14 were found positive, with minimum infection rate (MIR) of 5.8 per 1000 mosquitoes. Among the 18 study areas, 11 localities were found positive for dengue virus infection. Low income group (LIG) areas showed highest mosquito infectivity (9.8), followed by medium income group (MIG), *i.e*. 6.2; while least was observed in high income group (HIG), *i.e*. 1.3. No vertical transmission of dengue virus could be detected in 1206 *Ae. aegypti* males collected. **Interpretation & Conclusion**: The study concludes that there was high MIR in the identified localities of low and medium income groups. Estimation of MIR in a female *Aedes* mosquito in the existing arsenals for dengue surveillance would be an added advantage for early warning of dengue outbreak. The presence of infected mosquitoes in identified localities of Delhi was alarming and require rigorous vector surveillance so that thesevere outbreaks can be prevented.

KEYWORDS: *Aedes aegypti*, dengue virus, immunofluorescent assay, minimum infection rate.

256. **Sharma D**, **Lather M, Dykes CL**, Dang AS, **Adak T**, **Singh OP**. Disagreement in genotyping results of drug resistance alleles of the *Plasmodium falciparum* dihydrofolate reductase (*Pfdhfr*)

gene by allele-specific PCR (ASPCR) assays and Sanger sequencing. ***Parasitol Res*** **2016 Jan; *115*(1): 323-8.**

ABSTRACT

The rapid spread of antimalarial drug resistance in *Plasmodium falciparum* over the past few decades has necessitated intensive monitoring of such resistance for an effective malaria control strategy. *P. falciparum* dihydropteroate synthase (*Pfdhps*) and *P. falciparum* dihydrofolate reductase (*Pfdhfr*) genes act as molecular markers for resistance against the antimalarial drugs sulphadoxine and pyrimethamine, respectively. Resistance to pyrimethamine which is used as a partner drug in artemisinin combination therapy (ACT) is associated with several mutations in the *Pfdhfr* gene, namely A16V, N51I, C59R, S108N/T and I164L. Therefore, routine monitoring of *Pfdhfr*-drug-resistant alleles in a population may help in effective drug resistance management.Allele-specific PCR (ASPCR) is one of the commonly used methods for molecular genotyping of these alleles. In this study, we genotyped 55 samples of *P. falciparum* for allele discrimination at four codons of *Pfdhfr* (N51, C59, S108 and I164) by ASPCR using published methods and by Sanger's DNA sequencing method. We found that the ASPCR identified a significantly higher number of mutant alleles as compared to the DNA sequencing method. Such discrepancies arise due to the non-specificity of some of the allele-specific primer sets and due to the lack of sensitivity of Sanger's DNA sequencing method to detect minor alleles present in multiple clone infections. This study reveals the need of a highly specific and sensitive method for genotypingand detecting minor drug-resistant alleles present in multiple clonal infections.

KEYWORDS: allele-specific PCR, *Pfdhfr* gene, *Plasmodium falciparum*, pyrimethamine.

257. **Das A**. The distinctive features of Indian malaria parasites. ***Trends Parasitol*** **2015; *31*(3): 83-6.**

ABSTRACT

Malaria and factors driving malaria are heterogeneous in India, unlike in other countries, and the epidemiology of malaria therefore is considered 'highly complex'. This complexity is primarily attributed to several unique features of the malaria parasites, mosquito vectors, malaria-susceptible populations, and ecoclimatic variables in India. Recent research on the genetic epidemiology of

Indian malaria parasites has been successful in partly unraveling the mysteries underlying these complexities.

KEYWORDS: India, *Plasmodium falciparum*, *Plasmodium vivax*, genetic epidemiology, malaria.

258. Adjuik MA, Allan R, **Anvikar AR**, Ashley EA, Ba MS, Barennes H, Barnes KI, Bassat Q, Baudin E, Björkman A, Bompart F, Bonnet M, Borrmann S, Brasseur P, Bukirwa H, Checchi F, Cot M, Dahal P, D'Alessandro U, Deloron P, Desai M, Diap G, Djimde AA, Dorsey G, Doumbo OK, Espié E, Etard JF, Fanello CI, Faucher JF, Faye B, Flegg JA, Gaye O, Gething PW, González R, Grandesso F, Guerin PJ, Guthmann JP, Hamour S, Hasugian AR, Hay SI, Humphreys GS, Jullien V, Juma E, Kamya MR, Karema C, Kiechel JR, Kremsner PG, Krishna S, Lameyre V, Ibrahim LM, Lee SJ, Lell B, Mårtensson A, Massougbodji A, Menan H, Ménard D, Menéndez C, Meremikwu M, Moreira C, Nabasumba C, Nambozi M, Ndiaye JL, Nikiema F, Nsanzabana C, Ntoumi F, Ogutu BR, Olliaro P, Osorio L, Ouédraogo JB, Penali LK, Pene M, Pinoges L, Piola P, Price RN, Roper C, Rosenthal PJ, Rwagacondo CE, Same-Ekobo A, Schramm B, Seck A, Sharma B, Sibley CH, Sinou V, Sirima SB, Smith JJ, Smithuis F, Somé FA, Sow D, Staedke SG, Stepniewska K, Swarthout TD, Sylla K, Talisuna AO, Tarning J, Taylor WR, Temu EA, Thwing JI, Tjitra E, Tine RC, Tinto H, Vaillant MT, **Valecha N**, Van den Broek I, White NJ, Yeka A, Zongo I. The effect of dosing strategies on the therapeutic efficacy of artesunate-amodiaquine for uncomplicated malaria: a meta-analysis of individual patient data. ***BMC Med*** **2015, Mar 31; *13*: 66. doi: 10.1186/s12916-015-0301-z.**

ABSTRACT

Background: Artesunate-amodiaquine (AS-AQ) is one of the most widely used artemisinin-based combination therapies (ACTs) to treat uncomplicated *Plasmodium falciparum* malaria in Africa. We investigated the impact of different dosing strategies on the efficacy of this combination for the treatment of *falciparum* malaria. **Methods:** individual patient data from as-aq clinical trials were pooled using the worldwide antimalarial resistance network (wwarn) standardised methodology. risk factors for treatment failure were identified using a cox regression model with shared frailty across study sites. **Results:** Forty-three studies representing 9,106 treatments from 1999-2012 were included in the analysis; 4,138 (45.4%) treatments were with a fixed dose combination with an AQ target dose of 30 mg/kg (FDC), 1,293 (14.2%) with a non-fixed dose combination with an AQ

target dose of 25 mg/kg (loose NFDC-25), 2,418 (26.6%) with a non-fixed dose combination with an AQ target dose of 30 mg/kg (loose NFDC-30), and the remaining 1,257 (13.8%) with a co-blistered non-fixed dose combination with an AQ target dose of 30 mg/kg (co-blistered NFDC). The median dose of AQ administered was 32.1 mg/kg [IQR: 25.9-38.2], the highest dose being administered to patients treated with co-blistered NFDC (median= 35.3 mg/kg [IQR: 30.6-43.7]) and the lowest to those treated with loose NFDC-25 (median= 25.0 mg/kg [IQR: 22.7-25.0]). Patients treated with FDC received a median dose of 32.4 mg/kg [IQR: 27-39.0]. After adjusting for reinfections, the corrected antimalarial efficacy on day 28 after treatment was similar for co-blistered NFDC (97.9% [95% confidence interval (CI): 97.0-98.8%]) and FDC (98.1% [95% CI: 97.6%-98.5%]; $P = 0.799$), but significantly lower for the loose NFDC-25 (93.4% [95% CI: 91.9%-94.9%]), and loose NFDC-30 (95.0% [95% CI: 94.1%-95.9%]) ($P < 0.001$ for all comparisons). After controlling for age, AQ dose, baseline parasitemia and region; treatment with loose NFDC-25 was associated with a 3.5-fold greater risk of recrudescence by day 28 (adjusted hazard ratio, AHR= 3.51 [95% CI: 2.02 -6.12], $P < 0.001$) compared to FDC, and treatment with loose NFDC-30 was associated with a higher risk of recrudescence at only three sites. **Conclusions**: There was substantial variation in the total dose of amodiaquine administered in different AS-AQ combination regimens. Fixed dose AS-AQ combinations ensure optimal dosing and provide higher antimalarial treatment efficacy than the loose individual tablets in all age categories.

259. Toure OA, Rulisa S, **Anvikar AR**, Rao BS, Mishra P, Jalali RK, Arora S, Roy A, Saha N, Iyer SS, **Sharma P**, **Valecha N**. Efficacy and safety of fixed dose combination of arterolane maleate and piperaquine phosphate dispersible tablets in paediatric patients with acute uncomplicated *Plasmodium falciparum* malaria: a phase II, multicentric, open-label study. ***Malar J*** **2015 Nov 25; *14*(1):469. doi: 10.1186/s12936-015-0982-y.**

ABSTRACT

Background: The World Health Organization (WHO) recommends artemisinin combination therapy (ACT) for the treatment of uncomplicated*Plasmodium falciparum* malaria. The present study investigated the efficacy and safety of fixed dose combination (FDC) of arterolane maleate 37.5 mg and piperaquine phosphate (PQP) 187.5 mg dispersible tablets in paediatric patients aged

6 months to 12 years. **Methods:** Male and female patients aged 6 months to 12 years who were confirmed cases of *P. falciparum* mono-infection with fever or documented history of fever in the previous 24 h were included. The patients were administered FDC of arterolane maleate and PQP as single daily doses for three consecutive days based on their age. The primary efficacy outcome was proportion of patients with polymerase chain reaction (PCR)-corrected adequate clinical and parasitological response (ACPR) on day 28. Safety was analysed based on adverse events (AE), laboratory abnormalities and abnormalities on electrocardiograph. **Results:** A total of 141 eligible paediatric patients received FDC of arterolane maleate and PQP in a 42-day follow-up study. All the enrolled patients (141) were included in intention to treat (ITT) and safety analyses, and 126 patients were considered in per protocol (PP) population. The PCR-corrected ACPR on day 28 was achieved in all patients (100 %; 95 % CI 97.11–100) included in PP population. The median parasite clearance time (PCT) and fever clearance time (FCT) were 24 h (95 % CI 18.0–24.0) and 10 h (95 % CI 4.0–18.0), respectively. The most frequently reported clinical AE was vomiting. Majority of the AEs were mild to moderate in severity and resolved without sequelae. No patient was discontinued for any QTc (corrected QT interval) prolongation. No deaths or serious AEs were reported during the study. **Conclusion:** The findings from this study showed that FDC of arterolane maleate and PQP effectively cures *P. falciparum* malaria and attains acceptable level of cure by day 28 in paediatric patients. The efficacy and safety results observed in children warrants further studies on FDC of arterolane maleate and PQP dispersible tablets.

