VECTORS AND VECTOR-BORNE DISEASES:

Pathobiological Insights, Public Health Challenges and Management Strategies

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DEDICATED TO VICTIMS OF VECTOR-BORNE DISEASES

CONTENTS

ACKNOWLEDGEMENTS

We would like to thank all members of the scientific community and particularly the scientists who have worked in the area of vectors and vector-borne diseases for decades to bring us to this point in time when a large amount of the fruits of their labor is now being implemented in control of vector born diseases. We are particularly thankful to our Keynote Speaker, Plenary and Invited Speakers; Oral and Poster presenters including co-authors for presenting their research work and share their knowledge in *13th Conference on Vectors & Vector-Borne Diseases*. The conference is supported by South East Asia Regional Office of the World Health Organisation (SEARO-WHO), Indian Council of Medical Research (ICMR), Department of Science & Technology (DST), Defence Research and Development Organisation (DRDO) and National Vector-Borne Disease Control Programme (NVBDCP), Government of India. We also acknowledge with sincere thanks, the support of a great cadre of sponsors from industry such as Vestergaard Frandsen, Clarke, Tagros Chemicals, Sumitomo, Ajay Biotech, and Bayer India. The support of all memebres of the National Academy of Vector-Borne Diseases (NAVBD) and support of faculty, officers, staff and students of the Central University of Tamil Nadu (CUTN) are sincerely acknowledged.

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Dated, the 3rd February, 2017

MESSAGE

It is heartening to know that Central University of Tamil Nadu and National Academy of Vector Borne Diseases are jointly organizing the 13th International Conference on Vectors and Vector Borne Diseases. The conference theme 'Novel technologies: renewed hopes for elimination' is appropriate when we talk of eliminating various vector borne diseases in next decade.

Vector borne diseases are major public health problem in India. Apart from the major VBD like malaria, dengue, chikungunya, filariasis, Japanese encephalitis and visceral leishmaniasis; there is threat of emerging vector borne diseases like zika. There is need to address various challenges when we talk of elimination like knowing the burden of disease, quality diagnosis, effective treatment, vector control, human resource, etc. There is need to tackle these challenges and stay ahead in science and technology to combat various VBD.

I hope that the conference will provide a forum to bring together academicians, researchers, policy makers, industrialists around the world and deliberate on the modalities, research needs for elimination of vector borne diseases.

I convey best wishes to the organizers of the conference and hope that it will be a grand success.

Soumya

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03/02/2017

MESSAGE

It gives me immense pleasure to welcome our eminent speakers and all the delegates participating in the *13th Conference on Vectors & Vector-Borne Diseases* at Hotel Crowne Plaza, Chennai. I am particularly thankful to our Guests of Honour, Keynote Speakers, Plenary and Invited Speakers for having accepted our invitation.

Vector-borne diseases account for ~17% of the estimated global burden of all infectious diseases, according to the WHO reports. Emergence and spread of zika virus and scrub typhus are also of serious concern. Through this conference, participants would like to engage with all of you in an open and constructive discussion on various aspects of vector science and vector-borne diseases. It is also an opportune time to renew contacts and address issues of mutual interest. It is gratifying to note that the programme of the conference covers a wide range of very interesting areas relating to vectors and vector-borne diseases, and their management.

My sincere hope is that this conference shall provide a constructive stage to academicians, researchers, programme officers and professionals, industries and, above all, our young scientists to exchange their highly critical research findings in the field of vector-borne diseases resulting in tangible outcomes to overcome the challenges.

We are fortunate to have the support of the South East Asia Regional Office of the World Health Organisation; the Indian Council of Medical Research; Department of Science & Technology, Defence Research and Development Organisation, National Vector-Borne Disease control Programme, Government of India.

We also acknowledge with sincere thanks, the support of a great cadre of sponsors like: Vestergaard Frandsen, Clarke, Tagros Chemicals, Sumitomo, Ajay Biotech, Bayer India and others; whom of course you will meet during the conference.

The collaboration of the advisory committee members of National Academy of Vector-Borne Diseases (NAVBD) and support of faculty, officers, staff and students of the Central University of Tamil Nadu (CUNT) are acknowledged.

We look forward to your active participation and welcome you all to this conference.

(Prof. A.P. Dash)

CHAPTER 1

INTRODUCTION

Vector-borne diseases (VBDs) represent a major public health problem particularly across the tropical and sub-tropical regions of the world. Many VBDs are considered diseases of the poor since it is endemic in the low-income groups or in areas where the vicious cycle of diseases and poverty exists. While most of the VBDs are preventable and curable, the disease burden and economic impact are very high and unacceptable in this rapidly advancing modern era. Some of these diseases are fatal if not treated, while others may leave afflicted individuals disfigured and disabled. They account for ~17% of the estimated global burden of all infectious diseases. An estimated 207 million cases with 627,000 deaths due to malaria in 2012 was reported across the globe. Furhter, to be noted is the world's fastest growing VBD, dengue, with a 30-fold increase in disease incidence over the last 50 years.

Japanese Encephalitis (JE) is a leading cause of viral encephalitis, and its distribution is significantly linked to irrigated rice production combined with pig rearing. Chikungunya occurs in Africa and Asia, including the Indian sub-continent. An estimated 120 million people in 73 countries are currently infected with lymphatic filariasis. Kala-azar is endemic in more than 60 countries, with a total of 200 million people at risk. Many VBDs and arboviral infections are emerging and re-emerging. Efficient and effective implementation of current tools will lead to significant reduction of malaria, kala-azar and lymphatic filariasis, and these diseases are now being targeted for rapid elimination. However, additional tools and innovative delivery mechanisms to implement existing interventions and those that may be available in the near future are urgently needed to accelerate control towards elimination and prevent resurgence of transmission. Wolrd Health Organizaztion (WHO) has celebrated World Health Day on 7th April 2014 with the theme of “Preventing Vector-Borne Diseases”.

VBDs like malaria, dengue, Japanese encephalitis, chikungunya, lymphatic filariasis and kala-azar etc. have emerged as serious public health problems which have created impact on socioeconomic development in India. In addition, emergence and spread of Zika virus and scrub typhus are of serious concern. VBDs occur in epidemic forms, causing considerable morbidity and mortality in our country and as well as other tropical country. VBDs accounts for 17% of the estimated global burden of all infectious diseases.

It has been reported that 3.4 billion people live at risk of malaria in globally, whereas more than one million cases of malaria has been reported in India. More than 40% of the global burden of lymphatic filariasis has been reported. Most important mosquito-borne viral disease such as dengue is emerging as a serious public health concern in new areas and its incidence has increased 30-fold globally, resulting in 20,000 deaths annually. After a gap of more than three decades many states of India, such as Andhra Pradesh, Andaman and Nicobar Islands, Delhi, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu and Odisha has been affected by chikungunya.

Developmental activities without adequate environmental concerns have increased transmission of these infectious diseases. These diseases affect poor living conditions – particularly lack of access to adequate housing, safe drinking water and sanitation of urban, peri-urban and rural communities. In the recent past, the scientific community has experienced a wide spread expansion of insecticides resistance mosquito vectors.

Vector control plays a vital role to prevent these infectious disease outbreaks. Many existing interventions, such as insecticide treated bed nets and indoor spraying, are simple and proven. These vector-control tools can be particularly effective in combination with mass drug administration interventions such as involving large-scale treatment of affected communities. Vector control programmes will require increased research to develop a sustained approach to ecological and environmental changes in the years ahead.

THE BOOK STRUCTURE

The purpose of this book is to give an overview of vector, vector borne diseases and recent research on vector control programmes.

CHAPTER 2 describes molecular pathogenesis of vector borne diseases such as cytokine profile of malaria, pathophysiology of cerebral malaria and identifies target molecules for its diagnosis and treatment.

CHAPTER 3 addresses the burden of vectors and vector borne diseases globally and Indian scenario such as out break such as Dengue & Chikungunya.

CHAPTER 4 covers the social, climate and environmental determinants of Vector born diseases.

CHAPTER 5 emphasizes use of new drugs for vector and vector borne diseases management such as treatment of Post Kala-azar Dermal Leishmaniasis (PKDL), Malaria and Tick-Borne Diseases in animals.

CHAPTER 6 covers insecticides resistance and its management.

CHAPTER 7 describes Genomics and Proteomics aspects of vector, host and vector born diseases such as Malaria and dengue.

CHAPTER 8 addresses the biology and ecological habitat vector of vector born diseases.

CHAPTER 9 highlights transmission biology of vector borne diseases such as transmission of Novel Emerging Chikungunya Virus in Aedes mosquitoes, and Japanese Encephalitis Transmission in Odisha.

CHAPTER 10 describes the practice, challenges, opportunities and development of new tools including nano-metallic formulations for vector control.

CHAPTER 11 covers topics related elimination of neglected tropical diseases such as lymphatic filariasis, trypanosomiasis, arboviral diseases and scrub typhus.

CHAPTER 12 focuses the community involmenet in disease control and elimination.

CHAPTER 13 deals with public healthg challenges, health policy and management strategies.

CHAPTER 2

VECTORS AND VECTOR-BORNE DISEASES: DIAGNOSIS AND PATHOBIOLOGICAL INSIGHTS

Sphingolipids in the pathogenesis of dengue

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Cellular lipids play an important role in several aspects of viral replication, but their implication in dengue is not known. To better understand the effect of sphingolipid metabolism in the pathogenesis of dengue fever, the study measured the activity of sphingomyleinase (SMase) in the platelets obtained from dengue cases in comparison to other febrile illness cases (OFI). At the first instance, assay was done using 15 dengue cases and 12 OFI samples collected at two intervals: at the time of admission and around defervescence (3rd day post admission). Around 30,000 platelets/mL of each sample were used to determine the SMase activity as per the instructions given by the manufacturer (Biovision Inc. USA). The preliminary study observed an increased activity of SMase in dengue cases around defervescence compared to base-line, whereas no such difference was appreciated in the OFI samples. This implicates the participation of sphingolipids in dengue viral infected platelets during the course of infection. Studies are being carried out in a larger cohort using samples from both severe and non-severe form of dengue. Samples will be analyzed for the levels of Sphingosine-1-phosphate and ceramides using ELISA and LC-MS.

Role of translation research in diagnosis of Zoonotic diseases and its impact on public health of India

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The incidence of emerging infectious diseases among human population has increased in the recent past and possibility exist of increainge threat in the future. Over 30 new infectious agents have been detected worldwide in the last three decades; 60 per cent of these are of zoonotic origin. Developing countries such as India suffer disproportionately from the burden of infectious diseases given the confluence of existing environmental, socioeconomic, and demographic factors. In the recent past, India has witnessed outbreaks of emerging and re-emerging diseases in various parts of the country, the majority of these are of zoonotic origin.

Diagnosis of high-risk group pathogens is a major concern in India, where a few Biosafety Level 3 (BSL-3) laboratories and only one BSL-4 laboratory exist. Therefore, there is a need for development of safe diagnostic tests that can be used at primary health centers, medical colleges, and all other health settings across the country. Considering the ‘One health approach’, National Institute of Virology, Pune has enhanced diagnostic capabilities for survey, outbreaks and epidemic investigations of high-risk group of viral pathogens such as Crimean-Congo hemorrhagic fever (CCHF), Kyasanur Forest disease virus (KFDV), Ebola and Nipah.

Two ELISA kits had been developed for anti CCHF IgG detection from bovines and sheep - Goats. Survey of anti-CCHFV IgG antibodies in livestock would be better indicator to detect the presence of CCHF in any study area. A cross-sectional serosurvey of CCHF among livestock in 22 states and 1 union territory of India was carried out. A total of 5,636 samples from bovines, sheep, and goats were screened for CCHF virus IgG. IgG was detected in 6.28 % samples[1].

Anti CCHF Human IgM antibody detection assays was used for monitoring and confirming the CCHF suspected cases and the findings could make it possible for public health authorities to develop proactive preparedness programs that would enable them to send alerts and develop precautionary measures.

Molecular diagnostics has been extremely successful in the area of infectious diseases. Recent reports of KFD human infections have reached an alarming level, in spite of the availability of a vaccine. Real time based RT-PCR assays and ELISA developed by NIV were used for providing diagnosis on referred and ourbreak samples. In recent years we have reported presence of KFD to various newer areas of Karnatka (2012), Tamil Nadu (2013), Keral (2014), Goa (2015) and recently in Maharashra state (2016) [2,3,4].

Henipaviruses are naturally harbored by *Pteropoid* fruit bats. The presence of Nipah virus and associated encephalitis outbreak in West Bengal State was documented by NIV, Pune. Recent survey in north eastern region of India revealed the presence of Nipah in Pteropus bats from West Bengal and Assam States. Presence of NiV in the bat population in a naïve region is a matter of serious concern. Importance of these diagnostics provided, warrants implementation of necessary steps for survey of Nipah virus in other States of Northeast to determine circulation of Nipah virus [5].

Translational research has tremendous potential as a tool to reduce health disparities. Laboratory research has always provided new technologies and will continue to be the foundation for advances in development of translational research for the public health of India.

Laboratory Transmission Studies on Zika Virus with Indian Strain of *Aedes aegypti*: Effects of Concomitant and/or Super-Infections of Dengue and Chikungunya Viruses on Its Replication

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Dengue serotype-2 (DENV) and Chikungunya (CHIKV) diseases are highly endemic in India. With the advent of monsoon and post-monsoon season, country experiences the resurgence of these diseases and consequently, a large proportion of *Aedes aegypti* mosquitoes become infected either with DEN and/or CHIK virus. Encompassing this scenario study was conducted to understand perspective of the Zika virus (ZIKV) infectivity to such vector mosquitoes that are already infected with DENV or CHIKV. Laboratory data on experimentally infected mosquitoes with ZIKV showed in a high level of replication of this virus. Concomitant ZIKV infections with DENV and CHIKV in mosquitoes showed simultaneous growth of all three viruses. High titers of ZIKV observed in the salivary glands of infected mosquitoes. In mosquitoes with primary CHIKV infection followed by ZIKV, the immunofluorescence test on individual head squashes showed 7% positive for both the viruses whereas primary infection of ZIKV followed by CHIKV showed 8.3% dual infection positivity. When ZIKV was the primary infection followed by DEN, only 8.3% showed dual positivity but when DEN was the second infection about 5% showed dual infection. Attempts to determine occurrence of transovarial transmission phenomenon in small number of progeny (n=920) from infected females was not successful. Histopathology and immunohistochemistry of the

experimentally ZIKV infected infant mice organs showed significant lesions and viral antigens in the brain by 4-5th post infection day. The study has relevance to the preparedness for ZIKV in our country; this indicates the possible risk of establishment of ZIKV infection when already two *Aedes* borne infections i.e. CHIK & DENV are prevalent in country. This is first report on the replication of ZIKV with concomitant infection with other *Aedes* borne viruses. Data suggests the possible scenario of ZIKV that may have a relative advantage in replication and transmission dynamics. This study also demonstrates first time natural ZIKV transmission to mice through infected mosquito bite and supports this can lead to significant central nervous system lesions.

Immunological Profiling During Malaria Disease Manifestation and Its Severity

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Malaria is a major public health problem in India, and contributes significantly to the overall malaria burden in Southeast Asia. Severe malaria is the major cause of morbidity in India. However, the pathogenic basis of severe malaria is not well understood. Disease manifestation differs from person to person ranging from mild to severe, and may even extend to development of cerebral malaria. The cause of such differential responses and the degree of severity may be a result of pathogenic burden and host immune responses. Cytokines in malaria are reportedly the major players of immune responses during malaria. It is associated differentially with appearance of disease symptoms, levels of parasitaemia, degree of severity and complications. The balance between various cytokines plays a pivotal role in the regulation of immune responses and pathogenesis during complicated and uncomplicated malaria, although their role in disease pathogenesis and relationship to host protection has remained unclear. Here, we aimed to understand the ratio between the cytokine regulation and its level concentration in the patient suffering from complicated as well as uncomplicated malaria. As the pathogenic burden increases from mild to severe cases, the cytokine level as well as its gene expression pattern changes, this might play a major role in the overall host immune responses.

Genetics of Cerebral Malaria

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Malaria has exerted great evolutionary pressure on the human genome resulting in the natural selection of numerous polymorphisms in genes, encoding erythrocyte membrane proteins, hemoglobin, and other effectors of immunity. We in our study have analyzed the association of ABCA1 and NOS gene polymorphisms with the development of cerebral malaria. For the first time our study has not only shown novel sequence variations in the promoter regions of the ABCA1 gene but also significant association of – 477 C/T and – 320 G/C polymorphisms with uncomplicated malaria. Based on earlier reports, it is presumed that these polymorphisms might be leading to reduction of ABCA1 gene expression. The effect of reduced expression will be discussed in the light of efflux of microparticles, which are highly pro inflammatory and pro coagulative in nature. Genetic analysis of three isoforms of human NOS genes (iNOS, eNOS and nNOS) which influences the production of NO in *in vivo* reveals a significant association of genetic variants of iNOS (13 ≥

repeats of CCTTT pentanucleotide microsatellites) and eNOS, (the Glu^{298}→Asp substitution and "C-b-Asp" haplotype) which are known to enhance NO production with mild malaria and the variation of nNOS (-84A) which lowers NO production with severe malaria. It is known that the endothelial nitric oxide (NO) inhibits the exocytosis of Weibel–Palade bodies in the endothelium and also decreases tissue factor (TF) expression. The reduction of endothelial NO is associated with an increase in an angiogenic factor stored in Weibel–Palade bodies and angiopoietin-2 in cerebral malaria. Endothelial activation can thus be explained, since the van Wilbrand factor is copackaged with angiopoietin-2 in Weibel–Palade bodies. These effects can explain the dysregulation of coagulation in cerebral malaria along with acute endothelial activation in the disease pathology. Our study therefore suggests that low NO production may be a contributing factor for the progression of cerebral malaria. These studies possibly will help to understand the pathophysiology of cerebral malaria and identify target molecules for its treatment.

Maternal Filarial Infection Influences the Development of Regulatory T Cells in Children from Infancy to Early Childhood

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Lymphatic filariasis caused by thread like filarial worms involves asymptomatic to acute and/or disfiguring chronic conditions like lymphoedema, elephantiasis and scrotal swelling. Infection occurs when filarial parasites are transmitted to humans through mosquitoes. Adult worms lodge in the lymphatic system and disrupt the immune system that causes the disease. Nonetheless the infection if present during pregnancy, it affects the immune system of the unborn child in such a way that they become more susceptible to infection. But how the immune system of a fetus is affected by the maternal filarial infection is not known. Since regulatory T cells are responsible for development of hyporesponsiveness, a condition that supports the active filarial infection, and develops in thymus at an early stage of the human fetal development, we hypothesized that maternal filarial infection might be affecting the development of Tregs cell. Because Tregs secret IL-10, a regulatory cytokine, we have also measured its level in children born to infected and uninfected mother and correlate it with Tregs. We have observed a significantly high as well as a positive correlation between Tregs and IL-10 levels in children born to infected mother than the children of uninfected mother at the time of birth as well as early childhood indicating that Tregs and IL-10 contribute to immune modulation during pregnancy. Since ongoing MDA excludes pregnant mothers and children below 2 years of age, hence implementation of supervised therapy at the time of adolescent through MDA may help the programme in achieving the target of global elimination of LF by 2020.The results will be discussed.

Role of Parasites in Autoimmune Diseases

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There has been a silent epidemic of autoimmune disorders across the world. The interaction between genes and environmental factors play a key role in the causation of diseases and if it results in immune dysregulation the consequence is an autoimmune disease. The cause of an increased incidence and prevalence of autoimmune diseases is the focus of large number of studies. The dramatic increase could not be ascribed to genetic factor. Therefore, the role of environmental triggers have been under investigation. Nematode infections have been inversely associated with autoimmune disorders. The higher prevalence of autoimmune diseases in western countries compared to the tropical regions are believed to be associated with nematode load in respective populations which is the basis of Hygiene hypothesis. The basis of Hygiene Hypothesis is strongly supported by epidemiological as well as experimental evidence. Worm products play a crucial role in modulating the immune system which includes both the innate as well as the adaptive immune system. It down-regulates the inflammatory response by acting on various immune cells and cytokines and up-regulates an anti -inflammatory response. One of the key cells that gets a boost is the T regulatory lymphocytes which plays a major role in balancing the Th1 and Th2 lymphocytic response. The basis of of Hygiene Hypothesis has been experimentally proves in several diseases like CIA (collagen induced arthritis), inflammatory bowel disease, diabetes mellitus, experimental autoimmune encephalitis and allergic disorders. The products of several helminthes have been tested. Results from human studies have been very exciting and already in different phases of trial. The ‘worm capsule’ could be on its way for many autoimmune ailments and may be a novel method to treat diseases.

Center for the Study of Complex Malaria in India: Epidemiology, Transmission, Drug Resistance, and Pathogenicity Studies

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Malaria is an endemic infectious disease of major public health importance in India. The Center for the Study of Complex Malaria in India (CSCMi), one of ten International Centers of Excellence in Malaria Research funded by the U.S. National Institutes of Health, is a partnership between scientists at Ispat General Hospital, London School of Hygiene and Tropical Medicine, the National Institute of Malaria Research/Indian Council of Medical Research, New York University, and Pennsylvania State University. Over the past seven years, we have undertaken projects looking at the epidemiology, transmission, drug resistance, and pathogenicity of malaria at several sites in India, while building capacity and training the next generation of malariologists. An overview of some of these research findings will be presented, and how they may be used to help interpret and interrupt malaria trends in India as the country commences its framework for malaria elimination.

Chronic Malaria Protects from Endotoxemia, Typhoid and Polymicrobial Sepsis in a Murine Model

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It is observed that in malaria endemic population only 1-2 % of the individuals suffer from clinical malaria, while 20-30% individuals' display infection without clinical manifestations and serve as parasite carriers fostering transmission. Host/parasite factors that contribute to this carrier state of persistent infection are yet to be elucidated. We used a murine malarial parasite *Plasmodium chabaudi* that causes non-lethal infections followed by a phase of persistent parasitemia as a model to study status immune cells, their receptors and secreted cytokines and effects when challenged with TLR agonists or with live *Salmonella enterica* serovar typhimurium, an obligate intracellular pathogen or after Caecal Ligation and puncture (CLP) as a model for polymicrobial sepsis.

The salient features of the study are: 1. During acute phase of malaria most of the surface receptors (TLR2, CD80, CD86, MHC-II and CD14) were decreased on monocytes while TLR4 was significantly increased 2. During chronic malaria the animals were hyper susceptible to LPS challenge as shown release of increased plasma cytokines but were tolerant to LPS mediated mortality, 3. Mice with chronic malaria were resistant to challenge infection with *S. typhimurium* as shown by decreased mortality as well as low bacterial count (CFU) in circulation, liver and spleen. 4. The chronically malaria infected mice are tolerant to polymicrobial sepsis as shown by decreased mortality from CLP. More over absence of malaria parasite in the blood of non malarial septic patient from the tertiary care hospital that probably chronic malaria protects from genesis of sepsis in human. Decreased bacterial Phagocytosis, and autophagy in macrophages and decreased PAMP mediated inflammation was observed in chronic malaria infected mouse. The composition of Gut microbiota is significantly different during chronic malaria infected mouse in comparison to control as revealed by Principal component analysis of Gut microbiota. The ability of mice with chronic malaria to resist and clear lethal challenge with *S. typhimurium* and other microbes suggests an evolutionary mechanism for successful transmission of malarial parasites into arthropod vector.

Immunological Cross-Reactivity between *Plasmodium falciparum* and *Plasmodium vivax* – Implications for Cross Species Immune Protection and Vaccine Design

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Infections caused by *Plasmodium falciparum* and *P. vivax* account for more than 90% of global malaria burden. Exposure to malaria parasite elicits immune responses during natural infection and it is generally assumed that the immunity is not only stage specific but also species specific. It is

therefore believed that species-specific immunity may be needed to target different species of *Plasmodium*. Pfs48/45 and Pvs48/45 are well established targets in the sexual stages of the malaria parasites, and are being pursued for the development of malaria transmission blocking vaccines. Comparison of their sequences reveals 61% and 55% identity at the DNA and protein level, respectively raising the possibility that these two target antigens might share cross-reacting epitopes. Having succeeded in expressing recombinant Pfs48/45 and Pvs48/45 proteins, we hypothesized that these proteins will not only exhibit immunological cross–reactivity but also cross-boost immune responses. Immunization studies in mice revealed strong cross-reactivity of Pvs48/45-immune sera to full length Pfs48/45 protein and majority of this cross reactivity was in the amino–terminal and carboxyl-terminal sub-regions of Pfs48/45. In cross-boosting experiments Pfs48/45 and Pvs48/45 antigens were able to cross-boost each other in mouse immunization studies. In more recent studies, we have analyzed serum samples collected from children who were permanent residents of a *P. falciparum* transmission area in Zimbabwe for antibody reactivity against Pfs48/45, a *P. falciparum* gametocyte antigen and Pvs48/45, a P. vivax homolog of Pfs48/45 using ELISA. Twenty-seven percent (n=49) of the participants were found to be sero-positive for Pfs48/45 and 77% (n=36) of these Pfs48/45 positive participants also showed reactivity with Pvs48/45. Immune cross-reactivity revealed by ELISA was also confirmed by Western blot analysis using a panel of 23 Pfs48/45 and Pvs48/45 positive samples. Nested PCR analysis of blood samples confirmed infection with *P. falciparum* and generalized absence of *P. vivax* except for a single sample which revealed PCR positivity for both *P. vivax* and *P. falciparum*. Our studies with sera samples from a predominantly *P. falciparum* transmission area in Zimbabwe confirm immunological cross-reactivity with Pvs48/45, thus raising the possibility of partial cross-protective immunity and cross-boosting of immunity during co-infection with *P. falciparum* and *P. vivax*. These studies may have significant implications for immunity targeting transmission of both the species of malaria parasites.

Diversity Analysis of *P. falciparum var* Gene Family among Uncomplicated and Complicated Malaria Patients from Central India

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Plasmodium falciparum is the most virulent malaria parasite and its virulence is attributed to its ability to evade the human immune system by modifying infected host red blood cells to adhere to the vascular endothelium and to undergo antigenic variation. Genetic variation in parasite is one of the factors that might influence the disease outcome and disease severity. *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) appears to play key pathogenic role in disease severity. It is the main antigenic ligand and is encoded by *var* multigene family ~ 60 *var* genes per genome of a parasite, which plays a major role in pathogenicity and immune evasion. DBLα region of *var* gene was selected for the study and for the study four samples, two each from uncomplicated and complicated category were carried out for molecular analysis. PCR amplification was done using universal primer (DBLαAF and DBLαßR) for DBLα region of *var* gene. PCR amplicons were gel purified and were cloned in pGEM-T Easy vector system and transformed in E.coli DH5α competent cell. Recombinants were selected by blue white screening method. Positive clones were sequenced by using T7 and DBLαßR primers. A total of 60 positive clones, 15 from each sample were analyzed for *var* sequences and classification of DBLα sequence were done according to cys/PoLV groups. Out of 60 sequences majority of sequences were from Group 4. One motif in each PoLV was major motif with LYLG in PoLV1, LRED in PoLV2, KAIT in PoLV3 and PTYF in PoLV4.

Understanding the mechanisms and patterns of sequence variation may help in designing control strategies against this parasite as, worldwide repertoire of polymorphic malarial surface antigens is major hurdle in the vaccine development.

Genetic Predisposition of TLR Variants to Severe *Plasmodium falciparum* Malaria

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Toll-like receptors (TLRs) are critical mediators of inflammatory response to malaria infection and gene polymorphisms affecting TLR function may be in part responsible for inter-individual variation in disease manifestation. Of the ten functional TLRs described in human, activation and changes in expression of TLR2, TLR4 and TLR9 by malaria antigens are thought to potentially affect the disease outcome. Owing to the fact that only TLR2 form heterodimers with its co-receptors TLR1 or TLR6 during signaling, the TLR2 mediated outcome might be altered depending on the prevalence of TLR1 and/or TLR6 functional variants. We performed a genetic association studies analysing functional variants in TLR1 (I602S), TLR 2 (5' UTR I/D), TLR4 (D299G), TLR6 (P249S) and TLR9 (T-1237C and T-1486C) in 200 samples each from uncomplicated malaria (UM) and severe malaria (SM) obtained from VSS medical college and Hospital, Burla, Odisha. Further, SM was categorised into its sub-clinical groups (CM: cerebral malaria and NCSM: Non-cerebral severe malaraia or SOD: single organ dysfunction and MODS: multi-organ dysfunctions) and analysed. Besides, meta-analysis was performed for TLR4 (D299G) and TLR9 (T-1237C and T-1486C) to obtain more precise estimates of single effect of these variants. The TLR6 '249S' variant appeared to be the dominant determinant for genetic predisposition to SM and that its association with either TLR2 'D' or TLR1 '602S' modulates for CM development. The TLR9 -1486C variant was observed as a risk factor for SM and poor clinical outcome in malaria was found to be apparently modulated by its LD with TLR9 T-1237C variant. We did not find any difference of TLR4 D299G variant between UM and SM in all possible genetic model of analysis. The present study opens up several new avenues for their exploration and validation in future studies in different global settings for malaria.

Shikimate Pathway in Malaria: A Role in Host Pathogenesis?

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Shikimate pathway involves formation of aromatic ring from purely aliphatic precursors, and thus it is of widest biological significance. Its end product chorismic acid (Greek, "fork") is an important metabolic branch point and a precursor for biosynthesis of aromatic amino acids, folates, ubiquinone and various secondary metabolites in bacteria, fungi, plants and apicomplexan parasites. Humans (as other vertebrate hosts) lack Shikimate pathway and its essential role in pathogens' physiology makes it an attractive target for drug intervention in several diseases including malaria. This presentation would provide: (i) details of molecular and functional studies of Chorismate synthase from malaria parasites; (ii) review of the significance and druggability of Shikimate pathway in malaria parasites; and (iii) a compelling hypothesis about how Shikimate pathway in malaria maybe responsible for complications observed during severe malaria.

Indigenous Transmission of Leishmaniasis with Cutaneous Manifestations Among the Kani Tribes Settled in the Hill Tract of the Western Ghats

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Leishmaniasis with cutaneous manifestation (CL) is a skin infection caused by the protozoan parasites of the genus *Leishmania* that are transmitted by the bite of P*hlebotomus* species of sandflies. The stigma and trauma associated with this skin infection emphasize the social impact on the affected people. The tribes (1,444 as per 2011 census) have scattered in 28 settlements located at different altitudes, ranging from 267 to 2,425 ft (08037'49.7"N 077011'29.7"E, 08036'51.2"N 077009'54.9"E). While majority of them inhabit in huts (72.6%) made of forest materials such as dry leaves, wooden logs, grasses, bamboo and mud, the remaining in dwellings with roof made of either RCC or asbestos. A total of 27 CL cases were recorded and of which 5 were confirmed with CL infection due to *Leishmania donovani* when PCR assay was made. A total of 15,940 sandfly specimens, comprising of 19 species was recorded in this area. *Phlebotomus argentipes* (18.64%) was found predominant indoors. A total of 919 female specimens, comprising of *P. argentipes* (n=793), *P. colabaensis* (n-123) and 3 *P. stantoni* (n=3) were examined for natural infection, using PCR assay. Five female *P. argentipes* specimens were positive for *Leishmania donovani* parasites. The phylogenetic analysis of the sequences showed that the parasites were genetically identical to *L. donovani,* detected from the skin aspirates of the confirmed CL cases. The tribes domesticate dogs to alert them from the movement of wild animals. Blood samples collected from 47 out ~194 dogs and *L. donovani* parasite DNA was detected from 3, using PCR-RFLP analysis. Through sequencing, *L. donovani* infection among dogs was confirmed. The Kani tribes infected with *L. donovani* are the native of the settlements and have no travel history outside Thiruvananthapuram District, Kerala during the last 2-3 years, before the commencement of symptoms of CL. The study indicates the indigenous CL transmission in the area.

Pathophysiology and Complications of Surgical Treatment of Grade IV Filarial Lower Limb Lymphedema

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Lymphatic filariasis is a debilitating and disfiguring scourges among all diseases. Globally 1.3 billion people are estimated to be infected and 40 million are disabled physically and psychologically. 90% of hydrocele, adenolymphangitis and lymphedema can progress to elephantiasis. The chronic venous hypertension with lymphatic failure is a coexisting feature of lymphedema. It causes overload of lymphatics through a direct effect of increased capillary filtration and inflammation. The lymphatic channels become dilated and flow is impaired. Lymphedema need to maintain venous flow in order to prevent overloading of the venous system. Surgical treatment of Grade IV filarial lower limb lymphedema is largely disappointing. Two case studies described below indicate the failure of surgery. In this case medical treatment alone could lead a manageable life and prevent death.

Incidence of Malarial Acute Kidney Injury in Odisha

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Malaria is a major public health problem in tropical countries. About 500 million people suffer from malaria, leading to death in 1 to 3 million cases. Malaria is a mosquito-borne parasitic disease caused by the eukaryotic protists of the genus *Plasmodium*. There are five species of the parasite that affect humans, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. *Plasmodium falciparum* is the lethal one. Malarial Acute kidney injury (AKI) is one of the most threatening complications of severe malaria in adults and malarial acute kidney injury is the major contributor to the mortality of *P. falciparum* malaria in nonimmune adults. The study aims at determining the incidence of malarial acute kidney injury cases due to *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* infection reported in Odisha .Patients suffering from AKI with serum creatinine concentration > 3mg/dl (>265µmol/L) and/or 24-hour urine output < 400 ml despite rehydration and positive for asexual forms of malarial parasite on their peripheral blood smear are included in the study ,prior hospitalization for any other reason, pregnant women and having other chronic diseases are excluded. All the enrolled malarial acute kidney injury cases were screened for AKI as per KDIGO guidelines.Out of 163 complicated Malaria cases, AKI detected in 44% cases .Out of malarial kidney injury cases 51% cases were found to harbor *P. falciparum* mono infection, 34% cases *P. vivax* mono infection, 6.9% cases *Pf+Pv* mixed infection, 2.7% cases *Pf+Pm* mixed infection and 4.16% cases *Pf+Pv+Pm* mixed infection. 68% males predominantly affected by malarial acute kidney injury followed by 31% female. In malarial acute kidney injury males had a higher incidence rate than females. *P. falciparum* showed renal involvement though mono and mixed infections with both *P. falciparum P. vivax* and *P. malariae*. The early detection and prompt intervention can help in the rate of curing the severity of the disease.

In Silico Analysis of Differential Gene Expression with Reference to Infection with *Japanese encephalitis* Virus

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Till date, only a few studies have been done on *Japanese encephalitis* (JE) using microarray data. Gene expression data, GSE57330, was downloaded from the Gene Expression Omnibus (GEO) database, which contains 12 human microglial cells (CHME3) samples, constituting 6 uninfected and 6 infected with Japanese encephalitis virus (JEV) P20778 strain, for screening out key genes associated with the disease. *Insilico* approach was taken up for identification of differentially expressed genes and pathway enrichment analysis of these genes. The result obtained from this study shows that only 1 differentially expressed gene, namely, *STAT1*, which gets down-regulated during JE infection. Further analysis shows that *STAT1* interacts with Tyrosine protein kinase family members and cause JE through the JAK-STAT pathways. The transcription factors and their associated binding sites in the promoter region of the hub gene may make accessible potential directions for possible future drug design.

Malaria and Duffy Blood Groups among Jarawas: The Primitive and Vanishing Tribe of Andaman and Nicobar Islands, India

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Andaman and Nicobar Islands, union territory of India were inhibited by 14 aboriginal tribes. Some of these tribal population have already become extinct and the number of the existing ones are also dwindling. This group of Island being highly endemic for malaria, it was considered worthwhile to study the hunter-gatherer primitive tribe, Jarwas for their malaria and Duffy blood group phenotype. The Jarwas are a primitive Negrito tribe of Andaman Islands, India. The member of this tribe have been geographically and socially isolated from the other inhabitants of the Islands. No malaria had even been reported in the tribe until 2001, when an outbreak of febrile illness triggered a malariological survey. Malarial parasites all identified microscopically as *Plasmodium falciparum,* were detected in blood smears of 30 out of the 179 Jarwas investigated. Although most malaria among the non Jarwas inhabitants of the Island was caused by *P. vivax,* only *P.falciparum* was detected when blood serum from 26 of the subjects were investigated in PCR-based assays. Genetic diversity studies, based on msp1 and msp2 Polymorphic markers, also revealed a relatively low level of polymorphism in the *P.falciparum* parasite infecting the Jarwas compared with that seen in other areas of India. It seems possible that malaria parasite have only recently reached the Jarwas, as a result of weakening of tribe's isolation from other human in the Andaman Islands. Analysis of Duffy blood groups revealed a high frequency of Fy(a-b-) in the Jarwa tribe along with total absence of *P. vivax* infection suggests the selective advantage offered to (a-b-) individual against *P.vivax* infection. In addition to this malaria in Nicobarese tribe of Car Nicobar Island, its malaria vector *An.sundaicus,* its control and the first time description of *P.knowlesi* in the primitive tribes of Andaman Nicobar Islands will be presented.

Optimization and Testing of LAMP Assay for the Diagnosis of Malaria

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Since the launch of the Roll Back Malaria Initiative by WHO in 1998, and particularly in the past few years, malaria control has been intensified in endemic countries. As a consequence, malaria is going to be eliminated in 34 countries in near future. The low levels of parasitaemia during the elimination phase possess a challenge to early diagnosis and prompt treatment of the disease at individual as well as community level because of low efficacy of microscopy and currently available RDTs. To tackle this situation loop-mediated isothermal amplification (LAMP) of nucleic acids seems to be a promising technique. We have optimized a LAMP assay using a new proportion of primer mix and DNA extracted by heat treatment that can detect *P falciparum*, P *vivax* and *P malariae* in naked eye by color distinction. The sensitivity and specificity of this assay is similar to the PCR assay and the detection limit of parasite is significantly high than the microscopy. The test can be performed by a technician at CHC level hospital in developing countries like India.

A Paper Strip-Based Point-of-Care Assay for the Diagnosis of Dengue

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Dengue fever is an emerging global public health problem. In India, many states have become hyper-endemic to dengue viruses and its control faces real challenges as no commercial vaccine or specific treatment strategy is available yet. Timely diagnosis essentially complements the ongoing control measures as well as helps clinicians for identifying and confirming the cases, as many diseases are having clinical presentations like dengue, such as Malaria, Japanese encephalitis and Chikungunya, Leptospira etc, which are co-circulating in the country. Though ELISA and Polymerase chain reaction (PCR) are routinely used for dengue diagnosis, these technologies are not available in the resource-poor-settings. Hence, there is an urgent need for the development of a simple, robust, and cost effective diagnostic tool for rapid diagnosis of dengue. In our previous attempt, we have successfully expressed the recombinant protein of dengue virus NS1 and developed anti dengue NS1 specific monoclonal antibody and demonstrated its potential in diagnostic applications. Subsequently, we have explored the development and application of indigenous POC assay, based on colloidal Gold conjugates employing direct and indirect methods for the detection of NS1 antigen in clinical sera.

Evaluation of Biomarkers in *Plasmodium falciparum* Malaria Infected Patients

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Malaria is a major health problem and a serious issue in terms of morbidity and mortality among all infectious diseases. Most serious forms of the disease are caused by *Plasmodium falciparum*. The onset of complicated malaria is not always reflected by significant changes in standard laboratory and parasitological parameters. A biomarker is a substance or a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or responses to a therapeutic intervention. Biomarkers have been used to diagnose and prognosticate the progress and outcome of many diseases. Blood biomarkers and surrogate host markers for malaria can be used for early diagnosis, discrimination from other infections with overlapping clinical manifestations. It also acts as an aid in scrutinizing response to therapy and predicting outcomes. Moreover, investigation on the plasmodium induced alterations in human proteome can provide valuable information regarding malaria pathogenesis and host-parasite interactions. Understanding the mechanisms that trigger transition from non-severe to fatal severe malaria is clinically very important. The aim of this study is to predict the risk of developing neurological and other complications squeal post recovery. Prognostic biomarker will help in predicting consequences of disease for patients at high risk. It may be also helpful in judge the adjunctive therapy for patients to prevent the adverse outcome of the disease.

