Annual Report 2009 & 2010

Regional Medical Research Centre
Indian Council of Medical Research
Bhubaneswar

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Contents

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Preface

During the period 2009 and 2010, the centre has focused attention in expansion of research dimensions to newer areas, besides continuing its research endeavor in areas of vector borne diseases, diarrhoeal disorders and nutrition. Recently, the centre has got the laboratory established for virology and tuberculosis culture. Research activity has also been initiated to translate the important research findings of our scientists to help its implementation in public health settings. In addition, the centre has played a significant role to check some of the health problems at the community level during the period in close collaboration with state health department. In order to strengthen human resources of this region the centre has been undertaking Ph.D and M.Sc dissertation (six-month) programmes on regular basis.

Out of 21 scientific projects undertaken by this centre during 2009-10, 17 are extramural in nature and sponsored by Gates Foundation, DST, DBT, ICMR, CSIR Task Force or NVBDCP. Of these 16 are ongoing and 5 have been completed with logical conclusions. The centre has published 27 research papers during the year 2009, and in the year 2010 till date 14 in indexed journals. The average impact factor of the publications is 2.11 for 2009 and 2.44 for 2010 till date. Total 34 foreign print journals and 30 Indian print journals have been subscribed for the library during 2009 & 2010. Besides the print journals, library subscribes more than 3000 online journals through ICMR E-Journal Consortia, ERMED Consortia, JCCC@ICMR, and Science Direct that are being at reffered by scienctist of the center as well as scienctists and health professional of this region.

The centre has generated Rs 1.5 crore through sponsored research in year 2009 and around 4 crore in year 2010. During this period 21 research scholars have registered for Ph.D in different universities under the guidance of our scientists, while one of the students has been awarded Ph.D and four have submitted their thesis for examination after completion of the works. Six month M.Sc dissertation work was undertaken by 30 students during 2009 and 6 during 2010 sponsored from various Universities.

Research product of this Centre with high potential for community use were identified and streamlined to initiate translational research activity. This year two such research products were identified and translational research activity was initiated with standardization of each component of the research tool. These are (i) Mapping of vectors and their vectorial attributes by transfer of molecular technique for field use. (ii) Quadruplex PCR assay and other attributes for diagnosis of *V.cholerae* 01 and or 0139 serogroups causing cholerae. The technique can help diagnosis of *V.cholerae* with less time and cost while showing important information on

the cholera pathogen so detected. Besides above two products, attempt has been taken to identify other product or strategy having potential and so developed by our scientist to process for translation.

The vector borne disease research was addressed on lymphatic filariasis and malaria that formed the major research activities of the Centre. Besides above, vector borne diseases of viral aetiology like chikunguniya and dengue infection that appeared in sporadic form were also investigated. Lymphatic filariasis and malaria constitute important public health issues in Orissa. The priority issues of these diseases were addressed through research during the period. The key issues addressed in lymphatic filariasis were (i) identifying candiadate antigen for immunoprophylaxix through studies on protective immunity, (ii) evaluation of appropriate new drug regimens for use in Mass Drug Administration (MDA) to help national programme for elimination of filariasis through series of clinical trials and (iii) to study the clinicopathological correlation in early filarial infection to identify risk involved in young children that can be used as a strong advocacy tool for use in improving drug compliance in children during MDA. Risk map identifying the endemic regions of filariasis was developed through GIS and remote sensing for 2 districts of Orissa outlining the geographic factors responsible that may cause indigenous transmission.

Since Orissa reports high incidence and mortality due to cerebral malaria with multi organ involvement, hence series of studies were addressed to impart insight in to pathogenesis and development of cerebral malaria. The role of nitric oxide and angiotensin II in offering protection and CR-1 gene in rosetting phenomena observed in cerebral malaria were analyzed and reported through series of publications. The genotype expression of *P.falciparum* was incriminated to that of clinical severity of malaria. The co-existence of *P. malariae* mixed with *P falciparum* or *P. vivax* were also reported. Vector studies were carried out outlining the potential vulnerable geographic areas through GIS and remote sensing by mapping of 3 districts for malaria that may carry high potential to cause malaria Besides, studies on the vectorial attributes and bionomics that can help in vector control strategy of this region were studied in 13 districts of the State and reported to the programme. Vector susceptibility status of *A. culicifascies* indicated prevalence of SR gene in Kdr region which was observed in only 2.5% of the vectors showing resistance with WHO susceptibility test kit. Mapping of *A. annularis* was carried out using micro satellite markers to find out population dynamics of the vectors in different regions of India.

High prevalence of under nutrition and anaemia in vulnerable groups of population was earlier studied by this centre and reported from tribal dominated districts of Orissa. Hence interventional study using five arm regimens including nutrition education is being carried out amongst adolescent girls of Soura tribe in Gumma block of Gajapati district in an open trial to identify appropriate weekly regimen as prophylaxis for control of anaemia.

The Centre has been reporting the etiological agents for severe diarrhoeal disorders to the state health department through fortnightly surveillance of cases admitted with severe diarrhoea disorder in health facilities of Puri district of Orissa as well as during sporadic and epidemics of diarrhoeal disorder surfaced in coastal as well as tribal districts of Orissa over a decade. The current studies indicated persistence of El tor variant *Vibrio cholerae* 01 Ogawa strain in 13 districts of Orissa from tests conducted periodically from environmental water samples and rectal swabs of affected cases. Recent epidemic of severe diarrhoeal disorder reported at Rayagada district of Orissa in September 2010 was also investigated by the scientists of this Centre identifying aeitio-pathologic agent that provided timely assistance to the State Health Department.

The centre has added new area of research during the year like TB culture facility and Virology laboratory with support from Council. The construction of laboratory for TB culture has been completed, the equipment procured, while the scientist were trained at Tuberculosis Research Centre, Chennai. The sputum testing as well as culture of TB bacilli was initiated. Virology lab activity has been initiated to address sporadic or epidemics of diseases of viral origin reported from time to time in the State. The sporadic outbreak of Dengue, Hand foot & mouth disease & Chandipura virus infections that surfaced in the region were investigated by this centre and reported. The Center has established BSL-2 lab during the period and carried out H1N1 lab diagnosis test on throat swab samples referred from suspected cases from State. So far, out of 380 throat swab samples tested, 95 samples were confirmed with diagnosis of H1N1 and reported on same day the samples received to the State Nodal Officer.

The centre also addressed investigation into several other sporadic out breaks of Jaundice, Diarrhoeal disorder, Malaria, Chikunguniya infection that helped to assist local Health Dept. in taking timely public health measure.

The centre has established linkages with other ICMR and non-ICMR institutions of the Country in upgrading the expertise in the Centre, sharing scientific informations and in collaborative research programmes. The collaboration was also established with International Research Organizations like International Vaccine Institute (IVI), South Korea to undertake pilot introduction of Oral Cholera Vaccine in Orissa which will be initiated soon.The linkage with national programme was strengthened through NVBDCP, Delhi in imparting training to 243 Malaria Technical Supervisors (MTS) appointed by five States - Orissa, Jharkhand, Chhatisgarh, MP and Andhra Pradesh. Collaboration with state health department was strengthened in form of consultancy, undertaking evaluation of health programmes, diagnosis of referral cases in areas of Centre's expertise and for investigation of epidemics and disaster management.

Human resource development activity of this Centre focused on imparting training to M.Sc students, sponsored from various universities, to complete their six month dissertation work, and Ph.D scholars sponsored from UGC-CSIR and ICMR through their research fellowship as JRF. Of the total staff strength of 102, 93 are in position at the Centre. Out of sanctioned strength of 18 scientists, only 12 scientists are in position and the vacant positions are under active process of filled up.

The Centre had organized several scientific meetings and symposia during the period to strengthen scientific interaction with local medical colleges and expertise from various other fields. The Human and Animal Ethical Committee meetings were held regularly. The Centre undertook regular journal clubs, seminars and institutional review committee meeting to discuss research output and update on recent scientific issues.

During the year several developmental activities of the Centre were undertaken. The newly constructed Auditorium, Guest House and Hostel for Ph.D scholars and new animal facility were taken over from CPWD and now being made operational. New canteen facility, scooter and car shed for staff, internal road, annual maintenance of staff quarters, lift pump facilities were constructed in the campus through CPWD with support from Council during the period. Efforts have been taken to establish additional research facilities through construction of a new BSL-3 facility and an OPD for augmenting clinical research and lab studies.

The scientists, research scholars and staff of this Centre have made continuous effort and contributed to significant output of this Centre. I sincerely thank scientists, students and staff for their endeavor and contributions. I am also thankful to the State Health Department and other agencies, collaborating Institutes and experts of SAC, ethical and other technical committees for their assistance, support and cooperation. I extend my deep gratitude to DG, ICMR and the Council for their continuous support, guidance and encouragement. With all around support, the Centre can continue with its endeavor to achieve its goal.

DR.S.K. KAR
Director

Highlights of Research Activities

(April 2009- March 2010)

The Centre addressed to various research issues on lymphatic filariasis, malaria, hepatitis, diarrhoeal disorders, haemoglobinopathies and nutritional disorders during the period 2009-10. To support the state government the centre has established the H1N1 diagnostic facility at a war foot during 2009-10 and a new facility to detect MDR and XDR tuberculosis is being established.

Studies on human lymphatic filariasis are continuing with multidisciplinary approach. The ongoing programme of Mass drug administration (MDA) to eliminate LF from the country shows low rate of MDA regimen in certain areas due to fear of side reaction and confusion during distribution in three different doses to various age groups. To increase the drug compliance the Centre has undertaken an open clinical trial in three villages with matched population groups to find out the efficacy and tolerability of single dose DEC of 100mg, 200mg and 300mg strength given uniformly irrespective of age groups in filariasis endemic community of Orissa. Three rounds (2007, 2008 &2009) of supervised mass administration of DEC have been implemented annually in three villages accordingly. It has been observed that DEC compliance was around 75% in all three-regimen sites. Frequency and intensity of side reactions were significantly lower and the micro filarial clearance after 24th month was 83% among population receiving 100mg DEC, as against 81.4% in 200 mg and 87%in 300mg dose of DEC. The Mf densities, frequency of antigenaemia and vector transmission parameters were found to be comparable in three dose groups. Since above results are encouraging that show potential for translation to programme, the study is continuing to observe the effect in next rounds of MDA. The studies on protective mechanism against filariasis indicated that the children born from filarial infected mothers are more susceptible to infection compared to those children borne from infection free mothers. In a separate study it has been observed that B-1 cells are playing crucial role in protection against the disease and influence the outcome of human filarial infection. Quantification of IgM antibodies reacting to the SS-DNA and lipopolysacharides (LPS) were significantly low in microfilariarmic cases compared to endemic normal and chronic cases.

The intra-specific variations in nucleotide sequences will be used for development of species diagnostic techniques for identification of the sibling species.

As a part of referral diagnostic services in the year 09-10 total 372 referred cases were tested for detection of haemoglobinopathy disorder. Amongst them 9% were found to cary sickle cell disease gene, 8.10 % a thalassemia major, 1.90 % I Ib E/ a thal and 2.20 % sickle / a thal gene. Evaluation of Vitamin A deficiencies among a subset of cases have shown that about 60% of cases with a thal major were having Vitamin A deficiency followed by 57% among sickle cell trait cases and 40 % among sickle cell disease cases.

The State Health Department reported sudden onset of cases presenting with motor weakness, convulsion followed by unconsciousness with/ without fever in a tribal village of Kandhamal district Orissa. Most of them were young (below <10 yrs of age) who succumbed to death within 10 – 12 hrs of onset of symptoms. Serological tests revealed the presence of antibody to Chandipura Virus and survey made in collaboration with RMRI, Patna has shown presence of sand fly(P argentepes) in the affected village of Kandhamal district. This is the first report from the area.

Cases of paediatric age group presenting with fever and vesicular rash of distal part of extremities and pharyngeal lesion were reported from Bhubaneswar, an urban setup. Clinically cases were suspected to be hand, foot & mouth disease. This was confirmed to be due to entero virus (CA16) as tested by PCR and sero diagnosis done in collaboration with NIV, Pune.

The country faced a new challenge of pandemic Swine flu (influenza H1N1) infection affecting various States including Orissa. During this period (2009-10) total 71 swabs were tested at our centre's BSL-2 laboratory. Nine cases were diagnosed to be H1N1 influenza and report submitted to State health Dept on the same day of the receipt of the referred sample, which helped timely treatment and prophylaxis.

The Centre has organized a series of (5 batches: 25 trainees in one batch) training program of 10 days duration for Malaria Technical Supervisors of Madhya Pradesh, Chhatisgarh, Jharkhand and Orissa.

Highlights of Research Activities

(April 2010 - Dec 2010)

The centre continued to address various research issues on lymphatic filariasis, malaria, hepatitis, diarrhoeal disorders, haemoglobinopathies and nutritional disorders and extended support to the state health authorities during outbreaks or epidemics and natural calamities. The Centre also conducted various HRD programmes to strengthen the manpower of this region of the Country through its PhD programme and six month MSc dissertation training. During the period under report a new Animal House facility has been established.

While filarial infection in paediatric age group of endemic population was reported to be around 30% by detection of circulating filarial antigen and microfilaria; overt clinical manifestation usually appear in late adolescence and adulthood. It is well known that adult filarial parasites reside in the human lymphatics, where they can elicit an inflammatory response resulting in acute lymphangitis that appear usually in adolescent age group. To understand any development of lymphatic pathology during this clinically silent phase in infected children, a study was undertaken to examine the status of lymphatic channel and presence of adult filarial parasite in human host infected with filariasis with or without symptoms. Twenty microfilaremic children between 5 to 18 years of age group with or without overt clinical filarial manifestation were subjected to lymphoscintigraphy. Around 50% of children demonstrated delay in lymph flow and in two cases adult parasite was visualized (filarial dance sign). The interim study revealed distortion of lymphatic channel and delay in lymph flow in limbs where adult parasite was visualized. This finding can serve as a strong advocacy tool for MDA to improve compliance amongst children and asymptomatics in the community The Mass Drug annual administration (MDA) with DEC in combination with Albendazole (400mg) being undertaken in the Country aims to eliminate filariasis within 5-6 years. National Health Policy of Govt of India aims to achieve filariasis elimination by 2015. While DEC is known to be a powerful microfilaricide, Albendazole is believed to have sterilizing effect on adult worms that possibly suppress the resurgence of Mf. Hence effort has been made to identify a more effective MDA regimen by increasing the dose or rhythm of Albendazole used in MDA through randomized open clinical trial. The comparative efficacy and tolerability of four-armed regimen is being studied recruiting 104 microfilarimics. The

group receiving 800mg of Albendazole with DEC (300mg) given biannually achieved higher clearance of Mf at six month post drug. Clearance of adult worm at 12 months was found to vary from 20-75% in 4 regimens.