KEYWORDS: artemisinin combination therapy, arterolane maleate, piperaquine phosphat, malaria, paediatric PCT, FCT, PCR-corrected, ACPR, fixed dose combination.

260. **Kumar A**, **Moirangthem R**, Gahlawat SK, Chandra J, **Gupta P**, **Valecha N**, **Anvikar A**, **Singh V**. Emergence of *sulfadoxine-pyrimethamine* resistance in Indian isolates of *Plasmodium falciparum* in the last two decades. ***Infect Genet Evol* 2015 Dec; *36*: 190-8.**

ABSTRACT

Genotyping the *sulfadoxine-pyrimethamine* (SP) genes will help in identifying the genes under drug selection and the emergence of resistance in dhfr and dhps genes. India is an important hotspot for studying malaria due to the immense climatic diversity prevalent in the country. The central and eastern parts of the country are most vulnerable sites where malaria cases are reported

throughout the year. From different regions of the country 173 field isolates were genotyped at various loci in dhfr and dhps genes collected between 1994 and 2013. This encompasses the period before antimalarial resistance emerged and the period after the use of combination therapy was made mandatory in the country. We observed the rise of resistant SP alleles from very low frequencies (in the year 1994) to steadily rising (in the year 2000) and maintaining this increasing trend subsequently (in the year 2013) as shown by the sequence analysis of dhfr and dhps genes. This study assessed the prevalence of mutations in dhfr and dhps genes associated with SP resistance in samples indicative of increase in resistance levels of *Plasmodium falciparum* to SP even after the change in malaria treatment policy in the country.

KEYWORDS: drug resistance, mutations, *P. falciparum*, dhfr, dhps genes.

261. Conn JE, Norris DE, Donnelly MJ, Beebe NW, Burkot TR, Coulibaly MB, Chery L, **Eapen A**, Keven JB, Kilama M, **Kumar A**, Lindsay SW, Moreno M, Quiñones ML, Reimer LS, Russell TL, Smith DL, Thomas MB, Walker ED, Wilson ML, Yan G. Entomological monitoring and evaluation: Diverse transmission settings of ICEMR projects will require local and regional malaria elimination strategies. ***Am J Trop Med Hyg*** **2015 Sep; *93*(Suppl 3): 28-41.**

ABSTRACT.

The unprecedented global efforts for malaria elimination in the past decade have resulted in altered vectorial systems, vector behaviors, and bionomics. These changes combined with increasingly evident heterogeneities in malaria transmission require innovative vector control strategies in addition to the established practices of long-lasting insecticidal nets and indoor residual spraying. Integrated vector management will require focal and tailored vector control to achieve malaria elimination. This switch of emphasis from universal coverage to universal coverage plus additional interventions will be reliant on improved entomological monitoring and evaluation. In 2010, the National Institutes for Allergies and Infectious Diseases (NIAID) established a network of malaria research centers termed ICEMRs (International Centers for Excellence in Malaria Research) expressly to develop this evidence base in diverse malaria endemic settings. In this article, we contrast the differing ecology and transmission settings across the ICEMR study locations. In South America, Africa, and Asia, vector biologists are already dealing with many of the issues of

pushing to elimination such as highly focal transmission, proportionate increase in the importance of outdoor and crepuscular biting, vector species complexity, and “sub patent” vector transmission

262. Masur U, **Kumar H**, **Kumar A**. Evaluation of larvicidal effect of *Dysoxylum binectariferum* Hook against *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* L. ***Int J Bioassays* 2015; *4*(12): 4685-8.**

ABSTRACT

In the present study larvicidal activity of methanolic extract of leaf of *Dysoxylum binectariferum* Hook (Meliaceae) was assessed against 3rd & 4th instar larvae of 3 mosquito vector species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The bioassays revealed that *Cx. quinquefasciatus* was most sensitive species. The dose mortality data were subjected to log probit regression analysis to determine median lethal concentrations, LC50 and LC90 after 24-hour exposure. The highest mortality was observed in *Cx. quinquefasciatus* [LC50 = 340 ppm, (95% CI: 240 - 460 ppm); LC90 = 600 ppm (CI: 390- 1083 ppm)] followed by *Ae. aegypti* [LC50 = 3236 ppm, (CI: 2500-4180 ppm); LC90 = 55410 ppm (CI: 34720-103910 ppm)] and *An. stephensi* [LC50 = 13460 ppm (CI: 12840-14180 ppm); LC90 = 18010 ppm (CI: 16650-20360 ppm)]. As a larvicide, leaf extracts of *Dysoxylum binectariferum* could be explored further.

KEYWORDS: *Dysoxylum binectariferum* hook, larvicidal effect, leaf extract *Anopheles stephensi, Culex quinquefasciatus, Aedes aegypti*.

263. **Savargaonkar D**, **Nagpal BN**, **Srivastava B**, **Anvikar AR**, **Valecha N**. The footprints of relapsing malaria in southwest Delhi, India. ***J Vector Borne Dis* 2015 Dec; *52*(4): 287-92.**

ABSTRACT

Background & Objectives: Control of *vivax* malaria is challenging due to persistence of hypnozoites causing relapses and safety concerns with primaquine in G6PD deficient individuals. We present the epidemiology of malaria with emphasis on recurrence of *vivax* malaria over a period of four years in southwest Delhi among patients reporting to malaria clinic. Methods: Microscopic examination of stained blood smears of fever patients attending malaria clinic was performed. Confirmed malaria cases were treated as per the national treatment guidelines. The

epidemiological data of confirmed malaria cases including demographic characteristics, age, gender and past history of malaria were analysed. Patients were asked to report in case of occurence of fever. **Results:** From January 2011 to December 2014, 429 *Plasmodium vivax*, 24 *P. falciparum* and three mixed infection cases were reported to the Malaria Clinic at National Institute of Malaria Research, New Delhi. Malaria cases peaked in the months of August and September during all the four years. Recurrent episodes of *vivax* malaria were observed in 14.72% patients to whom primaquine was not dispensed, while the prevalence was 4.02% among those who received primaquine. The relapsing patterns observed were of both short as well as long latency *P. vivax* phenotypes. The entomological survey of area from where malaria patients reported, showed prevalence of *Anopheles stephensi*. **Interpretation & Conclusion**: The study showed presence of persistent *P. vivax* malaria with strains causing both frequent and long latency recurrences (probable relapses) in southwest Delhi. This highlights the need to evaluate primaquine regimens against both these strains and formulate strategies to improve compliance to 14-days primaquine treatment.

KEYWORDS: Delhi, malaria, *Plasmodium vivax*, relapse.

264. **Tripathi M**, **Das A**. Genotyping malaria parasites with DNA barcodes. ***Trop Med Int Health* 2015 Dec; *20*(12): 1636-8.**

265. Price RN, von Seidlein L, **Valecha N**, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant *Plasmodium vivax* - Authors' reply. ***Lancet Infect Dis* 2015 Jun; *15*(6): 630-1.**

266. **Ghosh SK**. Human dirofilariasis: a fast emerging zoonosis in India. ***Indian J Med Microbiol* 2015; *33*: 595-6.**

267. Quinones ML, Norris De, Mzilahowa T, Conn JE, Moreno M, Burkot TR, Yan G, Rosas A, Palomino M, Donnelly MJ, Mawajje HD, **Eapen A**, Coulibaly MB, Beier J, **Kumar A**. Insecticide resistance in areas under investigation by the International Centers of Excellence for Malaria Research: A challenge for malaria control and elimination. ***Am J Trop Med Hyg* 2015; *93*(Suppl 3): 69-78.**

ABSTRACT

Scale-up of the main vector control interventions, residual insecticides sprayed on walls or structures and/or impregnated in bed nets, together with prompt diagnosis and effective treatment, have led to a global reduction in malaria transmission. However, resistance in vectors to almost all classes of insecticides, particularly to the synthetic pyrethroids, is posing a challenge to the recent trend of declining malaria. Ten International Centers of Excellence for Malaria Research (ICEMR) located in the most malaria-endemic regions of the world are currently addressing insecticide resistance in the main vector populations, which not only threaten hope for elimination in malaria-endemic countries but also may lead to reversal where notable reductions in malaria have been documented. This communication illustrates the current status of insecticide resistance with a focus on the countries where activities are ongoing for 9 out of the 10 ICEMRs. Most of the primary malaria vectors in the ICEMR countries exhibit insecticide resistance, albeit of varying magnitude, and spanning all mechanisms of resistance. New alternatives to the insecticides currently available are still to be fully developed for deployment. Integrated vector management principles need to be better understood and encouraged, and viable insecticide resistance management strategies need to be developed and implemented.