A Novel Single Step PCR to Diagnose *Plasmodium falciparum* and *Plasmodium vivax*

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Malaria is one of the devastating diseases of tropical and sub tropical region caused by Plasmodium species. In 2015, it was estimated that there were 429000 deaths from malaria globally. Research on malaria has been done on wide range but still accurate and trouble-free diagnosis of malaria is difficult. A novel single step PCR was standardized for the detection of *Plasmodium falciparum* and *Plasmodium vivax*. Molecular detection of Plasmodium species based on different genes has been targeted and compared to the gold standard microscopy. Plasmodium species -specific nested PCR based on 18S rRNA gene has been compared with cytochrome c oxidase genes of *P. falciparum* (cox III) and *P. vivax* (cox I). Multiplexing PCR for both the cytochrome genes have also been done for the easy and fast detection of mixed infection. PCR amplicons were obtained with the minimum parasite count which was upto 0.1p/µl in both multiplex and individual reactions with sensitivity upto 100%. Traditional methods of diagnosing malaria is time taking and tedious. The molecular detection approach targeting cytochrome gene offers much higher sensitivity and time saving method and is amenable to high-throughput scaling up for large sample size.

Quality Assurance of Malaria Rapid Diagnostic Tests

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Malaria is one of the most widespread parasitic diseases all over the world. Early diagnosis, followed by prompt and effective treatment is the key to reducing malaria mortality and morbidity. Evaluation and validation of RDTs is essential for their improved sensitivity and specificity. To ensure accuracy of malaria RDTs quality assurance and adequate monitoring of laboratory services at the peripheral level are important. The major components of the quality assurance of RDTs for malaria include preparation of Quality Control (QC) panels, pre-dispatch QC, post-dispatch QC, External Quality Assurance Scheme (EQAS) and Internal QC. Malaria patients from different areas were selected as donors. Parasitized blood was diluted with O +ve blood cells and AB+ve fresh frozen plasma to attain parasite density of 200/µl (low positive) and 2000/µl (high positive). On the same lines, negative controls were also prepared. Panels were transported in liquid nitrogen containers and have been stored in -80^{0}C deep freezer. In initial testing, 58 RDTs from each lot, 10 negative and 4 sets of positive panels for *P. vivax* and *P. falciparum* are tested using the WHO protocol. RDT samples are also drawn from representative Primary Health Centres / Sub-centres / ASHAs and tested for their quality (post dispatch). 49 QC panels have been prepared till date. As a part of pre-dispatch QA, 3248 RDTs were tested in year 2015 and 1134 RDTs in year 2016 till date. Post-Dispatch testing was carried out on, 489 RDTs in year 2015 and 816 RDTs in year 2016 till date. These RDTs were received from 45 districts of 11 states. Conclusively, the result shows that the malaria RDTs being used in the country are satisfactory.

CHAPTER 3

EPIDEMIOLOGY AND DISEASE BURDEN INCLUDING ECONOMIC BURDEN

Vector-Borne Diseases Burden in Animals and Their Public Health Significance

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Blood sucking arthropods viz., mosquitoes, certain species of ticks, flies, fleas and bugs acts as vectors while taking a blood meal from an infected host and later transmit the pathogens into a new host during their successive blood meal causes havoc to the animals and humans. Vector-borne diseases (VBD's) account 17% of the estimated global burden of all infectious diseases. Every year, more than 1 billion people are infected and more than 1 million die from vector-borne diseases. In case of animals, vectors and VBD's causes huge economic loss to livestock industries and the incidence of infection is increasing every year. Amongst the major vector borne animal diseases, the impact of trypanosomiosis, theileriosis, babesiosis, *Rickettsiosis, blue tongue viruses,* Listeriosis, *Borreliosis,* Dirofilariosis is considered to be very high. The other important Vector and VBD's include, African horse sickness (orbivirus), Nairobi sheep disease (Nairovirus), Arboviruses, *Onchocera* spp. transmitted by *Culicoides* spp., viruses of Ephemeral fever and Vesicular stomatitis transmitted by *Phlebotomus* spp. , the viruses of Eastern equine encephalitis, vesicular stomatitis and myxomatosis transmitted by Simulium spp. and viruses of Bovine leukaemia, Hog cholera , Equine Infectious anaemia , Bovine viral diarrhea vectored by different species of Tabanus. The Fleas transmit Pseudo -tuberculosis, Erysipelas, Listeriosis, Q-fever, Tularaemia in domestic animals and lice are also act as vector for *Dipylidium caninum,* Swine fever virus, *Rickettsia prowazekii*, *R. quintana and Borrelia recurrentis.* Although it is very difficult to estimate the economic impact of VBD's as a whole on livestock sector, a conservative estimate of the economic impact of trypanosimiasis and blue tongue has been reported to be US$ 1-1.2 billion and 3 billion US$ /annum, respectively. Amongst the different factors, increased urbanization, globalized travel and trade, pollution, improper water storage and irrigation are responsible for increasing the disease incidence of VVBD's. Besides, impact of climate change and its anomalies that have been occurring in the past decade definitely have role in enhancing the transmission rate and geographical distribution of vector-borne diseases. It is predicted that global warming will increase the area of malaria transmission from 45% of the world's population to 60%. In view of life threatening nature, public health concern and economic burden involved with the VVBD's, it's time to understand the eco-epidemiology of each disease. Efforts to control these diseases have been focused mainly on the use of chemical pesticides, but arthropod resistance to pesticides is now an immense practical problem. To combat this challenge, integration of several strategies *viz*, integrated vector management, disease management in animals or humans, Proper treatment regime and comprehensive epidemiological surveillance systems are need of the hour. Concerted planning and collaboration across sectors including health, agriculture and the environment is required. Where, population is at risk, Coordination of disease surveillance along with vector surveillance, advice on use of personal protection measures (clothing, insect repellents, etc.) need to be done to prevent outbreaks of diseases. Moreover, vector control programme need to adapt to match the changing epidemiological patterns of new emerging threats. This will require focused multidisciplinary research planning to develop a sustained approach to ecological and environmental changes in the coming years.

Health Promoting School Model Interventions for Prevention of Vector Borne Diseases in Odisha, India

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Introduction: Schools serve as a gateway to the community. This World Health Organization-Tropical Disease Research grant aimed to examine if a ‘Health-Promoting School model’ could be used for prevention and control of vector borne diseases (VBDs) through behavioral changes among students in Odisha, India.

Method: 1236 students of VIII and IX class from eight co-ed schools (4 controls and 4 interventions) of two endemic districts in Odisha were covered in the study. Intervention included 12 hours of class room teaching and practical with activities (audio-visual show, problem ranking, social mapping and role play) focusing understanding the mosquito dynamics, source identification and control, prevention and management of VBDs (Malaria, filarial, dengue and chikungunya). Impact was assessed by looking at change in knowledge and practice through pre and post test. Control schools were only provided with the leaflets and posters covering the objectives.

Results: Intervention schools demonstrated 60% increase in knowledge and 44% increase in practice of vector control compared to 10% and 12% in control schools. Statistically significant difference was observed in all parameters between both the arms.

Conclusion: The model was well accepted by the students and community, which can be used as effective setting-based health promotion measure for vector control.

Keywords: Vector borne diseases, School, health promotion

Prevalence of Trombiculid Mites, the Vectors of Scrub Typhus Pathogen in Areas Reported with Human Cases of Acute Encephalitis Syndrome (AES) in Gorakhpur District of Uttar Pradesh

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Scrub typhus is an acute, febrile illness caused by the bacterium *Orientia tsutsugamushi* (Family: Rickettsiaceae). It is transmitted among small animals and humans by some species of larval trombiculid mites (chiggers). The disease has been reported from 18 states in India. If left untreated for over a week, scrub typhus may lead to meningoencephalitis or multiple organ dysfunction. Incidence of acute encephalitis syndrome (AES) of unknown etiology with considerable mortality and disability is reported every year in Gorakhpur district of Uttar Pradesh. A recent investigation during August-September 2014 identified scrub typhus as the etiology of AES in Gorakhpur. Consequently, survey of trombiculid mites was carried out in 13 villages of Gorakhpur district recently reported with human cases of AES during 2015. Mites were collected directly from the trapped rodents/shrews. Rodents/shrews were trapped by Sherman traps. All the study locations were characterized by the presence of shrub and bushy vegetation. The traps were set in peri-domestic areas of the selected villages one hour prior to sunset and retrieved the next day morning. During the survey, a total of 165 rodents/shrews were trapped using 829 traps, set in 13 villages. Of the total 165 rodents/shrews trapped, 85% were infested with trombiculid mites. *Suncus murinus*, the index animal of scrub typhus, was the predominant species (78.8%) followed by *Musbooduga* (15.8%) and *Rattus sp.* (5.4%). A total of 2794 mites belonging to 15 species of trombiculids were recovered from the trapped rodents/shrews. *Leptotrombidium (L) deliense*, the established vector of scrub typhus in India was the predominant species (81.7%) followed by *Schoengastilla ligula* (8.1%). The Chigger *(L. deliense)* index ranged from 0.71 to 16.3 in different villages surveyed and the overall Chigger index was 13.8 per rodent/shrew. In all the surveyed villages, the Chigger *(L. deliense)* index was well above the critical level of 0.69 per rodent/shrew indicating that the villages are receptive for scrub typhus outbreaks.

Surveillance of Dengue, Chikungunya and Zika virus in hospitalized fibril illness patients in tertiary care unit at Jabalpur

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This study includes samples from hospitalized fibril illness patients suspected of Dengue (DEN), Chikungunya (CHIK) virus infections. Samples were collected from, Netaji Subhash Chandra Bose Medical College, Jabalpur (Tertiary care unit in central India). Samples were diagnosed with NS1 ELISA for Dengue, IgM ELISA Kit [NIV] for DEN and CHIK and Trioplex real time RT-PCR kit (CDC, USA) for DEN, CHIK as well as Zika virus depending on their days of onset. The clinical and demographic data was collected from the hospital. A total of 387 samples tested of which 105 samples were positive for DEN and CHIK: 70 for Dengue, 25 for Chikungunya and 10 sample were found positive for both DEN and CHIK by IgM ELISA. However, no sample was found positive for Zika virus. Between these two diseases in hospitalized febrile illness patients, the highest percent cases were positive for DEN (17.96%) where as CHIK cases were 6.25%. It is interesting to note that at least in 10 cases DEN and CHIK co-infections detected based on IgM detection. It is difficulty to rule out these had both the acute infections at the same time or not. More males were positive for both DEN and CHIK as compared to females. Among the clinical sings the retro-orbital pain was more common in DEN cases than in CHIK patients. However, prolonged muscle pain was more common in CHIK than in DEN patients. Zika virus was not detected so far in any of these samples tested.

The Pillars and Pitfalls of Malaria Elimination in India: Need for Tailored Research

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Malaria is one of the major vector borne diseases prevalent in India with about One million cases reported annually. There is heterogeneity and variability in the risk of malaria transmission between and within the states of the country as many ecotypes/paradigms of malaria have been recognized. The pattern of clinical presentation of severe malaria has also changed and while multi-organ failure is more frequently observed in falciparum malaria, there are reports of vivax malaria presenting with severe manifestations. The high burden populations are ethnic tribes living in the forested pockets of the states like Orissa, Jharkhand, Madhya Pradesh, Chhattisgarh and the North Eastern states which contribute bulk of morbidity and mortality due to malaria in the country. Drug resistance, insecticide resistance, lack of knowledge of actual disease burden and new paradigms of malaria pose challenges for malaria control and elimination in the country.

India has responded well to these challenges and there had been introduction of malaria RDTs, artemisinin based combination therapy, long lasting insecticide nets and other novel vector control measures. When the country heads towards elimination, more and more research inputs are needed to address the issues like disease burden asymptomatic parasitaemias, *P. vivax* relapses, G6PD testing, new species, changing vector behavior, managing severe malaria, access etc.

Dengue Prevention - A Stitch in Time Saves Nine

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Public health threat of dengue is expanding geographically, the disease which was prevalent is most of the metropolitan cities and towns in India, has not only shown outbreaks from rural areas of Karnataka, Maharashtra and Haryana but also transmitted to immune population. The disease burden is exaggerated due to the absence of drug or vaccine, and to assuage this threat prevention is the lone choice. With early detection and proper symptomatic treatment greater mortality can be prevented at individual level. However, management of this disease like other vector borne diseases involves not only treatment of individual patient in terms of medical line, but also comprehensive management focussing prevention and control of *Aedes aegypti* mosquito. Thus preventive program involves participation of larger groups, strategically facilitating personal protection; biological and chemical control; environmental management and source reduction; health education and community participation. In order to bring about pan India awareness Government of India for the 1st time announced May 16th as National Dengue Day from this year, with the intention to bring about larger awareness on the magnitude of the disease prevalence and control measures. The aim of National Vector Borne Disease Control Programme – of Dengue is to sensitize huge population – community, school children and other stakeholders for ameliorating the disease menace. Considering the importance and timely need for community participation the dengue control programme is linked with the ambitious - Swacch Bharat Abhiyaan, of Ministry of Drinking water and sanitation. This paper presents comprehensive methods adopted by the Government of India under the banner National Vector Borne Disease Control Program to control the *Aedes Ageypti* and prevent Dengue.

Containment of Malaria Outbreak in erstwhile endemic village Manamelkudi of Aranthangi Health Unit District in Tamilnadu

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Malaria is one of the public health problems in Tamil Nadu. Despite considerable success of Malaria control in the state, still it continues a public health problem in some of the coastal areas of Ramanathapuram, Kanyakumari and Thoothukudi and riverine area of Dharmapuri and Corporation of Chennai. Tamil Nadu has long been demonstrated significant achievement in Malaria control with a progressive decline in total number of cases by implementing Integrated Vector Control activities in the Malaria problem areas.

During the Year 2014, Malaria focal outbreak occurred in erstwhile endemic villages namely Ponnagaram/Antoniarpuram of Aranthangi HUD having a population of 2200. These are coastal villages of Ammapattinam PHC of Manamelkudi block. The duration of this outbreak was 4 months (August – December) and due to the movement of fisherman community to Ramanathapuram which is a Malaria endemic place. All the detected 252 cases were P.*vivax* Malaria and given complete Radical Treatment. Mobile clinic was conducted in the outbreak site with diagnostic and treatment facilities. Integrated Vector Control measures such as special rounds of Indoor Residual Spray (IRS) with Alphacypermethrin 5%WP in two Health Sub-centres with a spray coverage of 89% and weekly anti-larval measures such as source reduction, Temephos/Bti-AS application were carried out. Gambusia affinis were introduced into wells. Thermal fogging operation with Pyrethrum 2% extract was undertaken. IEC was given to all the people. Government hospitals and Private Hospitals were fully involved in following NVBDCP treatment protocol. By all these intensified control measures the outbreak has been contained.

Dengue in Karnataka: Epidemiological and Entomological Analysis

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Dengue, a mosquito borne viral disease is prevalent in all the districts of Karnataka. Dengue outbreak was first reported during 1996 with 196 positives and 5 deaths from few districts viz., Bangalore Urban, Kolar, Chitradurga, Mysore and Dakshina Kannada which gradually spread to all the districts of Karnataka. Unplanned urbanization, narrowing down the margin between urban and rural areas, migration of labour population, improper water storage practices and solid waste management are known reasons facilitating spread of dengue. During 2016, a total of 64862 samples were tested with IgM ELISA of which 6083 were positive for dengue. The testing is done by 32 Sentinel Surveillance Hospitals (SSH) including IDSP and one State Apex Laboratory – NIMHANS, Bangalore. Karnataka experiences 2 peaks of Dengue incidence viz., pre monsoon (April- June) and post monsoon (July-October). The serotyping further revealed that three serotypes DENV- 1, DENV- 2 and DENV- 3 are reported in Karnataka whereas, 8 districts have reported all 3 serotypes. All the health facilities under private sector also report dengue cases regularly to District health authorities as Dengue / DHF/ DSS is a Notifiable Disease under Karnataka Public Health Act.

The primary vector for dengue transmission in Karnataka is *Ae. aegypti*, however, *Ae. albopictus* is prevalent in coastal belt of State. Regular vector surveillance is carried out in all districts by District Entomologist & Zonal Entomological Team. The involvement of ASHA, Domestic Breeding Checkers & other community volunteers helps in detection and elimination of *Aedes* breeding sources at village level. Local bodies and Grama Panchayath are necessarily involved in solid waste management. During 2016, a total of 43054856 containers were inspected of which 878770 were positive for aedes breeding. The average values of HI, CI and BI were 4.4, 2.0 and 6.7

respectively. The entomological survey conducted so far indicated major potential Aedes breeding in water storage containers like Cement tank (63%), Drums / Barrels (22%) & solid waste materials (15%). Sixty five pools of *Aedes* mosquitoes from various dengue outbreak areas were tested for presence of Dengue virus but none of the pool was found positive. The *Aedes* larval survey is also undertaken in Airports of Bangalore and Mangalore and surrounding villages within radius of 5 km (extended area) and in Sea ports of Karwar & Mangalore. The breeding noticed were destroyed in order to minimize the risk of Dengue, Chikungunya and also Zika. Strengthening case detection its management along with IEC activities and vector control activities through Integrated Vector Management has been instrumental in keeping case fatality rate around 0.13.

Detection of focus of *Brugia malayi* in odisha

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In India, *Bancrofti*an filariasis transmitted by the *Culex quinquefasciatus* contributes about 99.4%. Though, historical records indicate the prevalence of *Brugia malayi in* Sriharikota of Andhra Pradesh, Cachar in Assam; Durg in Chattisgarh; Alappuzha, Ernakulam, Kollam, Kottayam,Thrissur and Trivandrum of Kerala; Balasore, Puri of Odisha; Kanyakumari of Tamil Nadu and Midnapur East of West Bengal, it has been limited in small pockets of Kerala only.

In the year 2015-16, during additional night blood survey before Transmission Assessment Survey (TAS), presence of seven *B. Malayi* infection was detected at the time of cross checking of smears by Regional Office for Health and Family Welfare, Bhubaneswar in two districts of Odisha namely Bhadrak and Balasore. Out of 10000 slides collected from 10 additional random sites each, 4 and 3 smears were positive for *B. malayi* infection from two sites of Baleswar and one site of Bhadrak respectively which were reconfirmed by the VCRC, Puducherry. Odisha is endemic for *Bancrofti*an filariasis with only reports of B. *malayi* in 1989 from district Balasore and in 2005 from Puri. The presence of B.malayi in recent survey during 2015-16 necessitated the investigation. The district is located in the East Coast of India and bordering to West Bengal state. The people are fish loving, hence almost all houses are having a small pond in their backyard for fish culture which are congenial for Mansonia breeding. The vector Mansonia species were also collected but none as found positive. This is third report of *B.malayi* after 1955 in Odisha.

Zeroing of malaria cases in eight low endemic coastal districts of Odisha

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Odisha, an east coast state of India is a high malaria endemic state with Annual Parasite Incidence (API) 9.97. The malaria endemicity in the state is characterized by uneven distribution of disease with API varying from <1 to > 50 among the districts. At sub-district level the variation is high with wider API range. Under the National drive for malaria elimination by 2030, the state of Odisha envisages strategic intervention for achieving zero indigenous transmission at sub state level. Under the National Framework for malaria elimination, Odisha comes under the category 3 (i.e. API>1). Although the state is high endemic; there are few very low endemic zones. The eight coastal districts having around 27% of the total population of the state, have average API 0.34 (0.12 to 0.73), ABER (Annual Blood Examination Rate) remaining at 11 ± 2.0. These districts contribute only 0.8% of malaria cases (3727 out of the state total 449697) to the state's malaria burden with average case sharing of 465 by each district.

Eight coastal districts are Balasore, Bhadrakh, Cuttack, Jajpur, Jagatsinghpur, Kendrapada, Khurda and Puri. These districts have been taken into the study for strategic intervention for malaria elimination. Individual case based investigations have been carried out for 590 malaria cases in three lowest endemic districts of the state: Kendrapada, Puri, Jagatsingpur. Intensive surveillance, identification and clearing of residual foci by complete radical treatment and focal vector control measure along with constant vigilance for maintaining malaria free status in the area are the key activities undertaken for zeroing of indigenous cases from these districts. Programmatic attention with above activities led to substantial increase in ABER (1.3% more than previous year) with identification of transmission foci. Around 48% of cases have been found to have travel link and migratory. Other 44% potential addition is from Tribal residential Schools, thus only 8% of the cases are identified as having indigenous origin. This study will help in developing special strategic action plan for taking focused action for malaria elimination in eight coastal districts at sub- state level. This may generate evidences for malaria elimination strategy in similar areas in the country.

Sub-Microscopic Malaria in Chennai: The Hidden Parasite Reservoir

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Sub-microscopic malaria acts as a transmission reservoir in endemic areas and poses a major challenge in control efforts towards malaria elimination. The objective of the study was to find the burden of submicroscopic and asymptomatic malaria at Besant Nagar, Chennai. Of 1054 symptomatic patients visited clinic, 186 (17.6%) were by positive by microscopy and 222 (21.1%) by PCR. In survey, infections were low, 8 (0.9%) by microscopy and 24 (2.6%) by PCR out of 928 sampled. Majority of infections detected by PCR was submicroscopic, 70.8% (17/24) in survey and 18.9% (42/222) in clinic study. Submicroscopic infections detected in survey was 100% (17/17) asymptomatic. These data shows that submicroscopic infections were hidden in the population, which can act as a reservoir and hinders the control programme. Better interventions targeting the infection reservoirs are needed to scale down malaria to pre-elimination level.

Study of Entomological Indices and Outbreak of Dengue & Chikungunya in City Zone of Delhi

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Dengue is emerging as a major public health challenge in world. Delhi, the capital of India has witnessed dengue outbreaks in 1996, 2003, 2006, 2010, 2013 and 2015 whereas the first outbreak of chikungunya has been reported during 2016. *Aedes aegypti* is predominant vector for transmission of both dengue and chikungunya. High larval indices of *Aedes aegypti* were reported in Delhi during transmission period but a phenomenon of viral competition was observed between dengue and chikungunya virus. City zone is situated in walled city of Delhi and spread over an area of 25 Km^2. with a population of 579724 and population density of 23189 persons /Sq Km. High population density associated with water storage practices and cultural habits of local residents make this area more vulnerable for dengue and chikungunya outbreaks. City Zone reported 49 dengue and 137 chikungunya cases in 2016. High larval Indices were recorded from ward No 81 (Minto Road) of City Zone where 31 Dengue and 83 Chikungunya cases were reported this year. Entomological surveillances were carried out in Educational Institutions including Medical Colleges and Govt. Offices located in city zone, on regular basis. All types of water holding containers were inspected for the presence of mosquito breeding and larval index i.e. container index was calculated and analysed. During study period, the strong relationship between container index with dengue and chikungunya cases was observed. The positive cases either by NS1 (for dengue) or MAC ELISA (both dengue and chikungunya) notified by South Delhi Municipal Corporation (SDMC) were taken as confirmed cases. Ward No 81 of city zone consistently reported highest container index as compared to other wards of city zone. In 2013 and 2015, reported container index was 8.7% and 9.8% respectively. During 2016, this ward followed rising trend i.e. 5.8% to 25.3% from April to September month of 2016. Index case of Dengue and Chikungunya from City Zone was reported on 1st Aug in Ward 81. On investigation of Index case of ward No 81, Container Index of 12.8% was reported from campus of a medical College. Maximum breeding was reported from Medical College Hostels, Educational Institutions, schools, Police Stations, different Govt Offices and markets. Ward No 81 contributed 60% of Chikungunya cases. 73% of Chikungunya cases were reported from Campus of two Medical colleges. Ward No 81 contributed 62% of Dengue cases of City Zone. 58% of Dengue cases were reported from two medical colleges. Container Index of 22.9% was reported from Hostel of Medical College in first week of August. Highest breeding was reported in solid waste lying in these complexes (14.5%) followed by water storage containers (5.4%). A positive correlation was observed in Chikungunya cases and Container Index reported. Solid waste and water storage containers are the preferred breeding sites in city zone. The study concluded that the targeted intervention including sustained vector surveillance could help in controlling the sudden upsurge of dengue and chikungunya cases in densely populated zones of Delhi. There is a need to carry out studies on detection of Chikungunya and dengue virus in vector in non transmission and transmission season.

Situation of Malaria in Dantewada District, Chhattisgarh

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Chhattisgarh is the second most malaria contributing (11.8%) state of the country. Dantewada district is the highest malaria endemic district of Chhattisgarh state. In order to assess the situation of malaria in district Dantewada, the present study was planned so that necessary steps can be taken by the state and national authority to understand the needs to control the problem of malaria. A field visit to the study area was undertaken during the month of April 2015. During this visit entomological and parasitological survey was done in the three blocks of the district namely Geedam, Katekalyan and Dantewada. Last three year epidemiological data for malaria was collected from the office of district malaria officer. Dantewada district is highly endemic for malaria and annual parasite incidence (API) ranged from 38.63 – 49.37 during the year 2012-2014. During the fever survey, a total of 91 persons were examined by RDK for malaria positivity and 18 persons were found positive for malaria parasite. Out of 18 malaria positives, 14 were *Pf*, 2 with *Pv* and rest 2 were mixed infections. When the blood smears collected from these persons were examined, *Plasmodium malariae* was also detected from the blood smear of patient of Hidpal village. In entomological surveys, four species of anophelines *Anopheles culicifacies*, *An. subpictus*, *An. vagus* and *An. splendidus* were collected from human dwellings. Infections of *Pv*, *Pf*, *Pm* and mix infections were detected during the survey. It was for the first time P. *malariae* infection was detected from Dantewada district with second time from Chhattisgarh state. The current study was conducted during the month of April which is the non transmission season month. Despite it, slide positivity rate of 19.7 was obtained which shows poor surveillance of malaria in the region by health workers. Further in depth studies are required in district Dantewada and all high malaria endemic areas of Chhattisgarh state to know the reason of persistence of malaria in the district which will help in improving the situation of malaria control in this area.

Global Malaria Burden: Re-Energized Efforts for Elimination in the Era of Sustainable Development Goals

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Though significant achievements have been made in malaria control during last decade as projected in World Malaria Report (2016), which highlights that malaria cases have come down globally from 262 million in 2000 to 212 million in 2015 and malaria mortality rates are estimated to have declined by 62% globally, still there are few pockets which contribute enhanced burden, as well as the emerging challenge of insecticide resistance in mosquito vectors and drug resistance in malaria parasite particularly to new artemisinin based combination therapies (ACTs) are significant barriers which may halt and restrict to sustain the achievements gained as well as pose threat to achieve universal coverage of interventions. India has also achieved tremendous success in recent past and cases have come down from 2.08 m cases in 2001 to 1.10 m cases in 2014 but there are certain hard core, tribal dominated difficult areas that continue to pose great challenge and become barrier in rolling out key malaria control interventions and sustaining the achievements gained. Globally Malaria control will now be shaped by 2 newly adopted strategic documents, the second generation Global Malaria Action Plan (GMAP) known as Action and Investment to defeat malaria (AIM) for a malaria free world as developed by Roll Back Malaria (RBM) partnership, which has been built on the success of GMAP-2008-15, describes the actions and investment needed to achieve the targets and the Global Technical Strategy for Malaria (2016-2030) as developed by WHO global malaria programme. The world has now shifted from MDGs to SDGs with 17 Goals and 169 Targets, which are more comprehensive and focus on reducing global inequities and ending poverty. The AIM as developed by RBM partnership and the WHO developed Global Technical Strategy for Malaria are aligned to contribute in achieving Sustainable Development Goals (SDGs). The documents target at least 90% reduction in malaria mortality rates and incidence by 2030 and focus at least 35 countries to eliminate malaria. Malaria reduction and elimination will contribute to benefit intervention measures towards SDGs. It has been shown that 10% reduction in malaria was associated with 0.3% rise in annual GDP, hence malaria control may help in reducing poverty, boost economy and improving school & work station absenteeism as well as addressing malaria in pregnancy may help to enhance mother and child health the key agenda of SDGs. India has also launched a national framework for malaria elimination (2016-30) and attempts are being made for malaria elimination in phased manner under public private partnership for a significant impact. A vision for malaria free world may be realized with concerted and re-energized efforts.

GPS-Assisted Dengue Surveillance in Najafgarh Zone

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Dengue is a serious public health problem in India. As per National Vector Borne Disease Control Program (NVBDCP) data, the number of dengue cases in India escalated from 40571 in 2014 to 99,913 in 2015. Delhi, the national capital of India is well known for Dengue Fever (DF) epidemics. During 2015, DF resurged in Delhi and a total of 15867 cases and 60 deaths were registered. In the absence of dengue vaccine and drugs, identification and elimination of the breeding sites of dengue vector i.e. *Ae. aegypti* is the only dengue control measure. Delhi has got 12 zones under MCDs out of which Najafgarh is one of the endemic zone with increasing number of dengue cases every year. Ward No 142 of Najafgarh is the worst affected as around 80% of the dengue cases in Najafgarh zone were recorded from this area. Therefore a GPS assisted dengue surveillance study was initiated during 2016 to identify risk factors prevalent in Ward No 142, propagating dengue and to suggest suitable control measures. Two rounds of surveillance were conducted starting from January –November 2016. Nearly 22680 breeding containers in 3200 households were checked and about 1070 households were found positive. Though the field team cleaned the breeding sites, it was observed that around 15% same households (with positive breeding sites) were positive during both the surveillance rounds. Intermediate results of the study also revealed that houses with tanker water supply and those within 20m radius of it are contributing around 60% of the dengue cases whereas other ecotypes contributed only 5-10%. IEC activities based on KAP were carried out by stakeholders and based on KAP study data target group were identified in the community for conducting IEC activities. It was observed that households with low income group were having multiple water storage containers and solid waste stored on roof tops and courtyards which are contributing most of the *Aedes* breeding. The identified risk factors will help the program in formulating suitable control strategies for prevention and control of dengue.

Dengue Epidemic – A Global Health Challenge

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Dengue is one of the most common arboviral infections, and its burden is poorly quantified. It is one of the most emerging tropical diseases of the 21st century. The worldwide distribution of the risk of dengue virus infection and its public health burden are poorly understood from the public health aspect. Currently, there are no vaccines available and vector control efforts have not curtailed the global spread of the menace. Our study undertakes to investigate into some of the available records of dengue occurrence worldwide to map the global distribution of dengue risk. The biggest challenge in dengue estimates include deriving the actual number of dengue cases and cost per case, even in countries where detailed studies have been done. It is more important than malaria in terms of morbidity, and inflicts a significant burden in the global infection control domain. It also pictures the major social impact in those countries where large epidemics occur, often disrupting primary care for hospitalized patients. The review on various aspects of dengue control has revealed an urgent need for permanent surveillance programs and controlling disease transmission by following an effective implementation of vector control programs.

Dengue in Chilka Lake, Odisha

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Chilka Lake is a brackish water lagoon spread over Puri, Khurda and Ganjam district of Odisha state. The mosquito diversity in the Ckilka lake includes 22 species of mosquito belonging to 6 genera including Aedes mosquito. Chilka Lake is set to be developed eco-tourism destination attracts migrated birds during the winter season. Apart from the migratory birds several species of domestic birds also lives at Mangalajodi. The dengue fever caused by flavivirus, is transmitted by the day biting *Aedes* mosquitoes. Accoring to the District Health Office, Khurdha total 152 samples were collected from Mrach to July 2016 and 19 of them were positive for dengue virus by ELISA. Four of the patients have died. The survey conducted on aquatic fauna of Chilka lake area and its adjoining areas indicates that the climate is hot and humid with wide spread paddy fields. The unused broken fishing boats were thrown in different places by the fisherman provides a favourable condition for the breeding and growth of mosquitoes.Besides the decrease of salinity of the Chilka lake reduce the PPT from 6.7 to 4.2 PPT. As a random survey from previous to present the spread of dengue gradually increase in the coast areas of chilka lake which alarming the spread of dengue in Chilka.

Distribution and Bionomics of *Culex vishnui* Group of Mosquitoes in Odisha

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Japanese encephalitis (JE) a mosquito borne disease has been a serious public health problem in Odisha since 2013. During the last two years, outbreak of JE occurred in different districts *i.e.*, Malkangiri, Jajpur, Puri, Keonjhar and Mayurbhanj of Odisha. JEV is spread by infected mosquitoes especially *Culex vishnui* group of mosquitoes. This menace is an ever spreading one, with casaulties mounting and no particular effective remedial action to check it. To understand and analyse this disease a thorough research is the need of the hour. Unfortunately, in the past it has not been given the required attention, but with cases mounting, it demands urgent attention. The entomological observation during study period showed a total no of 6508 mosquitoes collected at dawn and dusk hours belonged to 4 genera and 19 species including 7 species which are known to be JE vectors in India. Among 19 species Cx. *vishnui* was the predominant species (66.41%) followed by *An.annularis* (6.97%), Cx *.quinquefasciatus* (5.43%), *Ma.uniformis* (5.11%), *An.aconitus* (4.39%), *An.hyrcanus* (2.55%) *and An. barbirostris* (2.44%). The remaining 12 species, which formed 6.7 percent of the total catches, were *An.culicifacies*, *An. fluviatilis*, *An. jamsei*, *An.karwari*, *An. subpictus*, *An. vagus*, *Ma.indiana*, *Ma. annulifera*, Cx. *tritaeniorhynchus*, Cx. *gelidus*, Cx. *luchia* and *Armigeres*. The highest PMHD of Cx.*vishnui* was observed in rainy season when compared with other two seasons in Jajpur and Keonjhar districts (regular study area) whereas in Puri district PMHD of Cx.*vishnui* was high during outbreak of JE in summer season (2016). The per dip density of larvae of Cx.*vishnui gr.* was 24.28 in rice field followed by 23.21 in irrigation channel, 21.66 in ponds and 18.40 in pools in Jajpur whereas in Keonjhar the per dip density of larvae of Cx.*vishnui gr.* was 12.2 in rice field followed by 11.12 in ponds , 9.45 in irrigation channel and 8.9 in pools. This showed how seasonal distribution of vector mosquitoes varies in time and space depending upon environmental conditions and availability of breeding habitats. From our molecular investigation we found that Out of 98 pools of processed samples 8 pools showed positive result which are belonging to *Culex vishnui* group and *Culex gelidus* of different study areas. The present study confirmed the transmission of JEV infection and further investigations on the ecology and vector potential of JE transmission in Odisha is continuing. As our research progress, the role of the various mosquito vectors will become clearer and cycles involving previously unsuspected mosquito species will be exposed. Therefore, continuous monitoring and control measures are always essential for the prevention of JE. Here in our study, we represent the current knowledge of the JE vector bionomics and the transmission of disease in Odisha.

Prevalence of Etiological Agent of Acute Encephalitis Syndrome (AES) in Hospital Samples

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Acute Encephalitis Syndrome (AES) is a group of clinical neurologic manifestation caused by wide range of viruses, bacteria, fungus, parasites, spirochetes, chemical and toxins. The virus that causes AES is Japanese Encephalitis (JE), Dengue, Herpes Simplex Virus (HSV), Epstein Barr Virus (EBV), Measles and mumps. Rubella cannot make AES but the affected upspring can suffer from AES. We conducted a hospital study to know the frequency of AES in the area. A total 2,298 samples were collected during the period of January-June' 2016. Among those samples only 685 (29.81%) samples were marked for diagnosis as AES according to signs and symptoms. Out of these samples, only 177 (25.84%) had a viral aetiological symptoms. Viral based analysis from the total hospital samples provided the percentage of different virus is as follows- JE was 2.15% followed by EBV - 0.77%, HSV-I - 0.46%, HSV-II - 0.23%, Measles - 0.15%, Dengue - 1.77%, Mumps - 0.15% and Rubella - 6%. Further analysis of JE and Dengue samples, it was found that samples were collected from age of 27 days old child to 81years old patients but predominantly young children < 14 years old (86%) were affecting groups. There was no significant different in gender. Symptom wise, 100% patients had fever in both Dengue and JE, followed by Seizure (66%), Motor deficit (48%), Confusion (41%) and Change in mental status were only 14% in Je cases. In Dengue, except fever, all other symptoms are very low in percentage. The study suggested that Japanese Encephalitis and dengue are the predominant cause of viral AES in hospital samples.

Seroprevalence of Japanese Encephalitis among School Children of Tirunelveli District, Tamil Nadu

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Japanese encephalitis (JE) is the leading viral cause of Acute Encephalitis Syndrome (AES) in Asia. The disease primarily affects children under the age of fifteen years. Many individuals living in flavivirus endemic areas developed subclinical infections in childhood and gain a degree of protective immunity. Finger prick blood samples were collected from school children of age 5-10 years in 4 study villages of Tirunelveli district during November 2011, March 2012, November 2012 and March 2013. Same children were followed for four collections in two years. Blood specimens were transported on ice to the Centre for Research in Medical Entomology, Madurai where they were stored at -20°C till tested. Plasma samples were tested by Haemagglutination Inhibition (HI) test for JE HI antibodies. A total of 326 children samples collected per season were screened for JE HI antibodies. Of 326 plasma samples tested, 12.82% - 84.81% were found positive. There were statistically significant differences in seropositivity rates between seasons. Age wise, season wise, sex wise and village wise details on seropositivity are discussed in this paper. In JE, there is always a preponderance of subclinical infections and therefore, reporting only overt cases underestimates the total level of virus transmission in an endemic area.

Japanese Encephalitis Outbreak in the Southern Districts of Odisha in 2016: Emerging Threat to Odisha

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A series of deaths reported from Malkangiri a southern district of Odisha during September, 2016. To investigate the possible cause behind such an outbreak a team from RMRC, Bhubaneswar visited the affected area. House to house survey was made to enumerate the suspected cases. Demographic data and clinical features were recorded and samples of blood were collected after their consent. The district is a tribal dominated area and the outbreak was reported in the “KOYA” tribe of the affected area .There were 328 suspected cases of which samples were collected from 199 including samples sent to the virology laboratory of RMRC from district hospital of Malkangiri. The affected patients were children and age group of 1-3 years (92 suspected cases) was mostly affected. But positivity was higher in children above 5 years (59%). Male and females were more or less equally affected. Out of 199 suspected cases, the definition of AES fitted in 194 and JE IgM was found in 66 cases (34.02%). The disease spread to the 4 different blocks having 100 villages. The common clinical features were fever (55, 85.9%), vomiting (35, 54.6%), neurological involvement (17, 26.5%), convulsion (11, 17.2%) and cough (5, 7.8%). The case fatality rate was 27.2%. Mean period between onset of disease and death was 3.82 days (±0.9days). Apart from this, ecological aspects or environmental factors like vector population, reservoir, and paddy field were supportive to JE infection. The outbreak was suddenly reported with deaths of children& spread of such infection in neighbouring villages.

CHAPTER 4

SOCIAL, CLIMATE AND ENVIRONMENTAL DETERMINANTS OF VECTOR BORNE DISEASES

The Crucial Challenges that *Aedes aegypti* Poses in the Spread of Flaviviruses

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Highly pathogenic viruses, Dengue (DENV), Yellow fever, Zika, Japanese Encephalitis and West Nile which belong to Flavivirus family and Chikungunya Virus (CHIKV) which belong to Togaviridae family are causing major public health problem globally. All this viruses are transmitted to human by Ades species of mosquitos, namely, *Ae. aegypti* and *Ae. albopictus.* Though all of these Flaviviruses and CHKV were characterized more than 60 years ago, number of human clinical cases and fatality remained low until recently. Re-emergence and rapid spread of these viruses in all parts of the world is a major health issue. There are estimated 3.2 million people were infected with dengue viruses in 128 countries in 2015 alone, whereas more than 2000 and 4500 Zika cases reported in Brazil and US territory in 2015-2017. Re-emergence of yellow fever has also become major concern with 952 cases including 10 cases in China where the virus never seen before. Though vaccines are developed against dengue and yellow fever and vaccines against Zika is in trial, cross-reactivity between the closely related flavivirus antigens and antibody dependent enhancement (ADE) of secondary infections are major barrier in preventing the disease caused by these viruses. Cases of co- infections with multiple viruses with severe clinical outcome are also being frequently reported suggesting altered replication pattern and pathogenesis in host and also implicating changes in vector competence. In this situation, vector control is the possible way to restrict the spread of these viruses. Two major strategies for A. aedes mosquitoes could be implemented; one is outdoor fogging and another is using insecticides like pyrethroids which has insect repellent properties. Recently, an Australian group Incairs have used Wolbachia vector which has shown that when introduced into the *Ae. aegypti* mosquito, Wolbachia can stop these viruses from growing inside the mosquito and being transmitted to people. Rapid urbanization, climate change, global expansion of trade and travel to the countries with human cases are among the major factors that may contribute to vector spread which is associated with disease burden. In India both *Ae. agypti* and *Ae. albopictus* species are present and tropical climate is favourable for the spread of Flaviruses and CHIKV. In 2016 there are 60000 chikungunya and more than 100000 cases of dengue infection reported (including 227 death) according to report from National Vector Borne disease control report. Interestingly, there is no report of a single case of Zika and Yellow fever. Investigating the factors behind the barriers in yellow fever and zika spread in India in perspective of vector biology may help ii understanding in disease progression and way to prevent Flavivirus infection. Global vector control consortium need to be established to control the spread of Flaviviruses and CHIKV.