The impact of filarial infection of mothers on the immune competence of the neonate remains poorly understood. Our study indicates that filarial specific IgG1, IgG2 and IgG4 antibodies were significantly elevated in cord blood from infected mothers than uninfected mothers. Comparison of the percentages of CD4+ cells expressing CD25 in paired maternal and cord samples from filarial infected and uninfected groups shows significantly higher T regulatory ($CD4^+$ and $CD25^+$ cells in cord blood samples of filarial infected mothers compared to cord blood samples of uninfected mothers. These findings provide evidence for influence of maternal infection on the development of subsequent anti filarial immunity in offspring. Significantly low levels of IgM antibodies to Actin and LPS in microfilaria carriers (known to be immunologically hyporesponsive) compared to patients with chronic filarial disease and endemic normals (immunologically hyper responsive) raises the possibility of poly reactive property of these antibodies.

To support the National Programme of Malaria Control GIS based micro planning in high endemic areas of Orissa has been prepared from the data obtained from house to house survey in Papadahandi block of Nawarangpur, Ghatagaon of Keonjhar and Banpur of Khurdha district. In a recent survey conducted in 5 districts of Orissa during rainy season, *P malariae* infection was detected by PCR assay; the prevalence being as high as 44.6%. The factors contributing to the pathogenesis of cerebral complications in malaria are not precisely known. A recent study indicates that the parasite population can be stratified based on their association with severity of clinical expression. While analyzing the extent of duffy-binding-like (DBL) gene diversity and rosetting potential of the parasite population associated with severe malaria, a significant association between high parasite density and severe malaria and "b" variants of FR region of DBLá with high frequency of resetting was observed. The mechanism reported to be responsible for the pathogenesis of severe malaria. Therapeutic response to chloroquine sensitivity studied in 7 districts shows poor response to Chloroquine.

The State of Orissa experiences diarrhoeal outbreaks frequently that accounts for high morbidity and mortality. A large outbreak of cholera was reported in Kasipur, Bisamcuttack and Kalyansinghpur blocks of Rayagada district during the period (Sept.2010) under report. The causative organism was El Tor variant of *V cholerae* having similar antibiogram profile of

2007 epidemic. Timely diagnosis of rectal swab and environmental water samples and reporting helped the Health Dept of the State to take quick action.

Supplementation of iron-folic acid and deworming in combination with Vitamin B_{12} and nutrition education regimens in reducing iron deficiency anaemia among unmarried tribal adolescent girls in Serango sector of Gumma block in Gajapati district is being tested. A total of 994 adolescent girls aged 12-18 years were included in the study. Their baseline indicators such as socioeconomy, clinical examination and morbidity pattern were measured. Anaemia was found to be 90% by hemoglobin estimation, of which 36% had hypoferritinemia evidenced by test for serum ferritin with 20% having acute infections tested by C-Reactive Protien. Iron deficiency detected in 50% of the samples and 32% were found to be Vitamin-A deficient. These clusters were allotted randomly to 5 regimens with different combinations of iron, folic acid, Vitamin-B12, deworming and nutrition education which has been imparted in two allotted clusters. The levels of Vitamin-B12 and folic acid are being analysed for assessing the base status of study indicators.

For the first time the presence of A and D sibling species of *An culicifacies* and role of *A subpictus* (previously non vector) as an incriminating vector for malaria has been reported from this Centre from Orissa. Development of microsatellite marker of *An annularis* by RAPD and ISSR analysis has been reported, that reveals 25 RAPD and 15 ISSR with different types of banding patterns among the *An annularis* of Orissa and the neighboring State of Jharkhand. To detect the sensitivity of botanicals inducible GST has been tested against mosquitoes that can help finding potential mosquitoe repellants.

Evaluation of referral cases for haemoglobinopathies revealed that about 9.5%, 8%, 2% & >2% of the cases carry the sickle cell disease, â thal major, Hb E/ a thal and sickle- a thal gene respectively.

Investigation of an outbreak of dengue infection was carried out that emerged for first time in Orissa in Malkangiri district. Aedes larvae were found in different type of containers both in rural and urban localities with Breteau Indices varying from 70 to 190. From adult collection three species of *Aedes ieAedes aegypti, albopictus* and *vittatus* has been confirmed. Out of these three species *A calbopictus* was found to be dominant in this district. Out of 61 subjects screened during house to house survey of both rural and urban settings there were 28 symptomatics with fever. Fourteen out of 61 blood samples tested were confirmed for IgM antibody against dengue virus strains (1-4), tested with NIV kit. Out of 14 cases tested positive

11 were symptomatic with fever, myelgia, arthralgia, head ache, retroorbital pain, photophobia/ vomiting and three subjects had haemorrhagic rash. The Centre has provided all assistance to the state health authorities for the diagnosis of H1N1 influenza cases refereed from different hospitals. Out of 370 swab samples tested 95 were confirmed for H1N1.The cases were recorded from twenty one out of thirty district of the state and the epidemic curve has shown the peak during the month of August'2010. As requested by the Govt of Orissa a malaria epidemic survey was also conducted in Nilagiri block of Balasore district, where the slide positivity rate was 54.5% and all were *P falciparum*. Two mosquito vector species *A culicifacies* and *A annularis* were present and sporozoites were detected in both the species.

Under translational research the centre has developed two PCR based tools. One is to monitor the information of vector prevalence, incrimination of vector for malaria transmission, identification of the sibling species of vector and chloroquine (CQ) sensitivity of the parasite ingested by the vector. By another tool all different serogroups of *V. cholerae* causing cholera can be detected in a single PCR test. Effort is being made for translation of these techniques for field use.

TB Diagnostic Facility

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2009 & 2010

On Going Studies

Contents

1. Efficacy and tolerability of single dose DEC of 100mg, 200mg & 300mg strength in filariasis endemic community in Orissa.

Principal. Investigator	:	Dr. B. Dwibedi
Co investigator	:	Dr. S. K. Kar, Dr. N. Mahapatra
Starting date	:	March 2006
Closing date	:	April 2011
Funding	:	Intramural

Objectives

1. To compare efficacy of single dose mass administration of DEC in 100mg, 200mg & 300mg strength in three defined filarial endemic population.
2. To observe the side reactions in three dosage levels as above.
3. To observe the vector transmission in the three sites.

Background

Elimination of filariasis is now targeted through annual mass administration of DEC. But fear of side reaction and problems in durg distribution and consumption limited the compliance to the programme. Hence attempt was made to look for alternatives for enhancing MDA compliance. Three endemic villages from 2 districts (Cuttack & Khurda) with population around 2000 were selected after screening several endemic villages based on Mf status and clinical disease. The study population was comparable and covered all ages and both sexes. After baseline examination for microfilaraemia and antigenenaemia three successive annual rounds of mass DEC administration either 100, 200 or300 mg was undertaken in uniform dose to all age groups in three study villages for successive years in 2007, 2008 and 2009. The study population were subjected to reassessment of filarial infection status periodically following the annual round of DEC. It was planned to continue the assessment of effectiveness of the three dosages for four successive annual rounds of MDA while observing the side reactions. Vector survey and xenomonitoring was carried out in the three areas after MDA. *Cx.quiquefasciatus* mosquitoes were identified and dissected for the presence of infective larvae of the parasite. Detection of filarial DNA in the vector was done by PCR for xenomonitoring. Molecular xenomonitoring has been used recently as a sensitive marker for assessing the endemicity of LF and a useful tool for evaluating the success and progress of control programs.Molecular Xenomonitoring (MX) employs PCR to detect filarial DNA in wild-caught mosquitoesAlthough

dissection can be used to detect filarial parasites in mosquitoes, MX becomes particularly valuable after implementation of MDA programs, when mosquito infection rates are reduced to levels that cannot be accurately assessed by dissection.MX has the potential to be a sensitive method for detecting persistent filarial parasites in communities. It should also provide an indirect indication of the potential for ongoing filariasis transmission. The transmission parameters which have been noted were a) Infection rate: % of mosquitoes positive for any developmental stages (L1,L2 and L3) of the parasite, b)Infectivity rate: % of mosquitoes positive for infective larvae (L3), c) L3 load: Number of L3 per infective mosquito, d)Transmission intensity index (TII),and e)Number of L3/infective mosquitoes.

The DEC coverage, side reaction, microfilaria clearance and vector transmission indices at 4th, 12th and 24th month following the MDA rounds has been given in the previous annul report (2008-09).

Progress of work

Fourth round of MDA was instituted during May 2010 in the three sites. The populations were followed by night blood examination at 36 months to see the effect of three successive rounds. Microfilaria clearance among the individuals who were microfilaraemic at the baseline was noted to be 90%, 76% and 97% in the 100mg,200mg and 300mg regimen sites respectively. Figure 1 shows the microfilaria clearance at 4th,12th,24th and 36th month in the three regimen sites.

Reduction in geometric mean of microfilarial count at 36th month among above individuals were 86.5%,86.9% and 85% in the three regimens respectively.(Fig-2)

Microfilaria prevalence rates in the community were 3.04%,5.8% and 1.8% at 36th month against the baseline mf rates of 9.8%,8.83% and 5.86% in 100mg,200mg and 300mg sites respectively. Fourth round of annual MDA have been instituted in the three sites and the repeat mf survey will be carried out 48th month.

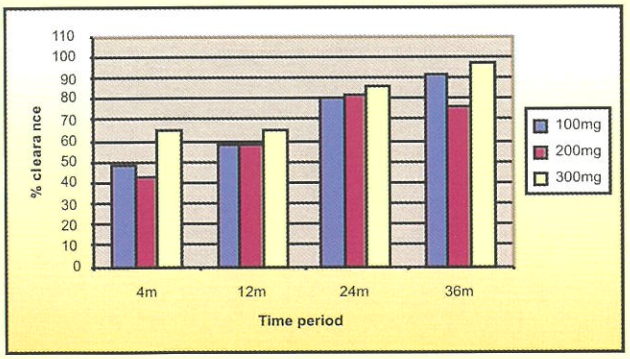


Fig 1. Mf clearance (%) in microfilarimics followed (GeoMean) in the microfilarimics at 4th,12th,24th and 36th month points.

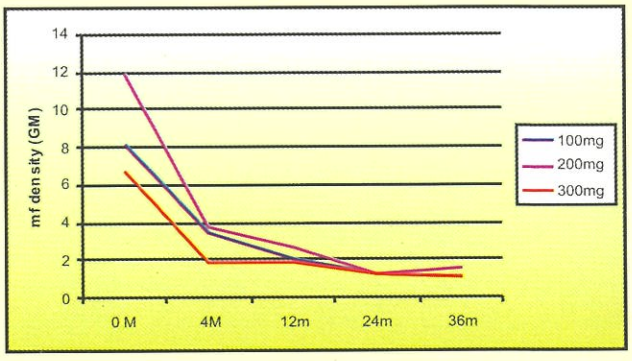


Fig 2. Mf count at different follow up

The mean infection rate after 1st round of DEC administration were 2.16 , 2.9 & 1.8%, which came down to 0.7, 0.8 & 0.7% in all in 100mg, 200mg & 300mg regimens respectively after 3rd round of DEC (fig 3) The mean infectivity rate were 2.16, 2.3 & 1.2% after 1st round of DEC, which came down to 0.6, 0.7 & 0.3% during the 3nd round in all the above mentioned three regimens(fig 4) Percentage reduction in infection rate were 67.6, 73.4 & 62.2% & of infectivity rate was 72.3, 69.6 & 75.7% and for L3 this was 54.3%, 33.4% & 35.8% in 100, 200 & 300mg regimens respectively (fig 5).

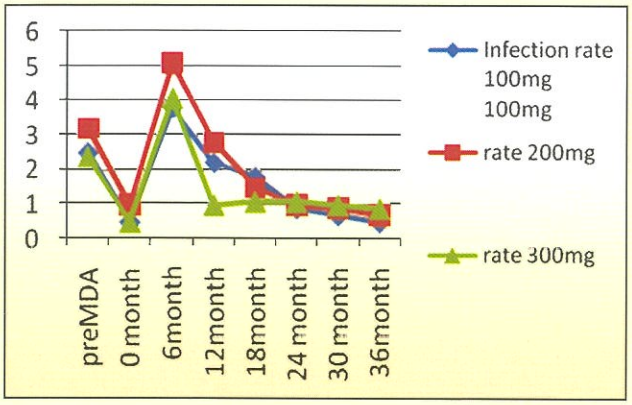


Fig:-3 Change in infection Rate

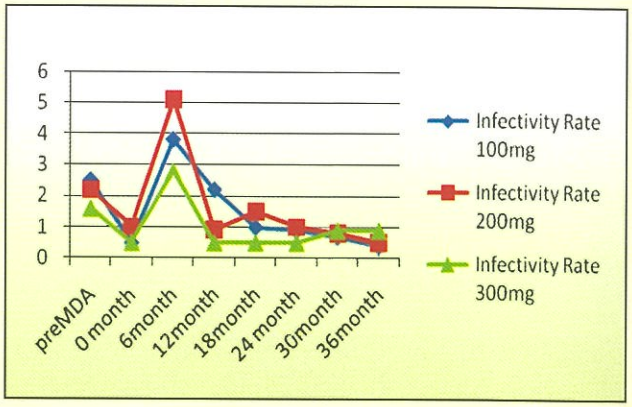


Fig:-4 Change in infectivity Rate

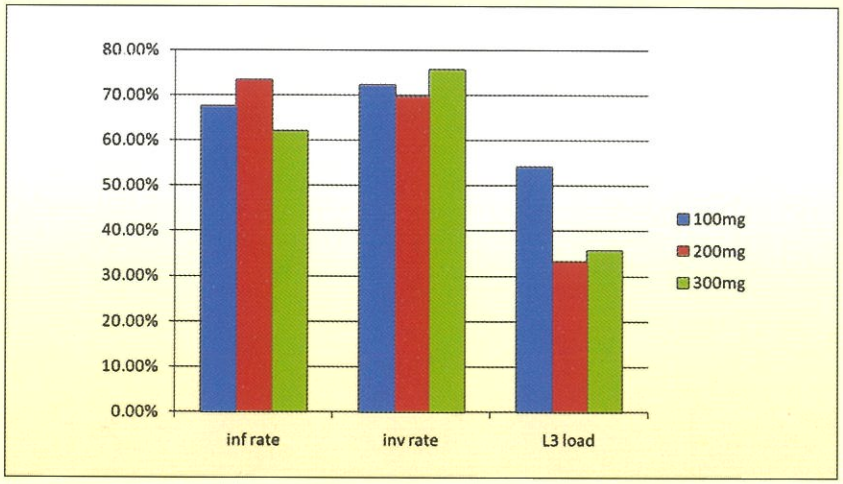


Fig:-5 Reduction in Transmission Parameters

Subsequent plan of activity

The study population will be subjected to night blood examination for microfilarimia and antigenimia at 48 months following fourth annual round of MDA for evaluation. Vector survey will be conducted in the three regimen areas to look for the vector infection and transmission parameters. The effect of fourth round of MDA will be assessed for the above parameters to check sustainability of the drug efficacy at three dosage levels.

2. Effect of Albendazole dose and interval on *Wuchereria bancrofti / Brugia malayi* microfilarial clearance in India: a randomized, open label study.

Principal Investigator	:	Dr. S.K.Kar
Co-Investigator(s)	:	Dr.B. Dwibedi, Dr. A.S.Kerketta Dr.S.S.Panda, Professor, Department of Medicine, KIMS
Starting date	:	October 2008
Duration	:	September 2011
Funding	:	Extramural (GATES Foundation, USA)

Aims & Objectives

1. To determine whether by increasing the dose or frequency of albendazole being used in the current MDA regimen is more effective in clearing microfilarimia as assessed by night time microfilarial counts in *Wucheraria bancrofti* microfilaria-positive patients.
2. To assess the effect on adult worm burden assessed by Doppler sonography detecting worm nests and adult worm antigen.