268. Kanchan K, Jha P, Pati SS, Mohanty S, Mishra SK, **Sharma SK**, Awasthi S, Venkatesh V, Habib S. Interferon-γ (IFNG) microsatellite repeat and single nucleotide polymorphism haplotypes of IFN-α receptor (IFNAR1) associated with enhanced malaria susceptibility in Indian populations. ***Infect Genet Evol* 2015 Jan; *29*: 6-14. doi: 10.1016/j.meegid.2014.10.030.**

ABSTRACT

Pro-inflammatory cytokines IFNγ and IFNα function through their cellular receptors IFNγR1 and IFNαR1, respectively to mediate immune processes during malaria infection. A total of 21 SNPs, 2 ins/del polymorphisms and a microsatellite repeat, selected on the basis of their reported association with infectious diseases including malaria in world populations, were analysed for association with *Plasmodium falciparum* malaria susceptibility in a case-control study with adult patients and ethnically-matched controls drawn from a disease meso- to hyperendemic and a nonendemic region of India. Among the five IFNG SNPs tested, an intron 3 and a 3'UTR SNPassociated with disease in the endemic region. In addition, large (CA)n repeats of IFNG intron

1associated with protection from severe malaria in the endemic region (severe vs. control, odds ratio=0.21, 95% CI=0.08-0.52, *P*=1.3 × 10(-4)). The TA11CAG haplotype (rs2069705 T/C, rs2430561 A/T, rs3138557 (CA)n, rs2069718 T/C, rs2069727 A/G, rs2069728 G/A) carrying a short CA^{11} repeat also exhibited very strong association with severe malaria, particularly in the endemic region (severe vs. control, OR=14.56, 95% CI=3.39-85.81, *P*=3 × 10(-5)). One SNP each from the IFNA8 and IFNA17 of IFNA gene cluster had a protective effect in the non-endemic region but not in the endemic region. A promoter and an intron 2 SNP of IFNAR1 were risk factors for disease and the IFNAR1 haplotype GCCAGG (rs2843710 C/G, rs2850015 C/T, +6993 C/T, rs2243594 A/G, rs1012335 G/C, rs2257167 G/C) carrying both the risk alleles strikingly associated with disease manifestation in the endemic region (severe vs. control, OR=27.14, 95% CI=3.12-1254, *P*=2 × $10^{(-5)}$; non-severe vs. control, OR=61.87, 95% CI=10.08-2521, *P*=1 × 10(-8)). The data indicates dissimilar contribution of cytokine and cytokine receptorvariants to disease in populations residing in areas of differential malaria endemicity.

KEYWORDS: cytokine, haplotypes, malaria susceptibility, polymorphisms, receptor.

269. **Lather M**, **Sharma D**, Dang AS, **Adak T**, **Singh OP**. Isolation and characterization of polymorphic microsatellite markers from the malaria vector *Anopheles fluviatilis* species T (Diptera: Culicidae). ***J Med Entomol* 2015; *52*(3)*:* 408-12.**

ABSTRACT

Anopheles fluviatilis James is an important malaria vector in India, Pakistan, Nepal, and Iran. It has now been recognized as a complex of at least four sibling species-S, T, U, and V, among which species T is the most widely distributed species throughout India. The taxonomic status of these species is confusing owing to controversies prevailing in the literature. In addition, chromosomal inversion genotypes, which were considered species-diagnostic for *An. fluviatilisspecies* T, are unreliable due to the existence of polymorphism in some populations. To study the genetic diversity at population level, we isolated and characterized 20 microsatellite markers frommicrosatellite-enriched genomic DNA library of *An. fluviatilis* T, of which 18 were polymorphicwhile two were monomorphic. The number of alleles per locus among polymorphic markersranged from 4 to 19, and values for observed and expected

heterozygosities varied from 0.352 to 0.857 and from 0.575 to 0.933, respectively. Thirteen markers had cross-cryptic speciestransferability to species S and U of the Fluviatilis Complex. This study provides a promising genetic tool for the population genetic analyses of *An. fluviatilis*.

KEYWORDS: *Anopheles fluviatilis* T, malaria, microsatellite marker, population genetics.

270. **Dykes CL**, **Kushwah RB**, **Das MK**, **Sharma SN**, **Bhatt RM**, Veer V, Agrawal OP, **Adak T**, **Singh OP**. Knockdown resistance (*kdr*) mutations in Indian *Anopheles culicifacies* Populations. ***Parasit Vectors*. 2015; *8*: 333. doi: 10.1186/s13071-015-0946-7.**

ABSTRACT

Background: *Anopheles culicifacies* s.l. is one of the primary vectors of malaria in India responsible for the highest number of malaria cases. This vector is resistant to DDT in most parts of the country with indication of emerging resistance to pyrethroids. Since knockdown resistance (*kdr*) is known to confer cross-resistance between DDT and pyrethroids owing to a common target site of action, knowledge of prevalence of knockdown resistance (*kdr*) alleles is important from insecticide resistance management point of view. **Methods**: Nine populations of *An. culicifacies* belonging to five states of India, representing northern, western and central-east India, were screened for the presence of two alternative *kdr* mutations L1014F and L1014S using PCR-based assays. Dead and alive mosquitoes, following WHO standard insecticide susceptibility test against deltamethrin and DDT, were tested for allelic association. **Results:** L1014F mutation was recorded in all populations studied except from Haryana and Rajasthan states in northern India, with low frequencies ranging between 0.012 and 0.076; whereas presence of L1014S mutation was recorded in five populations only belonging to central-east India, with allelic frequencies ranging between 0.010 and 0.046. Both the *kdr* mutant alleles were found mostly in heterozygous condition without deviating from Hardy-Weinberg equilibrium. Both mutations showed protection against deltamethrin whereas only L1014S mutation showed protection against DDT when tested using additive model. **Conclusions:** The two L1014-*kdr* mutations, L1014F and L1014S, co-occurred in five populations belonging to Chhattisgarh and Odisha states of India whereas L1014F was present in all populations studied except populations from northern states. Both *kdr* mutations were found

with very low allelic frequencies mostly in heterozygous condition and exhibited protection against deltamethrin.

KEYWORDS: *Anopheles culicifacies,* voltage gated sodium channel, knockdown resistance, Insecticide resistance.

271. Tennyson S, **Ravindran J**, **Eapen A**, William J. Larvicidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). ***Asian Pac J Trop Dis*** **2015; *5*(Suppl 1): S73-6.**

ABSTRACT

Objective: To evaluate the larvicidal activity of *Ageratum houstonianum* (*A. houstonianum*) crude leaf extracts against the immatures of vector mosquitoes. Methods: Bioassays were performed in the laboratory with hexane, ethyl acetate and methanol crude leaf extracts of *A. houstonianum* at concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 mg/L against the third instar larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. **Results**: Poor larvicidal activity was observed. The lowest LC50 value was noted in ethyl acetate extract against all three vector mosquito species studied and was 3377.84, 1952.12 and 3558.32 mg/L respectively after 24 h. The effect of toxicity was also manifested in a shorter period when compared to the other extracts *viz*., hexane and methanol. In *Anopheles stephensi*, more than 80% mortality was however observed at higher concentrations, after 24 h exposure in all the three extracts. In *Aedes aegypti* and *Culex quinquefasciatus*, this was observed by 3 and 24 h respectively in ethyl acetate extract. **Conclusions**: Screening of other parts of *A. houstonianum* with other solvents from different places for its larvicidal activity is recommended.

KEYWORDS: *Ageratum houstonianum,* crude leaf extracts, *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus,* larvicidal activity.

272. **Singh OP**, **Dykes CL**, **Sharma G**, **Das MK**. L1014F-*kdr* mutation in Indian *Anopheles subpictus* (Diptera: Culicidae) arising from two alternative transversions in the voltage gated sodium channel and a single PIRA-PCR for their Detection. ***J Med Entomol*** **2015; *52*(1): 24-27.**

ABSTRACT

Leucine-to-phenylalanine substitution at residue L1014 in the voltage-gated sodium channel, target site of action for dichlorodiphenyltrichloroethane (DDT) and pyrethroids, is the most common knockdown resistance (*kdr*) mutation reported in several insects conferring resistance against DDT and pyrethroids. Here, we report presence of two coexisting alternative transversions, A>T and A>C, on the third codon position of L1014 residue in malaria vector *Anopheles subpictus Grassi* (species A) from Jamshedpur (India), both leading to the same amino acid substitution of Leu-to-Phe with allelic frequencies of 19 and 67%, respectively. A single primer-introduced restriction analysis-polymerase chain reaction (PIRA-PCR) was devised for the identification of L1014F-*kdr* mutation in *An. subpictus* resulting from either type of point mutation. Genotyping of samples with PIRA-PCR revealed high frequency (82%) of L1014F-*kdr* mutation in the study area.

KEYWORDS: *Anopheles subpictus*, knockdown resistance, malaria, sodium channel.

273. Moss WJ, Dorsey G, Mueller I, Laufer MK, Krogstad DJ, Vinetz JM, Guzman M, Rosas-Aguirre AM, Herrera S, Arevalo-Herrera M, Chery L, **Kumar A**, Mohapatra PK, Ramanathapuram L, **Srivastava HC**, Cui L, Zhou G, Parker DM, Nankabirwa J, Kazura JW. Malaria Epidemiology and Control within the International Centers of Excellence for Malaria Research. ***Am J Trop Med Hyg* 2015: *93*(Suppl 3): 5-15. doi:10.4269/atmh.15-0006.**

ABSTRACT.

Understanding the epidemiological features and metrics of malaria in endemic populations is a key component to monitoring and quantifying the impact of current and past control efforts to inform future ones. The International Centers of Excellence for Malaria Research (ICEMR) has the opportunity to evaluate the impact of malaria control interventions across endemic regions that differ in the dominant *Plasmodium* species, mosquito vector species, resistance to antimalarial drugs and human genetic variants thought to confer protection from infection and clinical manifestations of plasmodia infection. ICEMR programs are conducting field studies at multiple sites with the aim of generating standardized surveillance data to improve the understanding of malaria transmission and to monitor and evaluate the impact of interventions to inform malaria control and elimination programs. In addition, these epidemiological studies provide a vast source of biological samples linked to clinical and environmental "meta-data" to support translational studies of interactions between the parasite, human host, and mosquito vector. Importantly,

epidemiological studies at the ICEMR field sites are integrated with entomological studies, including the measurement of the entomological inoculation rate, human biting index, and insecticide resistance, as well as studies of parasite genetic diversity and antimalarial drug resistance.