Climate, Ecology and Vector-Borne Diseases in Indian Context

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Vector borne diseases pose a considerable public health burden in India. With strengthened interventions using latest available tools, diseases like malaria, Visceral leishmaniasis and Lymphatic filariasis are decreasing while the diseases like dengue and chikungunya are increasing in spite of best available efforts. In addition, scrub typhus, Kyasanur Forest Disease and Crimean Congo Hemorrhagic Fever have re-emerged, the reasons of which have not been studied properly. In the wake of elimination goals, the understanding of climatic and ecological link of VBDs is essential otherwise possibilities of resurgence and /or new areas coming in the fold of transmission are always there. As documented in malaria, Climate change is likely to affect the spatiotemporal distribution of VBDs, therefore, the understanding of ecological epidemiology and assessment of impact of Climate change are warranted. With the advent of satellite remote sensing, ecological changes can be detected even at village level which provide landscape link to receptivity of the area to a particular VBD. Mapping of disease distribution and their vectors using Geographical Information System at fine resolution at different time slices can guide the National programmes in identifying the knowledge gaps and actionable sites. The present communication will highlight the work undertaken in the direction of climate, ecology and VBDs in India and the unaccomplished task ahead.

Impact of Climatic Factors on Dengue Incidence in India

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Dengue is the most important mosquito borne disease in the world, especially in tropical and subtropical countries. The incidence of dengue has grown dramatically around the world in recent decades. The recent study of the prevalence of dengue, estimates that 3.9 billion people, in 128 countries, are at risk of infection with dengue viruses. The transmission of dengue virus depends on the presence of *Aedes* mosquito. Studies reveals that the tropical and subtropical countries having high rainfall with varying high temperatures are more prone areas to vector borne diseases. India is one of the subtropical country which provides ambient climatic conditions for several vectors. In India according to “Integrated disease surveillance program” since 2010 to 2014 total 52,644 cases are reported and according to “Tropical Rainfall Measuring Mission” (TRMM) during this period the mean rainfall per year reported as 1249.2 mm and mean temperature ranges between 12.4°c to 30.8°c. After heavy rainfall, the stagnant water provides breeding habitats for mosquito and the warm temperatures positively influences the vectorial capacity, which is directly proportional to the disease occurrence. Sometimes the exposure event to the climatic factors does not produce immediate effect. The present work has been carried out to study the delayed effect of climatic factors such as rainfall and temperature on the dengue incidence in India. We used Quasi Poisson regression with distributed lag nonlinear model(R-language) while lag effect and nonlinear effect taking into consideration to identify the association between weather variables and disease incidence. The precipitation ranges 40mm-60mm at 8 to12 weeks lag period shows more positive impact on relative risk of dengue cases, while Precipitation ranges 60mm-80mm with more than 15 weeks lag period shows negative effect on dengue incidence. The weekly mean temperature range 25°c-30° c at 0 to 3 weeks lag shows more positive impact on relative risk of dengue cases while Mean temperature below 20°c and 26°-30°c more than 25 weeks lag shows negative effect on dengue incidence.

Microclimate Profile of Structure Types and Its Seasonal Variations – A Study from a Malaria Endemic Urban Setting in India

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Determining the range of microclimatic factors such as temperature and relative humidity experienced by malaria vectors in various micro-environmental field settings and their daily variation influencing the local malaria transmission are important in malaria control. Microclimatic temperature and humidity of the resting habitats of the urban malaria vector, *Anopheles stephensi* presumed to be experiencing were recorded using forty-two temperature and humidity data loggers (Onset HOBO data logger – U10-003) on hourly basis. They were equally distributed among seven structure types, indoor and outdoor positions of human dwellings (roof structures) besides, vegetation, wells and overhead tanks in a malaria endemic area, Besant Nagar in Chennai. The data from each logger was downloaded regularly for a period of one year. The maximum, minimum, mean values of temperature and humidity and diurnal temperature range (DTR) were calculated by logger, month and structure type wise. It was observed that minimum mean temperature of 26.64±0.44 (January) was recorded in outdoor thatched structure and the maximum mean temperature of 34.15±2.29 (May) in outdoor structure (OHT). Similarly, the minimum mean relative humidity of 57.65±8.01 (June) in indoor concrete structure whereas the maximum mean relative humidity of 96.14±3.44 (October) was recorded in wells. Indoor habitats were found to be warmer than outdoors. Of all structure types, the minimum values of both outdoor (26.64°C) as well as indoor temperature (27.63°C) was recorded from thatched structures. The temperature and humidity profile from the field setting is more reliable than the data from a distantly located meteorological station in order to determine the temperature or humidity dependant traits.

CHAPTER 5

PARASITE, RESISTANCE, NEW DRUGS AND DISEASE MANAGEMENT

Plasmodium knowlesi: Past, Present and Future

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Plasmodium knowlesi, a malaria parasite of long-tailed and pig-tailed macaques, was shown to be infective to humans by blood passage after it was first isolated in India from a long-tailed macaque imported from Singapore in 1931. Naturally-acquired human infections were thought to be extremely rare, until a large focus of human infections was reported in 2004 in Sarawak, Malaysian Borneo. Human infections have subsequently been described throughout Southeast Asia and in the Nicobar and Andaman Islands of India, resulting in the recognition of *P. knowlesi* as the fifth cause of human malaria. Most of the *P. knowlesi* infections have been diagnosed by microscopy as *P. malariae*, since these two species are morphologically identical and can only be correctly identified by PCR-based detection methods. *P. knowlesi*, unlike the benign *P. malariae*, has a 24-hour erythrocytic cycle and can lead to high parasitaemia and fatal human infections. Artemisinin combination therapy is recommended for the treatment of knowlesi malaria and severe cases require management and treatment as for severe falciparum malaria. The molecular, entomological and epidemiological data indicate that knowlesi malaria is a zoonosis in Southeast Asia and it has become the main cause of malaria in Malaysian Borneo. Methods that are currently used to prevent and control human malaria, such as the use of impregnated bed nets and residual spraying of houses, will be ineffective against knowlesi malaria since the vectors are outdoor feeding and forest-dwelling mosquitoes that belong to the *Anopheles leucosphyrus* group and macaques are a huge reservoir for *P. knowlesi* parasites. Whether *P. knowlesi* continues as a zoonotic infection or whether ecological changes due to logging or deforestation, with an associated increase in the human population and changes in mosquito biting behaviour, result in *P. knowlesi* switching to humans as the preferred host, remain to be seen.

Clinical and Molecular Monitoring of *Plasmodium falciparum* Resistance to Antimalarial Drug (Artesunate+Sulphadoxine-Pyrimethamine) in Madhya Pradesh, Central India

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The spread of *P. falciparum* resistant strain has led to a significant resurgence of malaria morbidity and mortality. The current cornerstone in malaria treatment in India is Artemisinin (Artemisinin + Sulphadoxine-Pyrimethamine) based combination therapy (ACT) for treatment of uncomplicated *P. falciparum* malaria since 2010. In the present study we assessed the therapeutic efficacy of ACT and molecular monitoring of antimalarial resistance. Therapeutic efficacy was determined by *in-vivo* method using 28 days follow-up. Molecular genotyping of *dihydrofolate*

reductase (*dhfr*), *dihydropteroate synthase* (*dhps*) and *kelch13* genes were analyzed. *msp-1* and *msp*-2 genotyping were used to differentiate recrudescence. Therapeutic efficacy of ACT was determined in 237 patients over the three year period (2012-2014). Most of the patients showed adequate clinical and parasitological response (99.6%). Molecular study revealed that 72% parasites were of mutant genotype (27.2% single mutants, 43.5% double mutants and 1.3% triple mutants) for *pfdhf*r while *pfdhps* showed 78.2% wild type alleles and 21.8% mutants (18.1% single mutants and 3.7% double mutants). Analysis of total 135 samples revealed mutation in *k13* gene along with non-synonymous single mutation at codon M579T (1.5%) and double mutations at codon M579T & N657H in 37%. ACT remains effective for the treatment of uncomplicated *P. falciparum* malaria in Madhya Pradesh, Central India. However, increasing mutation in *pfdhfr* (particularly triple mutations) and *pfdhps* may reduce susceptibility to partner drug SP and mutation in *k13 propeller* gene, highlighting the need for continuous monitoring of the efficacy of ACT.

Post-Kala Azar Dermal Leishmaniasis: Challenges in Diagnostics & Treatment

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Leishmania donovani causes visceral leishmaniasis (VL), which is endemic in the states of Bihar, Eastern U.P. and parts of West Bengal. Post kala azar dermal leishmaniasis (PKDL) is a dermal sequel of VL, reported in 5-15% of patients treated for VL, usually after an interval of a few months to years. PKDL cases play a pivotal role in maintaining parasite reservoir especially during inter-epidemic periods of VL.
Diagnosis of PKDL remains a major challenge as the parasite load may be scanty, particularly in macular and papular cases. Approximately 26% cases of PKDL are misdiagnosed at primary health centers, thus differential diagnosis becomes critical.

Conventional diagnostic methods based on parasite detection by stained smears, histopathology and culture have low sensitivity (35-58%) and use invasive procedures. Serological tests like DAT, ELISA and rk-39 based tests are not conclusive because of the persistence of anti-leishmanial antibodies after VL. Such limitations have been overcome with highly sensitive and specific molecular tests including PCR and nested PCR. We have developed a real-time PCR assay based on kinetoplastid DNA for a rapid and reliable diagnosis of PKDL. The assay is applicable for assessment of cure and to monitor the efficacy of new antileishmanial drugs by evaluation of parasite burden. Recently, we have developed Loop-mediated isothermal amplification (LAMP) assay which showed a high sensitivity of 97%, and specificity of 100% for diagnosis of PKDL making it a promising tool for simpler, rapid and sensitive diagnosis of PKDL.

The standard treatment of PKDL remains unsatisfactory. Increasing resistance to antimonials has paved way for oral drug Miltefosine for PKDL as the first line drug. The efficacy of monotherapy with Miltefosine for PKDL has reduced to 85% with an increased relapse rate of 15%, indicating the need to develop a robust regimen using other drugs/combination therapy with Miltefosine.

The central role of Abscisic Acid in regulating egress of two different intracellular stages of malaria parasite Plasmodium falciparum

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Fundamental to blood-stage propagation and transmission of malaria parasite is eventually the release of intraerythrocytic merozoites for propogation of asexual blood stage and intraerythrocytic gametocytes for switch to the sexual stage. Although considerable advances have been made in our understanding of the unique exit mechanisms, the signal that triggers the egress of these different intracellular stages, i.e. the blood stage and the gametocytes are not known. Herein, we show the production and involvement of phytohormone abscisic acid (ABA) in regulating Ca^{2+} dependent egress of both invasive merozoites and activated gametocytes. Extracts of *Plasmodium fakiparum* analyzed by LC-MS/MS at different time course of the blood stage life cycle revealed a marked spike in ABA levels at the late asexual stage parasites. Use of herbicide FLU, an inhibitor of ABA biosynthetic pathway, reduced the levels of ABA and blocked egress of merozoites as well as activated gametocytes. Further, addition of exogenous ABA triggered intracellular Ca^{2+} in late stage schizonts, and also rescued egress of both gametocytes and merozoites from FLU treated, host erythrocytes. *P. fakiparum* perforin like protein (PfPLP) previously entailed with host cell membrane permeabilization during egress of both merozoites and gametocytes, was also not localized to the erythrocyte membrane in FLU treated infected erythrocytes. Also the fluridone-treated, stalled schizonts, when incubated with rPfPLP 1 protein, were able to egress. These results thus, indicate ABA synthesis in malaria parasite as a critical step for egress of both merozoites and activated gametocytes. The plant like nature of ABA production pathway can be explored as target for both limiting the growth and transmission of malaria parasites.

Larvicidal and Anti-Plasmodial Effects of a Novel Phthalate Derivative from *Loligo duvauceli* and Its Interaction with CRK-2 Cell Division Protein by *In Silico* Assessment

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Background: Irrational and extensive use of anti-malarial drugs propagates the resistance in malarial parasites. Hence identifying new targets and drugs is the need of the hour. In this view, the pigmented ink of the squid *Loligo duvauceli* has been explored in this study for its promising larvicidal/pupicidal effect, anti-plasmodial activity and as an eco-friendly vector control agent. **Methods:** Collection of eggs and maintenance of larvae: The eggs of *Anopheles* were developed as per standard entomological procedures for the larval and pupal stages. Chromatographic evaluation of squid ink: The pigmented ink of *L.duvauceli* was subjected to crude solvent extraction, TLC, HPTLC and GC-MS analysis. Larval/pupal cytotoxicity assay was performed to calculate the LC_{50} and LC_{90} from toxicity data by using probit analysis. Plasmodial culture: Achieved on human erythrocytes as per standard cell culture procedures from malaria positive blood samples. *In vitro* anti-plasmodial assays were performed by microtitre plate method and rapid card method with varying concentration of the compound. Cell viability determination: By MTT assay in normal murine fibroblast cell lines and checked for cytotoxicity. *In silico* analysis: CRK-2 cell division protein homolog from *Pl.falciparum* was docked with BEHP to evaluate and analyse the drug target. Statistical analysis: Using SPSS version 9.0, ANOVA and Duncan's multiple range tests were done to test the significance. **Results:** Mass spectrum and chromatographic evaluation yielded BEHP with *Rf* value 0.73. LC_{50} and LC_{90} for the larvicidal/ pupicidal effect and IC_{50} in human erythrocytes of BEHP were derived at varying concentrations with statistically significant results. MTT assay showed no cytotoxicity. In-silico analysis yielded a single hydrogen bond interaction with a docking score of -11.81. **Conclusions:** The results indicate the anti-plasmodial efficacy of the phthalate derivative from the squid ink as a potential, safe, cheap and effective candidate to eliminate the

menace of drug resistance in malaria with further purification and clinical trials and an ideal eco-friendly approach in the control of vector mosquitoes.

In Silico Computational Analysis of Analogs of Resistant Drugs Against Malaria

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Malaria in human caused by protozoan parasite *Plasmodium* is a major global parasitic disease. It is caused by five *Plasmodium* species, namely, *P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi*. Of these, *P. falciparum* is the causative agent of severe malaria and the major cause of malaria-related fatality. Widespread drug resistance against currently available anti-malarials warrants the identification of novel drug targets and development of new drugs that should be economical, fast effective and safe to all age group. Proguanil is a prophylactic anti-malarial drug, a biguanide derivative which stops the reproduction of the malaria parasite, *P. falciparum* and *P. vivax*, once it is entered in the red blood cells by inhibiting the enzyme, dihydrofolate reductase (DHFR) that halts the parasitic lifecycle. The mutations in the receptor proteins tend to resistance towards proguanil due to weak binding affinity which could be overcome by development of analogs of proguanil. In the present study an attempt was taken to find out the analogs of proguanil through an insilico approach. This could be a potential anti–malarial inhibitor that acts against malarial target *Pf* TS-DHFR that plays a key role in parasitic life cycle. The molecular docking analysis of *Pf*TS-DHFR with Proguanil shown a binding energy of -7.41 Kcal/mol, whereas the docking studies of *Pf*TS-DHFR with ZINC00001127 and ZINC16343331 (analogs of proguanil) was -9.14 and -8.7 Kcal/mol respectively. The results obtained from the above study suggests that analogs of Proguanil shows better binding efficiency in comparison to the proguanil drug. These analogs may be of therapeutic importance in the treatment of malarial patients which could be further analyzed through *in vivo* and *in vitro* methodology.

Gametocytocidal Screening of Compounds against *Plasmodium falciparum*

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Clinical studies and mathematical models predict that in order to win the uphill battle against malaria and achieve the goal of malaria elimination, drugs targeting sexual stages of malaria parasite *P. falciparum* needs to be incorporated in treatment regimen along with ACTs. The main impetus is to interrupt the indigenous transmission of malaria throughout the country. Primaquine, WHO's primary gametocytocidal drug, is recommended along with ACTs in low to moderate transmission settings but not extensively utilized because of safety concerns among G6PD deficient patients. Here, an effort has been made to establish Indian field isolates of *P. falciparum* as an *in vitro* model to screen compounds for their inhibitory activity against gametocytes. Further, known antimicrobial drugs and newly synthesized triazole based hybrid molecules are being investigated for their gametocytocidal properties *in vitro*. A thiazine dye, methylene blue has been proposed

as an alternative to primaquine for its ability to knock out mature gametocytes and is also being investigated in the present study with primaquine as standard. Other molecules including novel triazole derivatives, having low nanomole inhibitory activity against asexual stages also exhibited submicromolar 50% inhibitory concentrations for gametocytes upon preliminary testing.

Anti-Plasmodial Efficacy of *Sonchus brachyotus* DC. (Asteraceae) against Lethal Murine Malaria Parasite *Plasmodium berghei*

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Natural products have a long history as sources of therapeutically effective medications to cure various ailments in the traditional medicine systems. *Sonchus brachyotus* (Sadhi) is used for the treatment of chronic fever in the Himalayan region.The present study has been undertaken to assess the *in vivo* suppressive, preventive and curative potential of the ethanolic leaf extract of the *Sonchus brachyotus* (ELESB) against lethal rodent malaria parasite *Plasmodium berghei*. The extract tested positive for various bioactive phytoconstituents like alkaloids, diterpenes and phytosterols in qualitative phytochemical screening tests. Median lethal dose (LD50) of ELESB was >5 g/kg. Maximum chemosuppression of 95.82% was observed at a dose of 1000 mg/kg on day 5 in the suppressive test. ED50 of ELESB was determined to be < 250 mg/kg, which indicates the presence of high schizontocidal activity of the extract against early infection of *P. berghei*. Mean Survival Time (MST) of 25.33±3.77 days was observed at a dose of 250 mg/kg of ELESB, which was extremely statistically significant ($p<0.0005$) as compared to the infected control (8.6±1.5 days). ELEBC also exhibited considerable preventive and curative activity against the parasite with 81.89% and 46.92% chemosuppression respectively on day 7. Considerably low parasitaemia levels 3.97±0.55%, 4.11±1.22%, 3.48±1.20%, 2.17±1.03% respectively were recorded in the surviving mice (G4-G7) on day 28 in the preventive test while complete parasite clearance was evident at a dose of 500 mg/kg (G6) in the curative test. The study points to the presence of considerable inhibitory activity of the extract against *P. berghei in vivo*. Further studies are being carried out to isolate the active phytoconstituents, which are responsible for the observed activity. Alternatively, the extract can be developed into an herbal formulation, which can be used as a phytomedicine against the disease.

Genotoxic Effects of Potentized Homeopathic Medicines *Cinchona officinalis* 30C and *Chelidonium majus* 30C on *Plasmodium berghei*

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Homeopathic remedies and their claimed efficacies as potent antimalarials have been challenged time and again due to their unknown mechanism of action and lack of evidence based research. Present study holds the attention on the possible genotoxic effect of *Cinchona officinalis* 30C (Chin. 30C) and *Chelidonium majus* 30C (Chel. 30C) on rodent malaria parasite- *Plasmodium berghei*. These two homeopathic formulations have been reported for their antimalarial activities. This investigation aims at ascertaining the effect of potentized Chin. 30C and Chel. 30C on the DNA of *P. berghei* in erythrocytes of Swiss albino mice. Short term *in vitro* culture of *P. berghei* was done with 50µl of homeopathic medicines. The treated cells were checked through comet assay using ethidium bromide staining. The DNA damage was analysed in CASP software. Present study revealed that the 30C potency of Chin. and Chel. are capable of causing DNA damage in the parasite. The tail length of cells in both the treated groups (49 and 21 respectively) confirmed that these highly diluted homeopathic formulations are genotoxic and probably killing parasite by their DNA damage. This could be the possible mechanism by which Chin. 30C and Chel. 30C are disturbing the survival and virulence of the parasite resulting in complete clearance of infection from the peripheral blood of the mice. However, the exact mode of action of these remedies still remains doubtful. Further studies are required to confirm the role of specific genes and their expression to know the exact mechanism of these ultra-high diluted drugs.

Virtual Screening and *In Silico* Docking Studies of Bioactive Natural Compounds from Plant Extracts against Malaria

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Malaria is a major global threat that is caused by protozoan parasite of genus *Plasmodium* results in more than 2 million deaths each year. In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. The treatment of malaria is becoming extremely difficult due to the emergence of drug-resistant parasites, the absence of an effective vaccine, and the spread of insecticide-resistant vectors. Further, development of resistance against the frontline anti-malarial drugs has created an alarming situation, which requires intensive drug discovery to develop new, more effective, economical and safe to all age group. There are many reports on medicinal plants that are commonly used and have demonstrated anti-malarial activity through ethnobotanical survey. In the present investigation an attempt was taken to identify the potential anti–malarial drugs/phytochemical compounds of plant extracts against malarial targets namely *Pf*CRT, *Pf*TS-DHFR and *Pf*DHPS that play a key role in parasitic life cycle. An *Insili*co approach was undertaken to report the anti-malarial activity of 11,000 compounds from TIPDB, through virtual screening and molecular docking. The study reported a total of nine phytochemical compounds as the potential inhibitors of malaria out of the above. The binding energy of phytochemicals resulted from docking analysis depicted Bisisodiospyrin with *Pf*TS-DHFR as -10.62 Kcal/mol, cycloartocarpin A with *Pf*DHPS as -10.95 and Lolitriol with *Pf*DHPS as -12.72Kcal/mol, Fatouapilosin, Ergovaline, Lolitrem E, Paxilline, Kudzuisoflavone A and Podocarpusflavone A with *Pf*CRT as -9.81, -10.78, -13.16, -10.19, -10.91 and -9.69 Kcal/mol respectively. The comparative insilico analysis predicted phytochemicals with best docking scores in comparision to existing drugs. The information generated from this investigation could be breakthrough in development of novel anti-malarial drugs. Further, *invivo* and *invitro* analysis of these phytochemical compounds are required to confirm their efficacy and anti malarial drug potency.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of Ethanolic Extract of *Calotropis procera* Exhibiting Anti-Plasmodial Activity

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Traditional medicinal plants have played an important role in the treatment of malaria. *Calotropis procera* (Asclepiadaceae) is known to exhibit anticancerous, anthelmintic, antimicrobial and anti-inflammatory properties. The present study has been undertaken to explore the antiplasmodial potential of the ethanolic extract of C. *procera* flowers (EFECP) and to analyze its phytoconstituents by GC-MS. The *in vitro* antiplasmodial activity of the plant was assessed by schizont maturation inhibition assay. Peters 4 day test was employed to evaluate the *in vivo* schizontocidal activity of EFECP. The various phytoconstituents present in the extract were analyzed using GC-MS. EFECP exhibited moderate activity (1C50=15.4 µg/ml) against RKL-9 strain of *P. falciparum* but showed promising activity (1C50 =7.9 µg/ml) for MRC-2 strain. The selectivity index (SI) of the extract was calculated to be >10. When tested *in vivo*, administration of EFECP (500mg/kg and 750mg/kg) and its flavonoid fraction (100mg/kg) resulted in considerable parasite clearance by day 28, with significantly ($p<0.0005$) enhanced Mean Survival Time of the host. GC-MS analysis of EFECP led to the identification of total 21 constituents. Friedelan-3-one was identified with the highest peak area (36.17 %) at retention time of 27.31. The present study points to the presence of considerable *in vitro* and *in vivo* antiplasmodial activity of EFECP. It also provides an insight into various phytoconstituents present in the extract. Friedelan-3-one can be a major component responsible for the observed activity of EFECP either singly or in combination against the disease.

Molecular Docking Analysis of Isopongachromene (C22H12O4) and Glabone (C18H12O4) with Odorant-Binding Protein (30GN) of *Culex quinquefasicatus*

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Odorant-Binding Proteins (OBPs) are highly expressed in insect olfactory tissues and involved in the first step of odorant reception and olfaction is essential in guiding mosquito behaviors. The structure of secondary metabolites of *Pongamia pinnata* was retrieved from Pub chem database. The inhibitory molecules were retrieved in standard 3D SDF format and selected compounds activity were predicted using PASS online server and VEGA tool. The retrieved molecules were prepared for docking using LigPrep module from Schrodinger suit. Analysis of molecular description of this compound was shown by using scatter plot MLogP against response values. Molecular descriptors of Isopongachrome and Glabone revealed that LogP is directly correlated to the log BCF value. In this scatter plot, the experimental values are reported for the training set and predicted values for the studied compound. Light blue dots represent values of compounds from training sets and red dot was the value of the studied compound. Interaction of 30GN with compound Isopongachrome and Glabone. The glide score of the compound isopongachromene was -11.33 and one number of H-bond interactions with distance of 1.877 and Glabone was -8.44 and 2 number of H-bonds with distance of 2.304 and 2.276.The target protein 30GN were obtained from Protein Data Base(PDB). The selected two secondary metabolite, Isopongachromene and Glabone were docked to the respective site of 30GN mosquito odorant proteins (PDB id: 30GN) and the interactions of the compound were observed. The selected secondary metabolites were considered as potential inhibitor molecules for mosquito odorant binding protein.

Laboratory Evaluation of Silver Nanoparticles Synthesized Using *Allium fistulosum* Linnaeus (Amaryllidaceae) for Toxicity

Against *Aedes aegypti* Linnaeus, *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Mosquitoes, referred to as 'flying syringes', 'tiny assassins' and 'public enemy number one' are the worst enemy of mankind since dawn of time and act as a vector of several diseases *viz*., dengue fever, chikungunya, zika virus fever, filariasis, Japanese encephalitis and malaria which are transmitted by the three genera of mosquitoes, *viz*., *Aedes*, *Culex* and *Anopheles*. The effect of past advocated synthetic chemical insecticides led to a search for alternative ways of countering these disease causing insects. To bridle these problems, safe and effective plant stratagem is in focus against the vectors and vector-borne diseases. The use of biopesticides especially botanicals continues to be a subject of keen interest and are considered as a more convincing strategy for the management of insect pests especially mosquitoes as they compete effectively with other types of insecticidal products. The potential uses and benefits of nanotechnology are enormous and include management of insect pests through the formulations of nanomaterials based pesticides. In addition to the direct use of phytoextracts for mosquito control, in recent days, biosynthesized phytonanopesticides gained momentum as biocontrol agents against mosquitoes besides holding promise for reducing the environmental footprint left by conventional pesticides. In the present study, the larvicidal activity of silver nanoparticles (AgNPs) synthesized using *Allium fistulosum* bulb aqueous extract against the three medically important genera of mosquitoes *viz*., *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* was determined by WHO protocol with minor modifications. Range of concentrations of synthesized AgNPs (1.25, 2.50, 5.00 and 10.00mg/L) were tested against the laboratory reared F_1 generation of third instar larvae of the above mentioned vector species. Maximum mortality was observed in the larvae of *Aedes aegypti* followed by *Culex quinquefasciatus* and *Anopheles stephensi* and their respective LC_{50} values were 5.10, 11.22 and 6.31mg/L after 24 hours of exposure. No mortality was observed in treated and untreated control. These results suggests that the green synthesis of AgNPs using the aqueous bulb extract of *Allium fistulosum* can be used as an eco-friendly biodegradable candidate for the control of vector mosquitoes.

Larvicidal and Pupicidal Effects of *Leucas aspera* Leaf Extract and Bacterial Insecticide, *Bacillus thuringiensis* Against Malarial Vector, *Anopheles stephensi* Liston. (Insecta: Diptera: Culicidae)

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The efficacy of leaf extracts of *Leucas aspera* and *Bacillus thuringiensis* has been proven against larvicidal and pupicidal activities of the malarial vector, *Anopheles stephensi*. The present study investigated the larvicidal and pupicidal activity against the first to fourth instar lavae and pupae of the laboratory-reared mosquitoes, *A. stephensi*. The plant extract showed larvicidal and pupicidal effects after 24 h of exposure. All larval instars and pupae have considerably moderate mortality; however, the highest larval and pupal mortality was the methanolic extract of leaf *L. aspera* against the first to fourth instar larvae and pupae values of LC_{50}=Ist instar was 93.45 ppm, IInd instar was 123.74 ppm, IIIrd instar was 167.17 ppm, and IVth instar was 216.15 ppm, and pupae was 256.66 ppm, respectively and bacterial insecticide, *B. thuringiensis* against the first to fouth instar larvae and pupae had the following values:LC_{50} Ist instar was 53.47 ppm, IInd instar was 62.09 ppm, IIIrd instar was 79.15 ppm, IVth instar was 95.39 ppm, and for the pupae was 105.76 ppm respectively. Moreover, combined treatment of values of LC_{50}=Ist instar was 85.09 ppm, IInd instar was 90.51 ppm, IIIrd instar was 111.91 ppm, and IVth instar was 137.61 ppm, and pupae was 154.40 ppm. No mortality was observed in the control. The present results suggest that the leaves of methanolic extracts of *L. aspera* and *B. thuringiensis* provided an excellent potential for controlling of larvicidal and pupicidal properties of against malarial vector, *A. stephensi*.

Human Skin Emanations for the Management of Mosquito Vectors

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Mosquitoes use the chemical cues released from potential hosts to locate them for blood feeding. Human sweat comprises of several volatile chemicals that guide the mosquitoes towards attractive hosts, and also repels them from un-attractive hosts. Chemicals that elicit positive orientation towards the source from responding mosquitoes could be utilized as attractants, while some other chemicals that elicit negative orientation, i.e., away from the source could be utilized as repellents that are natural origin. Thermal desorption-gas chromatograph coupled to mass spectrometer analysis of various individuals who differed in their degree of attraction and repulsion to dengue vectors revealed that geranyl acetone, methyl hexadecanal, dodecanol, cyclohexyl oxamide, dodecyl diglycol, etc were present in higher quantities in repulsive hosts than who were attractive to mosquitoes. In subsequent behavioural analysis it was found that fewer mosquitoes oriented towards the odor of repulsive individuals than attractive blood hosts. It is feasible to develop a cocktail of attractive molecules for use in traps as well as repulsive chemicals for development of repellents against biting from mosquitoes.

Investigation of Spinosad-Based Larvicides as Candidates for Resistance Management

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Spinosad is one of the few new active ingredients to enter the global public health vector control industry in decades. Spinosad has a unique mode of action compared to other products that are currently in use as larvicides. In 1999, the Country of Brazil began a rigorous program called (Network for monitoring resistance – MoReNAa – 1999-2013). In this program, besides monitoring resistance in field populations, there was a constant evaluation of products for resistance management. Since 2012 researchers evaluated Spinosad against a number of selected populations of *Aedes aegypti* known to have developed different profiles of metabolic resistance. Additional studies to investigate possible cross resistance in laboratory (estimation of Resistance Ratio). Finally investigations were conducted to determine formulation performance in the field.

Four populations representing different regions and diverse profile of metabolic resistance throughout Brazil were chosen to compare the product performance among them. The final conclusion was that there was no cross resistance between temephos and spinosad and field efficacy tests showed similar results among the four different *Ae. aegypti* populations. The conclusion was Spinosad was a good alternative as a tool for resistance management. Clarke is in the process of registering three Spinosad based larvicide products in India for use in control of important mosquito vectors of human disease such as *Aedes aegypti*.

Studies on the Effect of Curcumin on *Theileria* Infected Cattle Lymphocytes

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Curcumin, a natural polyphenolic compound, is isolated from the rhizome of *Curcuma longa*. Various reports have shown anticancer activity of curcumin. Theileria, a tick borne parasite, causes theileriosis in cattle. Theileriosis is a lympho-proliferative disease and possesses hallmark of cancer. In the present study, we report for the first time the effect of curcumin on *Theileria* infected cattle lymphocytes. The cellular viability and proliferation were assessed by trypan blue exclusion assay and MTT assay, respectively. Apoptosis was analyzed by DNA fragmentation analysis, Annexin V staining and confocal microscopy analysis. Here we observed that curcumin treatment significantly inhibited the cellular viability and proliferation potential of *Theileria* infected cattle lymphocytes in a dose- and time-dependent manner. In addition, curcumin treatment showed no cytotoxic effects to the PBMCs. Further western blotting with caspase 3 and caspase 8 showed the activation of apoptosis pathway. Our studies demonstrate that the curcumin treatment induces apoptosis in *Theileria* infected cattle lymphocytes. Together, these data suggest that curcumin exerts anti-theilerial effects.

Appraisal of Intention to Accept Indoor Residual Spraying as per the Guidelines among Residents of Mewat District of Haryana

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Objectives: To assess the intention to accept indoor residual spraying (IRS) among the residents of areas of interest in Mewat District. **Methods:** The present study is part of a larger community based interventional study and was carried out in district Mewat of State Haryana. A cross-sectional baseline survey was carried out in among 1200 households (600 households each of both interventional and non-interventional groups. The households were selected by cluster sampling technique. For selecting 600 households in each group, 30 clusters were selected and 20 households were selected in each cluster. The heads of these selected households were interviewed using a self-designed, pre-tested schedule after obtaining written informed consent. The study was approved by institutional ethics committee, and the data collected were analysed using standard statistical software. **Results:** Of the 1200 participants, 20 did not complete the interviews. Hence, the response rate was good i.e., 98.3 percent. In the two groups separately, 294/589 (49.9%) and 286 out of 591 (48.4%) heads of households didn't intend to get the IRS done as per the

guidelines. Whereas on comparing with the past actual behavior regarding acceptability to IRS as per guidelines (65.7% & 63.3% respectively), these findings were highly significant (i.e., $p<0.000$). **Conclusions:** The acceptability to IRS as per the guidelines is less i.e. ~50%. Our study warrants the need to sensitize people for improving the acceptability to IRS as per standard guidelines.

Biological Activity of Stigmasterol Glucoside $C_{35}H_{58}O_6$ and Sitosterol Glucoside $C_{35}H_{60}O_6$ from *Memordica charantia*

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Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. In recent years, plant based products has been retrieved because of the development of resistance, cross resistance and possible toxicity hazards associated with synthetic insecticides and rise of their cost. Nature of biological activity depends on the structure and physic-chemical properties of the compounds. Any biological active compound has a wide spectrum of effects. Some of them are useful in the treatment for diseases, but the others cause various toxic effects. The biological activity of the compounds *Stigmasteral glucoside* and *Sitosteral glucoside* were predicted and the results revealed the probability of active and inactive of the compounds. In Quantitative Structure Activity Relationship (QSAR), IC_{50} value for *Stigmasteral glucoside* was 387.06µm and P ^{IC50} was 3.41 and *Sitosteral glucoside* was 63.3 µm and 4.19 respectively. The PASS Computer program was used in this study to predict the biological activity profile. The results infer that *Stigmasterol glucoside* has various biological and pharmacological activities like dermatologic hepatoproteetant, immune stimulant, antitoxic, respiratory analeptic, oxidoreductase inhibitor, proliferative diseases treatment, anti hyper cholesterolemic, cholesterol antagonist, chemopreventive and anticarcinogenic. *Sitosteral glucoside* also showed biological activities like anesthetic general, antiviral, antifungal and wound healing agent.From the above findings, these two compounds were used for pharmacological and clinical research.

Use of Cost-Effective and Re-Salable Ovitraps for Surveillance of *Aedes aegypti* in West Zone of Delhi

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Ovitraps are being used in surveillance and control of *Aedes aegypti* in many countries are being Singapore, Malaysia and Brazil etc. Delhi being a megacity has a high population pressure and limited resources. It is practically impossible to attain good entomological surveillance throughout the year. In view of recurring dengue and chikungunya and in absence of effective vaccine against these diseases only option left as on date is surveillance and control. The present study thus aimed for improving surveillance and control through innovative Ovitraps. The study is being conducted in west zone of Delhi. The ovitraps with modified substrate (Cellulose comb) which is tested in laboratory and field conditions for their feasibility earlier were used in randomly selected houses (n=30) in the Bagdola, village in West zone Delhi where each year cases of dengue are reported. Ovitraps were placed at different locations inside houses *viz.* Bed room, Stores, Drawing rooms, Lobbies etc. In 10 houses among the selected for study houses conventional ovitraps (substrate of wooden paddle) were used as control for effectiveness assessment of new ovitraps. Observations on eggs were taken after 7 days. For precaution water used in ovitraps was pre added with adequate amount of Diflubenzuron (De-Larvae, classified by WHO, 1992) as metabolic insecticide (used in national vector control program) to prevent any larval development into adult. With initial 20% house positivity (House Index HI= 20) Ovitraps showed about 36.7 % house positive (ovitraps positive Index (OI) = 36.7) in First follow up. About 75% positivity was observed in ovitraps which were placed in toilets followed by 58.8% by those placed in stores/staircase areas, living rooms (24.5%), Bedrooms (21.5%) and Lobbies (15%). The follow-up data also showed reduction in positivity HI=20; OI=36.6 in IInd follow up HI=23; OI = 33.6 in IIIrd follow-up HI=10; OI =30 and in IVth follow-up HI=13 & OI=16.7. Results showed that positivity in Ovitraps were significantly higher ($\chi^2=9.8$; $p>0.01$) then the positivity found through entomological surveillance except for the IVth followup which showed higher positivity but not significant due to the festival time due to which about 33% ovitraps were dried and removed from the sites they were placed. The control ovitraps did not show any positivity in all the 10 houses. The preliminary results of these ovitraps are good they not only serving as tool for surveillance and control but also helping in creating awareness about dengue. It is seen people accept them and use them without much resistance as they show during normal inspection surveillance. In the cities like Delhi people feel insecure due to insurgencies and do not allow the surveillance workers inside houses. It is also seen a number of houses remains closed during routine vector surveillance due to all office going members (both male & females working). This new ovitrap approach provides solution for such conditions. Using such modified ovitraps can also acts as an indicator of risk in the house if found positive to the inhabitant to think about vector control measures to be taken immediately. In addition to this assessment of effectiveness vector control measures can be assessed and monitored for prioritizing the control efforts.

Mosquito Larvicidal Activity of *Melia azedarach* Compound 2, 7-Bis[2-[Diethylamino]Ethoxy] Fluorence and Synthesis of Silver Nanoparticles Against Dengue Vector *Aedes aegypti* (Diptera: Culicidae)

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The residual spraying of insecticides is the most common method of vector control. In Chennai, more than 100 cases of dengue have been reported so far this year (2016), most of them from Adyar, Kodambakkam and Alandur zones (WHO 2015). In recent years interest in plant based products has been revived because of the improvement of resistance and possible toxicity hazards associated with synthetic insecticides and the rise of their cost. Larvicidal activity of 2, 7–bis [2-[diethylamino]-ethoxy]fluorence isolated from the *Melia azedarach* leaves against *Aedes aegypti*. The maximum mortality was found in fluorence against *A. aegypti* (LC_{50} = 7.94; LC_{90} = 23.82 mg/ml). Molecular docking studies were performed using the secondary metabolites selected from fluorence against the AeSCP-2 binding protein of the *A. aegypti* mosquito. Protein –ligand interactions were carried put with various phytochemical, as a result of virtual screening Alpha-mangostin and panthenol were found to be good analogs and were allowed to dock with the mosquito cholesterol carrier protein AeSCP-2. Synthesis of silver nanoparticles has the potential to be utilized as a good, eco-friendly approach for the control of mosquito population. In the purpose study, synthesis of silver nanoparticles using fluorence against mosquito larvae was carried out. The synthesized Ag NPs were characterized by UV, XRD and HRTEM. The maximum activity was observed in synthesized AgNPs against *A. aegypti* (LC_{50} = 4.27; LC_{90} = 12.61 µg/mL). Further

research on AgNPs could bring a very promising target drug which can be used for protecting mosquito control.

CHAPTER 6

INSECTICIDE RESISTANCE AND MANAGEMENT

Expanding IR Mapper: mapping insecticide resistance in *Anopheles* species, *Aedes aegypti* and *Aedes albopictus*

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The emerging and rapid spread of resistance to major classes of public health insecticides threatens current malaria vector control efforts, namely long lasting insecticidal nets and indoor residual spraying, which have contributed substantially to the reduction of malaria since 2000. Prevention of dengue, chikungunya, yellow fever and Zika virus also relies heavily on insecticide based control of the primary vectors, *Aedes aegypti* and *Ae. albopictus*. Launched in 2012, IR Mapper is built on a systematic review of peer-reviewed, published literature on insecticide resistance. The user interface enables filtering by year, country, vector species, WHO or CDC assay, insecticide class and type, and resistance mechanisms. In 2016, the mapping platform was expanded to geospatially display reports of insecticide resistance in *Ae. aegypti* and *Ae. albopictus*. Additional fields added for *Aedes* included vector developmental stage and resistance ratio, where reported. As of December 2016, the *Anopheles* mapping platform consisted of over 16,000 unique field records from 60 countries and 64 *Anopheles* species or species complexes. 80% of countries reported resistance to at least one of the four classes of insecticides used for adult mosquito control. 96% of the countries that tested for pyrethroids reported confirmed resistance. Examining the top ten countries with the largest burden of malaria today, more reports of confirmed pyrethroid resistance were recorded in the period 2008-2016 compared to 2000-2007. Insecticide resistance mechanisms in *Anopheles* were reported in 80% of the localities. The *Aedes* mapping platform contained over 3,000 unique field records from 45 countries and territories. 71% of the countries and territories reported confirmed resistance to at least one of the four main insecticide classes approved for public health use. The highest proportion of confirmed resistance reports was to organochlorines (90% of tests) followed by pyrethroids (68%). Insecticide resistance was more frequently investigated in *Ae. aegypti* (84% of localities) than in *Ae. albopictus* (18% of localities). The majority of data were available from Asia followed by Americas; very few data points were available from Africa. Insecticide resistance mechanisms in *Aedes* were reported in 57% of the localities. IR Mapper is a useful tool for visualizing insecticide resistance trends in both *Anopheles* and *Aedes* and can be used to assist decision making for deployment of the most appropriate tools.