Background

Lymphatic filariasis (LF) remains one of the leading causes of disability in tropical areas worldwide. Most cases of lymphatic filariasis are caused by the filarial nematode, *Wuchereria bancrofti* (Wb) although *Brugia malayi* (Bm) accounts for approximately 10% of the ~129 million estimated cases worldwide. Adult parasites reside in the human lymphatics, where they can elicit an inflammatory response that can result in lymphangitis acutely. Symptoms usually appear 5-18 months after exposure to an infected mosquito. Long-term exposure and repeated infections can lead to lymphedema, hydrocele, and/or elephantiasis. Microfilariae, produced by the adult parasites, circulate in the bloodstream.

Currently, single dose albendazole (400 mg) and DEC (6mg/kg) is administered annually for 4-6 years, a regimen approved by the World Health Organization (WHO), to interrupt transmission in all filariasis-endemic regions except Africa where ivermectin and albendazole is used.While DEC is a powerful microfilaricidal agent, Albendazole possibly plays role in sterilising adult parasite, hence suppressing resurgence of mf when applied in repeated intervals. The largest impediment to mass treatment lies in individual countries' inability to sustain mass treatment for 5-7 years. Since microfilarial levels in the blood are directly responsible for continued transmission, a more effective suppressive regimen could shorten the overall duration of the mass treatment programs, decrease cost, and increase compliance.

Progress of work

Screening

Field screening was conducted covering 1716 people between age group of 18-55 yrs for microfilarimia by finger prick blood which recorded 118 Mf positives.For study eligibility individuals giving consent for enrolment were investigated for eligibility by Mf count, Hb%, ALT, Creatinine and urine pregnancy test for females. 104 individuals satisfied inclusion criteria.

Enrollment

All the **104** eligible subjects (**88 male, 16 female**) have been enrolled at baseline. The patients were admitted to the hospital (KIIMS, Bhubaneswar) **in small batches of 3-5 persons** for drug treatment after obtaining their consent. Each subject underwent predrug baseline assessment by clinical examination, ultrasonography, microfilaria count, Og4C3 Ag and haematology parameters like haemoglobin and eosinophil count and stool test for helminth. Each of them **was** assigned to one of the **following** four drug regimen group as per the random number table.

Subject assignment to drug regimens(four)

The 104 subjects were assigned to drug regimens as given below.

Regimen	Drug Dose	No of subjects enrolled
S1	DEC(300mg) +Alb(400)Annual	26
S2	DEC(300mg) + Alb(400) Biannual	26
H1	DEC(300mg)+Alb(800) Annual	26
H2	DEC(300mg)+ Alb(800) Biannual	26
	Total	**104**

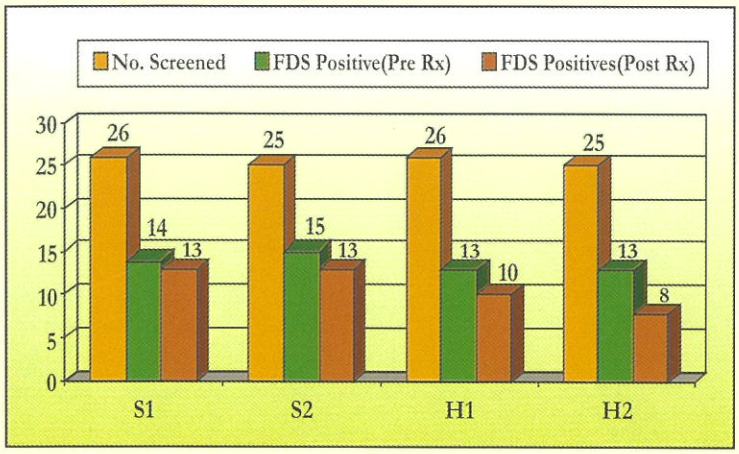
Microfilaria count: The baseline microfilaria count among the subjects ranged from 54 - 3000 mf /ml. The Geometric Mean of Mf count in different regimen groups were 455, 336, 383 and in 466 in S1, S2, H1, H2 arm respectively. (**Table 1**)

Density wise distributions of Microfilarimics in four arms are as under:

Treatmentarm	No. of individuals in the Mf Count range		
	50-100	101-1000	>1000
S1	5	13	8
S2	5	14	6
H1	6	13	7
H2	3	15	7

Ultra-sonography: The ultrasound examination for presence of filarial dance signs(FDS) **indicating the presence of** live adult worm in the lymphatic vessels was carried out in each of the subjects **at axillary, inguinal and scrotal (in males) regions bilaterally** . Out of **104** subjects 55 **had** nests showing FDS. How ever on repetition of USG within 3 days post drug (first dose) 44 subjects **demonstrated** FDS positivity. **Figure 1 demonstrates the observation in different regimens. The test was repeated after one year during follow up.**

Fig-1. Effect on adult worm FDS after first dose



Follow Up:

As per protocol the individuals were followed six monthly for investigation and/or treatment as per the treatment arm assigned.Till date **83** subjects were followed up for 6 months and **39** subjects for 12 months. The Mf count & Og4C3 were repeated at 6th & 12th month. USG was repeated within 3 days of baseline drug intake and at 1 year only on the cases showing positive FDS in baseline assessment. The percentage reduction in the mf count at 6th month was **75.6%** in S1, **78.0** % in S2, **87.7** % in H1 and **87.1** % in H2 respectively. Four subjects achieved total clearance of microfilaria.

Only **39** subjects have completed 12 months. The microfilaria clearance was noted in all regimen groups. **(S1:1 S2: 2, H1: 0, H2: 2). Table 1 and 2 show the changes in mf density and OG4C3 antigen in the four regimens.**

Table1. Mf density at 0, 6th and 12th month in the treatment cohorts

Treatmentarm	Mf Count (Geo Mean) (n=)			% reduction	
	O Day	6 M	12 M	6 M	12 M
S1	455(26)	111(19)	38.28(10)	75.6	91.5
S2	336(25)	74.1(24)	30.6(17)	78.0	90.8
H1	383(26)	46.8(23)	16.7(7)	87.7	95.6
H2	466(25)	60.30(17)	13.1(5)	87.0	87.0

Table 2. Change in Og4C3 titre (Mean) after intervention in 4 arms

Treatmentarm	Og4C3 titre (Mean) (n=)			% reduction	
	O Day	6 M	12 M	6 M	12 M
S1	12951(26)	1837 (19)	2953(10)	85.8	77.1
S2	15863(26)	2422(24)	3700(17)	84.7	76.6
H1	13011(26)	2876(23)	4954(7)	76.8	61.9
H2	6830(26)	2063(17)	1040(5)	69.7	84.7

Ultrasonography: Out of **39** subjects followed at 1st year, **25** subjects exhibited FDS at baseline **out of them 2** subjects showed FDS at the end of one year.

Adverse reaction

After administration of the different drug regimens, patients were followed up for 2 days in hospital and up to seven days in the village for any side reaction. However each subject was followed up periodically every fortnight at village level to ensure any clinical illness and **any other drug** used.

Out of total **104** enrolled subjects only **60** (57.6%) had side reactions (**after first dose**) like fever, headache, malaise, reeling head, drowsiness, nodule and testicular pain of mild to moderate grade. These symptoms started between **2 hrs to 3** days after the drug consumption. In each drug regimen group (both single and double dose Albendazole)more than **50%** subjects developed the side reaction.

Out of these 83 subjects **fourty one individuals received the second dose after 6 month.** Only 4(4.75%) out of **them** had manifestations of side reaction. At 1st year level 23 **were** followed up who consumed the drug, four had shown side reaction.

Plan for next year

All the individuals will be followed up six monthly with repeat drug administration and follow up investigations as per protocol following the treatment arm assigned to the enrolled individuals, to complete 24 month follow up period.

3. A study of Subclinical Lymphatic Manifestation in *W.bancrofti* Infection.

Principal Investigator	:	Dr. S.K.Kar
Co-Investigator(s)	:	Dr.B. Dwibedi, Dr.A.Maharana, Dr A.S. Kerketta
Starting date	:	October 2009
Closing date	:	November 2012
Funding	:	Extramural (GATES Foundation, USA)

Objective

1. Prevalence of sub clinical lymphatic pathology in population between 5-18 years with *W.bancrofti* infection in defined endemic community.
2. Effect of single annual and biannual dose of DEC plus Albendazole on lymphatic pathology in the identified group.

Background

Overt clinical manifestations of filarial disease have been mostly reported in young adults or older age groups, recent reports from various regions including Orissa indicate that in endemic areas around 25-30% of children (0-5 years) are already infected with filarial parasite as indicated by presence of circulating filarial antigen (Bal et al 2003). Earlier reports indicate detection of microfilariae (mf) even in infants aged 10 months. Data from endemic areas of India indicate that prevalence of filarial infection increases with advancement of age even without any overt clinical signs. Data on few asymptomatic carriers indicate morphologic distortion of lymphatic channels like dilatation and tortuosity, that are ascribed to the sub clinical pathology caused by the adult parasite. Although large group of paediatric population is infected, the clinical disease is clustered in later age groups. The preliminary data of ongoing study at Kerala in *B. malayi* infected population (Shenoy *et al* 2007) indicated the presence of live adult worms in 14% of children between age of 3-15 years (six microfilarimic children, one with filarial disease and 7 who were only positive for the filariasis specific IgG4 antibody test). Out of 100 children investigated lymphatic abnormalities was seen in 80 cases by lymphoscintigraphy (Shenoy *et al* 2007). Although distribution of *W.bancrofti* is found in 90% of endemic regions of India, no study has yet been done, that addressed to find out any evidence of subclinical lymphatic pathology in *W.bancrofti* infected children and adults before the appearance of clinical signs and there after.

Currently as per the global programme for elimination of lymphatic filariasis most endemic countries including India have initiated annual single dose mass administration (MDA) of DEC plus Albendazole for 5 to 6 yrs consecutively with a target towards elimination.

However the role of MDA in already infected children who have not developed symptoms is not addressed in MDA due to paucity of any evidence. Even effect of MDA on clinical filariasis is yet to be evaluated. As discussed, although the infection sets in at an early age the disease manifests in early adult hood or later. But the pathology in lymphatic system is likely to be progressive

during this gap. Detection and early intervention at this stage might prevent expression of disease or reverse early disease, for which evidence is lacking. Hence it is proposed to undertake an observational study to find out the sub-clinical lymphatic pathology in filariasis infected children and adolescents in *W.bancrofti* endemic area of the state; and to observe the effect of MDA with DEC and albendazole (alb) on the lymphatic abnormality.

Progress of work

Screening

Screening was done in 13 endemic villages from Khurda district, Orissa by night blood mf survey and antigenemia covering 526 children between 5 to 11 yrs of age and 796 children between 12 to 18 yrs of age. 66 subjects were identified as Mf positive and 16 were identified as OG4C3 antigen positive but mf negative in blood slide examination. The subjects were examined clinically for symptoms and signs of filarial disease (acute/chronic) Then eligibility screening test were conducted in 20 subjects for ALT, Hb%, serum creatine, urine pregnancy test and hematuria and all satishfied the eligibility criteria.

Enrollment

So far **20 (17 male, 3 female)** subjects were enrolled after obtaining their parents consent who satisfied eligibility criteria, of which **12** subjects were assigned randomly to annual and **8** to biannual drug regimen (with DEC + Albedazole 400 mg) group. Out of **20** subjects 4 were symptomatic rest of the children were asymptomatic.

Baseline investigation and drug administration

In the enrolled subjects, the initial microfilaria (mf) count ranged from **30 to 1540 mf/** ml (GM=**303.84**) The Og4C3 titre in the Baseline was **182 to 15107 units (Mean=5108).**

Lymphoscintigraphy of both upper and lower limbs was carried out by expert in nuclear medicine using radio labelled nanocolloid. The procedure was standardised before initiating the study.

Out of 20 subjects 10 had shown some abnormality in the lymphatic scan. Among them 8 had lymphatic flow obstruction,1 had increased collaterals and 2 had lymph node enlargement. Ultrasonography has shown filarial dance sign (FDS) of adult worm in 2 subjects.

All the enrolled children were given first dose of DEC plus Albendazole supervised by a physician and they were followed for any side reactions. Out of 20 subjects who received drug 9 reported with some side reactions like fever, headache, leg pain nausea, head reeling and cough. All were mild in nature and managed at home. No severe adverse event was noted

Plan for next yeart

It is planned to enrol eighty subjects into the study. To achieve this screening for microfilarimia and antigenimia will continue for seleting the possible study subjects, who will be further screened by the eligibility tests. Eligible subjects will be enrolled and follow up will be carried out six monthly as per protocol.

4. Role of $CD5^+$ B-lymphocytes in human lymphatic filariasis

Principal Investigator	:	Dr A.K. Satapathy
Co-Investigator	:	Dr B. Dwibedi , Dr P.K.Sahoo, Dr S.K.Kar
Duration	:	Three years
Starting date	:	April 2010
Close date	:	March 2013
Funding	:	Extramural (DST)

Objectives

1. To study the profile of B1 cell populations and its association with poly reactive antibodies in filarial infected human population.
2. To study the role of B1 cells in cytokine responses by filarial proteins and carbohydrates antigens in filarial infected human cells.

Background

The protective potential of T-lymphocytes in filarial infection is well documented. In contrast, the role of B cells in host protection against filariasis remains unclear. There are two major subsets of B-lymphocytes, B-1 and B-2 cells. In normal humans and mice, B-1 cells are committed to the production of polyreactive natural antibodies, mainly IgM, which bind to a variety of self-antigens. In contrast, conventional B cells (B-2) are mainly involved in the production of antigen-driven antibodies. Thus B-cells are a major component of the adaptive immune system. Several findings in literatures point towards a host protective role for antibodies in filariaisis. In human filariasis, anti filarial immune responses are profoundly influenced by infection status of the host. Several studies have shown that B1 subset of B cells play an important role in the outcome of infection in schistosomiasis, S. pneumonie and experimental filariasis. However, no serious effort has been made so far to study the status of B-1 cells and its role in outcome of human filarial infection.

Progress of work

Preliminary activity, which reflects in the previous year Annual report, was undertaken by using seed money from intramural fund. The project is started after receiving funds from DST. The objectives of the project are to study the profile of B-1 cell populations and its association with poly reactive antibodies in filarial infected human population. Since the study requires quantification of B-1 cells as well as poly reactive antibodies, blood samples of endemic normals, asymptomatic microfilariaemic carriers, cryptic and chronic patients were collected from a filarial endemic village. Cells were used for quantification of B-1 cell populations. Sera were separated and used for quantification of antibodies responses to a variety of self-antigens (e.g. ssDNA, actin,

tubulin, Lipopolysacharide). B-1 cells profile can be detected by flow cytometer from other cells by surface phenotypes. B-1 cells have been characterized in recent years and phenotypic markers on these cells have been identified. B1 lymphocytes are a subset of B cells that express the surface marker CD5 together with CD 19. The CD 5+ cells expressing CD 19+ (CD5+ CD19+) have been shown to appear to the right from the major population when B-cells are double stained with anti-CD5 and anti-CD19 antibodies (Fig-1). % of B-1 cells were quantified from the total B cells.