274. **Tiwari S**, **Ghosh SK**, **Satyanarayan TS**, **Nutan N**, **Uragayala S**, **Valecha N.** Malaria outbreaks in villages in north Karnataka, India, and role of sibling species of *Anopheles culicifacies* complex. ***Health* 2015; *7*: 946-54.**

ABSTRACT

Investigations on malaria outbreaks and role of sibling species complex of principal rural malaria vector *Anopheles culicifacies* were carried out in villages in north Karnataka, India from 1997 through 2014. Information regarding densities, resting and breeding habitats of malaria vectors prevalent in the area was also generated so as to formulate an appropriate vector control strategy. The Slide Positivity Rate (SPR), Slide *Falciparum* Rate (SFR) and *Pf* proportion was 43.1%, 35.9% and 83.3%, respectively. Three sibling species A, B, and C of *An. culicifacies* were found sympatric with cumulative percent composition of 63.7, 28.2 and 8.1, respectively. Per man hour and per structure densities of *An. culicifacies*, *An. culicifacies* and *An. culicifacies* varied from 0 to 27.5 and 0 to 56.0, 0 to 0.5 and 0 to 7.0 and, 0 to 2.5 and 0 to 7.5, respectively. The proportion of semi-gravid and gravid females was more as compared to fully fed and unfed females which indicated that most of the females rested indoor. Streams/river, wells, seepages and irrigation tanks are the major habitats supporting breeding of *An. culicifacies*. Integrated vector managements by indoor residual spraying of effective insecticide as per national guidelines along with biological control methods especially use of larvivorous fish *Gambusia affinis* and *Poecilia reticulate* are suggested to control malaria in the area.

KEYWORDS: malaria, *Anopheles culicifacies*, sibling species, biological control, north Karnataka.

275. Abdulla S, Binka F, Graves P, Greenwood B, Leke R, Malik E, Marsh K, Meek S, Mendis K, Schapira A, Slutsker L, Tanner M, **Valecha N**, White N, Alonso P, Bosman A, Cibulskis R, D'Souza B, Mnzava A, Ringwald P, Shutes E, Szilagyi Z. Malaria Policy Advisory Committee to

the WHO: conclusions and recommendations of seventh biannual meeting (March 2015). ***Malar J* 2015 Aug 5; *14*: 295.**

ABSTRACT

The Malaria Policy Advisory Committee to the World Health Organization held its seventh meeting in Geneva, Switzerland from 5 to 7 March 2015. This article provides a summary of the discussions, conclusions and meeting recommendations. Meeting sessions included: an update on the Greater Mekong Subregion elimination strategy; an update on the RTS,S vaccine; G6PD testing to support the safe use of anti-relapse therapy for *Plasmodium vivax*; update from the Vector Control Advisory Group; newly proposed evidence reviews or consultations on malaria terminology, malaria in pregnancy, and the feasibility of eradication; as well as updates from the World Health Organization Global Malaria Programme regarding their strategy update and policy setting processes. Policy statements, position statements, and guidelines that arise from the Malaria Policy Advisory Committee meeting conclusions and recommendations will be formally issued and disseminated to World Health Organization Member States by the World Health Organization Global Malaria Programme.

KEYWORDS: WHO malaria policy-making, mosquito control, drug resistance, surveillance, elimination, *Plasmodium falciparum, Plasmodium vivax,* background.

276. **Dev V**, **Adak T**, **Singh OP**, **Nanda N**, Baidya BK. Malaria transmission in Tripura: Disease distribution & determinants. ***Indian J Med Res* 2015 Dec; *142* (Suppl 1): S12-22.**

ABSTRACT

Background & Objectives: Malaria is a major public health problem in Tripura and focal disease outbreaks are of frequent occurrence. The State is co-endemic for both *Plasmodium falciparum* and *P. vivax* and transmission is perennial and persistent. The present study was aimed to review data on disease distribution to prioritize high-risk districts, and to study seasonal prevalence of disease vectors and their bionomical characteristics to help formulate vector species-specific interventions for malaria control. **Methods:** Data on malaria morbidity in the State were reviewed retrospectively (2008-2012) for understanding disease distribution and transmission dynamics. Cross-sectional mass blood surveys were conducted in malaria endemic villages of

South Tripura district to ascertain the prevalence of malaria and proportions of parasite species. Mosquito collections were made in human dwellings of malaria endemic villages aiming at vector incrimination and to study relative abundance, resting and feeding preferences, and their present susceptibility status to DDT. **Results:** The study showed that malaria was widely prevalent and *P. falciparum* was the predominant infection (>90%), the remaining were *P. vivax* cases. The disease distribution, however, was uneven with large concentration of cases in districts of South Tripura and Dhalai coinciding with vast forest cover and tribal populations. Both*Anopheles minimus s.s.* and *An. baimaii* were recorded to be prevalent and observed to be highly anthropophagic and susceptible to DDT. Of these, *An. minimus* was incriminated (sporozoite infection rate 4.92%), and its bionomical characteristics revealed this species to be largely indoor resting and endophagic. **Interpretation & Conclusions:** For effective control of malaria in the State, it is recommended that diseases surveillance should be robust, and vector control interventions including DDT spray coverage, mass distribution of insecticide-treated nets/long-lasting insecticidal nets should be intensified prioritizing population groups most at risk to avert impending disease outbreaks and spread of drug-resistant malaria.

KEYWORDS: *Anopheles baimaii*, *An. minimus*, malaria transmission, northeast India, *Plasmodium falciparum*, Tripura, vector bionomics.

277. **Tyagi S**, **Das A**. Mitochondrial population genomic analyses reveal population structure and demography of Indian *Plasmodium falciparum*. ***Mitochondrion*** **2015 Sep; *24*: 9-21.**

ABSTRACT

Inference on the genetic diversity of *Plasmodium falciparum* populations could help in better management of malaria. A very recent study with mitochondrial (mt) genomes in global *P. falciparum* had revealed interesting evolutionary genetic patterns of Indian isolates in comparison to global ones. However, no population genetic study using the whole mt genome sequences of *P. falciparum* isolates collected in the entire distribution range in India has yet been performed. We herewith have analyzed 85 whole mt genomes (48 already published and 37 entirely new) sampled from eight differentially endemic Indian locations to estimate genetic diversity and infer population structure and historical demography of Indian *P. falciparum*. We found 19 novel Indian-specific Single Nucleotide Polymorphisms (SNPs) and 22 novel haplotypes segregating in

Indian *P. falciparum*. Accordingly, high haplotype and nucleotide diversities were detected in Indian *P. falciparum* in comparison to many other global isolates. Indian *P. falciparum* populations were found to be moderately sub-structured with four different genetic clusters. Interestingly, group of local populations aggregate to form each cluster; while samples from Jharkhand and Odisha formed a single cluster, *P. falciparum* isolates from Asom formed an independent one. Similarly, Surat, Bilaspur and Betul formed a single cluster and Goa and Mangalore formed another. Interestingly, *P. falciparum* isolates from the two later populations were significantly genetically differentiated from isolates collected in other six Indian locations. Signature of historical population expansion was evident in five population samples, and the onset of expansion event was found to be very similar to African *P. falciparum*. In agreement with the previous finding, the estimated Time to Most Recent Common Ancestor (TMRCA) and the effective population size were high in Indian *P. falciparum*. All these genetic features of Indian *P. falciparum* with high mt genome diversity are somehow similar to Africa, but quite different from other Asian population samples.

KEYWORDS: historical demography, India, *Plasmodium falciparum*, population structure, mt genome.

278. **Gupta R**, **Mishra N**, **Kumar A**, **Rana R**, **Srivastava B**, **Tyagi PK**, **Anvikar AR**, **Valecha N**. Monitoring artemisinin resistance in *Plasmodium falciparum*: comparison of parasite clearance time by microscopy and real-time PCR and evaluation of mutations in *Pfatpase6* gene in Odisha state of India. ***Parasitol Res*** **2015, Sep; *114*(9): 3487-96**.

ABSTRACT

Antimalarial drug resistance including artemisinin resistance in *Plasmodium falciparum* malaria is a major concern in combating malaria throughout the world. Delayed parasite clearance time (PCT) is indicative of emergence of artemisinin resistance. Herein, PCT has been monitored with the help of gold standard technique microscopy accompanied by a more sensitive real-time assay for academic purpose. After the administration of artemisinin based combination therapy, artesunate + sulfadoxine pyrimethamine (AS + SP), all the subjects were followed up to day 42 for monitoring the therapeutic efficacy of AS+SP in Bisra Community Health Centre (CHC), Sundergarh district in the state of Odisha in India. Further, representative samples were analyzed

for L263E, E431K, A623E and S769N SNPs in *Pfatpase6* gene and copy number polymorphisms in *Pfmdr1* gene. Though all the samples were found parasite negative according to microscopy by the end of day 3 and attained adequate clinical and parasitological response (ACPR) at the end of day 42, real-time PCR showed day 3 positivity in 12 of the total analyzed samples ($n = 43$). This was further validated by end-point diagnostic PCR and correlated with high initial parasite load. E431K mutation was observed in 2 of the 12 samples (16.7 %) while the controls ($n = 18$) were all wild. L263E, A623E and S769N were wild in all the analyzed samples ($n = 30$). *Pfmdr1*copy number analysis showed no change in the said trait. Conclusively, real-time PCR could support microscopy for better monitoring of PCT and may provide as an additional but useful research tool for artemisinin resistance studies.

KEYWORDS: *Plasmodium falciparum* , delayed PCT, artemisinin resistance, *Pfatpase6*, E431K, real-time PCR.