Need for a Comprehensive System to Monitor Insecticide Resistance among Malaria Vectors and to Formulate Site-Specific Resistance Management Strategies in India

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Detection and monitoring of insecticide resistance in malaria vectors at an early stage are crucial so that the resistant management strategies could be designed and introduced in time for rationalizing the field operations to control malaria. Besides the level of resistance to the selective compounds, the type of resistance mechanism selected and consequently the spectrum and levels of resistance conferred to other compounds need to be considered while formulating such management strategy. Major mechanisms of insecticide resistance involve either mutation within the target site of the insecticide or an alteration in the rate of insecticide detoxification.

The conventional way of detecting resistance in field mosquito populations using WHO bioassay kit allows only detection of resistance but not the resistance level or mechanism. Also, the bioassay method may not detect resistance phenotypes at low frequencies. To overcome this problem, a number of biochemical tests have been simplified for field use with an ultimate aim of detecting the presence of particular resistance gene in an individual insect. Polymerase chain reaction (PCR) assay has been developed to detect a target mediated knock down resistance (kdr) in malaria vectors to DDT and synthetic pyrethroids (SPs). Metabolic resistance to organophosporous (OP) compounds is mainly due to quantitative or qualitative differences of carboxylesterases. Besides, an alteration in acetylcholinesterase (AChE) resulted in development of resistance to OP and carbamate insecticides. A single mutation (G119S of the ace-1 gene) alters AChE to become insensitive to the insecticide for binding thereby leading to resistance development. A PCR assay has also been developed to detect this altered AChE mediated resistance mechanism.

In India, of the six major malaria vectors, An. culicifacies and An. stephensi have shown wide spread resistance to DDT, BHC and malathion. Recently, resistance to SPs has been reported in *An. culicifacies*. *An. annularis* has developed resistance to DDT and BHC. DDT resistance in *An. fluviatilis* has also been reported in a few pockets of northern states. Even though, various reports summarize the gravity of resistance problem in malaria vectors and indicate the need to formulate resistance management strategies, the underlying mechanisms, a pre-requisite, have not been studied. There are reports that vectors develop resistance to DDT and SPs by adopting same mechanism but pyrethroids are used as a replacement to DDT. A carboxylesterase mechanism selected for malathion in *An. stephensi* and *An. arabiensis* produces a very narrow cross resistance spectrum, where as an alteration in AChE produces a much broader cross resistance spectrum which extends to many OPs and carbamates.

Further, besides adopting alternate or rotation or mosaic methods of using available insecticides, in the context of integrated vector management (IVM), it is necessary to explore the feasibility of using plant-based products for vector control. Additional benefit of using plant materials would be their reported capability of inhibiting certain enzymes responsible for biochemical resistance in insects. Also, in operational point of view, it has become essential to confirm whether mosquitoes develop resistance to DDT and SP using same mechanisms or not so as to justify the use of SP compounds as a replacement to DDT.

Bio-Efficacy of VEERALIN™ Long-Lasting Insecticidal Net (LN) Against Pyrethroid-Resistant *Anopheles gambiae*: An Experimental Hut Trial in Ivory Coast

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Long-lasting insecticidal nets incorporated with insecticides play a very important role in controlling malaria transmission. These nets are evaluated and recommended by World Health Organization Pesticide Evaluation Scheme (WHOPES). VEERALIN™ is a WHOPES recommended long-lasting insecticidal net incorporated with alpha-cypermethrin and piperonyl butoxide (PBO) into polyethylene monofilament yarns and has PBO in all the panels. VEERALIN™ is manufactured in Karur, Tamil Nadu, India. Bio-efficacy of VEERALIN™ LN was evaluated in experimental huts in Central Ivory Coast where the main malaria vector *Anopheles gambiae* is highly pyrethroid-resistant. The mechanisms conferring resistance include increased oxidases and estersases, in addition to *kdr*. Bio-efficacy of VEERALIN™ at various wash-stages namely, 5, 10, 15 and 20 washes were studied. The personal protection against *A. gambiae* bites conferred by VEERALIN™ LN is very high (71-88%). The personal protection levels are high even after successive washing up to 20 washes. Though there is a drop in bio-efficacy of VEERALIN™ after five washes there was no significant differences in efficacy between nets washed 5, 10, 15 and 20 times.

Studies on Insecticides Resistance Pattern and Plant-Based Control of *Anopheles* Mosquitoes

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Deltamethrin is one of the most commonly used insecticides. Therefore a preliminary assessment has been made to know the Deltamethrin resistance status of Anopheline mosquitoes. Larvae (late third to early fourth instar) of *Anopheles* of the Hooghly district of West Bengal were collected and treated with 0.05% Deltamethrin. Result shows 89% mortality. It indicates that they were intermediately resistant (WHO- 80-98%) to Delamethrin. For detection of *kdr* mutation, genomic DNA was isolated from *An.vagus*. Electrophoretic studies indicate that this species is intermediately susceptible to Deltamethrin. As plant based insectisides delay the development of resistance in mosquitoes because of its new structure, therefore an attempt has been taken to observe and compare the efficacy of specific parts of three commonly available plants in our locality.

CHAPTER 7

NEW BIOLOGY: GENOMICS, PROTEOMICS, GENETICS AND OTHER ASPECTS

Prevalence of pfhrp2 and pfhrp3 Gene Deletion in *Plasmodium falciparum* in India

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Rapid diagnostic tests (RDTs) for malaria diagnosis is used commonly in India particularly in difficult and remote areas. Most of the RDTs for *Plasmodium falciparum* diagnosis are based on histidine rich protein (HRP2). Deletion of pfhrp2 and pfhrp3 genes contributes to false negative test. This is the first prospective field study carried out in 8 highly endemic states of the country which contributes 80% malaria and 70% death due to malaria in the country. A temporary malaria clinic was established in two primary health centres (one highly endemic and one low endemic) in all these 8 states during peak transmission season (July- October). The results revealed that frequency of pfhrp2 deletion varied between the sites ranging from 0-25% (2.4%).The frequency of pfhrp3 gene deletion varied from 0-8% (1.8%). This study suggests that periodic surveillance is necessary for reliable use of pfhrp2 based RDTs.

Genetic Variation in *Plasmodium falciparum* Histidine Rich Proteins 2 and 3 in Indian Isolates

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Most of the commercially available RDTs detect histidine rich protein 2 (HRP2) which is produced during the asexual blood stage of the *P. falciparum* but not by other species of malaria parasites making PfHRP2 based RDT species specific. Some PfHRP2 based RDTs can cross-react with HRP3 encoded by *Pfhrp3* gene due to shared antigenic epitopes between these proteins. This study was carried out in eight malaria endemic states of India to assess the performances of PfHRP2based RDT kit. In this study, we analysed natural variations in *Pf*HRP2 and *Pf*HRP3 sequences and correlated these variations with RDT reactivity. A total 1392 *P. falciparum* positive samples were PCR amplified and sequenced for *Pfhrp2* and *Pfhrp3* genes. The deduced protein sequences were analysed for repeat variations and correlated with RDT reactivity. Out of 1392 PCR amplified samples, a single sample was *Pfhrp2* negative and two samples were *Pfhrp3* negative. Complete *Pfhrp*2 and *Pfhrp*3 sequences were obtained for 769 samples and 750 samples respectively. The size of the repeat region of exon 2 varied from 438 to 897 bases for *Pfhrp2* and 336 to 729 bases for *Pfhrp3*. This size variation was largely attributed to the variation in numbers of 27, 18 and 9 bp repeats. A total of 16 different types of repeats were found for *Pfhrp2* while 11 types of repeats were found for *Pfhrp*3, including some new repeat types. Overall, the level of diversity in the *Pfhrp3,* as measured by the proportion of sequence variations, is significantly lower than the *Pfhrp2* gene. No correlation was found between variations in the size of *Pfhrp2* repeat types 2 and 7 and performance of a commercial RDT at low parasite densities. The findings suggest sequence diversity in *Pfhrp2* and *Pfhrp3* genes in Indian isolates are not likely to negatively influence the performance of currently used *Pf*HRP2 RDTs.

Genetic Diversity Analysis of *P. falciparum* Apical Merozoite Protein Pf34 from Central India

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The genetic diversity and evolutionary plasticity are the major obstacle for malaria control. Diversity is the survival mechanism of *Plasmodium falciparum* which helps in the evasion of human immune system, which may leads to new forms of drug resistance and has become a major challenge to vaccine development. In this study genetic diversity of *P. falciparum* GPI anchored protein (Pf34) was assessed. Pf34 is encoded by a single exon gene (978 bp) located on chromosome 4 and expressed during erythrocytic stage of parasite having a molecular weight of ~34 kDa. It is also expressed in the liver stage of parasite and localized in the rhoptry neck of merozoite apex. Rhoptry proteins considered as potential vaccine candidate as they play important role in the invasion of host cell and formation of parasitophorous vacuole. Amino acid sequence analysis of Pf34 was done from reference strain of *P. falciparum* (PF3D7_0419700) and it was found that Pf34 is having amino acid repeats (NNI, NND, NNN). Samples were collected from Darbha CHC hospital, district Bastar, Chhattisgarh. Amplification of *Pf34* gene was done by using designed primers through polymerase chain reaction (PCR). Sequencing of PCR products was done by Dideoxy chain termination method using 3130 XL genetic analyzer. Out of 120 samples, 100 samples were successfully sequenced and analyzed. Total six types of variants of Pf34 protein (N54D, G60V, H77N, N90_ K91N, Q93H and S161R) were observed among 100 samples. The total (10%) isolates were harbouring variant Pf34. None of the variant was affecting amino acid repeats present in the protein. This study may help in estimating the potential of Pf34 as vaccine candidate or biomarker for further studies to develop malaria control measures.

Studies on Cytomorphological and Molecular Diversity with kdr Gene Mutation of Anopheles Mosquitoes in West Bengal

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Studies on the cytogenetics of Anopheles mosquitoes initiated through the preparation and the observation of banding and puffing pattern of polytene chromosome. Furthermore, investigation has been extended through the cytomorphological and molecular variation of Anopheles mosquitoes in natural as well as laboratory population. Cytological variations are manifested through the observation of inversion, translocation, asynapsis and transcriptional activity pattern in polytene chromosome. Furthermore, molecular studies have been extended by the studies on the ITS-2 sequence pattern, mutation in kdr gene sequences, gut microbial activity pattern.

The most malarial vector species are morphologically indistinguishable (sibling and cryptic species) in the present eco climatic condition. Now it becomes a burning problem not only to identify the Anopheles vector species but also to design and develop a proper control method to reduce the malarial vector species population. Earlier the malarial vector species are isolated and identified cytologically through fixed paracentric inversion in polytene chromosome. But the frequency of cytomorphological variation in Anopheles is very striking in rural & urban areas of West Bengal

.But this method has some problem, now PCR based diagnostic assay has effectively and widely been used for proper identification of Anopheles mosquitoes .

Many genes in mt DNA in Anopheles such as cytochrome subunit I (CO I), cytochrome subunit II (CO II), NADH dehydrogenase subunit 4 and subunit 5 are used to analyze the morphological, cytological and molecular variation in the population of Anopheles mosquitoes. The molecular method of PCR based diagnostic assay become effective and useful for the isolation and identification as well as to study the molecular variation of Anopheles vector species in different areas of West Bengal. Besides that, ribosomal DNA has also been extensively used for phylogenetic analysis of both closely and distantly related species of Anopheles mosquitoes. The ITS 2 evolves faster than coding sequences and has been used effectively for discrimination of closely related Anopheles mosquitoes PCR based molecular method has also been used to detect the kdr mutation in several insecticide resistant Anopheles mosquitoes. But the simple allele specific PCR assays are prone to lack of specificity and therefore primer induced restriction analysis (PIRA) PCR method has recently been developed to detect kdr mutation in Anopheles mosquitoes. But in West Bengal, morphological, cytological, molecular variation, detection of kdr mutation and gut microbial activity pattern are not so studied. Therefore, studies has been undertaken not only to know the cytomorphological and molecular variation but also to design a control measure for the Anopheles vector species in west Bengal.

Molecular Characterization of Densoviruses (DNV) Infecting Mosquitoes in North East India

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Densoviruses are single-stranded linear DNA viruses belonging to the genus Brevidensovirus of family Parvoviridae. These are the most common viruses found in different insect vectors especially in mosquitoes which cause mortality of mosquitoes through induction of Densonucleosis. Being pathogenic to the mosquitoes, these viruses could be used as potential candidate pathogens for biological control of mosquito vectors. Information available on this group of virus from India is very limited. In the present work, we aimed at molecular detection and characterization of Densoviruses in different disease carrying mosquito species belonging to the genus Aedes and Culex, collected from different regions of North East India. Mosquito specimens (adults and larvae) were collected from selected regions of North East India, using light traps and manual collection methods. Collected specimens were identified on the basis of established morphological characters, followed by pooling (5-10 specimens of the same species) and total DNA extraction. DNA samples from a total of 40 pools were further processed for quality assessment (PCR with housekeeping gene primers). DNA extracts passing the quality check were subjected to PCR with Mosquito Densovirus specific primer pairs, designed by us, using multiple sequence alignments of the Densovirus NS1, NS2, NS3 and EP genetic regions. Specificity of the amplicons of expected sizes were further checked by RFLP (Restriction Fragment Length Polymorphism) analysis using different REs. Amplicons confirming to the Densovirus specificity were further processed for cloning, sequencing followed by molecular evolutionary analysis based characterization.

Proteomic Landscape of Indian Vector of Malaria, *Anopheles stephensi*

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Anopheles stephensi is a major vector of malaria in India and neighboring Asian countries. Although substantial geographical variations in this vector in terms of vectorial capacity and behaviour have been reported, molecular level discoveries are needed for better understanding of vector-parasite interactions paving way for development of novel strategies for effective control of malaria transmission by this species. The genome of *Anopheles stephensi* has been recently sequenced. Precise identification of protein coding genes in this vector is still underway. To accelerate this process, we have carried out comprehensive proteomic analysis of *Anopheles stephensi* (Goa strain) to characterize organ specific proteome using high resolution LTQ-Orbitrap Velos mass spectrometer. By searching MS/MS data against a computationally predicted *An. stephensi* protein database, we have validated more than 7,000 predicted protein-coding genes. In addition to confirming 78% of predicted protein-coding genes, our proteogenomic approach has enabled identification and addition of 365 novel protein coding regions missed previously during genome annotation. Using a quantitative proteomic approach, we have identified more than 500 proteins abundant in the midgut of female compared to male *An. stephensi*. In addition, 30 putative novel genes were identified which appeared highly expressed in midgut of females of this vector. Unraveling organ specific proteome of *Anopheles stephensi* is first such tissue based attempt that can have wide applications. Besides better overall understanding of molecular biology of this vector, deeper proteomic analysis can help understand processes viz., vitellogenesis, compatibility and immunity to *Plasmodium* infection, resistance to insecticides, host attraction and feeding preferences.

Insecticide Management and Development of Resistance in Sandfly with Future Prospects

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Kala-azar is a major health problem in Indian Sub-continent. India, Nepal and Bangladesh are contributing 60 per cent of the global burden. The disease is under elimination mode to be achieved by 2017. Bangladesh achieved the target. However, Nepal and India are yet to get. There are different insecticides and various methods are under practice either in control programme or in research mode. Indoor residual spray of DDT 50% WP @ 1gm/m^2 was in practice under the programme till 2014. However, *Phlebotomus argentipes* developed resistance against it. Sandflies were found susceptible with synthetic pyrethroid i.e., deltamethrin after testing with insecticide treated WHO paper. The research on Long Lasting Insecticide treated nets (LLINs) like OLYSET®; polyethylene net, blended with permethrin 2% and PermaNet® 2.0, polyester fibres coated with resin containing deltamethrin with a target dosage of 55 mg/m^2 were not found effective in controlling sandflies at household level. Durable Wall Lining with deltamethrin 170 mg a.i. / m^2 in polyethylene was found effective in controlling sandflies in Bangladesh, Nepal and India. The alternative insecticide for indoor spray of α-cypermethrin (5% wp applied with 0.025g/m^2) is under programme since 2016. It was found effective in bringing down the population of sandflies to control the incidence of disease. There is evidence against development of resistance with synthetic pyrethroids in mosquitoes. The chances of development of resistance to sandflies against α-cypermethrin cannot be ignored. The development of resistance against insecticide should be mapped in common international online interactive platform. Data visualisation on insecticide resistance will be of high impact for research and promotion of programme in real time.

Understanding Complicated Vivax Malaria: Antisense, Vir genes & Systems Networks

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Malaria remains a very important vector borne disease in India, which is estimated as possessing the most disease burden amongst all the countries in the S.E Asia region. It has been projected that, outside Africa, half of the global population at risk from malaria, resides in India. Plasmodium falciparum has long been known to cause complicated malaria including cerebral malaria. It is only recently that P. vivax has also been accepted as causing the same. We along with our collaborators published in 2005 an article in **Emerging Infectious Diseases** on complicated P. vivax malaria from India which was one of the first to attract the attention of scientists and public health professionals to this phenomenon.

The significance of Natural Antisense Transcripts (NATs) in patient isolates of P. falciparum and P.vivax will be presented along with the probable significance of changes in sense/antisense ratios and some insights into mechanism and significance of generation of NATs using microarray experimentation. This part will be based on our publications in both the parasite species, and first reports in their own right.

The second part of the presentation, will provide highlights of certain aspects of the P. vivax transcriptome based on our analysis of microarray data of **cerebral** & **non-cerebral** complicated malaria. This analysis suggests the upregulation of some subfamilies of the vir genes which appear to be associated with these complications.

WGCNA (Whole Genome Co-expression Network Analysis), leading to the identification of disease specific modules and **hub** genes will be shown along with possible implications of the same.

Molecular Characterization and Serotyping of Dengue Virus and Its Correlation with Clinical Manifestation

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Dengue is a systemic viral infection transmitted by *Aedes* mosquito. It presents a dreadful economic and disease burden with around half the world’s population estimated to be at risk of this infection. The term ‘dengue viruses’ groups four genetically and antigenically related viruses that are known as serotypes DENV 1 to DENV 4, further each of them grouped into genotypes. In 2015, more than 15,000 cases of dengue were reported in Delhi with 60 deaths. Subsequently, in 2016, 4393 cases were reported with 10 deaths in Delhi. Every year, Delhi encountered with different serotypes. In 2003 all four DENV subtype were found to co-circulate in Delhi changing it to a hyperendemic state. Infecting dengue serotype and possibly genotype may play an important role in disease severity among adult dengue patients. The main objective of this study is to determine the circulating serotype and to co-relate its severity with clinical outcome. Blood sample were collected from suspected dengue patients at NIMR clinic, and used for RNA isolation. The extracted RNA template was transcribed into cDNA using reverse transcriptase which later used for serotyping by semi nested PCR. In 2015, out of 3493 fever cases, 935 were dengue positive (IgM and NS1). Twenty three out of 25 samples were DENV 2. In 2 samples Concurrent infection was observed, one patient had DENV 2 and DENV 3, while another had DENV 1 and DENV 2 infection. Patients with concurrent infection show severe complications.

Banding and Puffing Pattern of Ovarian Nurse Cell Polytene Chromosome of *Anopheles* Mosquitoes in West Bengal

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Most of our knowledge about the cytogenetics of *Anopheles* mosquitoes comes from the studies on the ovarian nurse cell polytene chromosome. Cytological variations are manifested through banding, puffing pattern, inversion, translocation, formation of asynapsis in polytene chromosome. A very little work has been done on ovarian nurse cell polytene chromosome of *Anopheles* mosquitoes in west Bengal. Therefore, studies have been made to know some aspect of polytene chromosome of most frequently available *Anopheles* (viz., *An. subpictus, An. vagus, An. barboristris* etc.) from different areas of West Bengal. The polytene chromosomes were prepared from the ovarian nurse cells of female *Anopheles*. A preliminary polytene chromosome map has been prepared by studying a number of temporary preparations. The polytene chromosome complements of *Anopheles* mosquitoes consists of five arms , the shortest of which represent the X chromosome and the remaining four autosomal arms (II and III). Studies have been made on the puffing and banding pattern, paracentric inversion of ovarian nurse cell polytene chromosome of different *Anopheles* mosquitoes inorder to get information about the cytotaxonomical and evolutionary aspect of *Anopheles* mosquitoes in West Bengal.

Design of Potential Epitope-Based Peptide Vaccine Candidates Targeting the Non-Structural Protein1 (NS1) of Japanese Encephalitis Virus: An Immunoinformatics Approach against Emerging Infectious Diseases

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Japanese encephalitis (JE), a vector-borne zoonotic viral disease caused due to *Japanese encephalitis* virus (JEV), which is the leading cause of viral encephalitis in more than 14 Asian countries due to its epidemic potential and high case fatality rate. Four classes of vaccines are currently available which elicit protective levels of neutralizing antibody, but they are highly expensive with adverse side effects. Despite of severe epidemic outbreaks on several occasions and endemic in many parts of Asia, not much progress has been made in the area of epitope-based vaccine design for JEV. With availability of genome sequences of JEV, immunoinformatics-driven approach to systematically search for key determinants of immunity, and design peptide based vaccine is considered as one of the promising approach to treat JEV. Herein, a computational approach was adopted to identify a multi-epitope vaccine candidate targeting the important non-structural protein NS1, which plays a crucial role in virus replication and elicits protective immune responses during infection. JEV genome sequences were collected and domain boundary of NS1 was delineated with *in silico* tool, to identify the most conserved immunogenic fragments. T cell immunity were checked for the peptides to ensure that they have the capacity to induce cell-mediated immunity. A total of 9 promiscuous (9 mers amino acids) peptides were found to be most potential epitopes targeting highest number of HLA alleles with high cumulative population coverage, ranging from 78.88% to 93.87%. The candidate epitopes were further tested for binding against the HLA molecules, using CABS-dock and GalaxyPepDock, to verify the binding cleft epitope interaction. The immunoinformatics study to design epitope-based peptide vaccine against JEV allows us to determine novel peptide antigen targets in NS1 on intuitive grounds, albeit the preliminary results, thereof require validation by *in vitro* and *in vivo* experiments.

Techniques for Visualization of LAMP Amplified Products

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Loop-mediated isothermal amplification (LAMP), a novel gene amplification method, enables the synthesis of large amounts of both DNA and RNA without thermal cycling and it is highly specific and sensitive. A visible byproduct magnesium pyrophosphate is formed in the end of the reaction. Isothermally amplified DNA/RNA can be visualized by gel electrophoresis or using other post-amplification detection steps that require opening of the reaction tubes and therefore significantly increases the risk of carry-over contamination. To reduce this risk and to increase applicability for simple field assays, single tube reactions are strongly recommended. We compared the direct and indirect methods to visualize LAMP by hydroxynaphthol blue (HNB), Calcein, SYBR Green I, Ethidium bromide (EtBr), and Lateral flow methods. All assays could be used to distinguish between positive and negative samples in visible or UV light. The use of HNB resulted in a colour change from violet to blue, whereas calcein induced a change from orange to yellow green. We also investigated SYBR Safe DNA gel stain as a nucleic acid specific dye that emits a fluorescence signal under UV light after a positive LAMP reaction. It has a comparable sensitivity to SYBR Green I. Based on our results, an optimal detection method such as SYBR Safe DNA gel stain can be chosen easily for isothermal real time or end point screening applications.

RNA Interference (RNAi): A Tool for Functional Studies of Genes in *Hyalomma anatolicum*

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Hyalomma anatolicum, a tick species acts as the vector of animal pathogen, *Theileria annulata* and human pathogen, Crimean-Congo Haemorrhagic Fever virus. Management of the vector by immunization of hosts is considered as one of the sustainable solution. However, identification of candidate cross-protective antigens is the major hurdle in the development of tick vaccine. Discovery of RNAi in *Caenorhabditis elegans*, revolutionized the molecular genetics and provided an efficient tool for the study of functional genomics. In tick, functional study of genes may helps in selection of novel vaccine /or drugs targets. In the present study, three conserved genes namely, subolesin (SUB), clareticulin (CRT) and cathepsin L (CathL) were targeted. The genes were amplified, cloned and sequenced. *In-vitro* transcription was carried out to produce high concentration of dsRNA using specific gene template containing T7 polymerase binding site at 5' end. For each dsRNA a group of unfed adult female ticks were inoculated with 0.5 - 1 µl of dsRNA containing 9.0-18.5 x 10^{11} molecules/µl using 34G, 1 inch needle and 10 µl Hamilton syringe. Following injection, the dsRNA inoculated ticks along with equal number of male ticks were released on the calves using ear bag for feeding and further development. Phenotypic and genotypic effect were recorded. The effects of dsRNA on female ticks was mainly found on the engorgement percentage where only 2.22, 6.66 and 18.0 % ticks were successfully engorged in SUB, CRT and CathL groups, respectively. Average engorgement weight of the ticks was recorded to be highest in CathL and CRT group and lowest in SUB group. The gene silencing effect on biological activity of tick is correlated with the expression of silenced genes in different stages of feeding. Statistically, a significant difference in expression of all the three genes was recorded in respective dsRNA injected tick groups at different feeding stages i.e., 72 hrs, 96 hrs and UTE (unable to engorge) in comparison to control. However, there was no significant differences in the suppression of expression of the CRT genes in engorged ticks was recorded. The entire protocol was standardized for successful screening of vaccine targets using minimum number of animals.

Evidence of Genetic Polymorphism in *Aedes albopictus* Population from North East India

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Aedes albopictus mosquito, the major vector of dengue and chikungunya in India. It is very difficult to control or eliminate *Ae. albopictus* mosquitoes because they have adaptations to the environment that make them extremely resilient, or with the ability to rapidly bounce back to initial numbers after disturbances resulting from natural phenomena or human interventions. Overall mosquito-borne diseases have re-emerged as a significant human health problem due to a number of factors, including the lack of progress in vaccine development, the emergence of drug resistance in pathogens and insecticides resistance in mosquitoes. An alternative strategy for vector control could be to exploit observed genetic variability in the vector populations. In the present study we have used RAPD primers to understand the genetic polymorphism present in the *Ae. albopictus* populations collected from various locations in northeastern India. A total of 20 RAPD primers were screened. Among these, the primer VT 09 was selected for further study on the basis of the clear, concrete and scorable fingerprint obtained by it. The statistical analysis was done with the help of POPGENE 1.31 using bands or loci representing each population. The consensus tree generated using the software showed different clusters which are branched as per their geographical distances. In conclusion we propose that our results on RAPD profiles provide evidence that there are enough and significant variations in the genomes of field collected populations from distant locations and the genetic pattern obtained in its various forms appears to be a major differentiating and orienting force for molecular changes in DNA across different populations.

Vaccine Studies on Various Cocktails of 31kDa, 36kDa and 51kDa Antigens of *Leishmania donovani* Against Experimental Visceral Leishmaniasis

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Visceral leishmaniasis is one of the most severely neglected tropical diseases recognized by the World Health Organization (WHO). The disease is associated with a spectrum of clinical manifestations, ranging from self-healing cutaneous lesions to fatal visceral infections. Chemotherapy is the only available treatment against visceral leishmaniasis. However, none of the available drugs can be considered ideal. Further, most commonly used drugs do not completely eliminate the parasites in infected individuals. A prophylactic vaccine would prove to be the most effective strategy for the control of visceral leishmaniasis. However, despite substantial efforts, currently there is no licensed vaccine available against human leishmaniasis. Till date, only one multicomponent vaccine LEISH-F3+GLA-SE has reached clinical trials. Although substantial number of antigens have been identified in the past, which could induce protection against the target parasite. However, very few achieve a degree of efficacy likely to make them candidates for single-antigen vaccines. Therefore, cocktail vaccines are proposed based on the assumption that such cocktails will show enhanced efficacy. The present study was designed to check the protective efficacy of low molecular weight antigens. Protective efficacy of different vaccine formulations i.e. 31+36kDa, 36+51kDa, 31+51kDa and 31+36+51kDa was revealed by significant decline in parasite burden and increased DTH responses. The antibody response was of IgG type with elevated IgG2a and decreased production of IgG1 whereas cytokine levels pointed towards the generation of protective Th1 type of immune response. Among all vaccine formulations, cocktail of 31+51 kDa was found to be most effective.

Screening of *Pfhrp2* & *Pfhrp3* Gene Deletion and False-Negative Malaria RDT in *Plasmodium falciparum* Isolates in India

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Rapid diagnostic tests are considered as the best tool for the detection of malaria after microscopy in remote areas. Most of the RDTs for *P.falciparum* malaria are based on the detection of *P. falciparum histidine-rich protein 2* (*PfHRP2*), but reports have questioned their sensitivity and reliability primarily due to parasite density and gene polymorphism. Along with *Pfhrp2*, *Pfhrp3* also affects RDT results due to its sequence homology and can be detected by monoclonal antibody coated for *Pfhrp2* in RDTs. False negative cases of *PfHRP2* based RDT has also been reported, leading to misdiagnosis of malaria and hence its control . Deletion of the *PfHRP2* and *PfHRP3* genes are one of the major causes of RDT false negative cases. The false negative results of RDTs may cause problem for malaria control and its management. The aim of the study is to screen the prevalence of *P. falciparum* isolates lacking *Pfhrp2* and *Pfhrp3* genes collected from different malaria endemic areas in India. The finding of this study may accentuate the need for new target antigens for malaria diagnosis.

Transcriptional Expression Analysis of Artemisinin-Sensitive and -Resistant *Plasmodium falciparum*

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The emergence of multidrug resistant *Plasmodium falciparum* parasites has made drugs e.g. quinolines (mefloquine, amodiaquine, chloroquine, quinine) and antifolate (pyrimethamine, proguanil, sulfadoxine) useless in many endemic areas. Artemisinin (ART) resistant *P. falciparum* is now firmly established in eastern Myanmar, western Cambodia and Thailand, and southern Vietnam and it is emerging in southern Laos and northeastern Cambodia. *In vitro* susceptibility testing and extensive transcripts analysis need to understand the molecular basis of *P. falciparum* artemisinin resistance. *P. falciparum* isolates were collected from different geographical malarial endemic areas of India and also from Malaria Parasite Bank (NIMR). Control strains of ART sensitive (3D7) and resistance (MRA-1241) were procured. Sensitive *P. falciparum* isolates (ART-S) were adjusted to 5% to 7% parasitemia and were grown in the presence of ART. Drug pressure was applied on ART-S strains from 1 nM to 10 nM. We have developed ART resistance on control strains (3D7) at 4 nM. There is no ART resistance observed in collected samples. Total RNA (ART sensitive and resistance strains) were extracted by using Trizol reagent. cDNA was extracted from all strains and will be used in further experiments (Microarray and Real time). This study will contribute towards understanding perturbed biochemical behavior of *P. falciparum* in response to high ART pressure. Such knowledge may be helpful in monitoring resistance in clinical isolates or to subsequently target major molecular interactions associated with ART resistance.

CHAPTER 8
BIOLOGY AND ECOLOGY OF VECTORS

Significance of Additional Taxonomic Attributes In Taxonomic Studies on Family Culicidae, Diptera

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Most of taxonomic studies in Culicine taxonomy are based on few traditional characteristics. The identification problems in family Culicidae are still enormous because of the existence of various biotypes, sibling species and species-complexes. Modern day taxonomists are exploring additional and new taxonomic attributes for updating and revising the status of various taxa in this economically important group. Use of scanning electron microscope and transmission electron microscope to study ultra structures of various species in Culicidae is the need of hour. In order to discriminate/ identify these sibling species/ species complexes, new and additional taxonomic attributes are of utmost importance. Keeping this in view, Scanning Electron Microscopic (SEM) studies have been conducted to explore new and additional taxonomic attributes of immature stages, structures like cibarium, mouthparts and genitalia of adult Culicine mosquitoes in the present studies. SEM Studies have been performed on mouthparts to study different features like variation in number of maxillary teeth, structure of hypopharynx and sensillae on labrum. SEM studies on immature stages of mosquitoes have yielded new features like different pattern of tubercles, shape of micropyle on the egg, varying number of teeth in mentum, processes of pectum in larvae and shape of trumpet and paddle hair in pupae. Many new taxonomic characters have come to light which will be incorporated to update the status of various taxa.

Seasonal Prevalence and Feeding Behaviour of *Anopheles* Species in Kalahandi District, Odisha

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Malaria is one of the major vector borne diseases causing serious health problem in the tropics and subtropics. Kalahandi district is known to be a malaria prone region with an Annual Parasite Index 18.6. A total of 1687 mosquitoes (*Anopheles culicifacies, Anopheles fluviatilis*, *Anopheles annularis, Anopheles pallidus, Anopheles vagus, Anopheles subpictus and Anopheles hyrcanus*) were collected from human habitation and cattle sheds of 12 villages of Kalahandi. The seasonal prevalence of *An. culicifacies, An. fluviatilis* and *An. annularis* was studied. In each season blood meal of 95 *Anopheles* sp. collected from human habitation and cattle sheds were analysed. Mammalian blood meals (human, cow, goat, buffalo) were identified from a single mosquito by using multiplex PCR assay. *An. culicifacies, An. fluviatilis* and *An. annularis* were found to be the dominant species (46.2%, 3%, and 9.8% of total collection respectively) in the studied area. *An. fluviatilis* and *An. annularis* were predominantly found more in and around human habitation than cattle sheds but *An. culicifacies* was found mostly in cattle shed. Total man hour density calculation revealed that *An. fluviatilis* and *An. annularis* were most prevalent during the winter season and *An.*

culicifacies was most prevalent in rainy season. Blood meal analysis showed that the *An. fluviatilis* and *An. annularis* preferred human blood than that of other animals. The result indicated the seasonal prevalence of *An. culicifacies*, *An. fluviatilis* and *An. annularis* in the area studied and the anthropophilic nature of *An. fluviatilis* and *An. annularis* might be the reason for perennial transmission and the cause in increase in intensity of malaria transmission in Kalahandi.

Characterizing the Habitats of Dengue Vectors Using Pupal Weight as an Indicator

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Entomological surveillance remains a key aspect in management of mosquito vectors. The vectors of Dengue and DHF *Aedes aegypti* and *Ae albopictus* are expanding the geographical distribution and abundance, increasing the probability of epidemic. The chances of viable population build up and the disease transmission potential corresponds to the resources acquired in course of larval development. Thus, characterization of the prospective population through the life history traits can prove helpful in management of these vectors. Pupal weight is being used as an indicator to predict individual and population level fitness of mosquitoes. The data obtained thereof will serve as an input to the models of life history theory to portray the prospective population size and fitness of the individual mosquitoes. In the present study this model was applied on the assessment of the relative abundance of dengue and DHF vector *Ae.aegypti* and *Ae.albopictus* in Kolkata, India. The observations reveal that the number of positive sites in each habitats differed with the months and between the mosquito species. Results of three-way ANOVA on pupal productivity revealed significant differences with respect to habitat, species and sex. The pupal weight of both *Ae. aegypti* and *Ae. albopictus* varied with the larval habitats. Although a wide range of spaces are utilized by the dengue vectors for breeding, most often, in urban context, the small containers of varied materials and shape serve as the larval habitats. Our results indicate that source reduction of the habitats should be done without discrimination of habitats but with special and effective emphasis on the one that supports production of pupa with higher individual biomass. Evaluation of the larval habitats and the pupal productivity thereof will enable precise regulation of the origin of the larval habitats and intervention of the mosquito-human link, to minimize the incidence of the disease at a spatial scale.

Importance of Cibarial Sensilla and Genitalic Features in Culicine Taxonomy

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Mosquitoes are of great medical importance and the most studied group across the world. Many species are occurring in species complexes and their taxonomic knowledge is far from complete. There are more than 3524 species of mosquitoes belonging to 113 genera recorded under two subfamilies i.e. Anophelinae and Culicinae (Harbach, 2012). Several species in the three principle genera i.e. *Anophleles* Liston, *Culex* Linneaus and *Aedes* Meigen are major vectors of malaria,

filariasis and arboviruses. As pointed above, the identity of various species of family Culicidae is still a major problem. In order to discriminate/ identify these sibling species/ species complexes, new taxonomic attributes have been studied. Scanning Electron Microscopic (SEM) studies have been conducted to find out more characters on cibarium and genitalia of Culicine mosquitoes in the present studies. SEM studies have revealed that variation in number and position of various papillae i.e. campaniform papillae, trichoid papillae, dorsal papillae and ventral papillae in cibarium. SEM studies have also led to provide new characters i.e. number and position of cercal setae, setae on basistyle, shape of dististyle claw, structure of phallosome, setae and shape of post genital lobe in male and female genitalia. Seven species belonging to four genera i.e. *Culex* Linneaus, *Aedes* Meigen and *Mansonia* Blanchard and *Armigeres* Theobald have been studied. These new and additional attributes will strengthen the diagnosis of various taxa in subfamily Culinae.

Over-expression of a Cytochrome P450 Gene in Pyrethroid Resistant Strain of *Rhipicephalus (Boophilus) microplus*

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Metabolic detoxification of acaricides is carried out by three specific enzyme families: Glutathione S-transferases, Esterases (or Carboxylesterases) and the CytochromeP450s (CYPs). Metabolic detoxification mediated by cytochrome P450 mono-oxygenases has been implicated in insecticide resistance in many insect species. However, little research has been carried out on the role of cytochrome P450 mediated metabolism and their subsequent association with resistance in ticks. Relative quantification of CYP41 gene expression was studied in deltamethrin resistant IVRI-IV strain of *R.(B.) microplus* to investigate the role of cytochrome P450 gene in pyrethroid resistance. Temporal expression profile of CYP41 gene was also determined after single exposure to LC_{50} concentration of deltamethrin. Primers were custom synthesized using Primer Express software and efficiency was worked out to 97.22% using SYBR green chemistry. The normal expression level was analyzed using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) using acaricide susceptible IVRI-I ticks as control while unexposed IVRI-IV ticks were used as control in temporal expression profile. Data analysis revealed that the IVRI-IV ticks exhibited highly significant ($P<0.0001$) fold change of 20.48±0.25 in CYP41 transcript relative to IVRI-I. After single exposure to deltamethrin it was observed that CYP41 transcript level was significantly downregulated at 3 hrs (0.24±0.34; $P<0.001$), followed by an insignificant rise in the transcript level at 6hrs (0.58±0.34) and 12 hrs (1.13±0.33) until significantly upregulated at 24 hrs with a fold change of 2.66±0.29 ($P<0.05$) relative to unexposed control. The findings clearly substantiate contribution of cytochrome P450 in metabolic detoxification of the acaricides in deltamethrin resistant IVRI-IV strain. This is the first report of association between over-expression of cytochrome P450 gene with acaricide resistance in India.

Exploration of Prey-Predator Assemblages in Selected Water Bodies along an Urban-Rural Gradient in West Bengal

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The aquatic bodies designated as mosquito larval habitats are diverse in size and species composition. These aquatic bodies serve as a congenial Biotope for mosquitoes to breed and thus pose a great threat from public health view point. The macroinvertebrate predators in these habitats influence the abundance of mosquito species, providing a basis for biological control. Although successful regulation of mosquitoes require prey preference and ecological interaction among existing community members, conservation of the insect predators can be achieved through their presence in different aquatic bodies. Assessment of species assemblage in these habitats will indicate the possible variations in the resource exploitation and trophic interactions and frame biological control strategies more appropriately. The present study was carried out at a spatial scale to identify and assess their relative abundance and diversity across the aquatic bodies with varying anthropological activities of 3 different areas located in Kolkata, Howrah and Hooghly. The relative abundance of the mosquito immature varied with the habitat and the number of useful predator taxa was higher in the larger habitats. The species composition in the habitats support that the abundance of coexisting macroinvertebrate species control the relative load of mosquito immature in the habitats. Thus environmental management policies should be framed to maintain insect diversities in aquatic bodies and promote conservation and biological regulation of vector mosquitoes. The findings of this study may be further tested to deduce the relative importance of the habitats in terms of the productivity of mosquito immature at a temporal scale.