Mononuclear cells from human blood were stained with anti CD5 and anti CD19 antibodies.

Quantitative analysis of B-1 cells in the clinical spectrum of lymphatic filariasis offered interesting leads in understanding the role of B-1 cells in the parasitological outcome of filarial infection. The investigations revealed a significant difference between microfilariaemia carriers and other clinical groups. B1 cells population was significantly low in microfilariaemic carriers in comparison to individuals with endemic normals and people displaying chronic manifestation (Fig-2). These observations point towards the possibility of B-1 cells playing a significant role in the parasitological outcome in the exposed population.

Fig-1

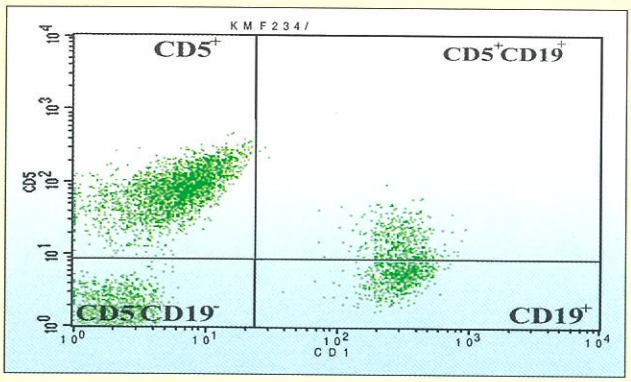
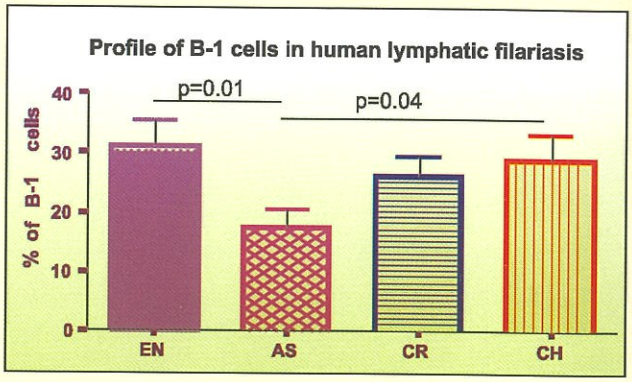


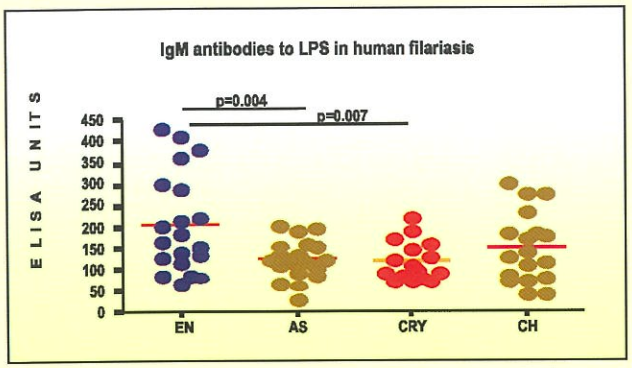
Fig-2

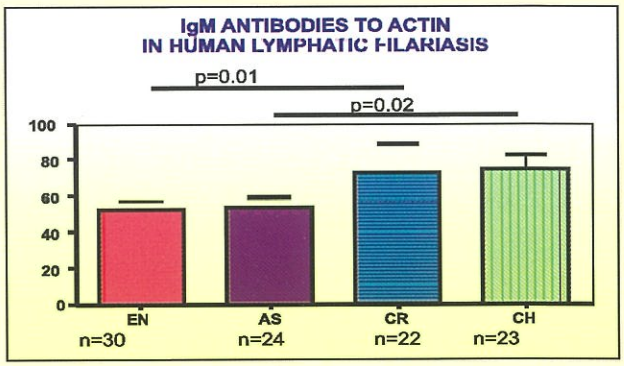


In normal humans, B-1 cells are committed to the production of polyreactive natural antibodies, mainly IgM, which bind a variety of self-antigens. The profiles of such antibodies that react with a wide variety of auto-antigens such as actin, myosin, tubulin, DNA etc were evaluated in human filariasis. Using a panel of sera collected from patient with chronic filariasis(CH), mf carriers(AS), Cryptic (CR) and endemic normals (EN) were tested for their response to a variety of antigens such as actin and ss-DNA and bacterial Lipopolysaccaharides. As reported in our earlier Annual report Ig M antibodies to ss-DNA were found to be significantly low in mf carriers cases in comparison to endemic normals and chronic patients. We quantified Ig G antibodies to ss-DNA in human filarial infection. Ig G antibodies reacting to ss-DNA were significantly low in mf carriers as compared to chronic cases. We next looked at the profile of antibodies responses in human filarial sera reacting to other antigens such as actin and lipopolysaccharide. Significantly low levels

of IgM antibodies to actin (Fig-3a) and LPS (Fig-3b) were demonstrable in microfilariae carriers (known to be immunologically hyporesponsive) in comparison to patients with chronic filarial disease and endemic normals (immunologically hyper responsive). The observation on decreased levels of Ig M antibody response to various antigens in mf carriers raise the possibility of polyreactive property of these antibodies.

Fig-3a





Pogress of work up to Sept 2011

Since B-1 cells are also committed to the production of polyreactive natural antibodies of Ig A, the levels of such antibodies reacting with a variety of antigens were evaluated in human filariasis. Significantly low levels of IgA antibodies to actin (Fig-4a) and LPS (Fig-4b) were demonstrable in microfilariae carriers in comparison to patients with chronic filarial disease and endemic normals. There was no significant difference between the groups when probed with anti-human Ig A conjugates against ss-DNA.

Fig-4a

Profile of IgA AnTIBODIES AGAINST ACTIN
IN DIFFERENT GROUPS OF CLINICAL CATEGORIES OF
HUMAN LYPHATIC FILARIASIS

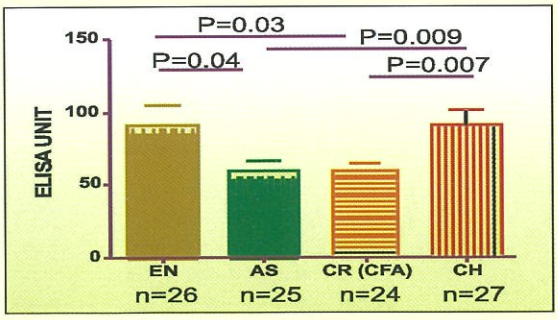
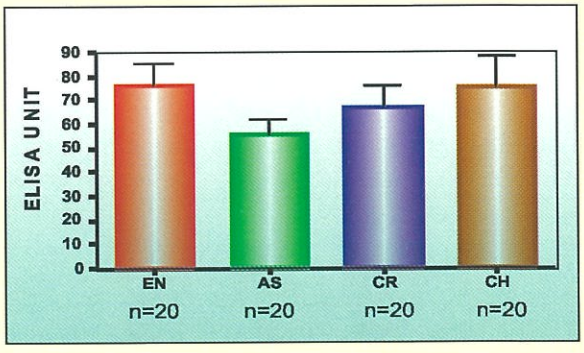


Fig-4b

IgA ANTIBODIES
HUM AN LYMPHATIC FILARIASIS



Since naturally occurring poly reactive antibodies have been demonstrated to play a role in innate immunity as well as in immunoregulation, investigations in our laboratory are now directed towards the study of the relationship between DNA-specific antibodies, anti-actin and anti-LPS antibodies during filarial infection.

5. Effect of maternal infection on neonatal immune responses in bancroftian filariasis.

Principal Investigator	:	Dr A.K. Satapathy
Co-Investigators	:	Dr M.S.Bal, Dr N.N. Mandal, Dr S.K.Kar
Duration	:	Three years
Starting date	:	Jan. 2010
Closeing date	:	Dec. 2012
Funding	:	Extramural (Immunology Task Force, ICMR)

Objectives

1. To study the B cell response (antibody isotypes) to filarial antigens in cord blood samples of offspring and in their corresponding mothers
2. To evaluate the influence of maternal filarial infection on mitogen and filarial antigen induced cellular responsiveness and cytokine production in cord blood of neonates
3. To compare the expression profile of T regulatory cells in cord blood of infected and uninfected mothers

Background

Lymphatic filariasis is caused by filarial nematode *Wuchereria bancrofti, Brugia malayi* or *Brugia timori*. It continues to be a major public health problem, in many tropical countries. The manifestation exhibits a wide spectrum and varies from asymptomatic infection to severe pathology. Although host genetic polymorphism and other environmental factor(s) may influence susceptibility to infection and disease, Filarial infection acquired through maternal origin has been considered a risk factor for increased susceptibility. A number of studies have shown that, the children of microfilaraemics mothers were more likely to be microfilaraemics than those of amicrofilaraemics mothers. Children borne of filarial infected mothers have been shown to impair filarial Ag-specific T cell responses. Children of infection free mother have been shown to respond vigorously to filarial antigen with lymphocytes proliferation, production of IL-2 and IFN-g. The above observations lead to the hypothesis that prenatal exposure may affect the subsequent immune responses and disease development.

Progress of work

Preliminary activity, which reflects in the previous year Annual report, was undertaken by using seed money from intramural fund. The project is started after receiving funds from ICMR. To find out the effect of maternal infection on transplacental transfer of circulating filarial antigens (CFA), cord blood along with the corresponding mother's blood samples were collected from District hospital, Khurda. Previously we have shown the evidence of transplacental transfer of

circulating filarial antigen from infected mother to their corresponding cord blood. Till now we have evaluated 171 pair of mother and their corresponding cord blood samples. An overall prevalence of CFA among mother was noted to be 47.3% where as it is only 10.5% in cord samples. None of the cord sample from CFA negative mother were found antigen positive but 22.2% of infected mothers have transferred CFA to their respective cord suggesting transplacental transfer of filarial antigens.

Immunological evaluation of humoral responses to filarial antigen was determined in paired maternal and cord samples to evaluate the influence of maternal infection on the development of subsequent anti filarial immunity in offspring. Antibody isotype (IgG, IgM, and IgE) to filarial antigen was determined in paired maternal and cord blood samples and shown in Table-1. No significant difference was observed in % positivity of antifilarial isotype (IgG, IgM, and IgE) between CFA positive and negative mothers. IgG positivity in cord blood did not vary significantly with the antigen status of mother (P=0.1239). Filarial specific IgM and IgE isotypes were significantly higher in cord blood (P=0.006 for IgM and P=0.0005 for IgE) of infected mothers than amongst uninfected mothers. IgM only could be detected in infants who were CFA negative, but born from CFA positive mothers.

Table-1. Filaria specific antibody isotypes (IgG, IgM, IgE) seropositivities in maternal and cord samples

Maternal Infection status	N	IgG Seronositive		IgM Seropositive		IgE Seronositive	
		Maternal sera N (%)	Cord sera No (%)	Maternal Sera No. (%)	Cord sear No. (%)	Maternal sera No. (%)	Cord No.
CFA Positive	53	32 (60.4)	8 (15.1)	34 (64.2)	6 (11.3)	29 (54.7)	13(24.5)
CFA Nagative	6	40 (60.6)	18 (27.3)	40 (60.6)	0 (0)	47 (71.2)	2 (3.03)

Fig –1 shows the analysis of filarial specific IgG subclass in paired maternal and cord blood samples. Filarial specific IgG1, IgG2 and IgG4 antibodies were significantly elevated in cord blood of infected mothers than amongst uninfected mothers. All IgG subclass response is higher in cord blood of infected mothers than non-infected mothers. All these findings provide evidence for pre-natal sensitization occurs during filarial infection.

The impact of placental filarial infection on the immune competence of the neonate remains poorly understood. Prenatal exposure to parasite antigens has been shown to induce tolerance in several experimental helminthic infections. It has been postulated that helminth worms stimulate regulatory T cells, which produce down regulatory cytokines to inhibit inflammatory responses and facilitate its own survival. We wished to determine whether maternal filarial infection influences

Fig-1. Filarial specific IgG subclass responses in cord sera of infected and uninfected mothers.

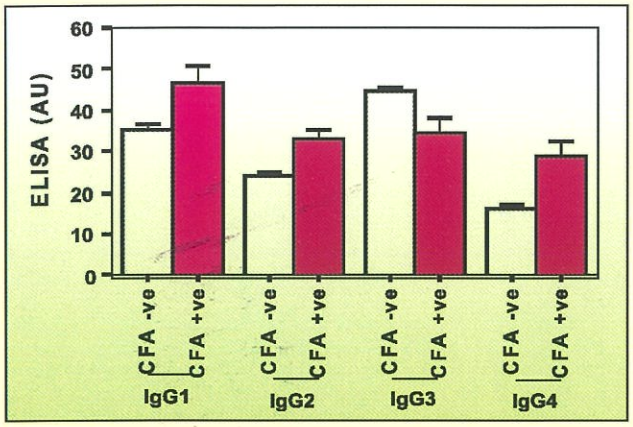
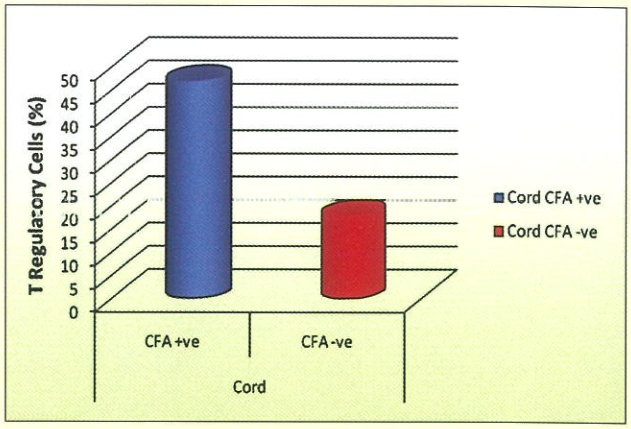


Fig-2. T Regulatory ($CD4^+CD25^+$) cells in filarial infected and uninfected cord samples.



T cell activation in utero. The presence of CD25 and CTLA-4 on T cells is generally associated with the extent of activation of both effector and regulatory T cells. We compared the percentages of CD4+ cells expressing CD25 in paired maternal and cord samples from filarial infected and uninfected groups. Our preliminary results are shown in Fig –2. T regulatory ($CD4^+$ and $CD25^+$) cells were found to be high in cord blood samples of filarial infected mother compared to cord blood samples of uninfected mother.

Drug administration to adolescent tribal girls for anaemia treatment.

6. Development of strategy for optimizing the accessibility and utilization of the government operated malaria control programme – an operational research study.

Principal Investigator	:	Dr A S Kerketta
Duration	:	Two years
Starting date	:	August 2007
Closing date	:	July 2009
Funding	:	Intramural

General objective

Identify the barriers in effective delivery of the available tools of the malaria control strategy EDCT, at community and different level of health providers & develop innovative strategy for bringing improvement in the accessibility and utilization of the government operated malaria control programme.