279. **Sharma VP**, **Dev V**, **Phookan S**. Neglected *Plasmodium vivax* malaria in northeastern States of India. ***Indian J Med Res* 2015; *141*(5): 546–555.**

ABSTRACT

Background & Objectives: The northeastern States of India are co-endemic for *Plasmodium falciparum* and *P. vivax* malaria. The transmission intensity is low-to-moderate resulting in intermediate to stable malaria. Malaria control prioritized *P. falciparum* being the predominant and life threatening infection (>70%). *P. vivax* malaria remained somewhat neglected. The present study provides a status report of *P. vivax* malaria in the northeastern States of India. **Methods**: Data on spatial distribution of *P. vivax* from seven northeastern States (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura) were analysed retrospectively from 2008–2013. In addition, cross-sectional malarial surveys were conducted during 1991-2012 in malaria endemic pockets across the States of Assam, Meghalaya, Mizoram and Tripura to ascertain the prevalence of *P. vivax* in different age groups. **Results**: *Vivax* malaria was encountered in all northeastern States but there existed a clear division of two malaria ecotypes supporting ≤30 and >30 per cent of total malaria cases. High proportions of *P. vivax* cases (60–80%) were seen in Arunachal Pradesh and Nagaland in the north with alpine environment, 42-67 per cent in Manipur, whereas in Assam it varied from 23-31 per cent with subtropical and tropical climate. Meghalaya, Tripura and Mizoram had the lowest proportion of *P. vivax* cases. Malaria cases were recorded in

all age groups but a higher proportion of *P. vivax* consistently occurred among <5 yr age group compared to *P. falciparum* (*P*<0.05). *P. vivax* cases were recorded throughout the year with peak coinciding with rainy season although transmission intensity and duration varied. **Interpretation & Conclusions**: In northeast India, *P. vivax* is a neglected infection. Estimating the relapsing pattern and transmission dynamics of *P. vivax* in various ecological settings is an important pre-requisite for planning malaria elimination in the northeastern States.

KEYWORDS: malaria burden, malaria control, *Plasmodium vivax*, spatial distribution, transmission dynamics.

280. Tennyson S, **Ravindran J**, **Eapen A**, William J. Ovicidal activity of *Ageratum houstonianum Mill*. (Asteraceae) leaf extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex* quinquefasciatus (Diptera: Culicidae). ***Asian Pac J Trop Dis* 2015; *5*(3): 199-203.**

ABSTRACT

Objective: To study the ovicidal activity of *Ageratum houstonianum* (*A. houstonianum*) leaf extracts against the eggs of vector mosquitoes and to develop additional tools for the control of mosquito-borne diseases. **Methods**: The ovicidal activity of crude hexane, ethyl acetate and methanol leaf extracts of *A. houstonianum* were assayed for their toxicity against the eggs of three important vector mosquitoes, *viz*., *Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus* at concentrations of 2.5, 5.0, 10.0 and 20.0 mg/L of the crude extract. **Results**: All extracts showed activity. The minimum concentration at which maximum egg mortality rate of 80% and above obtained was 10.0 mg/L in the case of methanol and ethyl acetate against *Anopheles stephensi* and *Aedes aegypti* respectively and 5.0 mg/L in ethyl acetate extract against *Culex quinquefasciatus*. One hundred per cent egg mortality was obtained only in ethyl acetate extract at 20.0 mg/L against *Aedes aegypti*. **Conclusions**: The crude leaf extracts of *A. houstonianum* did not exhibit potential ovicidal activity against the vector species studied. Among the crude leaf extracts tested, the activity of ethyl acetate extract was more effective. More research on the screening of phytochemicals as a potential ovicidal agent is warranted to add more tools in the control of mosquitoes.

KEYWORDS: *Ageratum houstonianum*, crude leaf extracts, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, ovicidal activity.

281. **Sharma D**, **Lather M**, **Mallick PK**, **Adak T**, Dang AS, **Valecha N**, **Singh OP**. Polymorphism in drug resistance genes dihydrofolate reductase and dihydropteroate synthase in *Plasmodium falciparum* in some states of India. ***Parasit Vectors*** **2015 Sep 17; *8*:471. doi: 10.1186/s13071-015-1080-2.**

ABSTRACT

Background: Sulfadoxine-pyrimethamine (SP) combination drug is currently being used in India for the treatment of *Plasmodium falciparum* as partner drug in artemisinin-based combination therapy (ACT). Resistance to sulfadoxine and pyrimethamine in *P. falciparum* is linked with mutations in dihydropteroate synthase (*pfdhps*) and dihydrofolate reductase (*pfdhfr*) genes respectively. This study was undertaken to estimate the prevalence of such mutations in *pfdhfr* and *pfdhps* genes in four states of India. **Methods:** *Plasmodium falciparum* isolates were collected from two states of India with high malaria incidence *i.e*., Jharkhand and Odisha and two states with low malaria incidence *i.e*., Andhra Pradesh and Uttar Pradesh between years 2006 to 2012. Part of sulfadoxine-pyrimethamine (SP) drug resistance genes, *pfdhfr* and *pfdhps* were PCR-amplified, sequenced and analyzed. **Results:** A total of 217 confirmed *P. falciparum* isolates were sequenced for both*Pfdhfr* and *pfdhps* gene. Two *pfdhfr* mutations 59R and 108N were most common mutations prevalent in all localities in 77 % of isolates. Additionally, I164L was found in Odisha and Jharkhand only (4/70 and 8/84, respectively). Another mutation 51I was found in Odisha only (3/70). The*pfdhps* mutations 436A, 437G, 540E and 581G were found in Jharkhand and Odisha only in 13, 26, 14 and 13 % isolates respectively, and was absent in Uttar Pradesh and Andhra Pradesh. Combined together for*pfdhps* and *pfdhfr* locus, triple, quadruple, quintuple and sextuple mutations were present in Jharkhand and Odisha while absent in Uttar Pradesh and Andhra Pradesh. **Conclusion:** While only double mutants of *pfdhfr* was present in low transmission area (Uttar Pradesh and Andhra Pradesh) with total absence of *pfdhps* mutants, up to sextuple mutations were present in high transmission areas (Odisha and Jharkhand) for both the genes combined. Presence of multiple mutations in *pfdhfr* and *pfdhps* genes linked to SP resistance in high transmission area may lead to fixation of multiple mutations in presence of high drug pressure and high recombination rate.

KEYWORDS: *Plasmodium falciparum*, sulfadoxine-pyrimethamine, *pfdhfr*, *pfdhps*, India.

282. Carlton JM, Volkman SK, Uplekar S, Hupalo DN, Pereira Alves JMP, Cui L, Donnelly M, Roos DS, Harb OS, Acosta M, Read A, Ribolla PE, **Singh OP**, **Valecha N**, Wassmer SC, Ferreira M, Escalante AA. Population Genetics, Evolutionary Genomics, and Genome-Wide Studies of Malaria: A View Across the International Centers of Excellence for Malaria Research. ***Am J Trop Med Hyg*** **2015 Sep; *93* (Suppl 3): 87-98.**

ABSTRACT

The study of the three protagonists in malaria—the *Plasmodium* parasite, the*Anopheles* mosquito, and the human host—is key to developing methods to control and eventually eliminate the disease. Genomic technologies, including the recent development of next-generation sequencing, enable interrogation of this triangle to an unprecedented level of scrutiny, and promise exciting progress toward real-time epidemiology studies and the study of evolutionary adaptation. We discuss the use of genomics by the International Centers of Excellence for Malaria Research, a network of field sites and laboratories in malaria-endemic countries that undertake cutting-edge research, training, and technology transfer in malarious countries of the world.

283. Roy M, Bouma M, **Dhiman RC**, Pascual M. Predictability of epidemic malaria under non-stationary conditions with process based models combining epidemiological updates and climate variability. ***Malar J*** **2015 Oct 26; *14*(1): 419. doi: 10.1186/s12936-015-0937-3.**

ABSTRACT

Background: Previous studies have demonstrated the feasibility of early-warning systems forepidemic malaria informed by climate variability. Whereas modelling approaches typically assume stationary conditions, epidemiological systems are characterized by changes in intervention measures over time, at scales typically longer than inter-epidemic periods. These trends in control efforts preclude simple application of early-warning systems validated by retrospective surveillance data; their effects are also difficult to distinguish from those of climatevariability itself. **Methods:** Rainfall-driven transmission models for *falciparum* and *vivax* malaria are fitted to long-term retrospective surveillance data from four districts in northwest India. Maximum-likelihood estimates (MLEs) of model parameters are obtained for each district via a recently introduced iterated filtering method for partially observed Markov processes. The

resulting MLE model is then used to generate simulated yearly forecasts in two different ways, and these forecasts are compared with more recent (out-of-fit) data. In the first approach, initial conditionsfor generating the predictions are repeatedly updated on a yearly basis, based on the newepidemiological data and the inference method that naturally lends itself to this purpose, given its time-sequential application. In the second approach, the transmission parameters themselves are also updated by refitting the model over a moving window of time. **Results:** Application of these two approaches to examine the predictability of epidemic malariain the different districts reveals differences in the effectiveness of intervention for the two parasites, and illustrates how the 'failure' of predictions can be informative to evaluate and quantify the effect of control efforts in the context of climate variability. The first approach performs adequately, and sometimes even better than the second one, when the climate remains the major driver of malaria dynamics, as found for *Plasmodium vivax* for which an effective clinical intervention is lacking. The second approach offers more skillful forecasts when the dynamics shift over time, as is the case of *Plasmodium falciparum* in recent years with declining incidence under improved control. **Conclusions:** Predictive systems for infectious diseases such as malaria, based on process-based models and climate variables, can be informative and applicable under non-stationary conditions.

KEYWORDS: epidemic malaria, India, transmission model, forecasting, prediction skill.

284. Rao MR, Padhy RN, **Das MK**. Prevalence of dengue viral and malaria parasitic co-infections in an epidemic district, Angul of Odisha, India: An eco-epidemiological and cross-sectional study for the prospective aspects of public health. ***J Infect Public Health*** **2015, Dec 1. pii: S1876-0341(15)00203-8. doi: 10.1016/j.jiph.2015.10.019.**

ABSTRACT

The co-existence of dengue and malaria infection in an individual and the primary and secondary dengue infection during co-infection were assessed. Over 1 year, 1980 blood samples were collected from suspected cases of dengue fever and analyzed by rapid diagnostic test (RDT), enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) methods to detect dengue infection. RDT and microscopic methods were used to detect malaria. Of the 1980 samples, only 22 (3.0%) cases were identified as dengue-malaria co-infection cases, out of which

13 were male and 9 were female. The highest number of confirmed cases were found during the hot and humid months of September and October (7 cases, 31.8%) and within the over 15 years age group. Of the cases of co-infection, dengue primary infection (21 cases, 95.5%) was significantly more common than dengue secondary infection (1 case, 4.5%) among all of the age groups. There were 12 cases of *Plasmodium falciparum* and 10 cases of *Plasmodium vivax* infection among malarial cases. A high prevalence of concurrence of dengue and malaria infection was recorded in this ecosystem. In light of the severity of co-infection and overlapping symptoms, a multidimensional diagnostic approach is suggested.