Species Composition and Diversity of Mosquitoes in Selected Areas of Coimbatore

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A study was conducted to assess mosquito species prevalent in the selected two areas, viz., Avinashilingam University campus and sennanur of Coimbatore city, Tamil Nadu, India. Fortnightly collections of adult mosquitoes were made from July 2010 to June 2011 using mosquito sweep nets during 5.30 pm to 6.30 pm. The different species of mosquitoes were collected and identified using the keys of Christophers (1933) and Barraud (1994). During the study period, meteorological parameters such as temperature, relative humidity and rainfall were also recorded to assess the influence of these factors on the mosquito population. Daily meteorological data were collected from the Meteorological Department, Tamil Nadu Agricultural University, Coimbatore. In order to study the impact of meteorological factors on mosquito population, simple correlation and multiple regression analysis were carried out. Further, the data were subjected to Canonical Correspondence Analysis (CCA) was carried out using the software PAST. Distributions of mosquito population in relation to seasons were used by neighbor joining clustering, which is an alternative method for hierarchical cluster analysis for two years. Multiple linear correlation and regression analysis showed negative relationship between mosquito populations and maximum temperature and there is no relationship between other meteorological parameters such as minimum temperature, humidity and rainfall in selected areas. Canonical Correspondence Analysis showed population of C.*gelidus* with humidity (14.22h), C.*vishnui*, A.*aegypti* and A.*subalbatus* with humidity (07.22h) and was C.*quinquefasciatus* with maximum temperature showed positive relationship in Avinashilingam University campus. The findings of this study help to assess appropriate and possible strategies to control different mosquito species.

Ecology and Distribution of *Aedes albopictus* with Special Reference to *Aedes albopictus* Subgroup Species in Kerala, India

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Aedes aegypti and *Aedes albopictus* are the vector mosquitoes of prime concern of Dengue and Chikungunya. Though reasonable literature on the ecology and distribution of *Ae. albopictus* is available, such information of the subgroup species in India is not known. Therefore, a study was carried out in 5 districts of Kerala: Wayanad, Eranakulam, Pathanamthitta, Idukki and Thiruvananthapuram based on the specific ecotypes in each district, favouring abundance and proliferation of *albopictus* subgroup besides, the incidence of Dengue and Chikungunya over the past few years. Extensive surveys were carried out in all the 5 districts to find out the distribution of *albopictus* subgroup species in the different ecotypes and geo-referenced. Both immature and adult collections were carried out following standard sampling techniques. Immature collections were made not only from the common container habitats, but also from various lesser examined habitats such as the leaf axils, bamboo cut stumps, rock pits etc. Adult collections were made using sweep nets and resting collections with mouth and mechanical aspirator. 266 *Ae. albopictus* and 36 *Ae. aegypti* adults were collected from Trivandrum district. 143 *Ae. albopictus* with two subspecies of *Ae. albopictus* namely, *Aedes subalbopictus* (2), *Aedes novalbopictus* (1) were collected from Wayanad; 173 *Ae. albopictus* and 15 *Ae. aegypti* mosquitoes from Ernakulam; 121 *Ae. albopictus* and 3 *Ae. aegypti* with one subspecies of *Ae. albopictus- Ae. subalbopictus* (2) from Idukki while 134 *Ae. albopictus* were collected from Pathanamthitta district.

Interspecific Competition between Larval Stages of *Aedes aegypti* and *Anopheles stephensi*

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Inter-specific competition occurs between members of two, or more, different species and can often have an influence on mosquito populations. Both *Ae. aegypti* and *An. stepehensi* are container breeding mosquitoes and co-exist which may result in larval competition. In this study interspecific competition between the above two species has been monitored under the laboratory conditions. Three sets of experiments were conducted with different stages of *Ae. aegypti* and *An. stephensi* larvae. First two experiments were set up with first/second instar and third/fourth instar larvae of *Ae. aegypti* and *An. stephensi* larvae respectively in the ratios of :-20:20, 20:40 and 40:20 in plastic bowls.. For third set of experiment 20 fourth instar larvae of *Ae.aegypti* were put with equal number of first instar larvae of *An. stephensi*. In the presence of food, 12.5-15 % mortality of Ist/IInd stage larvae was recorded in *Ae. aegypti* while in *An. stephensi* mortality ranged from 21-55%. Pupation commenced from 6-8 days onwards in *Ae. aegypti* while in *An. stephensi* it commenced from 11-16 days onwards. In the absence of food, there was no pupation in both the species but *Ae. aegypti* survived up to longer duration (7.5-18.5 days with 50% mortality) in comparison to *An. stephensi* (2-7 days with 50% mortality). When younger stages of *An. stephensi* (I/II) were put together with older stages of *Ae.* aegypti (III/IV) in the presence of food, 85% pupation was completed in *Ae. aegypti* mosquito while there was 100 % mortality of *An. stephensi*. The better survival and development of *Ae. aegypti* than *An. stephensi* under the same conditions exhibits interspecies competition showing competitive advantage of *Ae. aegypti* over *An. stephensi*. Further research is required to have a thorough understanding of the interaction between these two container inhabiting mosquito species in the nature.

Distance Pattern Analysis of Wing Shape in Mosquito Species, *Culex pseudovishnu* (Diptera: Culicidae)

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Vectors generally have high grade of morphological resemblance at adult stage and difficult to identify based on characteristics of adult females using the available morphological keys. Geometric morphometrics is a powerful tool for capturing the shape distinctiveness in several morphological aspects particularly head and wings. Insect wings are very suitable structure for studies because their two dimensional flattened shape bears many useful landmarks. Enormous variation in vein number, position and discrimination was observed in different insect orders and families. A geometry morphometric study was conducted to determine wing shape in male and female mosquitoes of *Culex pseudovishnu* collected from Coimbatore district. Twenty specimen of *Culex pseudovishnu* was identified and eighteen morphometric characters were examined. Results of the study confirmed that, the shape variation was high in the middle region of the female wing compared to male wing using the thin plate spline. Principal Component analysis (PCA) and Canonical Correspondence Analysis (CCA) revealed variation between female and male wing. The tps Relw program was used for calculating Eigen values for each principal warp of female (2.8323E+003) and male (2.2401E+003). Generalized F-test showed variation in male (F=0.763, df=42.336: P=0.8562) and female (F=1.2024, df=42,336: p= 0.1911). The tps small program was used for calculating the Procrustes distance and tangent distance. The relative warp analysis and Multivariate Analysis of Co-Variance (MANCOVA) proved to be very proficient in differentiating the variation of shape. It was clear that, variation between male and female wing is evident from the land mark data. This technique can also be employed to identify various species in one genus in taxon having problems in dichotomous keys which are currently available.

Oviposition Response of *Aedes aegypti* and *Aedes albopictus* Mosquitoes: Vectors of Dengue, Chikungunya and Emerging Zika Virus to *Dendrocalamus strictus* Bamboo Infusion

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Plant materials at water resources after fermentation releases various types of volatile chemicals which influence female gravid mosquitoes for oviposition. Oviposition response of container breeder mosquito *Aedes aegypti* and *Aedes albopictus* was assessed against bamboo leaf infusion prepared by fermenting the senescent leaves of common bamboo species *Dendrocalamus strictus*. Leaves were fermented for period of 1, 3, 7 and 10 days and tested for oviposition activity at different dilutions. Significant attractiveness towards the infusion was observed by both the mosquito species. The response of gravid females was varied with the fermentation period and its concentration. Gravid *Ae. aegypti* females were more attractive towards 5% than 10% dilution whereas *Ae. Albopictus* were comparatively more attractive at 10% dilution than 5%. At the

infusion fermentation time, 3-7 days was more attractive to Aedes mosquitoes with 7 days slightly superior. Residual activity of infusion and their efficacy in walk in cage bioassay also supported the results. The overall result suggested that the bamboo infusion act as chemical cues for gravid Aedes mosquitoes. The field performance of this infusion for monitoring and control of container breeder mosquitoes needs to be studied further.

Environmental Information System (ENVIS) – An Integrated Approach for Control of Vector Borne Diseases

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Vector-borne diseases take a heavy toll of life and are an annoyance especially in tropical and sub tropical regions of the world. By increasing developmental activities, industrialization, deforestation and rapid urbanization leads to epidemics of vector borne disease. The spectrum of the diseases threatens the mankind in full vigour and includes Malaria, Lymphatic Filariasis, Dengue, Chikungunya and Japanese encephalitis. The factors responsible for the emergence/resurgence of vector-borne diseases are complex. There is an urgent need for exploring new methods and technologies to devise ways to restrict these diseases. An integrated approach for the control of vector borne diseases will enable better sustainable control while helping to evade some of the problems associated with the use of conventional insecticides. Environmental methods and biological control are alternatives to chemical control and are key components of the integrated strategy. The Environmental Information System (ENVIS) centre on *Bioinformatics - Vector Control* hosted by CSIR- Indian Institute of Chemical Technology is dedicated dissemination centre on vectors and vector borne diseases. The objective of the centre is to collect, collate and disseminate the information on integrated vector control approaches and measures to be taken by the community to overcome the menace. Under this program Malaria, Filariasis and Japanese encephalitis databases were developed to store the information on various aspects like Epidemiology, Entomology, Socioeconomic and Meteorological details. The ENVIS portal also provides information on data mining applications like Self Organizing Maps (SOM) a clustering technique, Correlation and Regression Tree (CART), Bayesian network for prediction of Japanese encephalitis vector density (JEBNET) and VB Classif- Epidemiological data Classification Tool. Similarly the ENVIS centre also developed spatial applications like Malaria/Filaria monitoring and visualization system for identification of disease hot spot areas spatially. Apart this ENVIS centre is also focusing on climate based models for disease forecasting application for various vector borne disease in India. These applications help the public health authorities to take necessary control measures well in advance which in turn to reduce the mortality and morbidity occurring due to vector borne diseases.

Differential Olfactory Response of *Aedes albopictus,* to Human Skin Emanations from Various Body Parts

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The Asian tiger mosquito *Aedes albopictus* (Skuse), a freshwater and container breeder, is a new world vector of dengue and chikungunya. The scent of the human host functions as a kairomone for the mosquito in finding its host for blood meal. In this work, odour-mediated host-seeking behavior of *Ae. albopictus* in relation to human skin emanations was studied. Human skin emanations were collected on glass beads from facial (Non-axillary), from axilla (under arms) and feet of 10 volunteers and olfactory response of unfed (5-7 day old) *Ae. albopictus* were studied using Y-tube olfactometer to find out whether any variation exist in attractiveness of the body part from which sweat/emanations has been collected. Sweat collected from feet was most attractive followed by facial sweat and axilla. The kairomones from human emanations may be used to improvise the efficiency of the existing odour-baited traps used for the surveillance and control of day biters like *Ae. albopictus*.

Study on Container Breeding Mosquitos in Theni District, Tamil Nadu, India

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Breeding habitats have associated with distribution and abundance of mosquitoes and mosquito-borne diseases in many part of the world, especially in worm and tropical climatic regions. The present study is aimed to determine the distribution and abundance of mosquitoes in cess pits, drainage, ponds, septic tanks, containers, cess pools, tree holes in Theni district, Tamil Nadu, India. All the possible permanent and temporary water bodies were surveyed in selected sites during dusk and dawn hours. Sampling was made with 205 mL of breeding water and specimens were identified by taxonomic keys. Ten localities were randomly selected and sampled twice per month with 15 days interval for a period of six months, from July to December 2015. A total of 1021 mosquitoes of 19 species belonging to 8 sub-genera and 5 genera were identified. Among the collected specimens the *Culex* species were predominant in polluted water habitat. Diversity were estimated using Shannon-Weiner diversity index and Simpsons dominance index. The findings of this study highlight the importance of improperly maintained drainages, containers and unused things in study sites are engaged to mosquito development. This will be essential for designing and implementing mosquito control.

CHAPTER 9

TRANSMISSION BIOLOGY

Paradigm Shift in the Transmission of Vector-Borne Diseases

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Infectious diseases transmitted by insect vectors, otherwise known as Vector Borne Disease (VBDs) are causing havoc in the tropical and sub-tropical regions of the globe. Diseases like malaria, dengue, chikungunya, and zika are endemic to many of the countries. These VBDs not only cause mortalities, but some of them also cause high morbidity. Under a traditional model of disease transmission, symptomatic human hosts (patients) are considered to be the reservoir of pathogens which are then taken by the insect vectors and infect a new uninfected human host. This model therefore, assumes that the rate of transmission of VBDs is dependent on the number of symptomatic hosts in the population. However, in recent years this model seems not to hold true. In turn, recent research works have indicated that disease transmission is majorly contributed by otherwise underestimated high number of asymptomatic individuals in the population. In this communication, we have brought about the cases of high number of asymptomatic individuals infected with malaria, dengue and chikungunya in synthesizing and bringing into notice the importance of asymptomatic infection and the future direction to control VBDs through appropriate surveillance. We have also proposed a model to understand the relationship between symptomatic/asymptomatic infections and outbreak of epidemic/ inter-epidemic periods.

Transmission Spectrum of Novel Emerging Chikungunya Virus in Aedes mosquitoes

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Large scale Chikungunya outbreaks are primarily attributed to appearance of novel viral mutations as well as expansion of geographical territories of Aedes mosquitoes. There is a curiosity among scientific community to understand the transmission spectrum of CHIKV strains. The present study describes both horizontal and vertical transmission of chikungunya virus in Indian Aedes mosquitoes.

The mosquitoes were orally infected and subsequently, infection, dissemination and transmission rates were estimated. The infected mosquitoes revealed high viral replication in midgut, efficient dissemination, and substantial viral titres in saliva with a transmission rate of 60%. The 226V was highly selected in *Ae. albopictus*. In contrast, 226A was more transmitted by *Ae. aegypti* compared to *Ae. albopictus*. Further, the role of two important mutations (E1:K211E and E2:V264A in background of E1:226A/V) revealed remarkably higher fitness for *Ae. aegypti*, as indicated by significant increase in virus infectivity (13 fold), dissemination (15 fold) and transmission (62 fold) compared to parental E1:226A virus. These results indicate that similar to 226V, adaptive mutations in 226A are leading to efficient CHIKV circulation in *Ae. aegypti* endemic areas.

The potential for vertical transmission was studied over multiple gonotrophic cycles of *Ae. aegypti*. The results revealed that both the larvae and adults developed from the initial gonotrophic cycle were negative for CHIKV RNA. However, the larvae and adults developed after subsequent gonotrophic cycle were positive with minimum filial infection rates of 28.2 (1:35.5) and 20.2 (1:49.5) respectively. This study confirms experimental vertical transmission of ECSA genotype of CHIKV in *Ae. aegypti* from India, indicating the possibilities of occurrence of this phenomenon in nature.

The high vectorial competence of Indian Aedes mosquitoes, as evident through both efficient horizontal and vertical transmission might be attributed to sustained transmission of Chikungunya virus in India over last decade. These vector competence studies have the potential to predict novel viral mutants capable of causing severe epidemics.

*w*Mel Strain of *Wolbachia* Inhibits Dengue Virus Replication in *Aedes* Mosquitoes

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Dengue is one of the most widespread mosquito-borne diseases in the world posing a considerable threat to human lives. The causative agent, dengue virus (DENV), is primarily transmitted by the mosquito vectors *Aedes aegypti* and *Aedes albopictus*. The control strategy of *Aedes* has proved to be difficult using the conventional methods. Strategies for blocking pathogen transmission by mosquito vectors have been proposed as a means of augmenting current control measures for reducing vector-borne diseases. This ultimately has led to the introduction of *Wolbachia* in *Aedes* mosquitoes as a potential vehicle for dengue control strategies. *Wolbachia* is a gram negative endosymbiont that is responsible for various reproductive alterations like cytoplasmic incompatibilty, male killing, parthenogenesis and feminization. Polymerase chain reaction (PCR) was used to determine the presence of *Wolbachia* from field collected *Aedes* from various parts of the Odisha using *wsp* primers. *Aedes* transinfected with the *w*Mel strain of *Wolbachia* showed limited DENV replication that has led to field releases of these mosquitoes in Cairns, Australia as a biocontrol strategy for the virus. Field collected mosquitoes reared in the laboratory were transfected with *w*Mel and then challenged with DENV serotypes displayed limited rates of viral replication and dissemination as compared to uninfected controls. In our study it was found that *w*Mel was distributed in body tissues of laboratory transfected and reared mosquitoes, but, at seven days following blood-feeding, *w*Mel densities increased to a greater extent in these mosquitoes. Our results indicate that virus-blocking is likely to occur in *Wolbachia*-infected mosquitoes, suggesting that *Wolbachia* biocontrol may be a successful strategy for reducing dengue transmission in the field.

Japanese Encephalitis Transmission in Odisha

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Japanese encephalitis (JE) has been a serious public health problem in Odisha since 2013. During the last two years, outbreak of Japanese encephalitis (JE) occurred in three different geo - physiographical region of the state i.e., Malkangiri (Eastern Ghat), Jajpur (Coastal belt), Keonjhar and Mayurbhanja districts (Northern plateau). Entomological and epidemiological studies were carried out in the above district during the outbreaks between 2013 and 2016. Human blood samples were collected from 316 JE suspected cases from the affected districts ie, 78 from Malkangiri, 36 from Jajpur, 79 from Keonjhar, 42 from Mayurbhanj and 123 from Puri. Of these, 12 samples from Makangiri, 12 samples from keonjhar, 14 samples from Jajpur, 16 samples from Mayurbhanj and 51 samples from Puri were positive for JE virus. Entomological survey in the above affected areas showed a total of 6508 mosquitoes which revealed the presence of C*x.vishnui* group of mosquitoes along with *An.subpictus, An.vagus, An. culicifacies, An. fluviatilis,* Cx *gelidus* and *Mansonia uniformis.* Monoclonal antibody based antigen capture ELISA kit was used for detection of JEV from mosquitoes and out of 98 pools, four showed positive result one from Jajpur i.e., *Culex vishnui* group and two pools from Mayurbhanj district i.e. *Culex vishnui* group and *Culex gelidus* and one pool of *Culex vishnui* group was found positive for JE virus from Puri district during JE outbreak in 2016. Minimum infection rate (MIR) of *Cx.vishnui* was found to be 12 in Malkangiri, 10 in Jajpur, 2.72 in Keonjhar, 3.10 in Mayurbhanj and 0.48 in Puri during outbreaks across the different districts of Odisha. Blood samples were also taken from the pigs (amplifying host) of affected household by the expert veterinary surgeon. The blood samples were brought to RMRC lab and subsequently transported to NIV, Pune for serological tests, which showed that out of 45 samples 5 were positive for (IgG) against JEV. Our study shows that there is an active transmission of JE in Jajpur, Mayurbhanja and Puri, warranting specific control measures against JE transmission.

Effect of Urbanization on the Intensity of Malaria Transmission in Ghaziabad

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Urbanization has transformed environment by using natural resources for the rapidly growing population. Not only environment its affect can be seen on health of the people living in urban areas. Both communicable and non communicable diseases have increased in urban. Vector borne diseases like malaria, dengue, chikungunya cases can also be seen increasing recently. Malaria, a vector borne disease caused by anopheles mosquito is changing its malaria transmission dynamics due to change in ecology as a result of urbanization. Ghaziabad, a district of Uttar Pradesh is among the top 20 cities of India with large urban agglomeration (census 2011). Unplanned urbanization in Ghaziabad is supposed to effect anopheles (vector of malaria) mosquito's life cycle eventually affecting disease dynamics. Most of the studies done in Ghaziabad are related to insecticide resistance in *Anopheles culicifacies* a rural vector of malaria. Due to establishing urban ecology in Ghaziabad it is suspected that urban malaria vector i.e. *An.stephensi* should replace *An.culicifacies* (rural vector). Thus the purpose of our study is to find out the effect of increasing urban and peri-urban areas and its impact on breeding habitat of anopheles mosquitoes i.e. *Anopheles culicifacies*, *An.stephensi* and on malaria transmission in Ghaziabad. Remote sensing analysis of Ghaziabad was done from 2000 to 2015 to find out land use change. By analyzing data on land use it is found that urban built up area is increasing at the cost of agricultural, forested and other open spaces. Field collection of two seasons i.e. Pre-monsoon, Monsoon, Post monsoon from 2013 to 2015 has been conducted while stratification of Ghaziabad into rural, urban and peri-urban Ghaziabad in which larva, pupa and adult of malaria vectors *An. culicifacies, An.stephensi, An.subpictus, An. annularis, An.pulcherrimus* were found alongwith *Culex quinquefasciatus* and *Aedes* mosquito. In field collection the percentage of *An. culicifacies* was

13.8% in rural, 2.4% in peri-urban and 0.4% in urban while percentage of *An.stephensi was 2.4% in rural, 3.4% peri-urban, 11.53% in urban*. The above percentages indicate lesser rural and more urban vector in Ghaziabad. The field collected samples were then processed for presence of parasite Plasmodium through ELISA and confirmation of these samples is done by Nested PCR. The samples examined till now shows 0.41% (n=242) of *Plasmodium* infection in *Anopheles* mosquitoes of Ghaziabad. *Aedes* and *Culex quinquefasciatus* are also increased in study area. *Ae. aegypti* is increasing due to increase in population size resulting in increase of water storage containers by which cases of dengue and chikungunya are increasing in this area. C. *quinquefasciatus,* is found to be in high density due to open sewage, open wastes and improper drainage. Culex in Ghaziabad is not responsible for any disease but increasing nuisance, till now the Japanese encephalitis is not introduced in system.

Studies on Morphological Variation of *Aedes* Mosquitoes in Some Areas of West Bengal

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Dengue is an enigmatic vector-borne disease which is commonly known to pose a significant threat to public health and is transmitted by the day feeding *Aedes* (*Aedes albopictus* and *Aedes aegypti*) mosquitoes. *Aedes aegypti* and *Aedes albopictus* are closely associated with human habitations and adapted to feed on humans. India is the first leading country in representing a significantly larger dengue burden both in urban and rural environments. Re emergence of Dengue, Dengue hemorrhagic fever (DHF) and Chikunguniya in urban and suburban and rural areas of West Bengal along with the population abundance of *Aedes* mosquitoes have become a major health problem. Therefore, a preliminary attempt has been made to survey the breeding site preference pattern of *Aedes* mosquitoes from the different areas of West Bengal. In this context, both the larvae of *Aedes albopictus* & *Aedes aegypti* were collected from water (natural and artificial) filled container in and around the houses of different areas of West Bengal and reared in the laboratory colony culture cage. Morphological variations like white palp, mosaic palp, cup shaped proboscis and fringe spot of wings were observed from the emerged adult. These morphological variations may help to identify the vectors and its sibling species.

Effects of Variation in Amounts of Food and Density on the Life History Traits of *Aedes aegypti*

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Resource availability and density are essential factors that shape the life history of the mosquitoes. The larval effort in resource acquisition determines the adult features which in turn influence the disease transmission potential. On the basis of these propositions, variations in the life history traits of *Aedes aegypti* were evaluated through manipulation of the food and initial larval density. Synergistic effects of food and density on life history traits would allow assessment of the fitness of the individuals constituting the population as a consequence of intraspecific competition. Increased larval densities and reduced food availability led to delayed pupation, emergence of

adults with smaller biomass irrespective of sex. The females were comparatively larger than males in terms of pupal weight and wing length whereas males survived longer. These differences increased with the accessibility of resources, indicating the relative importance of these two traits for the fitness of each sex. The results of the present study indicate the impact of food amount (resource) and larval density regulated the outcome of the adult life history traits of both males and females of *Ae. aegypti*. Food amount available in larval habitats can therefore be considered as an important feature to decipher the population dynamics of *Ae. aegypti*. From vector management viewpoint, as density dependent intraspecific competition is an important factor regulating mosquito populations, further studies involving environmental conditions and a range of mosquito species should be initiated.

CHAPTER 10

VECTOR CONTROL: PRACTICE, CHALLENGES, OPPORTUNITIES, NEW TOOLS AND MANAGEMENT

Development of simple Insecticide Quantification Kits (IQK) for monitoring insecticide use for vector control programmes

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Tropical diseases spread by mosquitoes, sand flies and other insects contribute to global health problems with enormous economic and social impact. Insecticides are relied upon for vector control; however, with so few insecticides available for public health they are highly susceptible to rapidly evolving resistance. Careful use of insecticides is essential to ensure the correct doses are applied to ensure target insects are killed and prevent catastrophic control failures. Too little insecticide and the vector survives and pressure for insecticide resistance is increased, too much and there are increased chances of health and environmental damage, as well as a waste of expensive resources. New insecticide quantification kits (IQK) are being developed for DDT, cyanopyrethroids, carbamates and organophosphates that enable these insecticides to be easily monitored during spray operations (Russell *et al* 2014 Malaria J. 13, 178; Ismail et al 2016 PLoS NTD 10 (1), e0004324) Examples of these new tools and their potential impact on field operations to control disease transmitting insect vectors are presented. By making the analysis of insecticide dosing accessible to field operatives, routine monitoring of insecticide levels can be used to maximize the effectiveness of indoor residual spray operations (IRS).

Prevention and Control of Dengue by Targeting Key Containers – A Case Study in Delhi

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Dengue is emerging as one of the major health problem in Delhi, posing serious threat to public health. Since 1967, Delhi has witnessed various dengue outbreaks. *Aedes aegypti* is a principal vector of dengue virus in India. *Aedes* mosquitoes are container breeder and breeds in variety of natural and man-made containers.

An earlier study carried out by Nagpal *et. al.* in Delhi during 2007 –2012 with the objective to identify key containers for *Aedes* breeding. It was observed that overhead tank acts as Key Container of *Aedes* breeding as it shows a consistent breeding throughout the study period. The rest of the containers do play intermittent roles in supporting the breeding of *Aedes* mosquito. Subsequently, another study conducted in Delhi during 2012-2014 based on the hypothesis that whether continuous entomological surveillance of *Ae. aegypti* and simultaneous appropriate interventions in these key containers during non transmission (December – May) months would have any impact on breeding of *Aedes* and dengue cases during the following transmission months (June – November).

A total of 28 localities of West Zone of Delhi with persistent dengue endemicity were selected for the study. Out of these localities, 20 were included in study group while other 8 localities in control group. IEC and various *Aedes* breeding control activities were carried out in study group in both non-transmission and transmission season whereas control group did not have any such interventions during non transmission months as per guidelines of MCD. These activities were undertaken by a team of investigators from NIMR and SDMC, Delhi. In control group, investigators from NIMR carried out surveillance activity to monitor the breeding of *Aedes* mosquito in localities.

Comparison of baseline data revealed that all indices in control and study group of localities were comparable and statistically non-significant ($p>0.05$). In both study and control groups, indices were calculated after pooling data on seasonal basis i.e. transmission and non-transmission months for both years. The test of significance conducted on all the four indices i.e. HI, PI, CI, and BI revealed a significant difference ($p<0.05$) between the study group and control group during transmission and non transmission months except in HI. Due to consistent intervention measures undertaken in non-transmission months in study group, reduction in CI, HI, BI and PI was observed 63%, 62%, 64% and 99% respectively during transmission months as compared to control group where increase of 59%, 102%, 73% and 71% respectively. As a result of reduction in larval indices no dengue case (except one NS1) was observed in study group whereas 38 cases dengue were observed in control group. Through this pilot study, it is concluded that proper intervention in non-transmission season reduces vector density and subsequently dengue cases in transmission season.

Vaccines Targeting Sexual Development of Malaria Parasites in the Mosquito Vector: A Step Towards Malaria Elimination

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According to the WHO there were as many as 214 million cases and ~438,000 deaths from malaria worldwide, reported in 2015. Significant reductions in morbidity and mortality have been attributed largely to the implementation of insecticide-treated bednets and artemisinin combination therapies. However the emergence of artemisinin resistant parasites has threatened these positive trends and stresses the need for developing effective malaria vaccine(s) to help with the long-term goal of gradual malaria elimination and eradication. Vaccines represent an effective public health tool that have played a major role in curbing several infectious diseases. There are currently no licensed vaccines against malaria or any other human parasite. The only malaria vaccine, Mosquirix^{TM} (RTS,S/ASO1) based on *P. falciparum* circumsporozoite protein (PfCSP), and advanced through phase III trials, has shown <40% protective efficacy. Such a bleak outcome underscores the intricacy of malaria vaccine development and need for renewed vaccine efforts with better efficacy to realize the goal of malaria elimination via transmission reduction, which is a goal of our studies.

Malaria transmission is characterized by different development stages of the parasite, initiated by infected *Anopheles* mosquito injecting sporozoites which invade hepatocytes. Post hepatic asexual erythrocyte stages responsible for clinical disease differentiate into erythrocytic sexual stages, crucial for further development of the parasites in the mosquito vector leading to formation of sporozoites. Hence immune interventions aimed at blocking development of both the liver stage and sexual stage are expected to provide more effective strategy to protect against malaria. A transmission blocking vaccine approach targeting antigens in the sexual stages (i.e. male and female gametocytes and gametes) and the mosquito stages of the parasite (i.e. zygote and ookinete) is believed to be of central importance in malaria elimination efforts. Ingestion of antibodies elicited by transmission blocking vaccines effectively block sexual reproduction of the parasite in the mosquitoes, reducing further transmission potential. In *Plasmodium falciparum,* transmission blocking vaccine target antigens include Pfs25, Pfs48/45 and Pfs230, with known orthologs in *P. vivax*. In order to achieve effective interruption of malaria transmission, an optimal vaccine must prevent the development of intra-erythrocytic gametocytes and further reduce their development in the mosquitoes. While a hepatic stage vaccine will prevent or reduce the development of blood stage parasites including gametocytes in an infected person, a transmission blocking vaccine will block sexual reproduction of the gametocytes in the mosquito. A combination of vaccines targeting both hepatic stages and sexual/midgut stages is expected to provide effective ways for interruption of malaria transmission, critical for elimination goal. In our research we are employing vaccines based on recombinant proteins formulated using immunomodulatory adjuvants and DNA plasmids delivered by *in vivo* electroporation. The primary goal of our studies is to determine the potency of vaccine combinations: (1) comprised of target antigens from liver stages and sexual stages of *P. falciparum* and (2) vaccines targeting transmission of the two major *Plasmodium* spp. (*P. falciparum* and *P. vivax*). These studies will rationally develop and evaluate multi-stage (liver stage and sexual stages), multi-antigen (hepatic and sexual stage specific) and multi-species (*P. falciparum* and *P. vivax)* vaccine combinations to interrupt malaria transmission, a long-term and ultimate goal of our research, and an important goal of malaria elimination strategies.

Integrated Vector Management: A Potential Strategy in the Context of Prevention, Control and Elimination of Vector-Borne Diseases

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Vector borne disease continues to be a major public health concern in view of emerging and re-emerging infections. IVM is a rational decision making process to optimize the use of resources for vector control. World Health Organization has issued its position statement on IVM in 2008, encouraging member countries to promote IVM as an approach to improving vector control. Vector borne diseases are responsible for about 17% of the burden due to parasitic and infectious diseases. IVM concept is suitable in a different variety of situation. The problem due to vector borne diseases vary in space and time. Scope of IVM varies from simple vector surveillance to assess the risk to intensive control operations against the vector borne diseases that are targeted for elimination or control. A number of successful demonstrations of control of VBDs have been reported following one or combination of five key elements of IVM. A study carried out in Pondicherry to control bancroftian filariasis followed integrated approach in implementing appropriate methods and combinations, networking with other sectors and social mobilization. After five years there was significant reduction in infections levels. Another study carried out in the control of brugian filariasis in Shertalai in Kerala followed integrated method of vector management through income generating programme such as poly-fish culture. There was no new infection in the age class 1-7 years following IVM.

Promotion of mullti-sectoral approach is another attribute of IVM. A school based vector surveillance and reporting to the programme is another innovative method of risk assessment attempted in Puducherry for timely action. Students trained in assessing vector breeding sources could provide information for prevention of dengue epidemic. Legislation is one of the components of IVM and there are a number of successful demonstration by enforcing regulations.

IVM is highly relevant during post elimination surveillance which is aimed at ensuring that the gains on long term basis. Malaria elimination launched in many countries aims at long term use of insecticide treated bednets as personal protection measure with active community participation. With the new NTD based control/elimination strategies, vector control would benefit all the diseases including low priority diseases due low prevalence or lack of resources. IVM also permits to implement vector control programmes under decentralised system as VBDs are focal in nature and the decisions are made locally. Transformation of vector control activities to the local health system has been demonstrated. IVM aims at sustainability of control/preventive measures by linking with the health, administrative and social systems.

In Silico Screening of Phytochemical Compounds to Identify Novel Mosquito/Insect Repellent Compounds Targeting the Odorant-Binding Proteins (OBPs) of Malarial Vectors

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Odorant binding proteins (OBPs) are considered as important targets for structure-based rational approaches towards discovery of new repellent or other olfaction inhibitory compounds with desirable features. As of now little effort has been made to screen phyto-chemicals compounds with desired activity to design novel mosquito/insect repellents using high-throughput computational biology tools. Henceforth, in this study, an attempt was made to screen phytochemicals from 10 plants with mosquito repellant activity from published literature and public domain through theoretical modeling and molecular docking studies targeting odorant binding proteins (OBPs) of Anopheles gambiae and Anopheles stephensi (vectors of malaria). The widely used mosquito repellent N,N-Diethyl-m-toluamide (DEET) was selected as reference to check the binding affinity and specificity of the compounds against OBPs. A total of 40 compounds and DEET was docked to the active site of OBP models/crystal structures using the AutoDock. Among these phytochemical compounds, a total of 17 compounds showed higher binding energy along with more numbers of hydrogen-bonds as compared to DEET. Few compounds identified in this study i.e., azadirachtin, lycopersin, khusimol, khusimone and alpha-vertivone displayed high docking scores needs further investigation, which may aid in the design of safer and more effective insect repellents. The results from the present study is expected to steer the process of discovery of novel and effective repellant which will open better avenues to bring new possibilities in limiting the threat of malaria in near future.

Impacts of Complete Coverage of LLIN on Vector Density in Gadchiroli District, Maharashtra

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Malaria is a serious health problem in India. Long-lasting insecticidal nets (LLINs) have been introduced in India into the vector control programme for the containment of the disease. There exists a gap between receipt and utilization of LLIN in the community. The objective of this study was to determine the impact of LLIN on vector mosquito density in district Gadchiroli of Maharashtra. The three sub centers Kulbhati (four villages Rengagaon, Gota Tola, Tumdikasa and

Fulkoda), Pannemara (two villages Belgaon and Ampyali) and Yerkad (Sindesur and Tavetola villages) of Murumgaon PHC were selected for the study based on malaria incidence and LLIN distribution. Prestructured questionnaire were filled to know the distribution and use of LLIN as baseline, thereafter additional LLIN were distributed in selected villages for 100% coverage of population. Entomological surveys were undertaken to know the impact of LLIN on vector mosquito density. A total of 250 houses were surveyed in these villages and questionnaires were filled regarding bednet distribution and their use. 507 LLIN were distributed in study villages (population 1270) of Murumgaon PHC. In four selected villages, additional 409 LLIN were distributed for 100% coverage. During august month, MHD of *An. culicifacies* in human dwellings was 53 in experimental villages as compared to 146 in control villages. In cattle sheds collections, MHD of *An. culicifacies* was 333 in experimental villages as compared to 262 in control villages. In this study there was a significant impact of insecticide treated nets on vector mosquito density in human dwellings. Thus 100% coverage of human populations with LLIN may be an important tool in controlling malaria situation in endemic areas like Gadchiroli. Further longitudinal studies are required for more comprehensive results.

Impact of ICON MAXX-Treated LLINs on Malaria Incidence in Falciparum Endemic Tribal Area of Odisha, India

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ICON MAXX, an insecticide treatment kit for long-lasting insecticidal polyester nets containing lambda-cyhalothrin was evaluated for its effectiveness in reducing the density of malaria vectors and its impact in reducing the malaria incidence in five malaria endemic villages of Koraput district, Odisha. A census was carried out in all the five villages during June 2011. The nets were distributed in the last week of July 2011. Entomological and malariometric indices in all the five villages were assessed and compared during pre- and post-intervention periods for three years against *Anopheles fluviatilis*, the major malaria vector in the study area.

The per man hour density (PMD) of *An. fluviatilis* in human dwellings was found to be 6.8 in July 2011 i.e. before distribution of nets. The PMD became zero in human dwellings just after the distribution of ICON MAXX treated LLINs and maintained at zero level up to the end of the study. The malaria incidence was found to be reduced completely to zero level after six months of distribution of LLINs and remained zero thereafter up to two years. The slide positivity rate was 10.4 before distribution of nets in the villages. After 36 months of net distribution, the SBS results showed a significant reduction of SPR from 10.4 to 1.2. The study indicated that the regular use of ICON MAXX treated LLINs has a major impact on the density of malaria vectors and reduction of malaria cases. Therefore, high coverage and usage of ICON MAXX treated LLINs supported by a strong active case surveillance system would be a useful effective intervention strategy for the control of An. fluviatilis transmitted malaria in India.

Application of Eco-Friendly Method for Dengue and Chikungunya Control in Flood Affected Areas of Chennai, India

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The vector borne diseases like Dengue, Chikungunya focal outbreaks were expected at flood affected area in Chennai, Tamil Nadu during December 2015. The state health department had arranged intensive Anti larval measures to prevent and control disease transmission. A door-to-door survey was carried out in houses to explore vector mosquitoes breeding habitats. Larval surveillance was carried out and various indices have been analyzed as per NVBDCP guidelines. Source Reduction is an eco-friendly method applied to eliminate the vector mosquito breeding habitats without using chemical larvicides. Breeding preference ratio (BPR) calculated and discussed. BPR was observed that highest in discarded tires (0.76) followed by unused grinding stones (0.71), cement tanks (0.62) and plastic containers (0.52). Breeding Potential Ratio (BPR) study is very important tool in control measures. House index 57.4, Container index 60.0, Breteau index 126.0 and Pupal index 108.5 recorded in flood hit area for positive sign of focal fever outbreak. The cost effective and eco-friendly method of source reduction was carried out to eliminate all larval breeding habitats and the positive result was no Dengue and Chikungunya focal outbreaks reported in flood affected area, Chennai.

In-Depth Monitoring of Insecticides Susceptibility Status and Plan to Suitable Strategy for Control of Malaria Vectors in Gadchiroli District of Maharastra

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Determine the insecticide susceptibility of malaria vectors in eighteen villages of five high malaria endemic PHCs of Gadchiroli district of Maharastra state and selected on the basis of high malaria cases during the years 2010-14. Susceptibility tests were carried out at diagnostic concentrations of various insecticides as deltamethrin (0.05%), malathion (5.0%) and DDT (4.0%) against *An. culicifacies* and *An. fluviatilis*. Out of 18 populations, 10 populations of *An. culicifacies* were fully susceptible to deltamethrin, and other 8 populations were partial resistant /tolerant to deltamethrin. Populations of *An. fluviatilis* collected from 5 localities were fully susceptible to deltamethrin. All the populations of *An. fluviatilis* and *An. culicifacies* were resistant against malathion (5.0%) and highly resistant against DDT (4.0%). In addition cone bioassay tests were conducted for know the quality of indoor residual spray on surface wall of houses sprayed with Lambdacyhalothrin and cyfluthrin insecticide after 1 to 4 weeks of spray in 13 villages of 4 high malaria endemic PHCs of Gadchiroli district. It revealed that the spray was found satisfactory at only 5 villages. The spray was not uniform on wall of houses at all the villages. The spray was found incomplete and quality of spray was poor in study villages. The study indicates that there is a need to some improvements in strategy for control of *An. culicifacies* and *An. fluviatilis* malaria vectors in Gadchiroli district of Maharastra state.