Specific objectives

- Understand people's knowledge and perception on malaria and available treatment
- Understand the treatment seeking behaviour of the community
- Understand the knowledge and perception of providers in the area on malaria and its treatment

Progress of Work

The community perception on malaria & its control programme was done by household survey in two villages, amongst 142 households covering 1200 population. situated in bank of river Mahanadi. The community perception was assessed through the interview with the head of the household. The data shows that around 95% households have experience of malaria in their family. The cause of malaria attributed by the household was mosquito bite 71.1% and 28.9% for other causes like hard labour, exposure to cold & heat. The treatment was sought on day-1 by 28.4% of the people and by 64.8% on day 2, rest 16.6% of the family, the treatment was initiated on day 3 or 4. Around 47.2% had idea of free availability of anti- malarial drug with ASHA and rest 52.8% didn't know the availability of the drug. Regarding the option for treatment provider, none opined to go to the health provider rather sought the service of Quack or Pharmasist. Father was found to be the decision maker for treatment seeking in 49.6 families followed by mother in 25.4% and other elder members of the family in 24.0% households. Regarding the knowledge on the duties of ASHA by the villagers, 55.0% didn't have any idea for the duties of ASHA but 24.6% opined the slide collection,14.1% hygiene maintenance and 6.3% for holding meetings.

The findings of the community perception by in-depth interview was validated by illness narrative for the cases suffered from malaria in last 15 days and the reason shows 26.0% are avail the service of ASHA against none expressed availing the facility of ASHA in indepth interview, 48.0% from Quack, 12.0% from the government hospital and 16.0% had availed the service of the multiple provider depending on the severity of the disease. The people who went to Govt Hospital were mainly for the treatment of the patient of the child age group. The reason for obtaining different facility indicated in the table below.

Reasons for obtaining different facilities

Providers	ASHA	Quack	Govt Doctors
Distance	0.5-1KM	1-2KM	20KM
Faith	Less	Strong faith	Stong faith
Response	Immediate/delayed	Immediate	Immediate
Drugs	4tablets of CQ	Injection, saline, liquid formulation	Injection, saline, liquid formulation
Availability of drug	Uncertain	100% certain	100% certain

Plan for next year

The formative research will be completed and basing to the findings of formative research, the innovative strategy will be developed.

Preparation of clinical data sheet of Malaria patient

Field level training to BMC workers on vector control

7. Development of a LAMP assay for diagnosis of human malaria

Principal Investigator	:	Dr M R Ranjit
Co-Investigator	:	Dr A S Acharya
Starting Date:	:	July 2010
Closing Date:	:	June 2012
Funding	:	Extramural (DBT)

Objectives

1. To design species specific loop primers for detection of human malaria parasites.
2. To optimize the reaction conditions for easy detection of the LAMP derived products.
3. To find out the efficacy of the test compared to nested PCR and light microscopy

Background

Microscopy is the gold standard for diagnosis of malaria even though various rapid and simple tests have been developed in recent years. But loop-mediated isothermal amplification (LAMP) of nucleic acids seems to be a promising new technique, which enables to detect malaria parasites in a setting with limited resources. However, LAMP assay in its current form lacks sufficient accuracy in visualization of the end product. Therefore, optimization/standardization of the current method for visualization of LAMP end products is important. The proposed project will help to develop a suitable method for detection of end product.

Progress

The funding for the project has been received from DBT on 2/7/2010 and the work has been initiated. Three pairs of loop primers for each species of Plasmodium has been designed on the basis of the genus and species-specific nucleotide sequences of the 18S rRNA genes of *P falciparum, P vivax , P malariae* and *P ovale* by using LAMP design software Primer Explorer V3.The LAMP assay is being standardized by taking two different sets of archive blood samples: one PCR positive for *P faciparum* and the other PCR negative for *P faciparum*. Briefly parasite genomic DNA was isolated by following the standard chloroform isolation and phenol precipitation method and is being used as the template. The assay is being standardized in a total reaction mixture of 25ìl which consists of 7 ìl of primers (FIP and BIP 40pmol, Loop –F and Lop-B 20pmol and F3 and B3 5pmol), 12.5 ìl of reaction mixture (40mmol/L Tris-Hcl,20 mmol/L KCL,16mmol $MgSO_4$,20 mmol/L NH_4SO_4, 0.2% Tween 20, 1.6mol/L betaine, 2.8mmol/L dNTP each), 1 ìl Bst DNA polymerase, 2 ìl template DNA and distilled water to make the volume to25ìl. The reaction mixture is incubated in a hot water bath at 65^0 C for 30 minutes. This reaction mixture and reaction conditions are based on the previous publications for other organisms. The study is in progress and the results will be discussed in the SAC.

8. Molecular analysis of drug resistance genes and prediction of treatment outcome in *P falciparum* infections in Orissa.

Principal Investigator	:	Dr M R Ranjit
Co-Investigator	:	Dr A S Acharya
Duration	:	2 Years
Starting date	:	April 2008
Closing date	:	March 2011
Funding	:	Extramural (NVBDCP, Govt of India)

Objectives

1. To observe the frequency of the genotypes of Pfcrt/Pfmdr1 and DHFR-DHPS associated with Chloroquine and Pyremethamine-Sulphadoxine resistance in natural *P falciparum* parasite populations of Orissa.
2. To study the origin and spread of resistance alleles through the parasite population in this region.

Background

The WHO has outlined three ways of measuring drug efficacy (i) the clinical responses of patients to drug treatment (ii) the sensitivity of parasites to drugs in-vitro or (iii) accepted molecular markers as complementary tools for monitoring drug resistance. Though the first two methods are specific and quite sensitive, yet these are time consuming and sometimes raise ethical issues for its application. However, correlation of specific mutations in the genes that encode targets of the antifolate drugs and drug resistance, such as DHPS (targeted by Sulpha drugs) and DHFR (targeted by DHFR inhibitors), are well established; and certain mutations in the *P falciparum* chloroquine transporter gene (Pfcrt) and the *P falciparum* multidrug resistance gene analog (Pfmdr1) has been observed to be associated with the development of resistance to chloroquine in different studies. Despite certain difficulties the potential use of molecular markers as early warning signals and surveillance tool is clear. Since no systematic report is available on the frequency and distribution of CQ and S-P drug resistance markers in the state, the proposed study aims at generating a base line data on the frequency and distribution of CQ and S-P resistance markers in different physiographical regions of the state and predicting the origin and spread of these genotypes through *P.falciparum* populations in this particular regions of the country. This information will serve as a public health tool to develop a rational drug policy and combat spread of drug resistance.

Progress

During the period under report (April 2009 to March 2010) a total of 40 *P falciparum* isolates (of unknown CQ response) were analyzed for the presence of K76T mutation in the *Pfcrt* and N86Y in the *Pfmdr1* gene. For *Pfcrt*, only eight samples produced the expected 128 and136

bp amplicon when digested with *ApoI,* indicating the presence of wild K76. Rest of the 32 isolates resisted enzyme digestion thus, indicating the presence of T in 76th position (Table 1). Similarly, on restriction digestion of 603bp DNA fragment of *Pfmdr1* with *AflIII,* produced two fragments (253 and 250 bp long) in only those eight isolates that possessed K76, indicating the presence of N in the 86th position (Table 1). The presence of these two mutations was further confirmed in all the 40 isolates by DNA sequencing of these amplified fragments of the two genes.

Combining both the *Pfcrt* and *Pfmdr1* genes, we sequenced 867 nucleotides in each of the 40 *P. falciparum* isolates from Odisha, thus, in total 34,680 nucleotide base pairs were sequenced with 2X coverage. Separate sequence alignment for both the genes revealed six SNPs in the *Pfcrt* and two SNPs in the *Pfmdr1* genes. The number of haplotypes also varies between *Pfcrt* and *Pfmdr1* genes *i.e.* four in *Pfcrt* and three in *Pfmdr1*. Sequenced fragments of both the genes were translated into amino-acid sequences and five point mutations were observed in the 72nd, 74th, 75th, 76th and 97th positions of *Pfcrt* amino acid sequence, and two in 86th and 184th position of *Pfmdr1* (Table 1). No synonymous (or silent) substitutions were observed, meaning all the eight SNPs detected in both the genes were non-synonymous (amino acid changing nucleotide substitutions). The N86 and F184 were found together in 32 *P. falciparum* isolates and 184F was also present in two isolates that had N in 86th amino acid of the *Pfmdr1* polypeptide chain. Out of several haplotypes reported to be formed due to the presence of different amino acids from 72-76th amino-acid positions of *Pfcrt* gene, only three haplotypes (CVIET, SVMNT and CVMNK) were found in the presently studied Odisha samples. Proportion of CVIET (67.5%) was found to be the highest, followed by CVMNK (20%) and SVMNT (12.5%).

Table 1

	Pfcrt gene fragment						*Pfmdr1* gene fragemnt	
Position of codon	72	73	74	75	76	97	86	184
Wild type	TGT Cys	GTA Val	ATG Met	AAT Asn	AAA Lys	CAC His	TAT Tyr	TAT Tyr
No. of isolates with wild mutations	35	40	13	13	8	30	8	38
Mutant type	AGT Ser	GTA Val	ATT Ile	GAA Glu	ACA Thr	CTC Leu	AAT Asn	TTT Phe
No. of isolates with mutant mutations	5	40	27	27	32	10	32	2

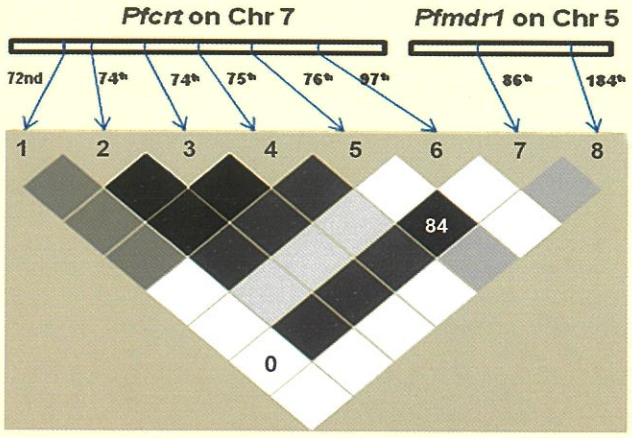
The two estimates of nucleotide diversity, as measured by π and θ_w, were found to be variable for both the genes (*Pfcrt* and *Pfmdr1*; Table 2). In general, the *Pfcrt* gene presented higher genetic diversity than the *Pfmdr1* gene (Table 2). It is clear from the Table 2 that the π values in both the

genes are a bit higher than the θ_w values, indicating more number of intermediate frequency mutations. We also conducted three tests of neutrality, and in none of them statistically significant results showing departure from neutrality were obtained (Table 2). However, in general, the data signify that both the genes are evolving under standard neutral model of molecular evolution in population sample of Odisha. Since, all the mutations detected in the study were non-synonymous, only *dN* values could be estimated which were found to be statistically significantly different from the silent polymorphisms (not detected in the study) in case of the *Pfmdr1* gene. However, in case of *Pfcrt*, no statistically significant difference was obtained (data not shown). Furthermore, LD estimates between all possible pairs of SNPs of both the genes (*Pfcrt* and *Pfmdr1*) was determined (Fig. 1). It is evident from the figure 3 that, three statistically significant intragenic associations between three SNPs (two SNPs in codon 74 and one SNP in codon 75) in *Pfcrt* gene and one intergenic association between SNP of *Pfcrt* (76T) and SNP of *Pfmdr1* (86Y) were observed.

Genes		*Pfcrt* (308526.....311620) 3096bp	*Pfmdr1* (957885.....962144)
No. of isolates		40	40
Size of the fragment (bp)		264	603
Nucleotide Position		308619.....308883	957885.....958488
No. of SNPs		6	2
No. of Haplotypes		4	3
Haplotype diversity		0.719	0.344
Nucleotide diversity	θ	0.00541	0.00078
	π	0.00866	0.00071
Tests of neutra lity			
Tajima's *D*		1.6583	-0.1789
Fu & Li's *D**		1.1919	0.77124
Fu & Li's *F**		1.5621	0.57422

Table 2

We have also isolated *P falciparum* genomic DNA from 245 malaria positive samples, collected from Sundergarh, Keonjhar, Mayurbhanj, Kandhamal, Rayagada, Angul, Kalahandi and Nayagarh districts, were extracted by phenol-chloroform and ethanol precipitation method. Ten different microsatellite markers flanking Pfcrt K76T mutations and four microsatellite markers flanking Pfmdr1N86Y in the upstream and downstream regions were also analyzed by 8% non-denaturing polyacrylamide gel electrophoresis and ethidium bromide staining,



to investigate the origin of this mutation in this region of the country. The flanking microsatellite size polymorphism of resistant Pfcrt and Pfmdr1 genes showed reduced genetic diversity around the gene indicating selection pressure on the target gene.

9. Mapping of *P.falciparum* susceptibility to Chloroquine, in malaria endemic districts of Orissa.

Principal Investigator	:	Dr.A S Kerketta
Starting date	:	October 2008
Closing Date	:	March 2011
Funding	:	Extramural (NVBDCP, Govt of India, New Delhi)

Objective

To assess the parasite susceptibility to Chloroquine (CQ) in treatment of uncomplicated P.falciparum malaria in seven high malaria endemic districts of Orissa.

Background

The study was initiated with the aim to map the susceptibility of P.falciparum to CQ in seven high malaria endemic district if Orissa, with the financial support from NVDCP, New Delhi. With of the first instalment of the grant for the period 2008-2009, out of seven study districts, four districts have been studied for the P.falciparum susceptibility to CQ. The study reported 58%-100% resistance of P.falciparum to CQ in the districts studied. The report was communicated to the funding agency. Keeping high prevalence of CQ resistance in the state as well as in other areas of country, the National Drug Policy was changed to Artemesinin Combination Therapy (ACT) as first line of drug for the treatment of uncomplicated P. falciparum malaria. Thus since 2009 ACT was implemented in the area reported having CQ resistance and its adjacent. But prior to it in some high malaria endemic districts like Mayurbhanj, Sundargarh and Dhenkanala the ACT has been used since 2008. Thus before releasing the second instalment of grant, the Funding Agency (NVBDCP) instructed to modify the protocol taking ACT instead of CQ in the initial protocol. Thus the protocol had been modified with change of the study districts where the ACT have already been used since 2008, Based on the modification the second instalment of grant has been released during June 10 for the period of April 2010 -March 2011.

Progress: The baseline information has been collected from the rest of the three study districts and a necessary arrangement has been made. The study on P.falciparum susceptibility to ACT will be initiated shortly in three high malaria endemic districts namely like Mayurbhanj, Sundargarh and Dhenkanala where the ACT has been used since 2008.