KEYWORDS: co-infection, dengue, malaria, Odisha, surveillance.

285. **Anushrita**, **Nagpal BN**, Kapoor N, **Srivastava A**, **Saxena R**, **Kumar V**, **Gupta S**, Jain Jk, **Valecha N.** Prevalence of vector mosquitoes of major mosquito borne diseases in areas of Indira Sagar Projection Madhya Pradesh, India. ***Int J Mosq Res* 2015; *2*(3): 182-7.**

ABSTRACT

Indira Sagar Project (ISP) is one of the highly ambitious projects designed for generating electricity and providing irrigation to many parts of Madhya Pradesh. A study to assess prevalence of major mosquito vectors was carried out since Jan 2013- Dec 2014 in ISP areas mentioned as Submergence (SUB), main canal mentioned as Command (CMD), and 2 Resettlement and Rehabilitation colonies mentioned as RR colonies. Malaria vector *An. culicifacies* was found to be dominating species in all the study areas, *An. fluviatilis* was found both in SUB and CMD areas and *An. stephensi* was found in RR Colonies. *Cx. quinquefasciatus* was observed as vector of LF in all areas. As a vector of JE, *Cx. vishnui* was observed from SUB and *Ae. aegypti* as vector of dengue and chikungunya was observed from all SUB, CMD and RR colonies. Larval densities showed significant differences in SUB; t (12) = 2.53, p=.016 (SUB), for CMD; t(12)= 3.97, *p*=.001, and for RR; t(12)= 2.17, *p*=.041. Regular surveillance of disease vectors in dam specific components will help to formulate new strategies of vector control. This study reports for the first time in India a comparative study in areas affected with both, reservoir of a dam and irrigation channel.

KEYWORDS: water development project, canal, *An. culicifacies*, *An. fluviatilis*, *Ae. aegypti*, *Cx. quinquefasciatus*, *Wuchereria bancrofti*.

286. **Kushwah RB**, **Dykes CL**, Kapoor N, **Adak T**, **Singh OP**. Pyrethroid-resistance and presence of two knockdown resistance (*kdr*) mutations, F1534C and a novel mutation T1520I, in Indian *Aedes aegypti*. ***PLoS Negl Trop Dis* 2015; *9*(1):e3332.**

ABSTRACT

Background: Control of *Aedes aegypti*, the mosquito vector of dengue, chikungunya and yellow fever, is a challenging task. Pyrethroid insecticides have emerged as a preferred choice for vector control but are threatened by the emergence of resistance. The present study reports a focus of pyrethroid resistance and presence of two *kdr* mutations—F1534C and a novel mutation T1520I, in *Ae. aegypti* from Delhi, India. **Methodology/Principal Findings:** Insecticide susceptibility status of adult-female *Ae. aegypti* against DDT (4%), deltamethrin (0.05%) and permethrin (0.75%) was determined using WHO's standard insecticide susceptibility kit, which revealed resistance to DDT, deltamethrin and permethrin with corrected mortalities of 35%, 72% and 76% respectively. Mosquitoes were screened for the presence of *kdr* mutations including those reported earlier (I1011V/M, V1016G/I, F1534C, D1794Y and S989P), which revealed the presence of F1534C and a novel mutation T1520I. Highly specific PCR-RFLP assays were developed for genotyping of these two mutations. Genotyping using allele specific PCR and new PCR-RFLP assays revealed a high frequency of F1534C (0.41–0.79) and low frequency of novel mutation T1520I (0.13). The latter was observed to be tightly linked with F1534C and possibly serve as a compensatory mutation. A positive association of F1534C mutation with DDT and deltamethrin resistance in *Ae. aegypti* was established. However, F1534C-*kdr* did not show significant protection against permethrin. **Conclusions/Significance:** The *Aedes aegypti* population of Delhi is resistant to DDT, deltamethrin and permethrin. Two *kdr* mutations, F1534C and a novel mutation T1520I, were identified in this population. This is the first report of *kdr* mutations being present in the Indian *Ae. aegypti* population. Highly specific PCR-RFLP assays were developed for discrimination of alleles at both *kdr* loci. A positive association of F1534C mutation with DDT and deltamethrin resistance was confirmed.

287. **Singh N**, **Mishra AK**, **Chand SK**, **Bharti PK**, **Singh MP**, **Nanda N**, **Singh OP**, **Sodagiri K**, Udhyakumar V. Relative Abundance and *Plasmodium* infection rates of malaria vectors in and

around Jabalpur, a malaria endemic region in Madhya Pradesh state, Central India. ***PLoS One*. 2015; *10*(5): e0126932.**

ABSTRACT

Background: This study was undertaken in two Primary Health Centers (PHCs) of malaria endemic district Jabalpur in Madhya Pradesh (Central India). **Methods:** In this study we had investigated the relative frequencies of the different *anopheline* species collected within the study areas by using indoor resting catches, CDC light trap and human landing methods. Sibling species of malaria vectors were identified by cytogenetic and molecular techniques. The role of each vector and its sibling species in the transmission of the different *Plasmodium* species was ascertained by using sporozoite ELISA. **Results:** A total of 52,857 specimens comprising of 17 *anopheline* species were collected by three different methods (39,964 by indoor resting collections, 1059 by human landing and 11,834 by CDC light trap). *Anopheles culicifacies* was most predominant species in all collections (55, 71 and 32% in indoor resting, human landing and light trap collections respectively) followed by*An. subpictus* and *An. annularis*. All five sibling species of *An. culicifacies viz.* species A, B, C, D and E were found while only species T and S of *An. fluviatilis* were collected. The overall sporozoite rate in *An. culicifacies* and *An. fluviatilis* were 0.42% (0.25% for *P. falciparum* and 0.17% for *P. vivax*) and 0.90% (0.45% for *P. falciparum* and 0.45% for *P. vivax*) respectively. *An. culicifacies* and *An. fluviatilis* were found harbouring both *P. vivax* variants VK-210 and VK-247, and *P. falciparum. An. culicifacies* sibling species C and D were incriminated as vectors during most part of the year while sibling species T of *An. fluviatilis* was identified as potential vector in monsoon and post monsoon season. **Conclusions:** *An. culicifacies* species C (59%) was the most abundant species followed by *An. culicifacies*D (24%), B (8.7%), E (6.7%) and A (1.5%). Among *An. fluviatilis* sibling species, species T was common (99%) and only few specimens of S were found. Our study provides crucial information on the prevalence of *An. culicifacies* and *An. fluviatilis* sibling species and their potential in malaria transmission which will assist in developing strategic control measures against these vectors.

288. **Uragayala S**, **Kamaraju R**, **Tiwari S**, **Ghosh SK**, **Valecha N**. Small-scale evaluation of the efficacy and residual activity of alpha-cypermethrin WG (250 g AI/kg) for indoor spraying in

comparison with alpha-cypermethrin WP (50 g AI/kg) in India. ***Malar J* 2015; *14*: 223. doi: 10.1186/s12936-015-0739-7.**

ABSTRACT

Background: Indoor residual spraying (IRS) with different formulations of insecticides is being used for the control of mosquito vectors in many countries. In the present study, residual efficacy and duration of effectiveness of IRS with alpha-cypermethrin WG-SB (250 g AI/m^2) formulation was compared with WP formulation (50 g AI/kg) in a small scale (Phase II) field trial. **Methods:** Two dosages, *i.e.* 20 and 30 mg AI/m^2, were used and the efficacy and duration of effectiveness was assessed on alpha-cypermethrin susceptible population of *Anopheles stephensi*. Four types of surfaces were selected, namely cement wall with distemper coating, cement wall with lime coating, mud wall with lime coating, and brick wall unpainted. Spraying was carried out with Hudson sprayer fitted with control flow valve. Bioassays were carried out at weekly and then fortnightly intervals. Chemical analysis of filter paper samples collected from the sprayed surfaces was done at Walloon Agricultural Research Institute, Gembloux, Belgium. **Results:** Alpha-cypermethrin WG-SB showed residual efficacy (>80 % mortality) for 13–15 weeks for the 20 mg/m^2 dosage and 13–16 weeks for the 30 mg/m^2dosage, whereas alpha-cypermethrin WP showed residual efficacy for 11–15 weeks for the 20 mg/m^2 dosage and 11–14 weeks for the 30 mg/m^2dosage on the surfaces tested. The average of the applied to target dose ratio ranged from 0.89 to 1.17 for alpha-cypermethrin WG-SB at 20 mg AI/m^2, 0.83–1.80 for the WG-SB at 30 mg AI/m^2, 0.87–1.66 for alpha-cypermethrin WP at 20 mg AI/m^2, and 0.68–1.06 for WP at 30 mg AI/m^2. No adverse events were reported, either by the spray men or the household inhabitants, during and after the spray operations. **Conclusions:** The results suggest that the dose of WG 30 mg/m^2 gave slightly longer effective residual action against malaria vector (16 weeks) on most common indoor surfaces and could be used for effective control of *Anopheles* mosquitoes. The WG formulation was found to be easy to handle, no smell was reported during the spraying and was found to be operationally acceptable for indoor residual spraying.

KEYWORDS: alpha-cypermethrin, *Anopheles stephensi*, indoor residual spraying, residual efficacy.