Achieving the Visceral Leishmaniasis Elimination Target in India with Effective Vector Monitoring

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Visceral leishmaniasis (VL) is a vector-borne neglected tropical disease of public health importance in Bihar and is transmitted by the bite of an infected *Phlebotomus argentipes* sand fly. Since the inception of the VL elimination programme in 1934, indoor residual spraying (IRS) has been conducted with dichlorodiphenyltrichloroethane (DDT). In 2015, in response to evidence showing a rapid decline in *Ph. argentipes* susceptibility, the elimination programme switched to the pyrethroid insecticide, alpha-cypermethrin (alpha). Entomological monitoring is crucial to ensure insecticide efficacy during the transmission season. Susceptibility tests were conducted to assess the current resistance status of *Ph. argentipes* across Bihar using WHO diagnostic tests. To assess the intensity of DDT and potential pyrethroid resistance, dose mortality curves were established using CDC bottle bioassays and F1 *Ph. argentipes*. The efficacy of DDT and alpha IRS was assessed by a testing process treating tiles made of three of the most common surfaces in Bihar (mud, brick and limewash). Susceptibility tests of F1 and field caught *Ph. argentipes* from Bihar showed 100% mortality rates when exposed to 0.1% alpha and 0.05% deltamethrin. Reduced mortality rates were observed when *Ph. argentipes* were exposed to 4% DDT (24.6-37.5%). The DDT CDC bottle bioassays showed that after 45 minutes of exposure at the diagnostic dose (100µg/bottle) 30% of sand flies were killed and 69% at 10x the diagnostic dose. Mortality breaching the 98% WHO limit for susceptibility was only found at 10x the diagnostic dose after 75 minutes and 5x the diagnostic dose after 150 minutes, demonstrating the intensity of resistance. However, with alpha, after 30 minutes of exposure at the diagnostic dose (12.5 µg/bottle), 89% of sand flies were killed, whilst mortality ranged between 96-98% for 2x, 5x and 10x the diagnostic dose. Efficacy of DDT on F1 *Ph.argentipes* sand flies exposed for 30 minutes, was 13.45-21.97% on tiles treated at 1g/m^2, conversely 0.025g/m2 treated alpha tiles was 33.33-83.33%. High levels of DDT resistance has been detected in field and laboratory settings, whilst alpha is shown to be effective. Monitoring entomological indicators across Bihar promotes informed decision-making, provides early indications of sub-optimal impact and could support the programme in achieving its 2017 elimination target.

Ushering malaria elimination with larvivorous fish in Karnataka

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Malaria elimination is a buzz word. Global Technical Strategy of WHO advocated that malaria would be eliminated from India by 2030. Many neighbouring countries including Sri Lanka have already achieved this goal. The important matter is to contain and eliminate the disease with the existing tools. Many strategies are being employed at different geo-ecological situations in the country. Bioenvironmental method is one of the strategies advocated by the National Institute of Malaria Research needs to implemented to sustain the elimination process. Use of larvivorous fish is one of the main components under the bioenvironmental method. In Karnataka, this strategy is used as one of the vector control methods for over two decades that has led to pre-elimination phase of malaria in most of the rural areas. Recent analysis indicated focal and limited cased are reported in new stone quarry areas where malaria was not present earlier. In Karnataka, it has been decided that larvivorous fish would be released in all potential vector breeding habitats in the state. Now a new initiative has been taken to eliminate malaria from Karnataka by 2020.

Effect of Neurotoxins on the Nervous System of Mosquitos: New Tools to Control Malaria Vectors

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Malaria remains as one of the priorities for researchers to work as a solution for its long-lasting control solution. In humans, malaria is caused by five different species of Plasmodium parasite which are carried and injected to human host by female Anopheline mosquito during blood meal. Developing a new line of therapeutics for malaria, in the context of emerging resistance to currently used antimalarials, is a big challenge and malaria vaccines are still under trial. For novel approaches to control malaria, mosquito physiology needs to be explored in detail as vector control measures. The opportunities offered by the mosquito's nervous system have not yet been investigated in detail. Current proposal focuses to investigate the molecular biology of synaptic transmission in mosquito's nervous system. Botulinum neurotoxin (BoNT) from Clostridium sp. specifically cleave the protein of SNARE complex [Soluble NSF (N-ethylmaleimide Sensitive Factor) Attachment protein Receptor]. The SNARE complex serves to release of neurotransmitters chemicals in synapses. Cleavage of these proteins by BoNT inhibits the neural signals thus resulting in paralysis followed by death of the insect. Cry toxin from Bacillus thuringiensis is also under research to serve as chemical insecticide. Cry toxin forms pore on cell membrane which lyse the cells results in death of insect (e.g. butterflies and moths; beetles and weevils and mosquitoes). In the mosquito's genome cart, several SNARE genes have been identified which encode different proteins have been reported, which could be the possible targets for Botulinum neurotoxin mediated cleavage. Outcomes from this work will help us to enhance our knowledge and understanding towards the mechanisms of neurotransmission in malaria vectors. Identification of SNARE proteins cleavage sites and their respective neurotoxin binding receptors will open opportunities to develop novel synthetic insecticide candidates targeting the neural system of mosquitos.

Spatio-Temporal Analysis of Malaria Endemicity in Visakhapatnam District, Andhra Pradesh, India

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Malaria is a fast emerging infectious disease that has peaked to an alarming rate in recent years, which is transmitted through the bites of infected female *Anopheles* mosquitoes. According to the latest reports there were 212 million cases of malaria in 2015 and 429 000 deaths globally. Malaria is a serious concern in most of the developing countries including India. Visakhapatnam is one of the major districts in the State of Andhra Pradesh receiving highest incidences of malaria. Malaria situation in Visakhapatnam since 1961 is under closed surveillance of National Malaria Programmes. Malarial incidence reduced dramatically with the help of National Antimalarial Programmes, till the resurgence in 1970's. Recent outbreaks in 1999 with the gradual increase of malarial cases till date in Visakhapatnam demands to study associations between demographics and the spatial distribution of disease for the prevention and control of malaria. To understand the epidemiology and spatial distribution of malaria, a geographical information system (GIS) based retrospective study using spatial statistical tools was conducted in the Visakhapatnam district of Andhra Pradesh, India during 1999-2014. The goal of this study is to stratify priority areas for malaria control in Visakhapatnam district. Quantitative analysis of spatio temporal data was performed using Spatial Statistics Tools like Global Moran's I index and Getis-Ord Gi^* and Spatial distribution maps of disease prevalence were developed at mandal level using ArcGIS 10.2.2 software. The analysis shows a constant presence of high risk areas (hotspots) of malaria infection like mandals of G K veedhi, Koyyuru, Dumbriguda and Araku valley, whereas Anakapalli, Kasimkota and Munagapaka are reported as cold spots of malaria incidence. Specific interventions targeting mandals with severe malaria epidemic are urgently needed.

Nano-Metallic Formulation as New Integrating Approach to Control Mosquito Vector

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Probability of upcoming epidemic of disease has been increased with the emergence of insecticide resistant mosquito species. Vaccines to mosquito borne diseases are yet to be established, due to the complex nature of parasites and viruses. In order to resist disease transmission, vector responsible for it should be controlled and larval stage of mosquito is mostly targeted to limit its population at the primary level. With the observation of antibacterial properties of silver nanoparticles, its larvicidal properties were also evaluated. Nanoparticles synthesized with the help of plant tissue extracts are now an eco-friendly approach to control mosquito vector population. The nano-metal formulation with different composition gives distinct mortality rates in laboratory test setups. Experimental data and results will be discussed with reference to monmetallic and bimetallic nanoparticles. In the present study we have synthesized nano-formulations with the help of plant tissue extract and the characterization study was done via visible colour change analysis, UV-Visible spectroscopy, electron microscopy etc. To test the larvicidal properties of nano-formulation standard protocol prescribed by WHO (2005) was followed and different test concentrations along with positive and negative controls in replicates were established from which lethal concentrations were determined with the help of probit method. In the conclusion we can say that the synthesized nano-metal formulations where effective against mosquito larvae in the laboratory setup.

Indigenous Medicinal Plant Extracts as Larvicidal Agents against Vector Mosquitoes

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Malaria, filariasis, dengue and chikungunya are prevalent in Tamil Nadu, India and their control depends largely on preventive measures against mosquito vectors. The present work aimed to investigate the larvicidal and oviposition deterrent effects of methanolic extracts from (PPLME) the

leaves of *Pongamia pinnata* against the mosquitoes *Anopheles stephensi* and *Culex quinquefasciatus* as a biological control means. The larval mortality was observed after 24 hrs. The LC_{50} (PPLME) values calculated were 386.50, 456.60 and 479.85 ppm against 2nd, 3rd and 4th larvae of *An. stephensi* and 695.15, 885.15 and 1065.68 ppm against 2nd, 3rd and 4th larval instars of *Culex quinquefasciatus*. The extracts showed oviposition deterrent effect against both species. Results reveal the crude methanol extract of *Pongamia pinnata* and possesses remarkable larvicidal and oviposition deterrent properties against both the tested species.

Insecticides Susceptibility Status of Invasive *Aedes albopictus* Collected from Endemic Districts of Odisha, India

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Asian tiger mosquito *Aedes albopictus* is currently the most invasive mosquito species in the world. Keeping in view the wide emergence of insecticide resistance, it is imperative to focus on the current susceptibility status for various insecticides in *Aedes albopictus* population. The present study is focused on understanding the mechanism of resistance in different populations of *Aedes albopictus* collected from endemic districts of Odisha. Insecticide resistance was evaluated by using standardized bioassay kits (WHO) and biochemical analysis. Mortality data were utilized to calculate lethal concentrations of exposed individuals. Alteration in activity of detoxification enzymes among susceptible and field populations were assessed by ANOVA. Larval bioassays revealed highest level of resistance form Jajpur population with RR_{50} of 15.3 and LC_{50} of 1.177ppm compared to LC_{50} of 0.077 for the susceptible strain LabS. Results indicated the presence of DDT resistance in majority of adult populations supplemented by elevated levels of GST. Altered activity of non specific esterases and P450s indicated resistance to organophosphates and pyrethroids. Molecular screening for common insecticide target-site mutations revealed the presence of the "knock down resistance" at high frequency. These findings revealed, *Aedes albopictus* from historical and new dengue sensitive areas displayed significant difference in their susceptibility to tested insecticides which could be probably due to prolonged exposure of insecticides in historically sensitive areas. In conclusion our study is first of its kind in investigating insecticide susceptibility status of *Aedes albopictus* in Odisha. It revealed the resistance of *Aedes albopictus* to temephos (larvae) and DDT (adults). This study is significantly important to develop management strategies and selection of suitable insecticide for mosquito control in endemic areas.

Larvicidal Properties of plant extracts of *Eucalyptus citriodora, Syzygium aromaticum* and *Ocimum tenuiflorum* against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*

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Vector borne diseases are highly pathologic in nature, mosquitos are one of the vectors which spread infectious diseases such as malaria, dengue, chikungunya, zika, etc. The synthetic insecticides have been used for long time in vector control. However their non-degradable in nature and creates immense disturbance to the environment. Hence, the research of vector control is shifted from synthetic to natural plant based insecticides. In the present study, we aimed to analyse the effects of various plant oil products against various species of mosquitoes such as *Anopheles stephensi, Culex quinquefaciatus and Aedes ageypti*. The plants used were, *Eucalyptus citriodora* (leaves), *Syzygium aromaticum* (buds), *Ocimum tenuiflorum* (leaves). The chosen parts of plants such as leaves, buds were subjected to oil separation by Clevenger method. Further, the active components of oil were analysed by GC-MS. It was found that the oil extract of above mentioned plants contains major components of Eucalyptol, Eugenol, Caryophyllene Oxide, Humulene, Phenol, Napthelene and α-Cubebene. These oils were tested against mosquito larvae using WHO test procedure to find out larvicidal activity. The mortality rate were observed after 24 hrs exposure. It was found that the *Syzygium aromaticum* and *Eucalyptus citridora* were found to be having high larvicidal activity in all three species. Hence, we propose that these plant extracts could be used as oil based insecticide against the mosquito larvae instead of synthetic insecticide.

Mosquito larvicidal and adult emergence inhibition effects of flower and seed extract from *Pongamia pinnata* against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*

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Mosquitoes act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya, filariasis, encephalitis etc., The continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non target organisms. Application of plant extracts derived active toxic agents as an alternative mosquito control strategy was available from ancient times. These are not toxic, easily available, biodegradable and show broad spectrum target specific against different species of vector mosquitoes. The present work aimed to investigate the larvicidal and adult emergence inhibition effects of methanolic extracts from flowers (PPFME) and seeds (PPSME) of *Pongamia pinnata* against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* as biological control means. The larval mortality was observed after 48 hrs. The LC_{50} and LC_{90} (PPFME) values calculated were 489 and 896 ppm against fourth instar larvae of *Culex quinquefasciatus*, 429 and 861 ppm against *Aedes aegypti* and 367 and 744 ppm against *Anopheles stephensi* respectively; LC_{50} and LC_{90} values of PPSME calculated were 437, 877; 412, 853 and 379, 768 ppm against IV instar larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* respectively. 50% of adult emergence inhibition (EI 50) were with PPFME 464.605, 429 and 367 ppm against 3rd larvae of the three mosquitoes the larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The extract inhibited pupal formation significantly. Results reveal that the crude extracts (PPFME & PPSME) of *Pongamia pinnata* possesses remarkable larvicidal and adult emergence inhibition properties against the three tested species; detailed results will be discussed.

Development of Indigenous Long-lasting Insecticidal Mosquito Net (LLIN), *Defender Net*™ for Protection from Mosquitoes

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Mosquitoes are irritating/annoying disease pests causing physical, psychological and monetary loss to the mankind. But they are harming more by spreading deadly and highly debilitating diseases like malaria, dengue, chikungunya, west Nile fever, JE, etc. in India and elsewhere and transmitting diseases to over 700 million people with millions of deaths annually world over. They also harm diary animals, horses and poultry birds. Therefore safer and sustainable, efficacious methods for mosquito control are being developed by DRDO. One of them is LLIN (Long lasting insecticidal net) named it as *Defender Net*™. LLIN developed by incorporation a chemical formulation including of synthetic pyrethroid (s) in the yarn of HDPE at the manufacturing stage and remain effective up to 6 years and even after 20 washings against adult mosquitoes of all important disease vectors in India.

LLIN repel and kill those mosquitoes which land on LLIN to bite inside sleeping person and is much better than the conventional net in efficacy and in service life. LLIN has been proved to be effective in reducing contact between man and mosquito. World Health Organization (WHO) has recommended the LLIN in malaria control as an important component. *Defender Net*™ is extensively field tested in the malaria endemic and non-endemic areas by the CRME, Madurai and DRL, Tezpur (Assam) after evaluation in the laboratory for its bioefficacy, toxicity and safety. Its technology is transferred to the Indian industry for commercialization.

Role of Physical Parameters on the Efficacies of Silver Nanoparticles against *Aedes aegypti*: Green Technology against Vector Mosquitoes

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Nanotechnology has played a tremendous role in the area of vector control technologies. Biosynthesis of the nanoparticles with the plant tissues is an environment friendly approach to control the vector mosquitoes of public health significance. *Aedes aegypti* is causative agent of various diseases of global health concern such as Zika, Dengue, Chikungunya. In the present study, we have synthesized the silver nanoparticles using the Aloe vera (*Aloe barbadensis)* leaf extracts by altering the two significant parameters temperature and pH. These nanoparticles have been characterized through Microscan reader, X-ray diffractometer and transmission electron microscopy. The effect of the altered key growth parameters was investigated over the efficacies of the Silver nanoparticles. It was observed from the study that by manipulating the parameters (temperature and pH) during the synthesis process of the Silver nanoparticles significantly affected the reduction of the mother compound into the nanoparticles which was validated by observing the absorption peaks of the $AgNO_3$ colloidal solution. By decreasing the concentration the change in the pH occurred, this resulted in the increase in the absorbance and the produced nanoparticles were found to be more potent against the target mosquitoes than the colloid of lesser concentration. Similarly the increases in the temperature lead to the decreased absorption peak which showed the reduction in the extracellular production of the Silver nanoparticles. These synthesised nanoparticles with change in the temperature, when tested against the target species exhibited a considerable change in their efficacies, increased efficacy with decrease in the temperature during the process of synthesis. With this study we assume that the alteration and manipulation of the growth parameters play a key role in the efficacies of the nanoparticles, and they can be proved very significant in vector control.

Changing Malaria Vector Species Composition in Jharkhand, India

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A change in landuse pattern has changed the dynamics of malaria in many parts of India. There is impact on distribution of mosquitoes species due to change in habitat type. The present study (2013-2016) was focused on three major districts of Jharkhand *i.*e Giridih, Ranchi and Latehar. These districts were selected for collection of mosquito species on the basis of ecology types and in the areas of change in ecology due to anthropogenic activities like foothill, plain, urban, forested, riverine, stream, dam etc. were selected for the study. Entomological surveys were carried in different seasons in these areas. During 2013-16, both adult and immature mosquitoes were collected using hand catch, total catch, light trap, evening collection, landing collection, larval collection from indoor/outdoor, human dwelling/cattle shed by standard WHO. During the study 56,760 mosquitoes of 18 species of Anopheles were collected. Of the vectors, *An. culicifacies* (32.0%) was most dominant followed by *An. fluviatilis* (6.7%), *An. stephensi* (0.2%) and *An. minimus* (0.2%). Among other non-vector species, *An subpictus* (17.5%) *An. annularis* (14.4%), *An. pallidus* (10.2), *An. vagus* (8.9%), *An. jeyporensis* (3.4%), *An. nigerrimus* (2.5), *An. splendidus* (1.7%), *An. barbirostris* (0.6%), were also recorded. Species collected from cattle-sheds was found significantly higher than human dwelling in all villages of three districts. The abundance of *An. culicifacies* in the almost all habitat suggest that the habitat is modified which might be favoring this species. Ranchi was found to be having 18 species (100%) of total collected species followed by Latehar (16 species) and Giridih (14). Among the habitat types forested habitat was found to be most species rich (17 species; Richness Index (RI)=16.89) followed by Plains (16 species; RI=15.9), foothills (16 species; RI=15.8) hill tops were least species rich (14 species; RI=13.8).All the habitats were dominated by *An. culicifacies. May be due to the deforestation and appearance of more plain that might be favoring this species* There was a change in distribution pattern of *An. culicifacies* it is now a dominating vector species, However, it has established itself long back in 1925 as per Cristopher but not found in abundance, Similarly, currently *An. stephensi* is increasing in plains which is an indicator of urbanization and in this area. The evenness of habitats has been altered due to excessive anthropogenic activities in Jharkhand. The present study suggest that there is a considerable change in distribution pattern of vectors such studies are important for planning species specific approaches for control of malaria in such district with vide varieties of eco-variations.

Vapor Toxicity of essential oil of coded plant nimr17 against mosquito vector *An. Stephensi*

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Essential oil was extract through hydro distillation using clevenger apparatus for six hours of coded plant nimr- 17 and screened against malaria vector *An. stephensi*. Ten percent solution of essential oil was prepared in acetone as a solvent. 100 micro-liter solutions were taken from 10% solution and spreading uniformly on the whatman paper, 5.5 diameters in circular shape. It was placed on bottom of the Petridis and covered by the net. The use of this net was keep filter paper out of reach from the mosquitoes and exposure time 3 minutes for one hour. The results showed that the

essential oil was more effective for vapor toxicity of mosquito vectors even at 10% doses evoking 92% mortality. Percent protection obtained against *An. stephensi* 92%, 88% and 84 at the dose of 10%, 5% and 2% vapor toxicity. The results demonstrate that the essential oil is potential tools for the mosquito control.

Combination Studies of Biological and Chemical Pesticide for Effective Mosquito Vector Control

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Development of pesticide resistance is a major hurdle in use of chemical pesticides for vector control that leads to high doses of different chemical pesticide which eventually leads to environmental adverse effects. Although use of biopesticide like Bt is the major substitute for chemical pesticides, however due to UV –visible light sensitivity and low environment stability due to enzyme mediated degradation, leads to resistance. These problems direct to designing of new strategies to fight against pesticide resistance. The use of multi target compound combinations was advocated for resistance free pesticides. The combined formulation of chemical and biopesticide may be a major solution of the problem but compatibility of pesticide is a major hurdle. In this investigation, local isolate of *Bacillus thuringiensis* was tested for its compatibility with various chemical pesticides i.e. Temophos, deltamethrin, cyhalothrin, cypermethrin and malathion. Of these, BTSV2 showed the compatibility with Temophos while other pesticides were utilized by BTSV2. Temophos + Bt (TBT) study revealed the synergistic effect with reduction in LC50 value and mortality time. Besides TBT combination show change in biochemical profile of mosquito larvae. The study revealed that the combination of these two pesticides reduces dose of chemical pesticides as well act as multitargetting controlling agent for mosquito larvae.

Characterization and Establishment of a Reference Deltamethrin and Cypermethrin Resistant Tick Line (IVRI-IV) of *Rhipicephalus (Boophilus) microplus* in India

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The problem of ticks and tick borne diseases is a global threat and growing reports of resistance to commonly used insecticides further aggravated the condition and demands for country specific resistance monitoring tools and possible solutions of the problem. Establishment of standard reference is prerequisite for development of monitoring tools. For studying possible role of different mechanisms involved in development of resistance in *Rhipicephalus (Boophilus) microplus* population and to develop newer drug to manage the problem of resistance, a deltamethrin exposed and selected tick colony, referred to as IVRI-IV, was characterized using reference

susceptible IVRI-I tick line as control. The RF values of IVRI-IV ticks against deltamethrin, cypermethrin and diazinon were determined as 194.0, 26.6, 2.86, respectively, against adults. The enzyme ratios of 2.60, 5.83, 3.77 and 2.24, were observed for α-naphthol esterase, β-naphthol esterase, GST and monoxygenase, respectively, with a comparatively higher activity of Est-1 esterase. Comparative analysis of IVRI-I and IVRI-IV carboxylesterase gene sequences revealed 13 synonymous and 5 non synonymous mutations, reported for the first time. The C190A mutation in the domain II S4-5 linker region of sodium channel gene leading to leucine to isoleucine (L64I) amino acid substitution was also detected in the IVRI-IV population. In the present study, monitorable indicators for the maintenance of the reference IVRI-IV colony, the first established deltamethrin and cypermethrin resistant tick line of India, were identified.

Larvicidal Potency of *Manilkara zapota* Seed Protein Extract Against Dengue Vector *Aedes aegypti*

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Mosquitoes are known to cause several diseases including dengue, malaria etc., to the human. Control of mosquitoe by using synthetic insecticides caused severe diverse effects to the environment and mammals. The present study was aimed to test the larvicidal activity of crude protein extract from *M. zapota* seed against all four instars of *Aedes aegypti* larvae and non target cladoceran (*Moina* sp.). The crude protein extract was prepared in 0.9 % saline and series of concentrations (1-10 mg/mL) were tested against first to fourth instars larvae of *A. aegypti* and mortality was recorded after 24 h. The highest mortality 100 % was observed at 6-10 mg/mL against first instar, 7-10 mg/mL against second instar, 9-10 mg/mL against third and fourth instar larvae after 24 h. The LC_{50} and LC_{90} value of *M. zapota* protein extract showed 2.64-4.68 mg/mL and 6.2-8.33 mg/mL against all instars of *A. aegypti* larvae. Moreover, the protein extract not showed any mortality against non target organisms of fresh water cladoceran (*Moina* sp.). Hemagglutination assay results revealed that the crude protein extract of *M. zapota* seed have ability lysis the erythrocytes of cow with hemolytic activity of 128 titer value. The result obtained from the present study suggested that *M. zapota* seed protein extract showed significant larvicidal activity against dengue vector *A. aegypti* and it can be used as low-cost and eco-friendly molecules to control mosquitoes.

Identifying the Normative Beliefs on Using Indoor Residual Spraying (IRS) as per Guidelines among Residents of Mewat District, Haryana

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Objective: To know the normative beliefs about using indoor residual spraying (IRS) as per guidelines among residents of district Mewat, Haryana. **Methods:** The study was part of a larger community based interventional study and was carried out in district Mewat of State Haryana. In-

depth interviews were conducted among 35 purposively selected heads of households who voluntarily agreed to participate in the study. Data were collected through interviewing the participants using a schedule / open-ended question guide after obtaining the verbal informed consent. Content analysis was done and the various normative beliefs that could influence using the IRS as per guidelines were identified. The study was approved by Institutional Ethics Committee. **Results:** Some of the identified normative beliefs with respect to using IRS as per guidelines were – daughters or daughter in laws, young children in the family, family members other than daughters/daughter in laws and young children, Maulvi ji/ religious leaders, neighbors, villagers other than neighbors, male Panchayat members, females Panchayat members, etc. **Conclusions:** The normative influence beliefs are known as identified above. The identified beliefs of the residents needed to be quantified, and adopting the appropriate strategies, they need to be changed in the desired direction wherever required so that IRS may be accepted and used as per guidelines.

Evalution of Larvicidal Effects of *Annona squamous L.* against *Aedes aegypti L. Anopheles stephensi* Liston and *Culex quinquefasciatus* Say

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Mosquito larvicidal activity and phytochemical screening of various solvent acetone, chloroform, hexane, petroleum ether and methanol extract of leaves and fruits of *Annona squamosal L.* belong to the family Annonaceae have been evaluated against the fourth instar larvae of *Aedes aegypti L. Anopheles stephensi* Listen and *Culex quinquefasciatus* say.Each solvent curde leaf and fruit extract was tested to determine the larval bio efficacy by making serial dilutions ranging from 1 to 5%.Among the extracts, methanol leaves and fruits extract showed maximum activity against *Ae. aegypti*. All the Extracts exhibited 100% mortality on *Ae.aegypti* at 5% concentration in 24th of inclusion. Among the species studied. The highest sensitivity if 4th instars larvae of *Ae. aegypti* to the methanol leaf and fruit extracts were also evident by their lowest LC_{50} and LC_{90} values. In the case of *A. Stephensi*, and C. *quinquefasciatus*, the maximum of 75% and 60% mortality of larvae respectively was observed in methanol leaf and fruit extracts. The least susceptibility of larvae of all the three mosquitoes was observed in petroleum ether is evident by the higher LC values.It is suggested that the leaves and fruits of *A. squanosa* possess larvicidal properties that could be developed and used as nature bio insecticides for *A. aegypti* mosquito control.

Role of Mobile Technology in the Management of Vector-Borne Diseases

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In spite of various control measures, vector borne diseases such as dengue and chikungunya are persisting problem due to untimely intervention in lack of innovation in control program. Presently, time delay in reporting is a major issue in intervention as most of data related to the surveillance and control of vectors relies heavily on paper-based field data collection. Transcribing large amounts of data from paper-based field records into a computer-based system is a time-consuming, tedious, and potentially error-prone undertaking. Therefore, there is an urgent need for introduction of improved tools such as Mobile Applications (Apps) for data management and analysis for early detection and prompt action and reducing time delay. Identification of the species of Anopheline mosquitoes is of paramount importance for the planners and administration involved in mosquito control operations in the field of malaria. To understand the responsible vector for disease transmission of disease, normally an experienced taxonomist is required who would go systematically through taxonomic characteristics of the species and try to identify it. In this reference several couplet keys were developed earlier which can identify up to 54 mosquito species against the present fauna of 58. A pictorial key by Nagpal *et al.* describing all the 58 species was developed. The problem with all the keys is that for identification, the user has to jump over different pages and at any point a slight oversight may result in misidentification of the species.Therefore, there is an urgent need for introduction of Apps for field data management and identification of mosquitoes which would be useful for entomologists, researchers, technicians and public health workers. National Institute of Malaria Research is in the development process of these Apps which will also serve a useful training tool for health workers in different organizations.

Impact of Deforestation on the Vectors of Malaria in North-Eastern States of India

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The unprecedented growth due to various development imperatives in India has affected the micro climate on a large scale resulting into ecological succession of the flora and fauna. In a recent study, it was observed that due to rapid and uncontrolled urbanization and developmental activities, forest cover is gradually declining. The forest cover in the NE region is about 64% of the geographical area and during the years 1993-95 maximum reduction was recorded. Forest cover in Assam got decreased by 32.87% during the period 1974-2012; in Meghalaya, it got decreased till 2003 and then gradually increased during 2008-09; in Manipur, it got decreased by 2.46% during the period of 1997-2013 while in Sikkim it got decreased by 52.66% during the period of 1988-2013. As deforestation of densely forested areas has drastically changed the bio-ecology of the vectors, hence a study was conducted to assess the impact of deforestation on the vectors of malaria in four endemic North-Eastern States of India viz. Assam, Meghalaya, Manipur and Sikkim in villages which had observed evidence of change in landaus pattern. Entomological and epidemiological surveys in pre and post monsoon seasons were conducted in 176 villages of 8 districts. In each district, 15 to 20 villages were selected based on earlier surveys carried out and according to different types of topography. In this study, it was observed that total deforested area in selected states namely in Assam was 560.98sqkm, Meghalaya 5.22sqkm, Manipur 1.77sqkm and Sikkim 1.56sqkm. However, in these four selected districts of Assam there was high land conversion (deforestation) for residential and for other developmental activities viz. in Lakhimpur (26.2sqkm), Nagaon (320.56sqkm), Sonitpur (200.28sqkm) and Goalpara (13.91sqkm). While in two districts of Meghalaya i.e., East Garo hill (2.31sqkm) and East Khasi hill (2.91sqkm) deforestation was occurred. In Chandel district of Manipur, deforested area was 1.77sqkm only while in East Sikkim district of Sikkim, 1.56sqkm area was deforested. Impact of deforestation was observed on the distribution and density of vector species. A total of 19327 specimens of 29 Anophelines were collected in which vector species *An. culicifacies* (93.4%) was found to be most dominating followed by *An. minimus* (4.2%), *An. fluviatilis* (1.7%) and *An. dirus* (0.63%) which is in contrast with earlier studies (before 2000), in which it was recorded that *An. minimus* (98.5%) was most dominating species followed by *An. fluviatilis* (0.6%), *An. culicifacies* (0.56%) and *An. dirus* (0.25%). Due to change in land use and other ecological changes the population of *An. culicifacies* has increased in all four NE States which should prompt the policymakers of malaria control to develop new control strategies for this region in light of new species composition.

Community Awareness, Perceptions, Acceptability and Preference for Using LLIN against Malaria in Rural Parts of Uttar Pradesh, India

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Long-lasting insecticidal nets (LLINs) have been introduced in India recently into the vector control programme. A study was undertaken to assess the community perception regarding use of LLIN, their acceptability and collateral benefits, in certain villages of District Gautam Budh Nagar, Uttar Pradesh, India where LLINs are in use by the community. A randomized community-based survey was undertaken covering 596 respondents in LLIN villages where LLINs have been distributed along with 307 respondents in untreated net villages where untreated nets were distributed using structured questionnaire. A substantial number of respondents had good knowledge of the symptoms of malaria. According to respondents, LLINs were very much effective in bringing down the malaria incidence in their families. About 98.3% of the respondents asserted the use of LLINs as their use not only reduced the number of mosquitoes as well as other non-target insects, but also reduced the malaria incidence. About 93.2% of the respondents were ready to purchase LLINs if available at nominal prices. All the respondents were satisfied about the performance of the LLINs in reducing the mosquito nuisance, safety of use and collateral benefits in LLIN villages. LLINs are safe, socially acceptable and should be promoted for vector control to reduce the disease burden in the communities.

Progress in Tick Control Strategy: Focus for the Management of Tick-Borne Diseases in Animals

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The importance of ticks has been realized due to its potential to transmit variety of pathogens like viruses, bacteria, protozoa and helminthes of human and animals. Ticks and tick borne diseases (TTBDs) affected 80% of the world cattle populations and tick-borne pathogens are believed to be responsible for more than 100,000 cases of illness in humans throughout the world. The TTBDs are ranked high in terms of their impact on livelihood of resource poor farming communities in developing countries including India. The annual cost of control of TTBDs of cattle in India has been estimated to be US$498.7 million (more than Rs.3000 crores). The different species of ticks involved in transmission of deadly pathogens *viz*, *Babesia* spp., *Theileria* spp., *Hepatozooan* spp., *Cytauxzoon* spp., *Aegyptianella* spp., many *Rickettsia* spp., *Francisella* spp., *Coxiella* spp., *Borrelia* spp. and several viruses includes, Nairovirus, Thogotovirus, Flavivirus, Asfavirus to animals. Many of

the tick vectors are involved in maintenance of infection and transmission of zoonotic pathogens to human where clinical form of diseases are not recorded in animals. Kyasanur Forest Disease (KFD) and Crimean-Congo Hemorrhagic fever (CCHF) are the wonderful example in this regards. The tick management is mainly focussed on use of chemical acaricides, but wide spread use has led to the development of multi-acaricide resistance, making acaricide use ineffective and limiting the available options of vector management. Further, adverse effect of acaricides on public and ecosystem triggers development of eco-friendly sustainable control methods. Accordingly, research is focusing towards development of phyto-acaricides and vaccines. The phyto-acaricides research has gained some momentum after getting funding support from funding agencies and a few effective antitick molecules were identified. However, there are a number of steps to be travelled before exploitation of the antitick molecules in suitable delivery format for effective application in the field situation. A number of candidate vaccine molecules have been identified in different tick research laboratories of the world but in most of the cases the antigens were not found to be cross-protective. Recently, five conserved molecules were characterized by RNAi technology and initial data were found to be encouraging. It is hypothesized that effective utilization of phyto-formulation and vaccine in integrated format will definitely help in tackling the multi-acaricide resistance scenario.

Castor Oil Nanoemulsion and Its Larvicidal Activity against Malaria Vector, *Anopheles culicifacies*

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Malaria is a vector borne disease caused by Plasmodium parasite that is transmitted to humans through the bite of the Anopheles mosquito leading to epidemic diseases in countries of Southeast Asia including India. The incidence of malaria in India accounted for 58% of cases in the South East Asia Region of WHO. Effective treatment and vector control are the only effective measure for eradication of malaria. There are various methods adopted by national vector borne disease control programme for vector population management: Source reduction, larvicidal/ biocontrol and adulticidal. Larvicidal control is advised in urban area only because here we have to focus on certain specific and small area with our pesticide, whereas in the case of adult control we have to consider large area. Unlike rural areas where both adulticidal, IRS and larval control are used. There are various synthetic larvicidal formulations available to control the larvae but they have resulted in the problem of insecticide resistance, non biodegradability and may pose toxicity to non target organism. The problem of persistent toxicity along with the growing incidence of insect resistance, has led to the shift from synthetic to green pesticide. Castor oil (*Ricinus communis*) is one of such potential green pesticide which is reported to have ant termite activity, various industrial and medicinal uses. We have formulated a castor oil (*Ricinus communis*) based Nano emulsion for large field breeding mosquitoes like *Anopheles culicifacies*. The stability of nanoemulsion was observed for 14 days at Room temperature and ATS (Accelerated temperature Studies) and was found to be stable. The average size of the nano droplet was found to be 80nm. This small size results in better delivery and stability of emulsion. We have screened the formulation for its efficacy against *Anopheles culicifacies* larvae using ICMR 2012 protocol. We have found that 10ppm of nano emulsion has resulted in 100% mortality within 24 hours, Lc_{50} was found to be 4 ppm. We have made botanical formulation which is biodegradable environment and user friendly. There is a need to conduct further studies on the mode of action of the formulation and its behavior with other synergistic compounds making this formulation as good candidate of bio larvicide for field application.

Green Synthesis of Silver Nanoparticles Using *Andrographis paniculata* Leaf Extract against *Aedes aegypti* in Thanjavur District

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Mosquitoes transmit serious human diseases, causing millions of deaths every year. Mosquito control is to enhance the health and quality of life of county residents and visitors through the reduction of mosquito populations. Mosquito control is a serious concern in developing countries like India due to the lack of general awareness, development of resistance, and socioeconomic reasons. Today, nanotechnology is a promising research domain which has a wide ranging application in vector control programs. These are nontoxic, easily available at affordable prices, biodegradable, and show broad-spectrum target-specific activities against different species of vector mosquitoes. In the present study, the activity of silver nanoparticles (AgNPs) synthesized using *Andrographis paniculata* plant leaf extract against first to fourth instars larvae and pupae *Aedes aegypti* was determined. Synthesized AgNPs and methanol leaf extract were prepared in various concentrations and tested against the larvae of *A. aegypti*. The synthesized AgNPs from *A. paniculata* leaf were highly toxic than crude leaf methanol extract in mosquito. The results were recorded from UV–Vis spectrum, Fourier transform infrared spectroscopy, scanning electron microscopy, and energy-dispersive X-ray spectroscopy analysis. Larvae were exposed to varying concentrations of aqueous extract of synthesized AgNPs for 24 h. The maximum mortality was observed in synthesized AgNPs, and methanol leaf extract of against *A. aegypti*. These results suggest that the use of *A. paniculata* synthesized silver nanoparticles can be a rapid, environmentally safer biopesticide which can form a novel approach to develop effective biocides for controlling the target vector mosquitoes.

Impact of Indoor Residual Spray on Sand Flies in Two Endemic Districts of Bihar, India

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Visceral leishmaniasis (VL) also known as Kala-azar in India is transmitted to man by *Phlebotomus argentipes*. In Indian subcontinent, Bihar state is endemic to VL. The main tool currently used in Bihar to control sand fly populations is Indoor Residual Spraying (I.R.S) using Synthetic Pyrethroids (S.P) in houses and cattle sheds. IRS is performed two times in a year. A Study was initiated for a year in twenty four villages, in two districts of Bihar, to study the impact of I.R.S using S.P. on sand flies. In each village twelve CDC light traps were installed in three types of locations which include houses, cattle dwellings, and vegetation. In each location type 4 traps were installed. Traps were activated every two weeks for 12 hours. Sand flies trapped in the trap baskets were transferred to the lab in Patna, identified, and recorded as to species of sand flies. Our trapping was started in February 2016 and will be continued up to January 2017. Sand flies were identified using morphologically and also using molecular techniques. All blood meals were identified from

trapped blood fed sand flies. We also tested sand flies for Leishmania parasite using molecular techniques. Of the 24 villages selected, 16 villages received IRS using S.P and 8 villages did not receive any spray. Impact of IRS on sand fly numbers were studied between IRS villages and non IRS villages and also sand fly numbers were compared between before and after IRS in these villages. Earlier studies suggested sand fly behavior was changed, they are more exophilic now, compared to past. So we need a new tool to control sand flies which are outside of houses and cattle sheds for achieving elimination of VL in India.

Effects of α-Amyrin Acetate a Novel Bioactive Compound on Oviposition Behavior, Egg Hatchability, Survival Rates and Its Synergism with Deltamethrin Against *Anopheles stephensi* (Diptera: Culicidae)

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Selective discriminating behavior of the ovipositing female for an appropriate oviposition habitat selection and the substances involved in oviposition site choice by vector mosquitoes have recently become a focal point of interest in the concept of integrated vector control management. In the current study, we isolated and identified α-amyrin acetate from *Catharanthus roseus* Linn (Apocynaceae) and assessed the oviposition deterrency, ovicidal, gravid mortality and deleterious delayed mortality against the malarial vector *Anopheles stephensi* Liston (Diptera:Culicidae). Water treated with the α-amyrin acetate had a high deterrent activity in ovipositing females: oviposition activity index values for the test species were –0.22, –0.38, –0.42 and –0.52 for α-amyrin acetate at concentrations of 0.007, 0.015, 0.025 and 0.050 p.p.m., respectively. High degrees of mortality were observed with various concentrations of α-amyrin acetate: 1.12 (control) to 7.20 for gravid females, and 0.62 (control) to 9.05 for oviposited females. The highest mortality in both gravid and oviposited females was observed soon after they came in contact with oviposition medium treated with the α-amyrin acetate, and this was found to be significant at doses higher than 0.015 p.p.m., suggesting possible contact toxicity of the α-amyrin acetate. The α-amyrin acetate caused moderate ovicidal activity against various age groups of *A. stephensi* but it inflicted delayed effects such as high larval, pupal and adult mortality. The age of the eggs and the duration of the α-amyrin acetate treatment influenced the ovicidal activity observed. It is clear that α-amyrin acetate of C. *roseus* can affect the oviposition cycle of *A. stephensi* Liston, thereby suppressing the vector population and adversely influencing transmission of the disease pathogen.

Larvicidal Efficacy of Certain Botanicals against Three Species of Mosquito Vectors

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Mosquitoes present an immense threat to millions of people worldwide, since they act as vectors for devastating pathogens and parasites, including malaria, filariasis, yellow fever, dengue, chikungunya and zika virus disease. The misuse and overuse of synthetic pesticides have led to the vast destruction of beneficial organisms along with detrimental effects on the environment. Botanicals are a promising alternative for mosquito control because of lower toxicity to non-target organisms and their innate biodegradation ability. In the present study, the crude hexane, diethyl ether, dichloromethane and ethyl acetate extracts of seven plants *viz*., leaves of *Abutilon indicum*, *Cleistanthus collinus*, *Leucas aspera*, *Murraya koenigii*, aerial parts of *Hyptis suaveolens*, whole plants of *Citrullus colocynthis* and *Sphaeranthus indicus* were tested for their toxicity against the third instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* at concentrations of 250, 500, 750 and 1000mg/L. Mortality was observed 24 hours after treatment. Amongst the different solvent extracts of the seven plants tested against *Anopheles stephensi*, ethyl acetate extract of *Leucas aspera* (LC_{50} 352.84mg/L) was found to exhibit high larvicidal activity. In the case of *Culex quinquefasciatus*, hexane extract of *Hyptis suaveolens* (LC_{50} 203.37mg/L) showed higher activity. While for *Aedes aegypti*, the ethyl acetate extract of *Sphaeranthus indicus* (LC_{50} 201.11mg/L) exhibited the highest activity. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use botanicals to control the immature stages of vector mosquitoes.