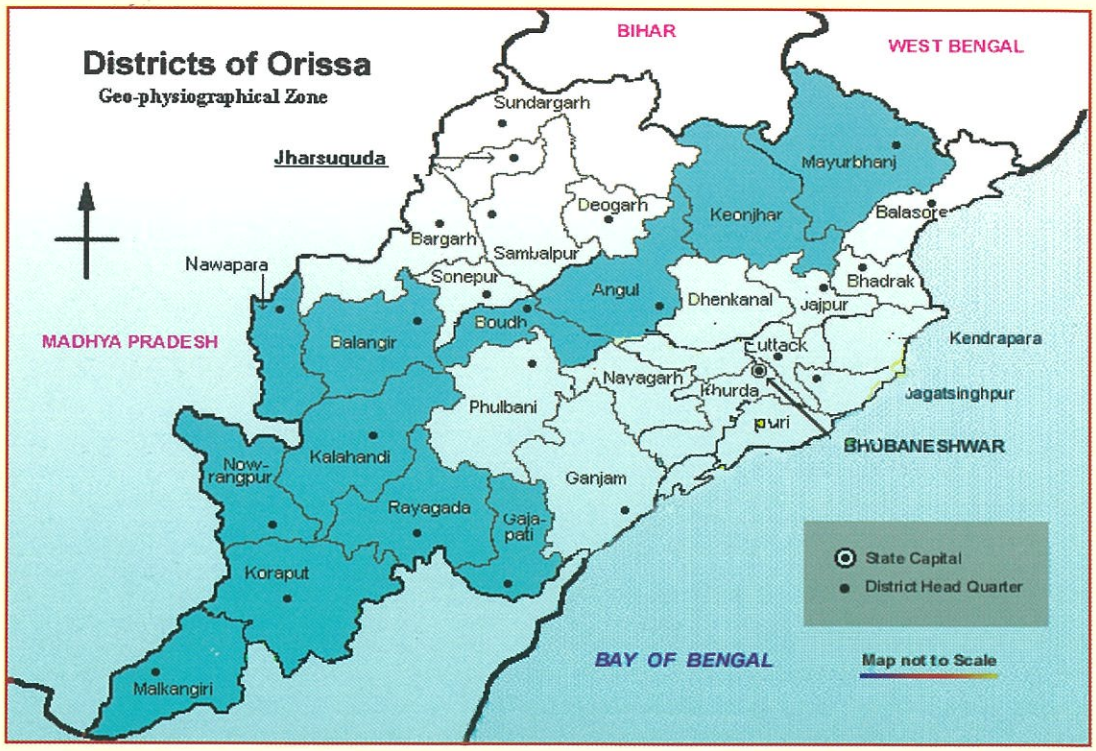
10. Molecular characterization of *Anopheles annularis* complex: Development of species specific diagnostic markers and microsatellite markers.

Principal Investigator	:	Dr. R.K.Hazra
Co .Investigators	:	Dr. N.Mahapatra
Starting Date	:	Dec 2007
Closing Date	:	December 2010
Funding	:	Extramural, CSIR

Objectives:

1. To compare cytotaxonomic technique with new molecular technique to establish the accurate identification of the sibling species.
2. To develop multiplex PCR technique to achieve simultaneous detection of sporozoite identification, blood meal analysis and sibling species identification from single mosquito.
3. To develop microsatellite markers for *Anopheles annularis* species for population genetics analysis.

Study area the study areas will be selected on the basis of different ecological condition.



Progress of Work

Development of Micro satellite Markers:

1. **PCR isolation of microsatellite arrays:** Here we developed a PCR isolation microsatellite arrays (PIMA), an approach to isolate and characterize microsatellite flanking sequences from small quantities of genomic DNA. This approach builds on previously described random amplified polymorphic DNA (RAPD) enrichment procedures but develops the use of repeat specific PCR to detect microsatellite arrays in contrast to standard radioactive hybridization techniques. The protocol is cheap and efficient, with the advantage that it requires minimum specialized equipments.

2. **Polymorphism in field populations:** *An. annularis* collected from different sites, where species A has earlier been found (Subbarao et al., 1994). *An. annularis* identified as species A cytotaxonomically and by molecular assays screened with selected microsatellite markers. Each selected marker has been screened for polymorphism in field-collected species for establishing polymorphism and allele frequency of each microsatellite locus.

Molecular analysis

Genetic similarity of the species Anopheles annularis were analysed by RAPD and ISSR primers. 25 RAPD and 15 ISSR primers gives different banding patterns. RAPD and ISSR analysis of *An.annularis* from different districts of Orissa showed different banding pattern ranging from100 bp-1500bp. From the gel between 200bp-800bp fragments were eluted and cloned into pGEM-T vector and sequenced. Banding pattern observed with in the anopheles species collected from different villages of same species. Significant changes observed 350bp in lane 3 and in lane 3,6 and 7 one unique band present at 200bp in fig 1. In fig 2 there is unique band present in lane 4 at 900bp and 100bp.

Molecular Characterisation of *Anopheles annularis* by RAPD and ISSR primers

RAPD gel photograph showed that different banding pattern show some difference within the species collected from different ecozones of Orissa.

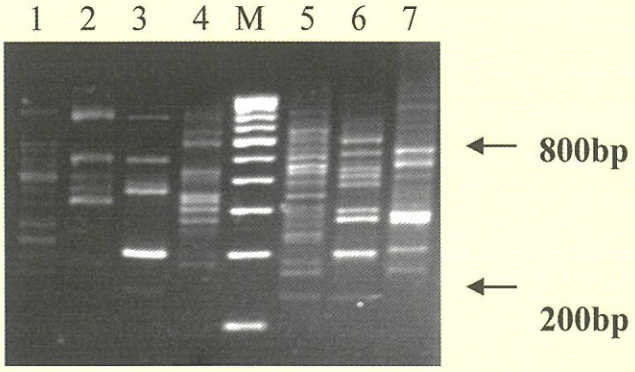


Fig 1. Ran 1 RAPD marker analysis of An. annularis collected from Mayurbhanj district.

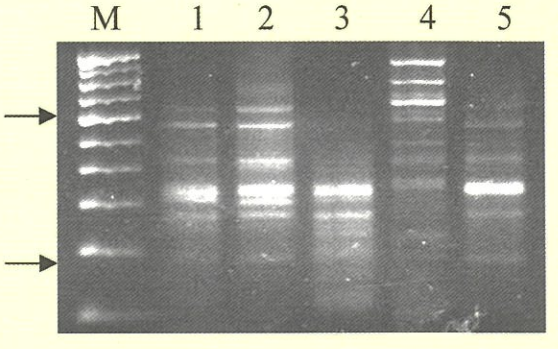


Fig2. Ran2RAPD marker analysis An. annularis collected from Keonjhar district.

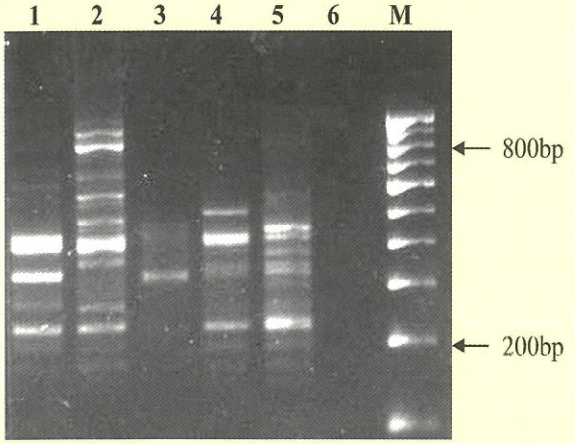


Fig-3. Gel photograph of Ran 5 RAPD marker analysis of An. annularis collected from Khurda district

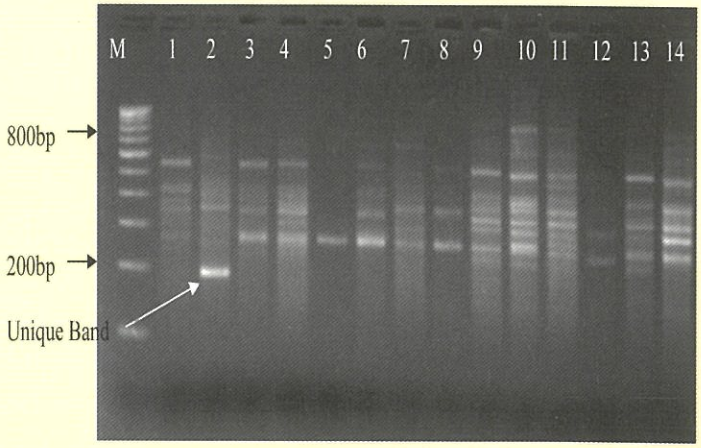


Fig-4. Gel photograph of $(GACA)_4$ ISSR marker analysis of An. annularis collected from 13 districts of Orissa

ISSR primers were amplified in the region between two microsatellite in the genome. So this region is highly conserved. There are 2 unique band in the sample number 2 and 10. After sequencing of the fragments the microsatellite regions were identified.

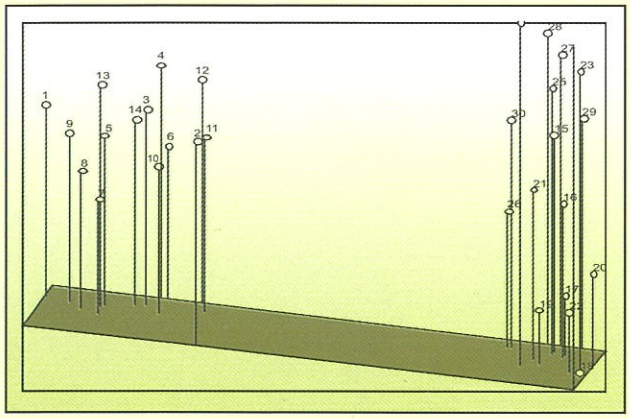


Fig 5. Dendogram showing similarity between An.annularis collected from three different geographical regions of Orissa.

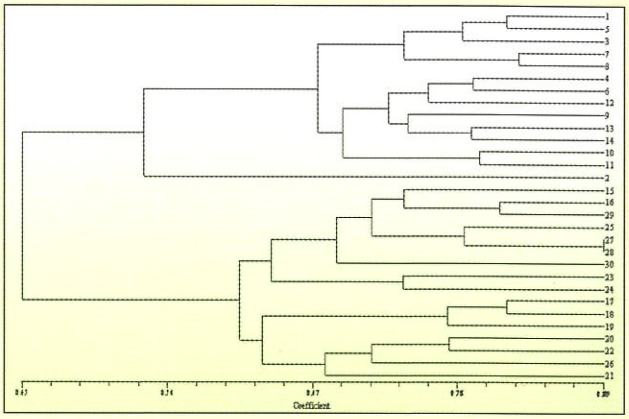


Fig 6. Principal component analysis of *An.annularis* collected from three different geographical regions of Orissa.

Isolation and characterization of Microsatellite loci:

High polymorphism and the relative ease scoring represent the two major features that make microsatellites of large interest for many genetic studies. The isolation of microsatellite markers began with a random amplified polymorphic DNA polymerase chain reaction (RAPD–PCR) enrichment. RAPD primers are already designed then after sequencing, the primers will be designed upstream and downstream of the repetitive DNA.

Microsatellite Repeats: $(CAA)_{5,}$ $(G)_{13,}$ $(CA)_4$

Microsatellite Repeats: (CAA) 5, (G) 11

Microsatellite Repeats: (GTG) 3, (CT) 3

Microsat repeat: (TTG)4, (CG)3, C6

Basing on the sequencing result primers will be designed for amplification of the microsatellite repeats. Primers were designed by Primer 4 software.

Out come of the project

The intra-specific variations in nucleotide sequences will be used for development of species diagnostic techniques for identification of the sibling species. Development of microsatellite markers would be useful tool for developing genetic markers, which will be used in mapping genes of interest, to study population structure, gene flow etc.

Correlation between cytotaxonomic and molecular technique

Cytogenetic analysis, in spite of some inherent difficulties has proved to be a powerful tool for the identification of sibling species of anophelines. The proposed study also planned to establish a correlation between cytotaxonomic and molecular technique for identification of sibling species which will help to used in mapping genes of interest, to study population structure, gene flow etc.

Training on PDA use for census data collection

11. Vector mapping with its susceptibility status to insecticides in seven high-risk districts of Orissa

Principal Investigator	:	Dr.R.K.Hazra
Co Investigators	:	Dr.N.Mahapatra
		Mr.H.K.Tripathy
Starting date	:	March 2008
Closing date	:	March 2011
Funding	:	Extramural (NVBDCP)

Objectives

1. To study the vector fauna, their habits and density and vector infection rate in the sample sites of seven high district of Orissa.
2. To study the susceptibility status of malaria vectors to different insecticides used in public health Programme.

As per the recommendation of SAC the vector mapping and susceptibility status should be done in all the seasons. As one season left and the fund received late of this year so the rest of the work will complete within March 2011.

Progress of the Project

Knowledge of baseline malaria transmission intensity in a given environment is important to guide malaria control interventions. In Orissa, recent information on malaria transmission intensity is insufficient. Therefore, an entomological study was conducted in seven ecologically different districts of Orissa to assess the seasonal patterns in malaria transmission intensity.

As identified by EMCP, operating in Orissa by Govt. of Orissa, following blocks of 7 districts are high risk for malaria transmission that are Nuapada (Kharia), Keonjhar (Ghatgaon), Khandamal (Khajuripara), Gajapati (Mohana), Boudh (Adenigarh), Rayagada (Muniguda) and Nabarangpur (Papdahandi).

All surveys were conducted during rainy and winter seasons in all the districts. The highest mosquito collection was made in Keonjhar area and lowest was in Nuapara area. There was more *Anopheles* species (77%) than *Culicine* (22.9%). The highest number of *Anopheles* was caught in Gajapati area (88.5%). A total of 6270 *Anopheles* mosquitoes of 23 species were obtained from adult collections was recorded during the study. The species recorded included *An. annularis, An. culicifacies, An. fluviatilis, An. minimus, An. philippinensis* and *An. varuna,* which have been recognized as malaria vectors in India. Most of the species (54.0% of the anopheline fauna) were

collected during the winter season. *An. culicifacies*, *An. annularis* was predominant vector in the seven regions and distributed as follows: Kandhamal (ann 46.7%, cul 31.9%, n=379), Rayagada (ann 18.6%, cul 33.8%, n=384), Gajapati (ann 32.8%, cul 11.8%, n=347), Nawarangpur (ann 19.7%, cul 28.5%, n=536), Nuapara (ann 27.6%, cul 39.1%, n=427), Keonjhar (ann 8.9%, cul 21.7%, n=578), Boudh (ann 21%, cul 24.3%, n=738).

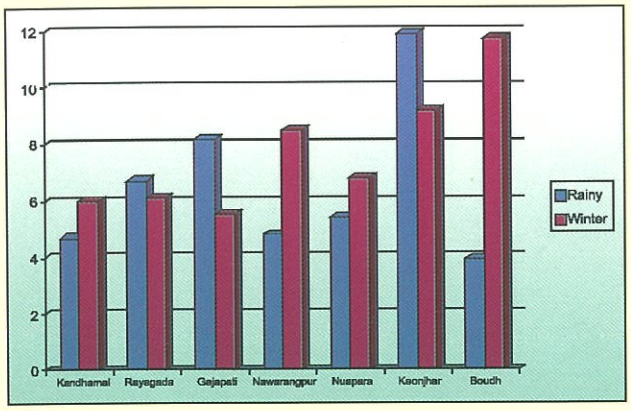


Fig.1 Percentage of Anopheles species collected during rain and winter season

In all, 4630 female anopheline specimens belonging to 5 species collected in the different study sites were processed for human blood index and *Plasmodium falciparum* infectivity by PCR. Out of these 28 sporozoite (Pf) positive were detrected in *Anopheles, in which An. culicifacies* (Hm 5.8%, Pf 1.3%), *An. fluviatilis* (Hm 47.8%, Pf 1.5%), *An. annularis* (Hm 2.7%, Pf 0.3%), *An. varuna* (Hm 1.4%, Pf 0%) and *An. subpictus* (Hm 0.44%, Pf 0%). The highest human blood index was found in the *An. fluviatilis* population at 47.8% while 5.8% of *An. culicifacies* had fed on human blood. Generally the mosquitoes particularly *An. fluviatilis* in the hilly area (Boudh) showed more propensity for human blood rather than those in the flat area. The main species i.e. *An. culicifacies*, *An. fluviatilis* and *An. annularis*, which are collected during winter season, showed high sporozoite positive than of the rainy season (Figure 2).