289. **Singh P**, **Dhiman RC**. Sporogonic cycles based on degree-days for malaria parasite development in different eco-epidemiological settings in India. ***Jpn J Infect Dis*** **2015; Jun 12. doi:10.7883/yoken.jjid.2014.549.**

ABSTRACT

In India, Malaria transmission is prevalent in diverse geo-ecological paradigms in India. Temperature is one of the key determinants for transmission of malaria, causing low endemicity in some areas while intensified transmission in others. Using a degree-day model, we estimated maximum and minimum possible number of days necessary to complete a sporogonic cycle and possible number of Sporogonic cycles for *Plasmodium vivax* and *Plasmodium falciparum* in two different ecological settings with low and high endemicity for malaria at different elevations. In Raikhalkhatta (Himalayan foothills) not a single sporogonic cycle was possible from November to February while in Gandhonia village (forested hills), only one month was unsuitable. A minimum of 6 days and maximum of 46 days were required for completion of one sporogonic cycle. Forested hilly area was found more suitable for malaria parasites development in terms of sporogonic cycles (25 versus 21 for *P falciparum* and 32 versus 27 for *P vivax*). Degree days also provided the climatic explanation for the current transmission of malaria at different elevations. The calculation of degree-days and possible sporogonic cycles can be useful for their application in regional analysis of transmission dynamics and management of malaria in view of climate change.

KEYWORDS: mosquitoes, malaria, parasites, sporogonic cycle, temperature, degree-days

290. **Kushwah RBS**, **Mallick PK**, Ravikumar H, **Dev V**, Kapoor N, **Adak T**, **Singh OP**. Status of DDT and pyrethroid resistance in Indian *Aedes albopictus* and absence of knockdown resistance (*kdr*) mutation. ***J*** **Vector *Borne Dis*. 2015; *52*(1): 95-8.**

ABSTRACT

Background & Objectives: *Aedes albopictus* is one of the vectors for dengue and chikungunya and emergence of pyrethroid resistance in this species could be of a major concern in controlling the vector. This study reports insecticide susceptibility status of *Ae. albopictus* to DDT and pyrethroids in some Indian populations and status of presence of knockdown resistance (*kdr*)

mutations. **Methods**: Three to four day old adult female *Ae. albopictus* collected from Delhi, Gurgaon (Haryana), Hardwar (Uttarakhand), Guwahati (Assam) and Kottayam (Kerala) were bio-assayed with DDT (4%), permethrin (0.75%) and deltamethrin (0.05%) impregnated papers using WHO standard susceptibility test kit. Mosquitoes were PCRgenotyped for F1534C *kdr*-mutation in the voltage-gated sodium channel (VGSC) gene. DDT and pyrethroid resistant individuals were sequenced for partial domain II, III and IV of VGSC targeting residues S989, I1011, V1016, F1534 and D1794 where *kdr* mutations are reported in *Ae. aegypti*. **Results**: Adult bioassays revealed varying degree of resistance against DDT among five populations of *Ae. albopictus* with corrected mortalities ranging between 61 and 92%. Kerala and Delhi populations showed incipient resistance against permethrin and deltamethrin respectively. All other populations were susceptible for both the synthetic pyrethroids. None of the *kdr* mutations was detected in any of DDT, deltamethrin and permethrin resistant individuals. **Interpretation & Conclusion**: *Ae. albopictus* has developed resistance against DDT and there is emergence of incipient resistance against pyrethroids in some populations. So far, there is no evidence of presence of knockdown resistance (*kdr*) mutation in *Ae. albopictus*.

KEYWORDS: *Aedes albopictus*, chikungunya, dengue, India, knockdown resistance, pyrethroid, voltage-gated sodium channel.

291. **Mishra N**, **Prajapati SK**, **Kaitholia K**, **Bharti RS**, **Srivastava B**, **Phookan S**, **Anvikar AR**, **Dev V**, Sonal GS, Dhariwal AC, White NJ, **Valecha N**. Surveillance of artemisinin resistance in *Plasmodium falciparum* in India using the kelch13 molecular marker. ***Antimicrob Agents Chemother*. 2015; *59*(5): 2548-53.**

ABSTRACT

Malaria treatment in Southeast Asia is threatened with the emergence of artemisinin-resistant *Plasmodium falciparum*. Genome association studies have strongly linked a locus on *P. falciparum* chromosome 13 to artemisinin resistance, and recently, mutations in the kelch13 propeller region (*Pfk-13*) were strongly linked to resistance. To date, this information has not been shown in Indian samples. *Pfk-13* mutations were assessed in samples from efficacy studies of artemisinin combination treatments in India. Samples were PCR amplified and sequenced from codon 427 to 727. Out of 384 samples, nonsynonymous mutations in the propeller region were

found in four patients from the northeastern states, but their presence did not correlate with ACT treatment failures. This is the first report of *Pfk-13* point mutations from India. Further phenotyping and genotyping studies are required to assess the status of artemisinin resistance in this region.

292. **Sharma P**, **Sharma S**, Mishra AK, **Thomas T**, **Das De T**, **Rohilla SL**, **Singh N**, **Pandey KC**, **Valecha N**, **Dixit R**. Unraveling dual feeding associated molecular complexity of salivary glands in the mosquito *Anopheles culicifacies*. ***Biology Open*** **2015 July 10; *4*(8): 1002-15 doi: 10.1242/bio.012294.**

ABSTRACT

Mosquito salivary glands are well known to facilitate meal acquisition, however the fundamental question on how adult female salivary gland manages molecular responses during sugar versus blood meal uptake remains unanswered. To investigate these responses, we analyzed a total of 58.5 million raw reads generated from two independent RNAseq libraries of the salivary glands collected from 3–4 day-old sugar and blood fed *Anopheles culicifacies* mosquitoes.Comprehensive functional annotation analysis of 10,931 contigs unraveled that salivary glands may encode diverse nature of proteins in response to distinct physiological feeding status. Digital gene expression analysis and PCR validation indicated that first blood meal significantly alters the molecular architecture of the salivary glands. Comparative microscopic analysis also revealed that first blood meal uptake not only causes an alteration of at least 12–22% of morphological features of the salivary glands but also results in cellular changes e.g. apoptosis, confirming together that adult female salivary glands are specialized organs to manage meal specific responses. Unraveling the underlying mechanism of mosquito salivary gene expression, controlling dual feeding associated responses may provide a new opportunity to control vector borne diseases.

KEY WORDS: malaria, mosquito, salivary gland, sugar and blood feeding, gene expression.

293. Wilson ML, Krogstad DJ, Arinaitwe E, Arevalo-Herrera M, Chery L, Ferreira MU, Ndiaye D, Mathanga DP, **Eapen A**. Urban Malaria: Understanding its epidemiology, ecology and transmission risk across diverse ICEMR sites. ***Am J Trop Med Hyg*** **2015; *93*(Suppl 3): 110-23.**

ABSTRACT

A major public health question is whether urbanization will transform malaria from a rural to an urban disease. However, differences about definitions of urban settings, urban malaria, and whether malaria control should differ between rural and urban areas complicate both the analysis of available data and the development of intervention strategies. This report examines the approach of the International Centers of Excellence for Malaria Research (ICEMR) to urban malaria in Brazil, Colombia, India (Chennai and Goa), Malawi, Senegal, and Uganda. Its major theme is the need to determine whether cases diagnosed in urban areas were imported from surrounding rural areas or resulted from transmission within the urban area. If infections are being acquired within urban areas, malaria control measures must be targeted within those urban areas to be effective. Conversely, if malaria cases are being imported from rural areas, control measures must be directed at vectors, breeding sites, and infected humans in those rural areas. Similar interventions must be directed differently if infections were acquired within urban areas. The hypothesis underlying the ICEMR approach to urban malaria is that optimal control of urban malaria depends on accurate epidemiologic and entomologic information about transmission.

BOOKS/CHAPTERS

A-Z: 2011-2015

294. Mathur A, Verma SK, Gupta V, **Singh SK**, Singh S, **Mathur D**, Bhat R, Prasad GBKS, **Dua VK**. Compaative studies on different vareties of apple (*Pyrus malus* L.) found in Kashmir (J&K) on the basis of PPO activity, Total Phenolic Content (TPC) and *in vitro* antioxidant activity. ***Pharma Sci Monitor* 2011; *2*(3) (Suppl 1): p. S1-6**.
ISSN: 0976-7908

ABSTRACT

India is considered the original home of apples (*Pyrus malus*.L). Phenolic compounds are believed to impart resistance to diseases in plants and Polyphenol oxidase (Catecholase and Cresolase) enzyme has been reported to be responsible for in vivo synthesis and accumulation of these compounds. In present study Polyphenol oxidase activity in different varieties of apple fruit samples (found in Kashmir) has been correlated with polyphenolic compounds and antioxidant activity. It was found that increase in Polyphenol oxidase (PPO) activity and total phenolic content in apple fruit samples of Ambri and Red Delicious varieties were maximum in accordance with antioxidant activity to that of Kessi and A. Trel which have reduced Polyphenol oxidase activity, total phenolic content and antioxidant activity. In the present investigation a relation between Polyphenol oxidase activity, Total Phenolic Content (TPC) and antioxidant activity has been established. Thus on the basis of PPO activity, polyphenolic content and antioxidant activity, the different varieties of the fruits can be differentiated. From the results of present study it can be concluded that apples having maximum antioxidant activity should be consumed more since these fruits will be much beneficial for health.

KEYWORDS: *Pyrus malus* L., PPO, catecholase, cresolase, Total Phenolic Content (TPC), antioxidant activity.

295. **Dua VK**, Verma G, Agarwal DD. Antiplasmodial activities of traditional medicinal plants from Garhwal region of north west Himalaya, India. In: Govil JN, Kaushik G, editor. ***Recent progress***

***in medicinal plants: Ethnomedicine and Therapeutic Validation*. USA: Stadium Press LLC 2012; *32*: 289–300**.

ISBN: 1-933699-22-1

ABOUT THIS BOOK

With growing economy, affluence and changes in lifestyle modern diseases like diabetes, obesity, cancer and cardiovascular diseases are on the rise especially in the developing countries. Conventional medicinal therapies are associated with high costs and prominent side effects. Medicinal plants offer suitable means to tackle the current grave situation on account of the growing recognition that these are natural products, free from side effects and are easily available at affordable prices. So there has been a spurt in demand for these plant based medicines. Such a backdrop has renewed the interest of scientists in medicinal plant research. However, it is also important that the scientific research in these aspects is documented in a condensed form for easy reference by students, budding scientists and general public.