Efficacy of *Diospyros melanoxyl* Extracts against *Anopheles stephensi* and *Culex quinquefasciatus* Mosquitoes

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The objective of the present study is to determine the bio efficacy of different crude extract of *Diospyros melanoxylon* against anopheline and culicine larvae. Larval susceptibility of crude n-hexane, ethyl acetate, methanol extracts of *Diospyros melanoxylon* leaves was observed against the malaria vector, *Anopheles stephensi* and the filariasis vector *Culex quinquefasciatus*. Among the extracts tested, n-hexane and methanol was the most effective and showed LD50 and LD90 value of 1.837 and 2.818ppm against the larvae of *Anopheles stephensi,* and LD50 and LD90 value 1.837 and 2.818ppm for *Culex quinquefasciatus* respectively. So it is concluded that methanol extract of *Diospyros melanoxylon* can be developed as potent larvicide.

Efficacy of Plant Extracts as Potential Mosquito Larvicides

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Botanical pesticdes are alternative to synthetic pesticides due to eco- friendly and non-toxic to parasites and predators, less polluting and cost effective. Screening of ten indigenous plants of south-eastern region revealed the mosquito larvicidal properties. The plants are *Calotropis gigantea Ocimum sanctum*, *Spilanthes acemela*, *Moringa oliefera*, *Mentha arvenis*, *Leucus linifolia, Tagates patula*, *Curcuma amada*, *Lantana Camera* and *Citrus reticulate*. The larvalcidal bioassay tests were carried out for ether extraction of leaf of *Calotropis gigantea* and roots of *Morninga*

oliefera, while steam distillation was further carried out in Cleavenger apparatus for the rest eight plants against third instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* in laboratory conditions. Lethal concentration values for each plant for both the species were calculated. LC 90 of ether extract of *Calotropis gigantea* was found to be most effective with values being 20.50 ppm and 30.7ppm for Cx. *quinquefasciatus* and *Aedes aegypti* respectively. The LC 90 values of rest nine species for the above two species were *Ocimum sanctum* (80.5ppm and 70.6ppm), *Spilanthes acemela* (90.9 ppm and 100ppm), *Moringa oliefera* (120ppm and 130ppm), *Mentha arvenis* (200.5ppm and 200.95ppm), *Leucus linifolia* (200.5ppm and 230.5ppm), *Tagates patula* (250ppm and 260.5ppm), *Curcuma amada* (280.8ppm and 270.6ppm), *Lantana Camera* (290.5ppm and 295ppm) and *Citrus reticulata* (300.05ppm and 310ppm). Out of 10 plant species screened in the present study, ether extract of *Calotropis gigantea* (leaf) was found to be the most effective. The study is going on for isolation of active compound which can be developed as a potent larvicide.

Evaluation of Mosquito Larvicidal Activity of *Jasminum* Species (Olaceae) Crude Flower Extracts against *Aedes aegypti* Linnaeus and *Culex quinquefasciatus* Say (Diptera: Culicidae)

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India is endemic to mosquito-borne diseases which are major health problems in tropical regions. Chemical pesticides have been used for several decades in controlling pests and vectors of various human diseases but resulted in several problems such as resistance and resurgence of pests, affecting human health and ecosystem being disrupted leading to the threat that their continued use may further harm the environment. This necessitated the need for environmentally safe and biodegradable indigenous method of vector control. Plant products are considered to be a potential alternative approach as they are environmentally safe, target specific and biodegradable. In the present study, the crude hexane and chloroform flower extracts of *Jasminum auriculatum*, *Jasminum grandiflorum* and *Jasminum officinale* were tested for the larvicidal efficacy against the third instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* at concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000mg/L. Mortality was recorded after 24 and 48h. Amongst the crude flower extracts of *Jasminum* species tested, the crude chloroform extract of *Jasminum grandiflorum* was found to be effective against *Aedes aegypti* and *Culex quinquefasciatus* showing 100% mortality at 1000 and 500mg/L respectively. The LC_{50} values were 300.47 and 212.10mg/L after 48h respectively. Further investigations are needed to elucidate the larvicidal activity of *Jasminum grandiflorum* crude chloroform flower extract against a wide range of all stages of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

Green Synthesis of Silver Nanoparticles Using *Syzygium cumini* (*Eugenia jambolana*) Seeds: Mosquitocidal, Anti-Malarial Activity (*in vivo* and *in vitro*) and Its Toxicity Effects on *Gambusia affinis* Predators

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Malaria is a disease caused by *Plasmodium* parasites and transmitted through bite of female *Anopheles* mosquitoes. Malaria is a serious global public health concern. The widely utilized of synthetic pesticides leads to harmful effects on medical and veterinary environment. Recently, plant based synthesized nanoparticles have been proposed as highly effectiveness in the control mosquito borne diseases. In this research, we synthesized silver nanoparticles (AgNP) using the *S. cumini* seed kernel extract as reducing and stabilizing agent. AgNP were characterized by UV–vis spectrophotometry, TEM, EDX, XRD, FTIR spectroscopy and ZETA potential. The *S. cumini* seed kernel extract and AgNP was toxic against immature stages of *Anopheles stephensi* (larvae and pupae), LC_{50} were 194.10 ppm (larva I), 214.08 ppm (II), 233.94 ppm (III), 256.54 ppm (IV), and 297.43 ppm (pupa). AgNP LC_{50} were 16.92 ppm (I), 18.93 ppm (II), 21.58 ppm (III), 24.27 ppm (IV), and 28.92 ppm (pupa). The antiplasmodial activity of *S. cumini* seed kernel extract and AgNP was evaluated against CQ-resistant (CQ-r) and CQ-sensitive (CQ-s) strains of *Plasmodium falciparum*. IC_{50} of *S. cumini* seed kernel extract were 63.42 µg/ml (CQ-s) and 69.12 µg/ml (CQ-r). *S. cumini* seed kernel synthesized AgNP achieved IC_{50}, of 84.18 µg/ml (CQ-s) and 87.24 µg/ml (CQ-r). However, in vivo antiplasmodial experiments conducted on *Plasmodium berghei* infecting albino mice showed moderate activity of the *S. cumini* extract and AgNP. In continuation, the predation efficiency of *Gambusia affinis* against *A. stephensi* I to IV instar larvae was 90.8 to 77.4 %, respectively. In AgNP-contaminated environments, predation was 97.4 to 81.8 %, respectively. Finally, we are carried out to investigate the acute and sublethal toxicity of *S. cumini* extract and AgNP on hematological and biochemical variables of a mosquito fish *Gambusia affinis* under laboratory conditions. Overall, this study showed that the *S. cumini* extract and mediated fabrication of AgNP is of interest for a wide array of purposes, ranging from IPM of mosquito vectors to the development of novel and cheap antimalarial drugs.

CHAPTER 11

NEGLECTED VECTOR-BORNE DISEASES TARGETED FOR ELIMINATION

A Perspective of Prevention and Control of Vector-Borne Diseases

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India is world's biggest democracy, second most populous country with extreme diversity and seventh largest by area, federal republic with parliamentary system having Health as a State Subject, consisting of 29 States, 7 Union Territories, 640 Districts and 640,930 villages. Various health programmes are implemented under Primary Health Care system for which technical guidance, policy formulation and logistic support are provided by centre whereas infrastructure and implementation is done by states. At independence in 1947, about one-fourth of country's population was estimated to suffer from malaria with 75 million reported cases and 0.8 million deaths annually. Launching of National Malaria Control Programme (NMCP) in 1953 and National Malaria Eradication Programme (NMEP) in 1958 was done exclusively for malaria which was made an integrated 'National Vector Borne Disease Control Programme' (NVBDCP) which is an umbrella National Health Programme for prevention and control of six VBDs viz., Malaria, Kala-azar, Lymphatic Filariasis, Dengue, Chikungunya and Japanese Encephalitis (JE). Out of these 6 VBDs, 5 are mosquito-borne, whereas, Kala-azar is transmitted by Sand fly. Three VBDs i.e. Malaria, Lymphatic Filariasis and Kala-azar are targeted for elimination. There has been remarkable success in reducing disease burden due to malaria, filaria and kala-azar during last decade by tackling various challenges through innovations and integrating various strategies with newer tools.

Malaria elimination has been targeted by 2030 for which a National Framework for Malaria Elimination was released by Hon'ble HFM on 11.2.2016. The whole country has been divided into 3 Categories (1-3) and the group of States falling in different Categories will be brought into Category-0 in phase manner ensuring that there is no indigenous transmission. The lowest endemic States falling in Category-1 are 15 and are targeted to bring down to Category-0 by 2020. Similarly, the Category-2 and Category-3 States will be subjected to intensified control measures so as to convert Category-3 into Category-2 and Category-2 into Category-1. These are targeted by 2026 so that in last 3 years, the efforts will made to sustained the zero indigenous transmission. The formulation of strategic plan for 3, 7 and 15 years respectively has been initiated for short-term, mid-term and long-term execution. The vector control strategy for prevention and containment will be maximum in Category-3 including IRS, LLIN, larviciding, Biological control and Environmental Management. Similar strategy will also be implemented in Category-2 with more focus on outbreak containment with focal spray. The Category-1 may not require regular round of insecticidal spray but the states will be prepared for focal spray to liquidate the foci in addition to other activities like source reduction and biological control. All the groups of states will be under entomological surveillance and advocacy for all VBDs including Malaria under Integrated Vector Management (IVM).

Lymphatic Filariasis and Visceral leishmaniasis are among other vector borne diseases targeted for Global/ Regional elimination. The success achieved in reducing the population at risk due to these diseases has been remarkable especially in relation with the magnitude.

Dengue is the most rapidly spreading vector borne infection and has become a major concern as it is being reported from all the states/UTs. Chikungunya is another very important arboviral disease transmitted by same mosquito which transmits dengue. This was included under programme subsequent to the re-emergence in 2006. Zika threat is also there as the same vector mosquito transmits it. These are made notifiable diseases in many states which facilitates in getting reports from private sectors too. Diagnostic facilities have been increased three times in last 5-6 years.

Japanese Encephalitis (JE) among arboviral diseases is known for outbreaks from different regions. Though the case fatality rate has declined in last few years due to improved diagnostics and health facilities, the number of cases in adults is the main concern.

In addition, other VBDs like Scrub Typhus (Mite borne), Tick typhus, Kyasanur Forest Disease (KFD), Crimean Congo Hemorrhagic Fever (CCHF–Tick borne), Plague (Flea borne), Cutaneous Leishmaniasis, Chandipura viral fever, Sandfly fever (Sand fly-borne), Lyme disease and other vector-borne viral, bacterial, and parasitic diseases, putting millions of people at risk for infection have also attracted attention in recent times and limited to areas of reports.

The recent spread of VBDs throughout the world including India are linked with globalization and modern transportation which also facilitates in spread of genetically changed virus strains with greater virulence. VBDs also cause significant economic impact in agriculture due to the importation of vectors and pathogens through crop movement (For Zoonotic diseases), and increased movement of animals and products. Moreover, poverty, social inequalities, and behavioral issues add to contributing factors in spread of VBDs. Breeding sites are now ubiquitous because of the proliferation of plastic bottles, cans, old tyres and earthen pots etc providing congenial sites for container-breeding mosquitoes in domestic and peri-domestic areas complicating source reduction and anti-larval operations.

From the success and challenges, it is a fact that preventive mechanism is far better than the cure. VBDs are directly linked with sanitation, therefore if it is linked with Swatch Bharat Abhiyan, Education, and dissemination of information through newspaper, social networking sites, it will bring a change. Further involving bureaucratic level at district, sub-district and block level etc for public meetings on these issues will boost the energy of programme and commitment will be transformed into ownership. NVBDCP has intensified entomological surveillance through existing state, zonal, district entomologists. The support is also taken from research organizations. Universities are being roped in this endeavor.

NVBDCP has further initiated work on concept of 'National Mosquito Control Mission' emphasizing its relevance to Public Health by giving relief to Public Health Programme through controlling the vector mosquitoes including nuisance mosquitoes thereby improving quality of life.

Lymphatic Filariasis Elimination: Microfilaria prevalence in tea garden worker population and vector infection status in endemic and non-endemic districts of Assam

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Lymphatic Filariasis (LF) is endemic in the state of Assam. Seven endemic districts of Assam are under Mass Drug Administration (MDA). After completion of six rounds of MDA, we have carried out LF prevalence survey in a tea estate covered under MDA of Dibrugarh district during 2015 and another tea estate not covered under MDA from a non-endemic district, Tinsukia of Assam during 2016. Over all microfilaria (mf) prevalence rate in tea estate worker population covered under MDA was 7.41 %, recording predominance in males (10.54%) than females (3.82%). In children of 2-9 yrs, mf rate recorded was 2.06%. Entomological collection and dissection revealed vector (Cx *quinquefasciatus*) infection and infectivity rates 13.20% and 3.70% respectively. In another tea estate, considered as non-endemic, overall mf prevalence rate recorded in 2016 survey was 4.30% having predominance in males 5.37% compared to females 3.40%. In children of 2-9 yrs mf rate recorded was 1.59%. Vector infection and infectivity rates were 5.88% and 1.96% respectively. Findings suggest that in both the situation, LF transmission is continuing. Unlike other states of India, LF is not uniformly distributed in the population of Assam. Microfilaria rate is fairly low (<1%) in urban population as well as in rural population. It is the tea garden workers population among the rural areas which accounts for LF burden. Hence, this target population needs full coverage of MDA for considerable period. This situation is unlike the hotspot areas of other states of India where such condition can be dealt under focal MDA. Assam has >765 tea estates across the state which constitute over 30 lakhs of workforce engaged in tea industry. At present, only 33% of tea estate population is under MDA coverage. Possibly, biannual MDA if adapted may hasten programme and make LF elimination successful in the state of Assam.

Malaria Targeted for Elimination in India: Roadmap and Challenges

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Malaria still continues to be one of the major public health problems in South East Asia and Africa Region. According to the latest WHO estimates, released in December 2013, there were about 207 million cases of malaria in 2012 (with an uncertainty range of 135 million to 287 million) and an estimated 627 000 deaths (with an uncertainty range of 473 000 to 789 000). The vision of Global Malaria Action Plan (GMAP) is of a world free from the burden of malaria. It is expected by GMAP that by 2015, the malaria-specific MDG is achieved, and malaria is no longer a major cause of mortality and no longer a barrier to social and economic development and growth anywhere in the world.

India malaria programme is also committed to achieve these goals and putting in all the efforts with the help of stakeholders i.e. WHO, Global Fund and World Bank. Currently, India has adopted stratified control strategies as the Annul Parasite Incidence (API) though is less than one per 1000 population, it varies widely from high (>10 per 1000 population) to very low (<0.1) in different states/Union Territories. But, it has planned in 12th Five Year Plan to reach pre-elimination state by the year 2017.

The incidence of malaria in India started halting and sustaining reversal of cases for last one decade. The malaria cases were brought down from 2.03 million cases in 2000 to 1.816 million in 2005 and further brought down to 1.067 million cases in 2012 and to 0.882 million in 2013. Country has achieved 56.6% reduction in incidence of malaria cases against the baseline (2000). It is important to note that out of this, there was 21.18 % reduction in 10 years (till 2010), and remaining 35.42% reduction has occurred in just three years when there was intensive inputs of Rapid Diagnostic Tests, Artemisinin Combination Therapy and Long Lasting Insecticidal Nets in the high risk areas covered under two projects supported by World Bank and Global Fund which showed the effectiveness and impact of these inputs. The API wise distribution of the states/union territories in year 2006, and 2014 reveals that the total number of States/UTs with API <1 has increased from 16 to 26. During 2014, out of 677 reporting units for malaria in the country, 527 units (78%) are showing annual parasite incidence less than one.

Malaria elimination is defined as zero incidence of locally acquired malaria infection through active control measures with continued measures in place to prevent re-establishment of transmission. Malaria transmission can be reduced within short to moderate timeframe with the implementation of proven malaria control measures bringing the malaria levels to very low levels. This can be the starting point for planning malaria elimination programmes with the aim to bring down it further up to zero level by 2030. Based on the intrinsic malaria burden, the levels of programme coverage that can be achieved and the available financial investment, assessment of possible reduction in transmission of malaria is required to be done carefully to evaluate whether elimination or reduction in malaria is feasible and appropriate goal or not in a specific area. The technical, operational and financial feasibility would be the essential components of malaria elimination strategy for the country.

All the States / UTs have been stratified into Category 1: States with less than1 API including all the districts in the state with less than 1 API, Category 2: States with less than 1 API with few districts reporting more than 1 API and Category 3: States with more than 1 API with either all the districts with more than 1 API or few districts with less than 1 API and few with more than 1 API. Area specific micro action plans and strategy would be implemented keeping in view the overall objective of case detection, complete treatment and vector control.

High level political support and involvement with good collaboration between sectors involved (agriculture, education, immigration, irrigation), community participation, cross border collaboration and adequate financial support from domestic budget as well as international funding sources will be required for it. The programme would require local committement, ownership and timely implementation of programme activities with strict monitoring and supervision. Roadmap towards malaria elimination included dissemination of NFME 2016 - 2030 to all States and UTs , release of Operational Manual for Malaria Elimination 2016, formulation of National Malaria Elimination Committee (NMEC) and National Malaria Elimination Taskforce (NMET), situation analysis for assessing feasibility of malaria elimination in states and UTs and making malaria a notifiable disease.

Further, actions have been initiated for the preparation of Strategic Action Plan for Malaria Elimination (2017-2022), estimated financial requirements for the first phase of malaria elimination activities, meeting with stakeholders for malaria elimination (May- June, 2016) , sensitization Meetings with states and UTs, finalization of State and UT action plans for malaria elimination, review the need for a revised Malaria Information System.

The key challenges include the difficult hilly and forested terrain and outreach areas, climatic conditions, drug and insecticide resistance, multiple vectors, migratory population, conflict areas, deforestation, developmental projects. Malaria Elimination in India is feasible with all concerted ongoing efforts thereby overcoming the challenges faced by the programme.

Lymphatic Filariasis in India: From Control to Elimination

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Lymphatic filariasis is one of the neglected tropical diseases caused by parasitic filarial worms *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. This infection is mosquito-borne and known from ancient period with significant social and economic consequences for affected individuals their families and communities. It is commonly known as elephantiasis. Many of the countries had control strategy of treatment of patients and feasible vector control either vertically or in synchronization with malaria control for collateral benefits. Global elimination of this disease was targeted and 83 countries were enlisted as endemic which later revealed that 73 countries needed to be covered with preventive chemotherapy for interruption of transmission. In South East Asia Region (SEAR), out of 9 countries only 7 are LF endemic out of which India contributes more than 40%. The disease is endemic in 21 states and UTs of India leaving areas like North-Western States/UTs and North-Eastern States. India has mainly *Bancrofti*an filariasis (99.4%) transmitted by the *Culex quinquefasciatus* whereas *Brugian filariasis* transmitted by *Mansonia* is limited in small pockets of Kerala and stray cases from odisha. Diurnal sub-periodic *W. bancrofti* infection is prevalent in Nicobar Group of Andaman & Nicobar Islands. This is transmitted by O*chlerotatus (Finlaya) niveus* group of mosquitoes formerly classified as *Aedes (Finlaya) niveus*.

Indian experience towards control and elimination of LF is very rich. The first pilot project in the world was taken up in Odisha for LF control during 1949 to 1954 using five day mass DEC therapy @4mg per kg body weight, indoor residual spray and anti larval measures. The project revealed a reduction of about 30% in transmission individually by each method. National Filaria Control Programme (NFCP) was launched in 1955 for the control of bancroftian filariasis to delimit the endemic areas, control measures in selected areas and develop skilled human resource. The programme assessment resulted into paradigm shift like withdrawl of mass DEC therapy and IRS due to poor coverage and precipitation of resistance among vectors and restricting weekly anti larval operations in urban areas. Selective DEC therapy to microfilaria carriers at a dose of 6 mg per kg body weight per day for 12 days was introduced.

Following the consultative meeting of WHO/CTD/TDR at University Sains Malaysia (22-24 August, 1994), recommending mass distribution programme should completely replace selective treatment, a two day WHO sponsored workshop to formulate guidelines for revised strategy for control of LF in India was organized on 4-5 January, 1996 at Delhi (jointly by NICD now known as NCDC and NVBDCP). The piloting in 13 districts of 6 states was started with revised strategy of annual mass drug administration with DEC in 1996. However, World Health Assembly Resolution in 1997, targeting this disease to be eliminated with India as one of the signatory led to formulation of global programme to eliminate lymphatic filariasis (GPELF). The commitment given by the then Union Health Secretary as Chair in the first meeting of global alliance in 2000 at San Diego led the National policy 2002 document with a target to achieve by 2015 though he announced that interim target is to be set before 2020 which is the goal for global elimination. To realize the goal, the revised strategy of annual MDA was expanded to all endemic states/UTs in 2004. The districts were selected as implementation unit (IU) on historical evidence of filaria endemicity, presence of Lymphodema and hydrocele cases and/or presence of microfilaria carriers. The MDA campaign was launched by Union Minister for Health & Family Welfare and by Union Minister of State for Health & FW on 5th June 2004 which was subsequently changed to 11th November considering the convenience of states and extreme climatic conditions in summers. Home based morbidity management and scaling up of hydrocelectomy were also initiated as 2nd pillar of the strategy. The programme while scaling up also introduced strip packing for DEC tablets as distribution of loose tablets was one of the issue for low drug compliance. The strategy of co-administration of DEC and Albendazole as decided by National Task Force in 2006 was also scaled up since 2007. The coverage of MDA has improved from 72% in 2004 to 89% in 2015. The overall microfilaria prevalence reduced from 1.24% in 2004 to 0.4% in 2015. The flexibility to the states was considered for implementation period of MDA which was extended from 1 day to 1 week and in certain states it was extended for about a month to ensure the drug compliance.

Drug compliance assessment of MDA was initially done which revealed large gap between reported coverage and actual drug compliance. This activity was therefore made as in built component of programme by involving medical colleges and research institutes to give insight of programme which also indicated the gap and accordingly programme improved the compliance in subsequent years in many states. Measuring the impact of MDA was initiated using Transmission Assessment Survey (TAS). Based on WHO guidelines, the National guidelines were developed for states to conduct TAS for which about 290 officials have been trained. The Indian Programme to eliminate LF is one of the largest programme with various challenges. Many of the challenges were already known through research and experience of scientists and health programme officials which were tackled, however, some of them are still being faced as challenge in different areas.

The successful examples of tackling challenges are: service providers reaching to the people of about 600 million, Mobilization of resources, capacity building of varying degree, creating movement for LF elimination and providing treatment even to the districts with population at risk with borderline microfilaria prevalence i.e. below 1%. This was done with the objective that no known foci should be left. The results were encouraging and districts from south, west and northeast started showing reduction of mf prevalence. Efforts made since 1996-97 on pilot basis and since 2004 on massive scale to cover about 600 million population with annual mass drug administration have shown the successful results in about 200 million population. The remaining population is yet to be subjected for TAS and part of it may show impact but hard core areas are the major challenge. The main issues are suboptimal drug compliance, irregular MDA in addition to people's perception, inadequate priority at grass root level and fatigue in implementation.

The focused attention can be paid if areas with actual risk in a large population is identified and to do so smaller units of 5-7 lakhs (2-3 blocks) can be formed for assessment and those requiring can be subjected for MDA annually or twice in a year along with National deworming campaign. DEC double fortified salt as adjunct with ongoing MDA in hard core districts is another alternative. The combination of ivermectin with DEC plus Albendazole is under pilot and it can be another choice but the key to success will be actual intake for which round the year, programme needs to focus both on drug compliance and morbidity management.

Elimination of Lymphatic Filariasis: Improved Consumption during Mass Drug Administration – The Key Factor for Success

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Lymphatic Filariasis (LF) caused by parasite *Wuchereria bancrofti* in India and transmitted by mosquito vector, *Culex quinquefasciatus* leads to manifestations in which, the infected person may develop swellings of limbs and genitals, which keep on increasing and making the person incapacitated. The patient, besides physical trauma, also becomes a victim of social stigma. Additionally such patients suffer from frequent attacks of lymphangitis, high fever, swelling and pain. There is no cure for the patient at this stage and person is forced to live with huge swellings exposed to secondary infections. National Filaria Control Programme has been in operation in India since 1955 with detection cum treatment and vector control strategy. However, India launched the elimination campaign in 2004, in pursuit to achieve its global elimination goal with main objective to progressively reduce and ultimately interrupt the transmission. To prevent and reduce disability in affected persons through disability alleviation and morbidity management was also augmented. In Karnataka state of south India, 9 districts were identified for MDA in 2004 and national strategy of ELF was intensified in these districts. Karnataka is one of better performing state especially with better governance in al health programmes. Three of the nine districts have also shown desired impact on microfilaria prevalence so as to stop MDA. An analysis of a) the reported MDA coverage and b) assessed drug compliance done independently over the years 2005 to 2015 was done to understand why only 3 out of 9 districts has shown promising results.

Though a gap between reported drug coverage and compliance has existed throughout these years, our study has shown that the gap has decreased consistently over these years. District wise analysis indicated that three districts have average consumption ranging between 65-89% which in other districts ranged below 65%-the cut off criteria of consumption at community level. The districts with low consumption could not demonstrate mf prevalence below 1% individually in all sentinel and spot-check sites thereby not qualifying for Transmission Assessment survey. This indicates that even in better performing states with better governance, results may vary and therefore intensive efforts and vigilant monitoring are required to achieve the goal of elimination.

Evaluation of Annual Mass Drug Administration in Chennai Tamil Nadu, India

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Chennai Corporation was known to have low endemic pockets for bancroftian filariasis adjoining waterways, Cooum river and other areas with polluted water. Since there was no filaria clinic to screen the patients as a routine, the surveillance was carried out by the Urban Health post as well as the Health staff which revealed low mf prevalence. The positive cases were treated with 12 days course of DEC at 6 mg/ kg body weight. The study done by Hyma et al (1989) from 1974–1986 in LF clinics in the city of Madras (Chennai), indicated disease prevalence with varying extents though there was no alarming situation for Lymphatic Filariasis. To avoid further risk due to expansion of the city and considering the adjoining districts under MDA, five rounds of annual Mass Drug Administration (MDA) from 2004 to 2012 was carried out in the endemic pockets covering a population of 3.46 lakhs. MDA could not be implemented continuously and was missed in 2005, 2006 , 2008 and 2011. The reported coverage ranged from 88.5% to 92.1% in different MDA rounds. Impact assessment in sentinel and spot-check sites has shown that in none of the sites microfilaria (mf) prevalence was more than 1%. Transmission assessment survey (TAS) was conducted in 2015 as per WHO protocol and National guidelines to make a decision whether to stop or to continue MDA. The target population of children 6-7 years of age was 44768 enrolled in 481 schools. School based survey was selected and Survey Sample builder tool was used for sampling school clusters and number of children to be tested. A total of 1843 children from 35 schools were screened for filarial antigen using ICT. 2 children were found positive for filarial antigen. As the number of positive children was below the critical cut off of 20, this evaluation unit (EU) qualified for stopping further rounds of MDA. Investigations of the two positive cases showed that one migrated within 2-3 years from neighboring districts and the other had history of visiting filariasis endemic areas. The area is under post MDA surveillance.

Human Trypanosomiasis in India

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Human trypanosomiasis encompasses two important disease conditions-human African trypanosomiasis, restricted to sub-Saharan Africa, and Chagas disease, restricted to central and South American countries. These diseases are restricted to specific geographical locations due to restricted distribution of the vectors responsible for their transmission. In the past two decades, a number of non-tsetse transmitted trypanosomes have been recorded in humans in Asian countries including India. Out of these, nine cases were reported from India. In all these cases, animal trypanosomes, viz., *Trypanosoma evansi* and *T. lewisi* have been incriminated as the causal agent and both of the organisms are transmitted by blood sucking arthropods. These animal trypanosomes are highly prevalent in domestic, wild and synanthropic animals. In spite of high prevalence in animals, humans never contact the disease due to innate immunity or host specificity of the parasites. However, there is now enough evidence that these trypanosomes have the ability of jumping species barrier and can establish in unrelated host species. Moreover, as the cases of HIV-AIDS increase, the chance of opportunistic infection with animal trypanosomes also increases. At least four cases have been reported from Maharashtra, two from Madhya Pradesh, one each from UP, Gujarat and West Bengal.

Current Scenario of Lymphatic Filariasis Elimination Program in Madhya Pradesh, India - Issues and Challenges

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To eliminate lymphatic filariasis (LF) as public health problem mass drug administration (MDA) is being carried out in 250 filariasis known endemic districts since 2004 in India in line with the global elimination of lymphatic filariasis by 2020. It is assumed that with >65% compliance mf prevalence will reduced to <1% and transmission cease to occur. In Madhya Pradesh MDA is being given in 11 districts and 12 rounds of MDA have been completed in Dec 2015. To assess the impact of MDA a cross sectional mf survey was also carried out in Panna district after 12th round covering three of the four sentinel sites, two spot check sites and two random sites in Nov 2016 and compliance rate was assessed as per the NVBDCP guidelines after 11th and 12th annual round of MDA in four districts. Overall MDA coverage (received drug) was 77.7% in 11th round while the corresponding rate was 69.3% in 12th the round. Highest coverage (99.5%) was recorded in Chindwara district followed by district Damoh (93%). MDA compliance rate was highest in district Chhindwara 75.7% in 11th round followed by district Damoh (72.8%). Consumption rate was > 65% in 11th round in three districts while in 12th only district Umaria had >65% compliance rate. Consumption rate was below <65% in Katni district in both the rounds. Overall mf rate was 6.9% (range 0.8% - 13.6%). Mf rate was 3.5% in sentinel sites, 10% in spot check sites and 8.8% in random sites. In panna district only 34.8% of the population consumed the drug in 12th round of MDA which does not leave any impact on mf prevalence. Poor compliance of MDA is the issue in LF elimination which need to be addressed to achieve the elimination goal.

Arboviral and Rickettsial Diseases with Confusing Presentation- A Common Panacea in Sight!

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India accounts for 21% of world global disease burden of infectious diseases and has highest burden of neglected tropical diseases (NTDs) in the world. Amongst the NTDs enlisted in the WHO list, vector borne diseases (VBDs) has remained a priority for elimination strategies in South Asia, including India. In India, a huge proportion of VBDs are caused by arboviruses. However, only Dengue has been listed under the WHO's list of NTDs. Apart from Dengue, other viral disease viz., Japanese encephalitis (JE), West Nile (WN) and Chikungunya have been able to gather attention in India as NTDs since the past few decades. More recently, rickettsial diseases viz., scrub typhus, spotted fever group and typhus group rickettsiae have been reported from different zones of the country. Rickettsial disease is considered as re-emerging disease in India, however, the lack of actual data on the true prevalence, lack of diagnostics facility and unawareness of its presence has envisaged as one of the most NTDs in current period.

Currently in India, JE has been the leading cause of acute encephalitis syndrome (AES). Among the AES cases, besides JE, scrub typhus (ST) occupies the second place with regards to causing high morbidity and mortality especially in Northeast (NE) India. Other AES etiologies causing substantial morbidity in the NE India are West Nile (WN), Leptospirosis, Spotted fever group rickettsiae (SFGR) and Typhus group rickettsiae (TGR). Despite constant implementation of disease control strategies like the use of Deltametrin treated long lasting nets, mass JE vaccination programmes among children (also included among routine immunization programme) and adults, vector control programmes, etc., there has been no success with regards to drug discovery to cure patients suffering from JE or West Nile. Although there has been a decline in the number of JE cases post vaccination, yet there remains a huge burden of AES cases to combat due to the confounding clinical presentation of the major AES etiologies. The absence of distinguishable clinical features among the contributing AES etiologies makes it difficult to diagnose the disease without laboratory investigations leading to delay in treatment. Therefore, it necessitates the incorporation of a more effective universal module of point to care diagnosis and treatment at health care levels to combat the disease burden.

Doxycycline, a tetracycline group of drug is recommended for treatment of scrub typhus and other rickettsial infections as well as Leptospirosis. Doxycycline is known for its bacteristatic effect which inhibits protein synthesis. An in vitro study was conducted to evaluate the antiviral activity of Doxycycline against JE. This study showed doxycycline inhibits JE virus pathogenesis by affecting virion structure and altering viral replication in a dose-dependent manner. This in vitro findings were also supported by an in silico study where virtual screening of JEV NS3 helicase/NTPase and candidate drugs (mainly kanamycin and doxycycline) showed favorable interactions in motifs I, II and VI of helicase suggesting possible viral replication inhibition.

Doxycycline has minimum side effects in children and pregnant women only. Therefore, this drug has the potential for treating major AES etiologies in adults. Inclusion of this drug along with the empirical treatment for AES might be instrumental in bringing down the case load caused by JE, ST, SFGR, TGR and Leptospirosis in endemic regions. Adopting a universal module for inclusion of Doxycycline in the treatment agenda of AES should be implemented from national to the local health care levels. This requires the sensitization among the clinicians about the presence of NTDs and also of use of Doxycycline as a therapeutic drug for treating febrile and AES cases in endemic regions. Recording of occupation details and ecological background of the attending patients, and identifying the persons from agrarian ecosystem could be a vital source for disease suspicion especially for rickettsial infections.

Prevalence of *Babesia* Parasite and Its Vector in Cattle from Southwest Gujarat, India

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Babesia is an intra erythrocytic apicomplexan protozoa parasite transmitted by a tick vector causes extensive economic loss to the farmer/dairy owner in the form of morbidity and mortality in animal. In India, babesiosis independently, accounts for annual losses to the tune of US$ 57.2 million. Usually, diagnosis of babesiosis is based on clinical signs and microscopy where, clinical signs of babesiosis are characterized by fever, anaemia and haemoglobinuria in infected animals. But, both methods have the limitations of their inability to detect the subclinical and carrier animals. On the other hand, PCR assays has added advantage to diagnose the parasite at levels far below the detection limit. Accordingly, the present study was conducted to assess the status of *Babesia* spp. infection among cattle population from southwest Gujarat by both microscopic examinations (ME) and Polymerase Chain Reaction (PCR). Randomly 144 blood samples collected from cattle were subjected to ME and PCR to compare the efficacy of two tests. While blood collection, animals were thoroughly examined for any tick infestations. ME revealed prevalence of *Babesia* spp. to be 22.81% in cattle. On the contrary, PCR assay was able to detect 40.97 % prevalence of *Babesia* spp. in cattle. The result shown that the PCR assay was 100 % sensitive and 82.9 % specific when compared with ME for babesiosis. On the other hand, 62.32 % of the cattle were found infested with *Rhipicephalus (Boophilus) microplus* ticks. Observation revealed that, 10.23 % of animals were have high intensity of infestations, 34.34 % with moderate intensity of infestations and 55.43 % with mild intensity of infestations. The investigation reveals the concurrent latent infection of babesiosis in bovines and presence of vector population in south western Gujarat.

Available Surveillance Tools for Routine Surveillance of Mite-Borne Scrub Typhus Disease Vectors

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Scrub typhus is a chigger borne typhus *Tsutsugamushi fever* an infectious bacterial illness caused by *Orientia tsutsugamushi*. Humans are accidental hosts in this zoonotic disease. An estimated one billion people are at risk for scrub typhus and an estimated one million cases occur annually. Scrub typhus is a re-emerging disease in India and an increasing trend of case incidence has been noticed in many states. However, data pertaining to the disease is not available. Though scrub typhus is considered as an important emerging public health problem, no systematic study has been conducted to map either the vector mites or the circulation of the causative agent O. *tsutsugamushi*. It is required to map the occurrence and distribution of different scrub typhus vector mites in different regions to demonstrate the circulation of the causative agent O. *tsutsugamushi*. In the vector surveillance chiggers will be collected and identified. Mite diversity, abundance and vector species can be identified. Chigger mites act as the primary reservoirs for O. *tsutsugamushi* and monitoring of these vectors is conducted by different methods. Chiggers can be collected from field

directly on human beings by walking in the field wearing stockings. Next some of the sentinel animas like white laboratory mice or rats in small cages containing food and water in the field overnight to attract the chiggers are used for the collection of Trombiculid mites from the field. The following morning chiggers can be collected from the body of the sentinel animals, from the external parts of the body of the host, usually the ears, the belly and thighs. By undertaking wild rodents trapping method using Sherman and Wonder traps are used to collect the animals from the affected areas. The other potential method without the usage of the vertebrate host is the Berlese or Tullgren funnel method which is used to segregate the mites from the sand and debris materials collected in and around the rat burrows. A light bulb is used to heat and light which will drive the mites downwards into the funnel into a container containing alcohol. A similar separator is the photoeclector or Winkler/Moczarski Elector in which a moist cloth is attached to the bottom for the separation and the mites can be preserved in 70% alcohol for identification. Chigger index 0.69 is an indicator for the implementation of vector control measures which is the average number of chiggers infecting a single host. Continuous surveillance of vector mites would help the programme personnel to identify the vector infested areas to initiate relevant early vector control measures.

Tick-Borne Diseases in India – An Epidemiological Approach

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Vector borne diseases are illness that are transmitted by vectors, which includes Mosquito, ticks and flies. Ticks as vectors of several zoonotic are ranked second only to mosquitoes as vectors. It accounts for about 20% of all emerging infectious diseases recorded up to 2004. Recent increase in Tick borne diseases includes Crimean –Congo hemorrhagic fever Virus, Kyasanur forest disease virus, Babesia spp, Theileria, Rickettsia conorii, Anaplasma marginale. Most tick- borne diseases are caused by tick borne viruses. Two major tick- borne virus zoonotic diseases, kyasanur forest disease (KFD), Crimean- Congo hemorrhagic fever (CCHF) are found in India and it shows high mortality rates. KFD was first identified in 1975 in Karnataka *Haemaphysalis spinigera* is the major vector for the cause of the disease. Human infection occurred in Bandipur through handling of dead monkeys that were infected. A human carrier was also detected in Wayanad (Kerala). It infects annually 400- 500 people. CCHF is distributed in Asia, Africa and some part of Europe; *Hyalomma* spp. Ticks are the main vectors. The existence of CCHF in India was first confirmed in 2011 in Gujarat state. In 2013, a non-nosocomial CCHF outbreak in Amreli district, as well as positive tick, animal and human samples in various areas of Gujarat state, India. Babesia (also called *Nuttallia*) is a genus of Protozoan apicomplexan piroplasms that infect the blood and cause a parasitic, hemolytic disease known as Babesiosis. In 1893, Americans Theobald Smith and Fred Kilborne identified the parasite as the cause of Texas cattle fever, the same disease described by Babeș. The first case of Babesiosis was seen in humans in 1957. The emergence of KFD and CCHF of various Indian states emphasizes the implementation of nationwide surveillance, improved diagnostic facilities and public awareness and thorough Tick control in affected areas.

Prevalence of Clinical Cases of Canine Ehrlichiosis at a Veterinary Hospital in Chennai

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Canine ehrlichiosis is a tick borne disease caused by Anaplasmataceae family of the Genus Ehrlichia. The etiological agent *Ehrlichia canis* is an obligatory intracellular parasite. It causes tropical canine pancytopenia. Canine rickettsiosis which infects leukocytes is also referred as monocytic ehrlichiosis. The brown dog tick *Rhipicephalus sanguineus* is the proven vector of *Ehrlichia canis. E. canis* transmission occurs during the warm season when the vector is active. Chronic phase of the diseases has a grave prognosis. It is exacerbated when other vector borne pathogens are concurrently present. Clinical manifestation of ehrlichiosis include fever, weight loss, anorexia, bleeding disorders and lymphadenomegaly. A total of 31 samples collected between August to October 2016 as suspected cases of canine ehrlichiosis at Madras Veterinary College Clinical department were analysed. The affected dogs exhibited symptoms of Fever (90%), Epistaxis (50%), enlargement of popliteal lymphnode (50%), respiratory distress (80%), anorexia (95%), anemia, dullness, depression with tick infestation or previous history of tick infestation as found in 100% of cases. Examination of leishman-stained peripheral thin blood smears revealed 16.12 % (5/31) positivity for the morulae of *E.canis*. The whole blood samples kept in ethylene diamine tetraacetic acid vial of canine were used for determination of haemoglobin concentration, total leukocyte count and total platelet count by utilizing a fully automated blood analyzer. Dog's hematological parameters showed monocytosis (90.32%) and thrombocytopenia {dogs with severe thrombocytopenia (<50,000 is 45.16% and dogs with moderate thrombocytopenia (50000 to 149000 is 54.83%), activated platelets (60%), hypochromasia (70.97%) among 31 dogs. Based on haematological references dogs were treated with Doxycycline 10mg/kg bodyweight for 28 days, Thrombup syrup 5ml/20 kg.bodyweight along with acaricidal treatment. *E. canis* was most prevalent in the summer followed by rainy season. It was least prevalent in the winter season. The highest infection was in adults (more than 1yr to 71/2 years) is (83.87%) and evenly distributed among all breeds (descript-25 and Non-descript- 6) of dogs and the incidence of infection has no variation among sex (12 Females and 19 males).