The *Anopheles* species were collected from different resting places. *An. fluviatilis* were collected less in numbers from different resting places where as *An. culicifacies* and *An. annularis* were captured at more relevant density and at different abdominal conditions from animal shelter. All species showed a distinct preference for animal shelter.

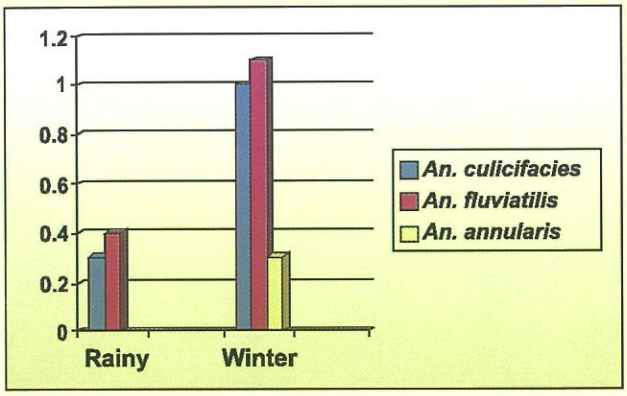


Fig 2 Impact of the season and the Plasmodium falciparum sporozoite positive rate during the entomological study.

Exposure to DDT induced significantly reduced mortality in *An. culicifacies* from all study localities, implicating resistance according to established criteria. The highest resistance of *An. culicifacies* was recorded from Kandhamal followed by Keonjhar and Boudh where as resistance of *An. annularis* was recorded from Keonjhar followed by Kandhamal. By applying WHO criteria (98-100% mortality indicates susceptibility, 80-97% mortality requires confirmation of resistance with other methods and < 80% mortality suggests resistance), it was found that field samples were resistant to

DDT. A total of about 749, female *Anopheles* mosquitoes (*An. culicifacies,* 560 and An. *annularis* 400) were exposed to DDT (4%) to determine their level of susceptibility. Over 75% and above of the malaria vector mosquitoes exposed was knocked down within an hour of exposure to the DDT. The genotype assay by PCR was done for the knock down and survivor' species of the susceptibility test. The *kdr* mutation was detected in Keonjhar district at frequency of 1% (Table1).

Table-1. Phenotype data using WHO susceptibility test and genotypes as assessed by PCR assay

District	WHO Bioassay test	*An. culicifacies*n=560				*An. annularis*n=400			
		Total	SS	SR	RR	Total	SS	SR	RR
Khandamal	Dead/ knock down	76%	76%	-	-	81.3%	81.3%	-	-
	Survivors	24%	21.5%	2.5%	-	18.7%	16.2%	2.5%	-
Rayagada	Dead/ knock down	90%	90%	-	-	91.2%	91.2%	-	-
	Survivors	10%	10%	-	-	8.7%	8.7%	-	-
Gajapati	Dead/ knock down	82.3%	81.3%	-	-	85%	85%	-	-
	Survivors	17.7%	16.5%	1.2%	-	15%	12.5%	2.5%	-
Nawarangpur	Dead/ knock down	86.3%	86.3%	-	-	88.8%	88.8	-	-
	Survivors	13.7%	12.5%	1.2%	-	11.2%	8.7%	2.5%	-
Nuapara	Dead/ knock down	95%	95%	-	-	91.2%	91.2%	-	-
	Survivors	5%	5%	-	-	8.7%	8.7%	-	-
Keonjhar	Dead/ knock down	81.2%	81.2%	-	-	80%	80%	-	-
	Survivors	18.8%	16.5%	1.3%	1%	20%	15%	3.7%	1.2%
Boudh	Dead/ knock down	85%	85%	-	-	86.3%	86.3%	-	-
	Survivors	15%	14%	1%	-	13.7%	12.5%	1.2%	-
Total		560		-	-	400			

12. Development of intervention strategies to reduce iron deficiency anaemia among adolescent girls through iron-folic acid, deworming, Vitamin B_{12} supplementation and nutrition education in a tribal block of Gajapati district, Orissa'.

Principal Investigator : Dr.G.Bulliyya
Co-Investigators : Dr.A.Moharana, Dr.B.Dwibedi
Starting date : April 2009
Closing date : March 2011
Funding : Extramural (ICMR Tribal Taskforce)

Objective

1. To carry out a comparative study on the efficacy of iron-folic acid and deworming in combination with vitamin B_{12} supplementation and nutrition education regimens in reducing iron deficiency anaemia among unmarried tribal adolescent girls in Gajapati district.

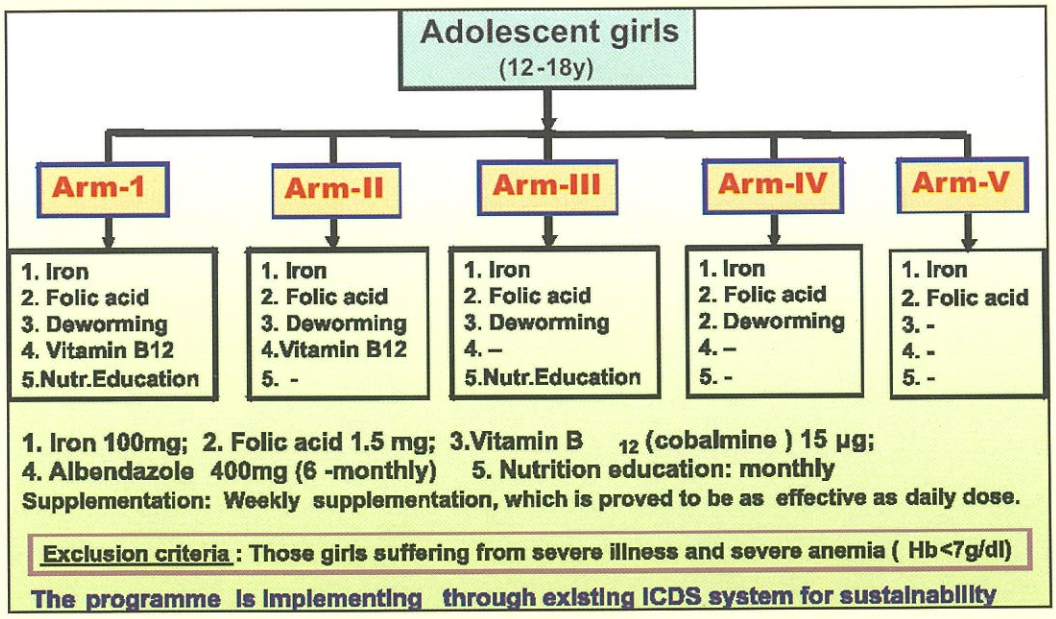
Background

Anaemia is a significant public health problem in Orissa and iron deficiency is considered as the major contributory factor. Orissa has the highest rates of infant mortality and maternal mortality and anaemia becomes the major cause. Prevalence of anaemia among pregnant women and adolescent girls reported 81% and 96% respectively. Despite the national anaemia control program, anaemia continued to be universal among pregnant women. Pregnancy is too short period of time to reduce pre existing anemia, when women do not seek prenatal care until 2-3rd trimester, although intervention channels already exist to target iron supplementation. This approach has been found not effective and possible reason could be the preexisting iron deficiency anaemia in women at the time of conception. Adolescence, as a period of growth and development, is considered the best time to intervene in order to assist in physical and mental development, and to prevent later maternal anemia, thereby determine the well-being of the next generation. To achieve the goal of controlling anaemia in adolescent girls, a 5-arm regimen approach is being adopted to compare the efficacy of iron and folic acid administration when combined with deworming, vitamin B_{12} and nutrition education through routine monitoring by the existing ICDS network.

Progress

Methodology for Baseline survey

A randomized controlled field trial is being implemented in assessing effectiveness of a combination of 5-arm regimens in reducing iron deficiency anaemia among adolescent tribal girls. Each arm comprises one or more combinations of regimens, namely 1) Iron supplement, 2) Folic acid, 3) Vitamin B12, 4) Deworming supplementation, and 5) Nutrition education.



Study population

The study population comprises adolescent girls aged between 12 and 18 years are included in the study. Calculation of sample size is based on a minimum anticipated increase in 1.0 g of haemoglobin (Hb) concentration by weekly supplementation of iron (100 mg elemental iron) for a period of one year. Considering mean Hb=10.7 g/dl and standard deviation [SD]=1.46 g/dl in adolescent girls (NNMB, 2003), 95% confidence and 80% power, the required sample in each group is 52. Adding a design effect of 2, to allow the variance between age groups (12-14y and 15-18y), expected 30% dropout rate to follow-up and exclusion of 5% severe anaemia, total of 150 girls will be enrolled in each regimen, thus a total 750 unmarried adolescent included in 5-arms.

Study area

Gumma, one among the seven revenue blocks in Gajapati district is selected for the study because it is predominantly with tribal population. The study got the permission from the District Collector, who is the Chairman, of Integrated Tribal Development Agency of Gajapati district, for implementing project at Child Development Project Office (CDPO) of the Integrated Child development Services (ICDS). Out of 5 sectors in Gumma Block, Serango Sector having highest tribal population is selected for the study, especially Lanjia Saura primitive tribe, one among the 13 primitive tribal groups in Orissa.

A pre-tested questionnaire was used to collect the household data, socioeconomic information of parents, adolescent girls, age, sexual maturity, personnel hygiene practices, morbidity, clinical examination, KAP on anaemia and its control measures and food consumption patterns. Anthopometric measurements were taken using standard equipment and procedures. Venous blood samples were collected for haemoglobin and blood smear (thin and thick) for detection of malaria. Blood was centrifuged and serum samples were preserved at -20^{0}C till analysis. Serum ferritin, soluble transferrin receptor, c-reachive protein folic acid and vitamin B_{12} levels were determined using ELISA techniques. Anaemia was considered to be present if the Hb value was below 12 g/dL for adolescent girls. Levels of anaemia were classified further as severe (<7 g/dl), moderate (7-10 g/dl), or mild (10-12 g/dl) category. Serum ferritin <15 ug/L and transferrin receptor <8.3ug/ml were considered to indicate depleted body iron stores and iron deficiency respectively. Stool samples were collected and examined for intestinal worm infestations.

Observations

A total of 891 adolescent girls aged 12-18 years were studied from four Gram Panchayats in Serango area of Gajapati district. Majority of study population belonged to ST 89%, while 8% SC and 3% other communities. Two thirds of the households had kutcha house. Electricity was available in 7% and piped water was available in 4% of households. Sanitary latrine was available in less than 2% of the households. Overall 41% of the adolescent girls reported that they had education, of which 37% had primary school, 3% had secondary school and 1% had college.

Study clusters

The study area is divided into 5-clusters considering adequate sample size of study population that include Ajayagada, Bhubani, Rungrumba, Serango and Tumulo. In each study cluster, four contiguous Anganwadi Centres were included in order to avoid cross contamination between regimen groups. In a study cluster, approximately 200 adolescent girls are available for study.

Clinical examination

Clinical examination was carried out and illness history recorded to find out the common illness, which might contribute towards the anaemia and to exclusive other diseases in adolescent girls. History of fever due to malaria was reported by 25-38% of girls. While features of recurrent malaria and hepatosplenomegally were not of high magnitude, intestinal helminthes as visible worm passage in stool was reported in 5% of individuals (Table 1). Diarrhea and upper respiratory tract infections were the other common disorders, the prevalence of which was 1.5% and 2% respectively. Three-fourth of adolescent girls had clinical sign of pallor. The 5 study clusters were almost similar in terms of clinical illness and of common disorders. Recurrent illness leading to anaemia was not predominant in the study population as per clinical impression.

Table-1. Cluster-wise clinical history of morbidity (%) in adolescent girls.

Clinical sign	Cluster-1 Rungrunba (168)	Cluster-2 Tumulo (172)	Cluster-3 Bubani (193)	Cluster-4 Serango (167)	Cluster-5 Ajaygada (191)
History of fever	28.9	38.4	24.9	32.9	36.7
History of malaria	31.4	20.8	22.6	19.9	19.3
Diarrheal disorders	1.0	-	1.5	1.0	2.0
RTI	3.8	1.6	-	0.6	2.6
Common cold	0.8	1.3	1.8	-	1.0
Joint pains	5.7	2.3	-	0.6	-
Pallor	28.6	20.0	31.3	31.7	28.7

Growth status

The cluster-wise mean anthropometric characteristics of adolescent girls are presented in Table 2. As expected mean height, weight, BMI-for-age and mid arm circumference increased consistently with increase in age. The mean height ranged from 144.4 to 146.4 cm, while their body weight ranged between 36.5 and 38.6 kg. The mean percent of age-specific weight and height of girls is less than the respective NCHS references. Mid-upper arm circumference is raged between 21.8 and 22.3 cm.

Table-2. Mean+SD anthropometric characteristics of adolescent girls (12-18y)

Anthropometry/	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5
nutritional status	(168)	(172)	(193)	(167)	(191)
Body weight kg	38.5+7.71	38.6+7.15	36.45+8.94	37.31+9.10	37.0+8.06
Height cm	146.2+7.64	145.4+7.04	144.4+9.36	146.0+9.20	146.4+9.28
BMI-for-age kg/m2	17.8+2.60	18.1+2.44	17.1+2.71	17.2+2.83	17.1+2.46
Mid-arm girth cm	22.3+2.51	22.3+2.72	21.8+2.75	22.2+2.80	21.8+2.61

Haemoglobin and anaemia status

The mean haemoglobin concentration of adolescent girls was 10.3+1.35 g/dl, ranged between 5.6 g/dL and 12.9 g/dL. Overall, 84.5% of adolescent girls had some level of anaemia, of which 44%, 32% and 3% had mild, moderate and severe grades of anaemia respectively. The proportion girls having moderate and severe anaemia was relatively higher in younger (12-14 y) than in older age group (15-18 y).