In this regard the Volume 32 titled *Ethnomedicine and Therapeutic Validation* of Recent Progress in Medicinal Plants will immensely contribute in discovery of new drugs from medicinal plants and also serve as a handy reference material for further research work. The highlight of the volume is an exhaustive compilation of scientific data from more than 17 countries world over, namely Italy, Argentina, India, Sri-Lanka, Brazil, Serbia, Germany, Cameroon, USA, Egypt, Saudi Arabia, Nigeria, Democratic Republic of Congo, Belgium, Iran, Portugal and Hungary.

The focus is particularly on medicinal plants which are important in treatment of cancer and neurodegenerative diseases. This compilation emphasizes the fact that it is important to preserve traditional medicinal system based on plant products but also to focus research on them in order to enhance our scientific knowledge pertaining to their pharmacological properties and active principles. It is further hoped that this volume would be of great use to the scientific league and would also enhance knowledge of general public about medicinal plants and their use in daily life.

296. **Mohanty AK**, Garg S, Dhindsa K, **Kumar H**, **Kumar A**. Phenotypic characterization of mosquito larvicidal lycinibacillus strains isolated from paddy field and mangrove vegetation. I edn. In: Barbudhe SB, Ramesh R, Singh NP, editors. ***Microbial diversity and its application***. **Delhi: New India Publishing Agency 2013; p. 49–58.**

ISBN 13: 9789381450666

ABOUT THIS BOOK

Microbial organisms occupy a peculiar place in the human view of life. Microorganisms represent the richest repertoire of molecular and chemical diversity in nature as they underlie basic ecosystem processes. Microorganisms are used for various purposes including food production and preservation, management of pests and pathogens, bioleaching of metals, increasing soil fertility, generating biofuels, monitoring pollutants, cleaning up of oil spills, waste water treatment, assaying of chemicals and serving as tools for medical research. Besides microorganisms are the major sources of antimicrobial agents and produce a wide range of other important medicinal compounds including enzymes, enzyme inhibitors, antihelminthics, antitumor agents, insecticides, vitamins, immunosuppressants and immunomodulators. The study of microbial diversity is thus important to solve new and emerging disease problems and to advance biotechnology. The book focuses on microbial applications in industry, agriculture and health which will help to update the knowledge on microbial diversity and its application.

297. Nayak P, Gaonkar T, **Mohanty AK**, **Kumar A**, Bhosle S, Garg S. Isolation and characterization of polyhydroxyalkanoates producing bacteria from coastal sand-dune ecosystem. I edn. In: Barbudhe SB, Ramesh R, Singh NP, editors. ***Microbial diversity and its application***. **Delhi: New India *Publishing Agency* 2013; p. 75–82.**
ISBN 10: 9381450668
ISBN 13: 9789381450666

ABOUT THIS BOOK

Microbial organisms occupy a peculiar place in the human view of life. Microorganisms represent the richest repertoire of molecular and chemical diversity in nature as they underlie basic ecosystem processes. Microorganisms are used for various purposes including food production and preservation, management of pests and pathogens, bioleaching of metals, increasing soil fertility, generating biofuels, monitoring pollutants, cleaning up of oil spills, waste water treatment, assaying of chemicals and serving as tools for medical research. Besides microorganisms are the major sources of antimicrobial agents and produce a wide range of other important medicinal

compounds including enzymes, enzyme inhibitors, antihelminthics, antitumor agents, insecticides, vitamins, immunosuppressants and immunomodulators. The study of microbial diversity is thus important to solve new and emerging disease problems and to advance biotechnology. The book focuses on microbial applications in industry, agriculture and health which will help to update the knowledge on microbial diversity and its application.

298. **Singh V**. Antimicrobial resistance. In: Mendez-Vilas A, editor. ***Microbial Pathogens and Strategies for Combating them: Science, Technology and Education*. Badajoz, Spain: Formatex Research Center 2013; *1*: 291–6.**

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ABSTRACT

A vast majority of pathogens, the etiologic agent for several diseases have become resistant to one or more microbial agents. It is pertinent to understand the evolution of antibiotic resistance in pathogens for several factors from the drug to pathogen to their genetics to their ecology; as they all interplay together increasing the complexity of this process. The wide range of pathogens which ranges from bacteria causing tuberculosis, the viruses causing influenza, the parasites causing malaria to the fungi causing yeast infections are becoming resistant to the various antimicrobial agents such as antibiotics, antivirals, antimalarials and antifungals used for their treatment. Due to this, the risk of losing the treatment of infectious diseases looms large. In this perspective, we discuss here the emergence of antimicrobial resistance in pathogenic organisms on the treatment of infectious diseases. The first section of the chapter discusses the main mechanisms of resistance - an important aspect of the emerging and re-emerging resistance with serious consequences on the treatment of infectious diseases. The rampant use/misuse of the antibiotics in human medicine is the major contributing factor in this prevailing phenomenon. The next section of the chapter elaborates the measures to be undertaken to combat the multidrug resistant pathogens and their spread worldwide, causing clinical failures adding more woes to the public health crisis. The last section of the chapter talks about the need for discovering new antibiotics/novel drugs and the importance of further research in this field, the importance of partnerships between governments, research institutions and public.

KEYWORDS: pathogens, antimicrobial resistance, clinical breakpoints, drug resistance mechanism, resistant strategies.

299. **Pandey KC**. Cysteine proteases of human malaria parasites: Proteases in health and disease. VII edn. In: Dhalla N, Chakraborti S, editors. ***Advance in Biochemistry in Health and Disease*.** **New York: Springer Science and Business Media 2013; *7*(1): p. 121–34.**
Hyperlink: DOI: 10.1007/978-1-4614-9233-7_8
Print ISBN: 978-1-4614-9232-0
Online ISBN: 978-1-4614-9233-7

ABSTRACT

There is an urgent need for new drugs against malaria, one of the most important infections of human which takes millions of lives annually. Cysteine protease inhibitors have demonstrated anti-malarial effects, and they are potential new drug targets, especially when current drugs are showing resistance. Falcipains and vivapains are best characterized cysteine proteases of *P*. *falciparum* and *P*. *vivax*, respectively. Using cysteine protease inhibitors and manipulating cysteine proteases specific genes have confirmed their roles in hemoglobin hydrolysis. In *P*. *falciparum*, falcipain-2 and falcipain-3 are major hemoglobinases that hydrolyzes human hemoglobin. Vivapain-2, vivapain-3 and vivapain-4 are important cysteine proteases of *P*. *vivax*, which shared a number of features with falcipain-2 and falcipain-3. Structural and biochemical analysis of falcipains and vivapains showed that they have specific domains for specific functions. These include trafficking domain, inhibitory domain, refolding domain and hemoglobin binding domain. Recent study also indicates the mechanism of auto-activation of falcipains, where salt bridges and hydrogen bonds between pro-mature domains play crucial role. Study indicates that cysteine and aspartic proteases work collaboratively to enhance the parasites' ability to hydrolyze host erythrocyte hemoglobin. Recent advances in cysteine proteases biochemistry and the complexes of cysteine proteases with small and macromolecular inhibitors provide structural insight to facilitate the drug design. Therefore, giving due importance to the cysteine proteases, this chapter will focus the recent advancement in the field of cysteine proteases of human malaria parasite and prospects for exploitation as drug targets.

KEYWORDS: hemoglobinases, drug resistance, protein–protein interactions, hot-spot, hemoglobin binding domain, refolding domain, anti-malarial.

300. **Dev V**, **Sharma VP**. The dominant mosquito vectors of human malaria in India. In: Sylvie Manguin, editor. ***Anopheles mosquitoes-New Insights into Malaria Vectors.*** **Croatia: InTech 2013; p. 239–71.**

Hyperlink: DOI: 10.5772/3392

ISBN 978-953-51-1188-7

ABOUT THIS BOOK

Anopheles mosquitoes are highly important insects due to their involvement in the transmission of human malaria and its devastating consequences in endemic countries worldwide. In 2010 alone, malaria was responsible for an estimated 660,000 deaths. As the study of *Anopheles* species and populations is a key element for reaching the goal of malaria elimination, an enormous amount of information has accumulated over the past century, and together in recent decades with the advent of novel technologies the acquisition of new knowledge has accelerated even further. The originality of this book is to offer the latest compilation on various research, new concepts, paradigms and innovative approaches for the control of anophelines using state-of-the-art methodologies and analysis. The 24 chapters, written by internationally recognized experts from 5 continents, cover the rich landscape for the understanding of *Anopheles* mosquitoes and the development of more effective weapons to control the vector of malaria.

301. Chandra G, **Ghosh SK**. Use of larvivorous fish in biological and environmental control of disease vectors. In: Cameron MM, Lorenz LM, editors. ***Biological and Environmental Control of Disease Vectors*****. Wallingford, UK: CABI 2013; p. 25–41.**

Hyperlink: DOI: 10.1079/9781845939861.0025

ISBN: 9781845939861

ABSTRACT

This chapter describes the effective use of predatory larvivorous fish as biological control agents of mosquito vector larvae. Approximately 315 larvivorous fish species belonging to 32 genera

under seven families are being used, with the family Cyprinodontidae contributing the most number of genera (15) and species (300). Their current use in the control of three important vector-borne diseases, *i.e.*, malaria, dengue, Chikungunya infection, are discussed, as well as important recommendations and future strategies.

302. **Sharma SK**. Long-lasting insecticidal nets in malaria control: A review**.** In: Kumar A, Rodrigues S, Dias A, editors. ***Major Tropical Diseases: Public Health Perspective*****. Broadway Publishing House 2014; p. 98-114.**

NIMR

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Advance in Biochemistry in Health and Disease (**1**) - 299

Advances in Parasitology (**1**) - 158

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Anopheles mosquitoes-New Insights into Malaria Vectors (**1**) - 300

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Asian Pacific Journal of Tropical Disease (**3**) - 216, 271, 280

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