Black Fever: A Vector-Borne Disease: Challenges Ahead

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Black fever is a deadly disease transmitted via the bite of an infected sand fly *Phlebotomus argentipes*. Indian visceral leishmaniasis (VL) (Black Fever) is an intracellular protozoal infectious disease caused by a haemoflagellete Leishmania donovani. Black fever is caused by a protozoa called Leishmania. It usually develops over several months or years. If black fever is not treated, it can be fatal. Black fever is also called kala-azar and leishmaniasis. The parasitic life cycle of leishamania includes the sand-fly and an appropriate host. Humans are one of those hosts. According to WHO If the disease is not treated, the fatality rate in developing countries can be as high as 100% within 2 years. Post-kala-azar dermal leishmaniasis (PKDL) is a complication of visceral leishmaniasis (VL) in areas where Leishmania donovani is endemic; it is characterized by a hypo pigmented macular, maculopapular and nodular rash usually in patients who have recovered from VL. Kala-azar is a major public health problem in the areas of its prevalence, principally India and its neighbors Bangladesh and Nepal, and Brazil and Sudan. In India about 1, 00,000 cases of VL are estimated to occur annually; of these, the State of Bihar accounts for over than 90 per cent of the cases. Diagnosis of VL typically relies on microscopic examination of tissue smears, but serology and molecular methods are better alternatives currently. In India the disease is found in Bihar, Jharkhand, West Bengal and pockets of eastern Uttar Pradesh. Diagnosis and treatment of Black fever are problematic because of a variety of reasons. While treatment is lengthy and relatively costly. The Napierís aldehyde test has been used for a long time. The test relies on the jellification caused by the binding of the serum globulins to the formaldehyde. The serum globulins increase in a variety of infections and thus this test is rather non-specific.

Lymphatic Filariasis: Mode of Transmission, Diagnosis and Treatment

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In the 21st century's world, mosquito vectors (class-Insecta, order- Diptera) are the most important single group of deadly insects which cause millions of death every year by transmitting pathogens of various life threatening diseases like dengue, chickungunya, yellow fever, lymphatic filariasis, Japanese encephalitis, malaria etc. *Culex* mosquitoes, especially C. *pipiens* and C.*quinquefaciatus* are the chief vector of bancroftian filariasis caused by *Wuchereria bancrofti* in many regions of the world. *Culex* also responsible for the transmission of several serious viral diseases like Rift valley fever (RVF), West Nile virus (WNV) and Eastern Equine encephalitis. Similar to other encephalitis viruses, it is cycled between humans and mosquitoes and transmitted to man by infected mosquitoes. Lymphatic filariasis is caused by nematode worm *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. However, 90% cases of LF are only due to the worm *W. bancrofti*. LF is transmitted by the infectious biting of *Culex* mosquitoes which breeds in association with human habitations, mainly in polluted water. When a *Culex* mosquitoes bites an infected person, the worms enter into the mosquitoes body, changes its stage from L1 to L3 stage and again enter into another healthy persons body during mosquito biting. In healthy persons body worms enter into the lymphatic system where they grow, mate and form nests which leads to swelling of lymph vessels. Lymphatic filiarisis is characterized by fever, inflammation of the arms, legs and brests, hydrocele (swollen and painful scrotum) in male in its actual stage; whereas elephantiasis i.e. huge limb (due to accumulation of lymph in tissue), chyluria (lymph excretion in urine) in case of its chronic stages. For its global prevention, recently new diagnosis aspects, treatment methods and control strategies are also adopted. This paper is the outcome of collective work from different secondary sources. As paper is a descriptive one, the information used in this collected from various research journals, official reports and published information from organizations.

Information Technology in Morbidity Management of Human Lymphatic Filariasis - A Promising Tool in Global Programme for Elimination of Lymphatic Filariasis

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India is a signatory to the 1997 World Health Assembly resolution for Global Elimination of Lymphatic Filariasis. The target for global elimination of human lymphatic filariasis is by 2020. The Global Programme for Elimination of Lymphatic Filariasis has two main components viz. interrupting transmission of lymphatic filariasis through mass drug administration and managing morbidity and preventing disability due to filariasis. Consorted efforts by the Public Health Department in Tamil Nadu for elimination of lymphatic filariasis was launched in the year 1997 concentrating on both the components of the programme. The data on the prevalence of filarial morbidity and its entire management is at present based on manual reports and registers generated by the field functionaries in the public health departments. Field surveys during the study indicated that interpretation and evaluation of morbidity management activities was based on manual formats compiled as non analytical reports and stored in computers. To overcome the constraints in the manual reporting system, PHP and MySQL facilities were used to generate modules highlighting the advantages of online reporting system in morbidity management of Lymphatic Filariasis; feasible for data entry, analysis and retrieval at the Health Sub Centre Level itself. Feed back from field functionaries was encouraging and supported the implementation of such web based reporting system to achieve the goals of morbidity management of filarial lymphodema; specially after MDA is withdrawn.

Assessment of the Immunoprophylactic Efficacy of Autoclaved *Leishmania donovani* Antigen alone and Together with Different Adjuvants against Experimental Visceral Leishmaniasis

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Visceral leishmaniasis, is one of the most severely neglected tropical diseases recognized by the World Health Organization. The threat of this debilitating disease continues due to unavailability of promising drug therapy or human vaccine. An extensive research is undergoing to develop a promising vaccine to prevent this devastating disease. Overall, protection against *Leishmania* species that cause visceral leishmaniasis depends on the development of a strong T-helper 1 (Th1) type of response. Therefore, attention to the immune responses elicited by vaccine candidates needs to be carefully considered in light of the balance between the need to control parasite replication and the responses involved in pathology. Although, there is still not an effective human vaccine for leishmaniasis, new antigens, adjuvants and improved understanding of the immune factors underlining effective control of parasite replication and pathology give hope for future success. Keeping that in mind the current study was carried out using Indian strain of *Leishmania donovani* viz; MHOM/IN/80/Dd8. Inbred BALB/c mice were immunized with Autoclaved *L. donovani* promastigote antigen alone (10^7) and in combination with four different adjuvants thrice at an interval of two weeks. Three weeks after last booster mice were challenged with 10^7 live promastigotes by intravenous route. Mice were sacrificed 15 days after last immunization and on 90 post infection/challenge day. Protective efficacy of different vaccine formulations was analyzed by assessment of the parasite burden and generation of cellular and humoral immune responses. Out of four adjuvants used in the present study, liposomal encapsulation contributed maximum to enhance the efficacy and immunogenicity of antigens.

Protective Efficacy of *Codonopsis clematidea* Against Visceral Leishmaniasis in BALB/c Mice

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The increasing number of drug resistance issues of *Leishmania donovani* to common drugs compels to develop new therapeutics against leishmaniasis with minimal toxicity. The search of new drugs from natural products possessing antileishmanial activity is ventured throughout the world. Several plant extracts have been found to show leishmanicidal activity via immunomodulation. As part of a hunt for new drugs, in the present study *in vivo* antileishmanial activity of *Codonopsis clematidea* in murine model has been evaluated. Inbred BALB/c mice were infected intravenously with promastigotes of *L. donovani*. After 30 days of infection animals were administered with 500 mg/kg body wt. of hydroethanolic extract of C. *clematidea* (HEECC) orally for 14 days and checked for parasite clearance, delayed type hypersensitivity (DTH) response, histopathology, biochemical and cytokine levels. Sodium stibogluconate (SSG) and Amphotericin B were used as positive controls. The animals treated with HEECC showed a significant reduction in parasite load with elevated levels of DTH response. No biochemical alterations in hepatic and renal enzymes were observed. The efficacy was well supported by strong Th1 immune responses and significantly suppressed levels of Th2 cytokines. Histopathological studies of liver, kidney and spleen revealed normal architecture in animals treated with HEECC but some alterations were observed in SSG and Amphotericin B treated animals. Thus, protection against *L. donovani* infection in the plant treated animals was due to the induction of cellular immunity via immunopotentiation without any adverse side effects. It demonstrates that *Codonopsis clematidea* could be considered as a relatively inexpensive alternative to the currently available chemotherapeutic drugs.

Efficacy of *Rhodiola imbricata* Edgew. in Experimental Visceral Leishmaniasis

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Visceral leishmaniasis (VL) is one of the most neglected tropical diseases and is a serious public health problem on the Indian subcontinent. It is lethal due to the secondary infections if left untreated. The antiquated chemotherapeutics in use for antileishmanial therapy are related to high toxicities and cost. So new therapeutic options which are safe and less expensive are required. Natural products offer an unlimited source of new drug modules and can be used to replace toxic synthetic drugs. The current study is primarily focused to evaluate the anti-leishmanial effects of hydro-ethanolic extract of roots of *Rhodiola imbricata* Edgew., which is an adaptogenic plant. Inbred BALB/c mice were infected with *Leishmania donovani* and after 30 days the animals were treated with hydro-ethanolic extract of roots of *R. imbricata* (500 mg/kg b.wt.). In positive control groups, animals were treated with sodium stibogluconate (SSG) and amphotericin B.The control of infection was assessed in the giemsa stained impression smears of liver. Delayed type hypersensitivity responses (DTH) responses and generation of Th-1 and Th-2 cells related cytokines in the serum were also studied. Further, to check any plant related toxicities, examination of various biochemical parameters of liver and kidney and histological study of liver, kidney and spleen was performed. The extract reduced the parasite load to a significant level and generated a protective immune response as evident from the enhanced DTH responses and elevated levels of

Th-1 cytokines. Moreover, the results of biochemical tests and histology indicated that the extract has no toxic effect as compared to SSG and amphotericin B. However, it should be further explored in higher animal models for control of leishmaniasis.

Synthetic Antigenic Peptides of *Orientia tsutsugamushi* and Screening its Usefulness in Developing Point-of-Care Assay for the Diagnosis of Scrub Typhus

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Scrub typhus is a mite borne zoonotic infection of human caused by the rickettsial pathogen *Orientia tsutsugamushi*. The organism is geographically distributed throughout South East Asia and Oceanic. Scrub typhus is a re-emerging public health problem in India and resurgence of cases has been frequently reported from different parts of the country Scrub typhus is a antibiotic treatable infection, however, delayed initiation of antibiotic treatment may lead to death of the patient. Timely diagnosis is very important for initiating antibiotic therapy. In untreated cases, CFR is estimated around 30%. Scrub typhus is routinely diagnosed by Weil-Felix, IFA and ELISA tests. But none of these assays can be used as a rapid diagnostics at the bedside of patient. Hence, a simple, rapid and reliable point of care assays is very much needed for early diagnosis of Scrub typhus. There is no indigenous kit available in India. Moreover, BSL-3 facility is an absolute necessity for handling rickettsial pathogens which hampers the research efforts, since the facility is available only in few laboratories in the country. Synthetic antigenic peptides are found promising as an alternate technology in developing antigens of highly infectious pathogens and have been successfully demonstrated in developing effective diagnostics and vaccines. Considering several advantages of synthetic antigenic peptides, the present study predicted potential B-cell epitopes from certain immune dominant protein encoding genes of the prototype strains of Karp, Kato and Gilliam strains of *Orientia tsutsugamushi* available in database domain using popular bioinformatic tools *i.e.*, BCpred, BCpred AAP, FBCpred and Bepipred. The bioinformatic tools have predicted both cross reactive and unique antigenic peptides of the prototype strains studied. The custom synthesised peptides were further exploited for its reactivity and analysed the usefulness in development of diagnostic assays by screening against known positive and negative sera collected during surveillance activity throughout the district. The current study presents the preliminary results of the work done and discusses the possible application of synthetic antigenic peptides of *O.tsutsugamushi* in developing safe, rapid and reliable diagnostics for Scrub typhus and for control of the disease in India.

Rickettsia Species in Adult Engorged *Rhipicephalus heamaphysaloides* from Kerala

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Molecular evidence for the presence of a *Rickettsia* sp. from a tick infested on a bovine male calf in Wayanad district of Kerala, South India is reported. Based on morphological features, the tick species was identified as *Rhipicephalus heamaphysaloides*. The *Rickettsia* specific PCR products were amplified from cDNA synthesized from the isolated RNA of an adult female *R. heamaphysaloides*. Phylogenetic analysis of citrate synthase (gltA) and outer membrane surface protein (OmpA) gene sequences revealed that the isolate has identity with, *Rickettsia slovaca / R. marmionii,* members of *Rickettsia rickettsii* clade of spotted fever group *Rickettsia*.

Medical Entomology: A Neglected Subject in Medical Colleges in India

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Vector-borne diseases (VBDs) afflict more than half of the world's population. In India Malaria, Filariasis and Kala azar are endemic, and Japanese encephalitis, dengue and chikungunya occur in epidemic form. About 1-2 million new cases of malaria and about 1000 deaths occur every year. JE is currently endemic in 135 districts in 5 states. There are other insects that transmit pathogens to animals and from animals to humans known as zoonotic vector-borne diseases. Entomological surveillance is an important activity in VBD control besides early diagnosis and treatment. For effective management medical person and an entomologist are the two important human resources are essential in the health system. In India Health and Panchayat raj systems are responsible for control of VBDs. Under the health system the medical officers in PHCs in rural areas and health officers in urban areas are responsible for VBDs Control. An indepth review was made whether they acquire capacity building in terms knowledge and skills in medical entomology during their MBBS course. For assessing this, faculty recommended by MCI, curriculum from the standard text books of medicine and community medicine are reviewed. Five years question papers in community medicine of four universities of south India are also reviewed. Entomologist is not in the Medical Council of India (MCI) faculty list, including AIIMS, JIPMER and CMC Vellore. Some government medical colleges in TN have entomologists. Other medical colleges this subject is dealt by assistant professors who had passed out without adequate exposure in Entomology. The standard text books have also not covered adequately. Last five years university question papers in community medicine reveals that questions were asked only 1 to 5% marks. Inspite of huge VBDs problem the MCI, universities and governments are not serious about capacity building for medical students the future doctors. VBD outbreak measures are taken in a fire fighting mode by Medical Officers who are not adequately taught Medical Entomology. I recommend through this 13 th conference on vectors and VBDs to GOI, state governments, MCI and Universities to take suitable action to include Entomologist in all the medical colleges as teaching faculty to renew the "Hope For Elimination" of Vector Borne diseases in India.

CHAPTER 12

COMMUNITY INVOLVEMENT IN DISEASE CONTROL

Community Participation in Disease Control

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The community involvement in solving their problems was spontaneous when the society as a whole had extensive control over the natural resources. However, this trend was lost during the industrial revolution when the social structure started disintegrating. Simultaneous discovery of antiparasitic drugs and powerful insecticides created a euphoria that all vector borne diseases could be controlled if not eradicated. Though the use of these tools resulted in control or eradication of vector borne diseases in many parts of the developed world, in poor developing countries the initial gains could not be sustained. This is partly due to non acceptance of technology by the beneficiary. Realizing that technology without the people is not going to solve the problem, the idea of community participation was revived. Since then, community participation has become a popular slogan and most programmes claim to have achieved community participation. The projects have not originated from the community and fail to differentiate between the gain achieved due to community effort and that due to the project personnel. The technical and material inputs provided are much higher than the national programmes can afford, carried out in limited areas for short periods and sustainability never demonstrated. Community participation in vector borne disease control has considerable potential, especially in source reduction, reduction in man vector contact or accepting antiparasitic or antivector measures. True sustainable participation is possible only when the present trend of top-down planning is reversed to bottom-up planning; decision making power is handed over to the community; the people involved in conceiving, planning, implementation and evaluation of all developmental programmes and if the programmes are responsive to local needs and priorities.

Detecting the Undetected: Unblocking the Bottlenecks in Malaria Elimination

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Malaria is one of the major vector borne diseases in India with about one million cases reported annually. The strategy for malaria control includes early diagnosis, complete treatment and vector control. Since year 2007, the Indian national drug policy for malaria has switched over from presumptive therapy to treatment after confirmation and recommends investigating suspected malaria cases. Clinical diagnosis is often inaccurate and nonspecific. Microscopy has been considered to be the gold standard for diagnosis of malaria but its availability and quality are major challenges. Rapid diagnostic tests have proved to be a game changer but they are also not free from challenges. There are other tools available like fluorescent microscopy, serological and molecular techniques and have value as research tools. Non-invasive methods to diagnose malaria from samples like saliva are under evaluation.

Asymptomatic parasitaemias have a great impact on malaria transmission. It is very difficult to tap these infections since they are asymptomatic. Strategies need to be developed to detect them. Further, there is an emergence of new species causing human malaria. Tools need to be developed for diagnosis of these species.

There are steps taken by India in improving diagnosis. The national programme and NIMR have a robust QA programme for malaria RDTs. QA of Microscopy also has been revamped. Improving malaria diagnostics is needed to ensure smooth road to elimination.

Prevention and Control of Dengue by Targeting Key Containers: A Case Study in Delhi

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Dengue is emerging as one of the major health problem in Delhi, posing serious threat to public health. Since 1967, Delhi has witnessed various dengue outbreaks. *Aedes aegypti* is a principal vector of dengue virus in India. *Aedes* mosquitoes are container breeder and breeds in variety of natural and man-made containers.

An earlier study carried out by Nagpal *et al.* in Delhi during 2007 –2012 with the objective to identify key containers for *Aedes* breeding. It was observed that overhead tank acts as Key Container of *Aedes* breeding as it shows a consistent breeding throughout the study period. The rest of the containers do play intermittent roles in supporting the breeding of *Aedes* mosquito. Subsequently, another study conducted in Delhi during 2012-2014 based on the hypothesis that whether continuous entomological surveillance of *Ae. aegypti* and simultaneous appropriate interventions in these key containers during non transmission (December – May) months would have any impact on breeding of *Aedes* and dengue cases during the following transmission months (June – November).

A total of 28 localities of West Zone of Delhi with persistent dengue endemicity were selected for the study. Out of these localities, 20 were included in study group while other 8 localities in control group. IEC and various *Aedes* breeding control activities were carried out in study group in both non-transmission and transmission season whereas control group did not have any such interventions during non transmission months as per guidelines of MCD. These activities were undertaken by a team of investigators from NIMR and SDMC, Delhi. In control group, investigators from NIMR carried out surveillance activity to monitor the breeding of *Aedes* mosquito in localities.

Comparison of baseline data revealed that all indices in control and study group of localities were comparable and statistically non-significant ($p>0.05$). In both study and control groups, indices were calculated after pooling data on seasonal basis i.e. transmission and non-transmission months for both years. The test of significance conducted on all the four indices i.e. HI, PI, CI, and BI revealed a significant difference ($p<0.05$) between the study group and control group during transmission and non transmission months except in HI. Due to consistent intervention measures undertaken in non-transmission months in study group, reduction in CI, HI, BI and PI was observed 63%, 62%, 64% and 99% respectively during transmission months as compared to control group where increase of 59%, 102%, 73% and 71% respectively. As a result of reduction in larval indices no dengue case (except one NS1) was observed in study group whereas 38 cases dengue were observed in control group. Through this pilot study, it is concluded that proper intervention in non-transmission season reduces vector density and subsequently dengue cases in transmission season.

Visceral leismaniasis elimination: Where does India stand?

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Visceral Leishmaniasis also known as Kala-azar is endemic in India sub-continent. In India VL is at present endemic in 54 districts in the states of Bihar, Jharkhand, West Bengal and Uttar Pradesh. An estimated 130 million population is exposed to the risk of VL. The peak annual incidence of VL was seen in 1992, when 77102 cases and 1419 deaths were reported. The target date for elimination in India is by 2017. The National Roadmap for VL Elimination (2014) document was developed for focused efforts at national, state, district and sub-district level. To achieve the target, programme is focusing both on case management and integrated vector management with effective IEC / BCC approach. Developmental partners are also helping the programme to improve access to and use of services, expanding the effectiveness, reach, training, community mobilization etc. Single day single dose treatment introduced during 2015. Incentive to patient for loss of wages and honorarium to Accredited Social Health Activist (ASHAs) has been provisioned. DDT has been replaced with Synthetic Pyrethroid for better community acceptance. District and Block level supervisory cadre are at place for supervision and monitoring. Programme has started diagnosis of all confirmed VL case for HIV test also. VL Health Management Information System (HMIS) has been generated on line. In 2007, the numbers of VL cases were 44533 and 203 deaths which were reduced to 8500 (81%) cases and 5 (98%) deaths in 2015. In 2016 as of December only 6221 cases reported with no deaths. 78% in 2015 & 85% in 2016 blocks have achieved less than one case per 10,000 population. Treatment with single day injection has improved treatment compliance up to 94%. Through active search and camps 569 positive VL cases and 519 new PKDL cases were treated in 2016. In 2016, 84 HIV VL co-infected cases were treated. With the concerted efforts, the number of VL cases are showing declining trend since 2012 in India. In 2016 there was a decline of 81% of VL cases and 94% reduction in deaths in comparison with the year 2011. The programme is of the view that VL cases will further reduce in 2017 as surveillance, case detection and treatment alongwith supervised monitoring and supervision has improved with the involvement of all the stakeholders and the target date of elimination is doable.

Strategies for Malaria Control and Elimination: Is CCMP An Answer?

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Odisha, high malaria endemic Indian state, have a large proportion of its population living in forested foothills. Many residents living in those remote areas face access barriers to timely malaria diagnosis and treatment. Comprehensive Case Management Project (CCMP) was piloted in Odisha from 2013 to increase the universal access to timely malaria diagnosis and treatment for. The present study aims to study the immediate effect of CCMP on malaria epidemiology of one of the study blocks of the CCMP. Dhenkanal was selected as a medium endemic district for CCMP intervention (API- 2-5), study blocks were malaria notification-matched one intervention and control block. Some of the important interventions of CCMP included intensive training of existing village-wise health activists, employing new activists in remote uncovered villages, strengthening supply chain management and monitoring. Annual blood examination and case notification data from the constituent health sub-centres (SCs) of the blocks (28 in intervention & 15 in control), were extracted from the routine state malaria programme surveillance system. Pre-post differences and difference-in-differences (DID) in and between intervention and control SCs, in terms of annual blood examination rate (ABER) and annual parasite incidence (API), were estimated– 2011 and 2012 representing the pre and 2013 and 2014 representing the post-intervention phases. Month-wise block-wise blood examination and malaria case notification rates were collected for interrupted time-series (ITS) (2009-2015) analysis. The proportion of intervention and control population exposed to malaria transmission risks were also measured to adjust for such differences in the regression model. The intervention and control SCs had different profile of risk-exposed populations. ABER showed a two times greater pre-post difference in intervention having a estimate of 1.33 (-2.54, -2.28; p=0.47). The corresponding DID for API was 4.05 (2.19, 5.86; p<0.01), signifying a sixteen fold greater pre-post rise of cases in intervention areas. In ITS analysis greater post pre difference in both MBER and MPI slopes was obtained in intervention blocks as compared to the control counterpart. The intervention areas showed a substantially greater increase in blood examination and case notification in the post-CCMP era. Future analyses should explore whether this initial increase has triggered a decline in malaria or not.

CHAPTER 13

HEALTH POLICY, PUBLIC HEALTH CHALLENGES AND MANAGEMENT STRATEGIES: A WAY FORWARD

Networking Governance Model for the Management of Vector-Borne Diseases: A Systems Framework

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Rapid economic expansion of industrial production system in India has not only led to urbanization but also increased migration. This consequentially led to development of slums as the urban infrastructure is incapable to accommodate the burgeoning urbanization. Poor housing, improper sanitation and lack of safe drinking water associated with rapid urbanization led by migration are the major factors contributing to the expansion of vector borne diseases like dengue. Dengue is emerging as the fastest growing infection globally as well as in India. Lack of systematic and timely surveillance network possibly leads to huge under reported cases and empirical studies suggest that it could be 282 times higher than the official report. Ironically 99% of Dengue deaths could be easily preventable. . The paper outlines the contextualization of Networking Governance (NG) in prevention and control of vector borne diseases. This analytical review is drawn on the basis of best practices in dengue surveillance in Singapore, Cuba and Brazil. The objective of the paper is to present multidimensions of NG, determinants for dengue mortality and policy framework and to critically review the role of networking governance on management of neglected tropical diseases particularly Dengue in India; to review factors contributing to the spread of dengue and stakeholder's participation for pragmatic vector control programme. Based on gaps present four strategic recommendations are drawn upon for the need based successful implementation of vector control programs for combating dengue in India: (i) relevance of interdisciplinary approach to understand the determinants of health and Dengue epidemics; (ii) to generate evidence based data on epidemiological information to guide policy making and implementation process; (iii) to promote accurate and timely public health surveillance components with a well- functioning and sustainable surveillance system.

Progress towards Malaria Elimination in Tamil Nadu, India 2016 – 2030

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Tamil Nadu is one of the Endemic States in India for Malaria, contributes < 1 percent of Malaria incidence of the Country. Tamil Nadu has achieved 96% decline in reported Malaria Cases since

1990. Mortality due to Malaria has been very low with only 42 total reported deaths over the past 2 decades and zero deaths from 2011 – 2016. Though Pv and Pf are prevalent in the State, only Pv accounts for 90 to 95% of the cases and the remaining are Pf. It could be attributed to low mortality rate in Malaria. Malaria transmission occurs year around with peaking in June to November during South-West and North-East monsoons.

Even though the map of malaria in Tamil Nadu is shrinking, it is still a public health problem in some of the Coastal line of Ramanathapuram, Kanyakumari and Thoothukudi, Riverine line of Dharmapuri and urban like Corporation of Chennai in the State. Only 2 Anopheles species namely An.*culicifacies* and An.*stephensi* are mainly contributed to malaria transmission.

Tamil Nadu is currently categorized under Pre-elimination phase for Malaria Elimination in India 2016 -2030 based on API 2014. Accordingly, out of 43 Health Unit Districts (HUDs) in the State 30 HUDs have no indigenous cases (Prevention of re-establishment phase), 5 HUDs in Elimination phase, 7 HUDs in Pre-elimination phase and 1 HUD in Intensified Control phase.

Out of total 8712 HSCs in the State, 58 HSCs with a population of 3.68 lakhs fall under Intensified Control Phase, in which strengthening of fever surveillance by sustaining MBER 2%, ensuring complete Radical Treatment, foci based vector control measures in addition to regular and special Indoor Residual Spray, regular monitoring and evaluation are initiated to bring down these HSCs to Pre-elimination Phase by 2018.

17 HSCs with a population of 1.03 lakhs fall under Pre-elimination phase and 29 HSCs with a population of 2.97 lakhs fall under Elimination phase will be brought down to Prevention of re-establishment phase by 2020 by means of additional surveillance in project areas, construction sites, migrant and slum population with MBER sustained at a minimum of 1%, mapping of potential vector breeding sites, regular adult vector monitoring, larviciding, biological control and foci based vector control interventions such as space spray followed by IRS.

Malaria in an Eastern Indian State: From Approach of Control to Phased Elimination

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Odisha, an eastern Indian state, is highly endemic for malaria Annual Parasite Incidence (API) 9.97. The state contributes around 38% of cases and 28% deaths to the national malaria burden. Plasmodium falciparum is the killer species and contributes 85% of the total malaria cases. The state had shown significant improvement during 2010 to 2013 with reduction of API from 9.29 in 2010 to 5.3 in 2013 & deaths from 247 to 67. This can be attributed to the intensified malaria control measures with newer tools like Long Lasting Insecticidal Nets (LLIN), Early Diagnosis and Complete Treatment (EDCT) using rapid diagnostics tests and Artemisinin combination therapy (ACT) at community level with strong involvement of trained community volunteers, Accredited Social Health Activist (ASHA). But this impact could not be sustained due to interruption in supply of LLIN as it was mostly externally supported. Currently, the estimated population at risk for malaria is 24 million in the state. 14 districts are high endemic with API >10 characterized by large forest area and major tribal population residing in inaccessible deep forest or forest fringe areas. Other problems include various socio-dynamic challenges. There are eight moderately endemic districts within API 2-10, having all vulnerable factors for case rise, if not given guided anti-malarial inputs. Other eight coastal districts with API<1 exhibit all positive features for elimination of malaria. Key focus is low laid on phased malaria elimination in alignment with the National Framework for Malaria Elimination 2016-2030.

With progressive improvement in coverage by quality EDCT with introduction of artemether-lumefantrine as the ACT for the treatment of uncomplicated falciparum malaria, preventive interventions like LLINs, IRS, escalated focus on disease & vector surveillance, improved supervision and monitoring, capacity building and institutional strengthening, the initiative would get a boost. The state has integrated inbuilt operational research activities to exercise T3 (Test, Treat and Track) in high burden areas in partnership with Institutions of National and International repute and civil society organizations. Focus is to work in tandem and especially to reach the unreached with community based diagnosis and treatment and empowering communities with information through BCC activities. For strengthening HMIS, data analyses and feedback on real time basis, higher platform like DHIS2 have been initiated. Procurement and supply chain being critical to programme success are being upgraded through State Medical Corporation.It is expected that, with all the above interventions and support and well geared management system the state will stride in the path of malaria elimination.

Health Impact Assessment of Indira Sagar Project: A Study on Water Development Projects

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Very limited studies on health impact assessment (HIA) of water development projects (WDP) in relation to mosquito borne diseases have been carried out in India so far. Using Health Impact Assessment as a tool for finding impact of Indira Sagar Dam Project (ISP) components in Madhya Pradesh (MP) in relation to mosquito borne diseases; it was emphasized to incorporate it as an integral part for any WDP. Entomological, epidemiological, socio-economic and knowledge, attitudes and practices (KAP) data related to malaria transmission in three dam components namely Submergence (SUB), Command (CMD) and Resettlement and Rehabilitation (RR) colonies were generated for the period of Jan' 2013-Dec' 2014. Statistical analysis was attempted to compare data among dam components and to identify risk factors. Component specific mitigation measures were suggested based on observations. *An. culicifacies* was found dominating species in all the three dam components and its MHD in CMD areas was found higher in comparison to SUB and RR. Odds of finding a positive malaria case was found much higher in CMD as compared to SUB (OR=1.24, CI-95%- 0.71-2.43) and RR (OR= 5.48, CI-95%- 0.73-40.63). Respondents of CMD stated more previous episodes of malaria (81.8%) as compared to RR (61.4%) and SUB (55.7%). Engineering manipulations may be carried out in CMD areas to control seepage and RR colonies should be established beyond 3 kms from reservoir/ Indira Sagar canal considering the flight range of *An. culicifacies*. Strengthening of surveillance with early detection and complete treatment (EDCT) was recommended for CMD areas. HIA should be an essential for planning better control activities.

Entomologists in DRDO and Their Role in Protection of Indian Armed Forces from Arthropod Vectors and Pests

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Indian Armed forces are posted in different parts of the country at varying degree of climatic conditions like deep jungles, rough terrains, snow bound high altitudes, and hot desert areas etc. They are continuously exposed to many problems due to arthropod vectors & vector borne diseases in the field conditions while protecting our nation against odds and stress. "Man behind weapon" is very important for all successful operations. Entomologist at DRDE, Gwalior & DRL, Tezpur both under DRDO, Ministry of Defence is working since 1958 and developed many products and technologies including a multi insect repellent Diethyl phenyl acetamide (DEPA), WOOLCARE, LLIN, slow release insecticidal paint (SRIP), ATTRACTICIDE, ROACHLINE, ROACHTOX, RATOX etc., to protect armed forces from arthropod vectors, other pests and also for protection of stores, materials and animals like horses, mules etc used by them. Many of these products have spinoff benefit to civilians also. Newer technologies like repellent impregnated cloth, odor baited traps etc are also explored.

Detecting the Undetected: Unblocking the Bottlenecks in Malaria Elimination

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Malaria is one of the major vector borne diseases in India with about one million cases reported annually. The strategy for malaria control includes early diagnosis, complete treatment and vector control. Since year 2007, the Indian national drug policy for malaria has switched over from presumptive therapy to treatment after confirmation and recommends investigating suspected malaria cases. Clinical diagnosis is often inaccurate and nonspecific. Microscopy has been considered to be the gold standard for diagnosis of malaria but its availability and quality are major challenges. Rapid diagnostic tests have proved to be a game changer but they are also not free from challenges. There are other tools available like fluorescent microscopy, serological and molecular techniques and have value as research tools. Non-invasive methods to diagnose malaria from samples like saliva are under evaluation.

Asymptomatic parasitaemias have a great impact on malaria transmission. It is very difficult to tap these infections since they are asymptomatic. Strategies need to be developed to detect them. Further, there is an emergence of new species causing human malaria. Tools need to be developed for diagnosis of these species. There are steps taken by India in improving diagnosis. The national programme and NIMR have a robust QA programme for malaria RDTs. QA of Microscopy also has been revamped. Improving malaria diagnostics is needed to ensure smooth road to elimination.

Are We Really Winning the Crusade Against Dengue? Time for Reality Check

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The WHO estimates that almost 40% of the world population are at risk of dengue infection. The dengue virus belongs to the family flaviviridae and occurs in four antigenetically distinct serotypes that have emerged / re-emerged throughout the world since the 17th century. There is genetic variation within each serotype in the form of phylogenetically distinc subtypes. 5th serotype has also been reported in the recent past from Malaysia. It was estimated that in the 2080s over 5 billion people would be at risk of dengue infection globally as a result of climate change and population increase. Today, the geographical distribution of dengue includes more than a hundred countries while dengue was endemic to only nine countries in the 1950s. Aedes aegypti and Aedes albopictus are the potential vectors of dengue virus in different parts of the world including India. Aedes albopictus is also regarded as 'Asian tiger mosquito', has been included within the hundred most dangerous species in the global invasive species database. The geographical expansion of these two highly invasive sympatric species are causing concern to the public health authorities globally especially in the context of global warming and globalization. There is also no effective vaccine available against the pathogen. In India the total number of dengue cases per year varies from18860 to75808 between 2010 and 2014. Despite various tools and techniques, projects and programmes, strategies and policies applied and adopted in controlling dengue, the outcome seems to be far from satisfactory. Hence, time has come to review and restructure our war strategy against dengue.

Geographical Information System (GIS) in Decision Support to Control Malaria

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Malaria is a major public health problem in India and its cases are around 1.5 million in a year. In tribal states, malaria problem is difficult to tackle because of vast tracts of forest with large tribal settlements. Besides ecological parameters which influence the disease there are some important local factors such as socioeconomic, socio-cultural and behaviour patterns of the community which play a major role in disease transmission. If these parameters are to be used effectively for decision-making, they must be well organized and managed. This goal can be accomplished by compilation of generated dataset and its integration within spatial infrastructure (SI) and introducing a geographical information system (GIS) for analysis and management. GIS is a set of strategies and tools capable of integrating, storing, editing, analyzing and displaying geographically referenced information from various sources. A GIS based study was carried out in Koraput, situated in southern part of Odisha for identification of risk factors based on ecological parameters for decision support in formulation of appropriate control strategies. The dataset specific to the study was derived from remote sensing (IRS-1D/ LISS III), topographic maps (1:50,000), surveys, ground-truth and epidemiological data from the district. This information was used to develop primary spatial infrastructure in collaboration with Odisha Remote Sensing Application Centre, Bhubaneswar. The thematic layers developed were: PHC map, geomorphological parameters, landuse, soil type, water bodies and drainage network. Land use map was further used to derive other thematic layers like forest cover and settlement. The analysis was done within Arc/View GIS to describe primary risk factors within PHC. Epidemiological data collected from district malaria office were linked to the PHC map to prepare malaria API map. Thematic maps of ecological parameters were overlaid on malaria API map to identify the parameters responsible for malaria incidence in each PHC. The current study clearly identifies the risk factors associated with high malaria transmission in different PHCs of Koraput and focused intervention based on these factors may be undertaken in these areas to control malaria transmission in this district.

Urban Malaria and Challenges for Control in Chennai, India

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Urban malaria control have become a challenge due to lack of inter-sectoral coordination; poor planning; mosquito control is practiced rather than species sanitation; inadequate finances; acute water shortage and erratic water supply in highly populated areas; water storage in a variety of containers; inadequate staff to tackle vector control operations and parasite surveillance; incomplete treatment; legislative measures and building bye laws either not in place or not enforced. Though UMS is under operation for many decades, the existing system of malaria control as practiced, is unlikely to eliminate malaria due to multifaceted reasons like rapid urbanization, increasing migrant labourers and other socio-ecological reasons. Malaria transmission in Chennai city is perennial with a peak between July and October. The city contributes 53.6-78.8% malaria cases annually to the malaria problem of Tamil Nadu. *Anopheles stephensi,* the vector breeds in clean/clear stored water such as overhead tanks, cisterns and wells besides, roof gutters, curing pits in construction sites, fountains and ornamental tanks. Urban vector control can be possible with total commitment of agencies responsible for the development and maintenance, their co-ordinated effort with appropriate measures for vector control, community participation and strict enforcement of bye-laws.

Point-of-Care Diagnostics as Field-Based and Low Cost Techniques: Renewed Hopes for Elimination of Vector-Borne Diseases

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The inability to diagnose numerous diseases rapidly is a significant cause of the disparity of deaths resulting from both communicable and non-communicable diseases. Existing diagnostic instrumentation usually requires sophisticated infrastructure, stable electrical power, expensive reagents, long assay times, and highly trained personnel which is not often available in limited resource settings. We analyze the current point-of-care technologies, which have made a major impact on diagnostic testing.The advantages offered by such systems, including low cost, ruggedness, short turnaround time, minimal manual input, portability, ease of use and the capacity to generate accurate and reliable results rapidly. Microdevices can be composed of microfilters, microchannels, microarrays, micropumps, microvalves, and microelectronics, and these mechanical and electrical components can be integrated onto chips to analyze and control biological objects at the microscale. To find out the classes of analytes which includes proteins, cells, nucleic acids, small molecules, the various methods used are Immunoassays, Signal Detection, Fluid Control, Fluid Actuation and Delivery Cell-Based Assays, Nucleic Acid Amplification Testing, Sample Preparation, Signal Amplification, Product Detection, Clinical Chemistry Assays, Hematology . For example, the use of a microfluidic platform for diagnostic of the Mycobacterium tuberculosis has also been reported on an automated microfluidic system that was able to detect the pathogen in 50 min, eliminating the need of a culturing step. Other researchers have introduced a magnetic nanoparticle/quantum dot-based immunoassay for the detection of pathogens responsible for water borne and food borne diseases (e.g., E. coli 0157: H7, Salmonella, etc.) using a microfluidic chip.

Public Health Significance of Dengue Viral Infection

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Dengue has become a global concern since the Second World War and has been found to be common in more than 110 countries across the globe. Annual estimates suggest that approximately 500 million people are infected and 10,000 - 20,000 succumb to the infection. We studied the causes and consequences of dengue infection as the incidence rates of dengue infection has drastically increased over the last few years. Dengue viral infection represents one of the most important vector-borne diseases, which appears to have re-emerged with a stronger magnitude in recent years. Here we present an overview on the public health measures required in the prevention and control of dengue relies on such as advocacy, social mobilization and legislation to ensure strengthening of public health organizations collaborations and capacity-building to necessitate strategic control of disease outbreaks. Improved understanding of the public health significance aspects will greatly help in disease control and prevention of dengue fever and the associated morbidity and mortality rates.

AUTHOR INDEX

SUBJECT INDEX

ABOUT THE BOOK

Vector borne-diseases (VBDs) are major public health problems particularly in tropical and sub-tropical regions. Many VBDs are considered diseases of poor since it is endemic in low income groups or in areas where the vicious cycle of diseases and poverty exists. While all these diseases are preventable and curable, the disease burden and economic impact are very high. The chapters presented here are the abstracts of keynote address, plenary lectures, guest lectures, invited oral presentations, oral presentations and poster presentations.

Vectors and Vector-Borne Diseases: Pathobiological Insights, Public Health Challenges and Management Strategies will be essential source book for Scholars and Practitioners in the field of Public Health, Molecular Medicine, Applied Health Sciences and Policy Studies. The source book is designed to be used as ready reference materials for basic, applied and operational research.

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