Table-3. Cluster-wise characteristics of haemoglobin, serum retiol and iron deficiency anaemia

Iron indicator	N	Cluster-1(168)	Cluster-2(172)	Cluster-3(193)	Cluster-4(167)	Cluster-5(191)
Mean haemoglobin g/dl	891	10.1±1.68	10.3±1.86	10.5±1.64	10.2±1.59	10.2±1.74
Anaemia Hb<12g/dl %	891	89.8	92.4	86.3	91.0	87.0
Iron deficiency (%) (ferritin <15 ug/l)	131	34.9(89)	41.8(89)	44.2(42)	35.4(99)	37.8(49)
Mean serum retinol (ug/dl)	121	29.1+10.9 (20)	25.4+11.2 (19)	23.2+10.9 (38)	25.2+10.9 (17)	29.6+11.3 (27)

Iron deficiency anemia

The serum ferritin provides a precise quantitative measure of storage iron. The mean serum ferritin concentration was 34+21.5 µg/L in a sample of 131 adolescent girls. The proportion of girls with inadequate iron stores (< 15 µg/L) was 36%, while a considerable proportion of 43% had ferritin values <50 µg/ml, which is being considered the cut-off value for defining hypoferritinaemia. Serum ferritin is an acute-phase protein and increases two-to fourfold in response to inflammation. C-reactive protein (CRP), a marker of acute inflammation, has been used to correct for the effects of infection in iron deficiency. The CRP levels get elevated in 27% of 75

anaemic girls indicating a considerable population had acute infections. Deliciencion of folic and (< 6 µg/ml) and vitamien B_{12} was 33% and 30% respectively.

Vitamin A status (retinol)

Serum retinol concentrations estimated in a sample of 121 girls ranged between 23.2 and 29.6 µg/dl, of which 32% had sub-clinical vitamin A deficiency (<20ug/dl). The concentrations of haemoglobin are directly correlated with serum retinol (r=0.235, P=0.009).

Confounding factors of anaemia

Confounding factors of anaemia such as malnutrition (underweight and stunting), malaria and intestinal worm infestations are almost similar in all the 5 study clusters.

Cluster-wise prevalence of confounding factors of anaemia in adolescent girls

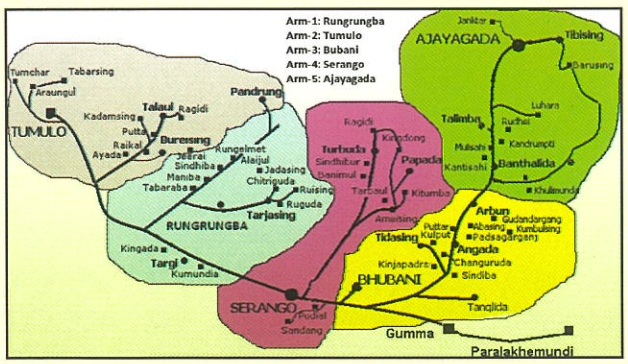
Parameter	N	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5
Underweight (%)	981	29.2	29.8	35.2	31.7	39.4
Stunting (%)	981	45.8	43.1	48.1	44.1	43.5
Malaria positive	346	13.6(78)	8.6(62)	12.5(36)	8.2(82)	9.4(88)
Worm infestations	407	16.6(99)	20.7(77)	18.7(82)	22.9(70)	18.5(79)

The prevalence of malnutrition in terms of underweight (29-39%) and stunting (43-46%), slide positive for malaria (8.2-13.6%), and stool samples for worm infestations (16.6-22.9%) are found to be within marginal ranges among the study clusters.

Randomization of study clusters

The defined clusters were enlisted in alphabetical order (1.Ajayagada, 2.Bhubani, 3.Rungrumba, 4.Serango and 5.Tumulo) and random allocation was made using a computer-generated table of random numbers by a statistician, who had no role in the implementation of the study. Each cluster is allocated one by one in order from 1 to 5-arms regimen groups. Accordingly,

Map showing stratification of study clusters



regimen arms 1, 2, 3, 4, and 5 are allocated study clusters 3, 5, 2, 4 and 1 respectively.

Training of ICDS functionaries and healthcare providers

The training session conducted with ICDS supervisors, Angnwadi Workers and helpers, members of community organizations

in defining the problem of health and nutrition, sanitation, personal hygiene, focusing at causes, signs and symptoms, prevention and control measures of anaemia. Prior to implementation of programme, each Anganwadi Workers is supplied with a booklet containing list of study population, education materials related to coverage and compliance. The primary objective of the training component is to develop ownership in the programme as well as to sensitize the stakeholders in facilitating the intervention strategy.

Nutrition education

Community education is provided in two study clusters (1and 3) covering all villages. The target population includes adolescent girls, their parents, village heads, and health workers. Promotional materials developed in conjunction with communication expert from NIN, Hyderabad such as folders, posters, flip-charts etc with the key messages on health, nutrition and sanitation and personal hygiene practices. Case-studies and focus group discussions are being conducted to promote good practices on balanced diet, nutrient-rich foods including iron-promoters such as vitamin C-rich foods (add tomato, lemon, raw salad or citrus with each meal) and avoid tea/coffee with a meal.

Implementation of the intervention programme

The 5-arm regimens are being implemented in combination of iron, folic acid, vitamin B12 (weekly supplement), deworming (six-monthly dose) and nutrition education (monthly interval). Intervention includes administration of respective regimen (iron-foilc acid or iron-folic acid with vitamin B12 tablets) and deworming in respective study clusters. Adolescent girls were enrolled for drug administration after obtaining written consent. Free monthly supply of iron/folic acid/ vitamin B12 tablets are given directly to girls in presence of Angawadi Worker and other village heads. The first dose of drug was administered to orally on the day of survey under direct supervision of a Physician, who is the Co-PI of the project. A monitoring card is supplied to each girl mentioning personal details and counselled to take the iron tablets regularly and consistently specified on a supplementation calendar. To aid compliance, girls are advised to take the tablet on specific day every week (Sunday) and instructed to swallow with water, not taking them on an empty stomach, and avoid tea/coffee 2 hours before or after taking the tablet. A poster explaining preventive measures of anaemia are displayed at each Anganwadi Centre, Panchayat Raj Institutions and Schools for educating the study population.

Monitoring compliance and coverage

A three-level monitoring system is being put in to the system, compliance to iron supplementation in secorded by each the girls using an independent monitoring card supplied and a supplementation calendar. Community-level monitoring by the local Angawadi Worker recording the coverage and compliance charts. Coverage includes number of girls accessing the service, number

of tablets received, adherence from the commune, adverse events. if any Prior to supplementation, Anganwadi Workers were trained for compliance monitoring. Periodic compliance monitoring is also being done by the investigating team at fortnight intervals.

Impact assessment

Impact of the programme will be assessed intermittently at 6th, months on a subsample of population that include evaluation of changes in hemoglobin, iron indicators, measured the extent of iron-deficiency anemia and behaviour changes from that of the baseline survey indicators. Comparisons will be made for assessing effectiveness of each of the regimen independently and in combinations of regimens in reducing iron deficiency and anaemia among adolescent girls and suggest recommendations. Impact assessment will be done after 12 months intervention period in May-June2011.

13. Diagnosis of pulmonary tuberculosis by culture and its drug sensitivity

Principal Investigator	:	Dr Dasarathi Das,
Co-Investigator	:	Dr H K Khuntia
Collaborator	:	TRC, Chennai
Funding	:	Intramunal
Starting Date	:	Feb 2009
Closing Date	:	Jan 2011

Background

Tuberculosis (TB) is a major public health problem in the state of Orissa. The state lacks TB culture facility. Only recently at SCB Medical College, Cuttack such facility is being initiated. Considering the load of Tuberculosis cases in Orissa one culture lab will be inadequate to address the issue. RMRC, Bhubaneswar can act as another Intermediate Reference Laboratory in Orissa for MDR and XDR TB and also look for the type of drug resistance prevalent in this area.

Objective

To establish the TB culture lab for isolation of MDR TB pathogen and to assess the drug susceptibility pattern of mycobacterium tuberculosis prevalent in this area.

Progress of work

The investigators of the project already had taken training at TRC Chennai on Mycobacterium culture and Drug Susceptibility Testing. Space for setting up the TB labs has been identified in consultation with TRC, Chennai and CPWD has initiated laboratory modification work. The majority of equipments required for tuberculosis work has been received/ ordered.

Initial standardization of ZN microscopy and fluorescent microscopy for identification of Mycobacteria has been done. Culture of M tuberculosis has been initiated in semisolid commercially available LJ media. Briefly sputum samples from tuberculosis OPD of Capital Hospital,

Table. Comparison between Sputum microscopy and Semisolid LJ culture

	Microscopy		
	Positive	Negative	Total
LJ			
Culture Positive	10	3	13
Culture Negative	3	1	4
NTM	0	1	1
Contaminated	0	0	0

LJ = Löwenstein-Jensen; NTM= Nontuberculous mycobacteria.

Bhubaneswar were collected and stained with fluorescent auramine dye for detection of Mycobacteria. The sputum samples further homogenized and decontaminated by modified Petroff's method. The processed samples were inoculated in two commercially available slants one with plain LJ medium and another with PNB. The cultures were incubated at 37^0 C and growth (rough cauliflower like colonies) was observed at weekly intervals up to 8 weeks. At the end of 8 weeks final reading was taken.

Out of 69 sputum samples collected from Capital Hospital, Bhubaneswar, 18.8% were found positive for AFB by Auramine staining and by culture in semisolid LJ. All of these 13 positive cultures were failed to grow in slants of LJ medium with PNB. Out of the Four sputum samples which were negative by Auramine staining three were found positive by culture. Work is in progress.

14. Impact assessment of the Janani Suraksha Yojana (JSY) on maternal health in Orissa.

Principal Investigator : Dr.A S Kerketta
Duration : 18 month
Starting Date : July 2010
Closing Date : Dec 2011
Funding : ICMR Extramural

Objectives

General objective: To assess the impact of Janani Suraksha Yojana on maternal health in Orissa.

Specific objectives

- To assess the quality of care provided to women delivering at health institutions
- To assess process of the JSY scheme functions
- To assess the impact on maternal and neonatal morbidity and mortality
- To assess population based coverage and accessibility of institutional deliveries
- To assess the actual costs to households during pregnancy, delivery and post-partum

Progress of work

The secondary data on the institutional delivery have been collected from NRHM, Orissa. The data of the key component of JSY on place is given in table-1. The study districts are selected

as earlier the recommendation taking different geographical areas basing on agro-climatic and geographic distribution. Two districts each from Northern plateau, Central tableland & Coastal plains and Southern Eastern Ghats are included. Since the , central tableland and coastal plains are same with respect to health facility, infrastructures, communication and accessibility both have been included in one zone. Thus a total six districts would be studied covering both tribal and non tribal population. The districts namely Sundargarh, Mayurbhanj from Northern plateau, Bargarh and Puri from central tableland and coastal plains and Kandhmal and Nabrangpur from Southern Eastern Ghats have included in the study. Out of the six districts the preliminary visits have been made to two districts.The project was briefed to CDMO's and discussions were made on the detail plan of the study. Basing to the secondary data of district and performance of institutional delivery, of the study PHC/CHC and sub-centre area have been selected for the study.

The data on place of delivery in study districts (Source-NRHM 2007)

Districts	% of Home Delivery (05-06)	%of Home Delivery (06-07)	% of Inst delivery (05-06)	% of Inst Delivery (06-07)
Sundargarh	58.47	41.07	37.45	80.53
Mayurbhanj	60.53	56.85	24.83	62.84
Bargarh	62.55	19.47	39.47	43.15
Puri	35.84	8.64	64.16	91.36
Kandhmal	75.48	59.63	12.94	51.64
Nabrangpur	87.06	48.36	41.05	81.44

15. Etiology of diarrhoea in 3 tribal districts of Orissa

Principal investigator : Dr. B. B. Pal
Co-investigator : Dr. H K. Khuntia
Collaborator : Dr. Bikash Pattnaik
Funding : Extramural, Tribal task force, ICMR
Starting Date : Oct 2010
Closing Date : Sept 2013

Objective

1. Phenotypic characterization of common enteric bacteria including the *Vibrio cholerae* O1 El Tor variants from diarrhoea patients from the tribal populations of Orissa.
2. To find out the antibiotic susceptibility test of the diarrhoeagenic *E.coli* (EPEC, ETEC, EHEC, EAggEC), Salmonella, different Shigella spps, Aeromonas spp and *V.cholerae*.

(3) To find out the correlation between clinical isolates of *V. cholerae* by different molecular techniques for the detection of biotype(tcpA-classical/El Tor), serotype (O1 /O139), virulence(ctxA) and regulatory genes(toxR) by Quadruplex PCR assay, mismatching amplification for mutation assay (MAMA) PCR for the detection of El Tor variants of *V. cholerae* O1 with ctxB gene of classical strains.(4) The clonality of all serogroups of *V.cholerae* isolates will be done by RAPD PCR, PFGE, sequencing and dendrogram etc. to track their migration from one outbreak area into other. Further a detailed analysis of the strains causing different outbreaks will provide the origin of new clones of *V.cholerae* strains.

Progress of work

The project was initiated during the month of May, 2010 in Kashipur, Dasmanthpur, Laxmipur and Mohana blocks with intramural funding. Attempts were done to collect the rectal swabs from diarrhoea patients and environmental water samples. From May-September 2010 a total of 201 water samples and 129 rectal swabs were collected for bacteriological analysis. 12 out of 201 water samples were positive for V. cholerae non 01 and non 139 strain collected from chua, river and stream from Kashipur, Dasmanthpur and Laxmipur area. Similarly out of 129 rectal swabs culture positive were 72 (55.8%).Out of culture positive samples 37 (51.4%) were V. cholerae 01 Ogawa, E.coli-32 (44.4%) Salmonella-2 (2.7%) Shigella species-1 (1.4%). The V. cholerae strains were sensitive to ciprofloxacin, norfloxacin, neomycin, azithromycin, gentamicin, chloramphenicol, Ofloxacin and resistant to tetracycline, co- trimoxazole, ampicillin, furazolidone, nalidixic acid. Presently there was a diarrhoea outbreak in the studied areas and it has spread to different villages of Kashipur, Kalyan singhpur, Bisam Cuttack areas of Rayagada district. Recently two villages were affected due to cholera outbreak in Mohana block. No diarrhoeal outbreak has been reported from Dasamantpur and Laxmipur areas. Consumption of contaminated water, unhygienic condition and poor knowledge on diarrhoea, low socio-economic status and migration were responsible for acquiring and spreading the infection. The MAMA PCR results indicated that most of the strains are El Tor variants of V. cholerae O1 Ogawa. showing multiple drug resistant to different antibiotics. Regular surveillance on diarrhoeal disorders is required to combat any future diarrhoeal outbreaks in these blocks.

Analysis of Water samples collected from different water sources (May, 2010 to September, 2010)

Block	Total water samples	No of positive for *V. cholerae* non O1 & non O139
Kashipur	120	8 (Chua- 3, River-3, Dugwell -1, Nala-1)
Dasmantpur	22	1 (River)
Laxmipur	41	3 (Stream-1, Chua-2)
Mohana	18	0
	201	12

Annual Report
2009 & 2010

TRANSLATION RESEARCH

Bhubaneswar

1. Transfer of a Molecular Technique from Lab based study to Field for Mapping of Malaria Vectors and their Vectorial Attributes

Principal Investigator	:	Dr. R.K.Hazra
Co- investigator	:	Dr. N.Mahapatra
Collaborator	:	VCRC, Pondicherry

Objective

- To standardize methodologies for different parameters for vector mapping
- To test the standardized methodologies from Phase-1
- To map the vectors at PHC level and identify operational issues
- To prepare a vector map at district level
- Transfer the laboratory based technology to the field.

Background:

Malaria poses a great challenge to the public health personnel of India. Out of 444 species of Anophelines found globally, 58 species are available in India and 9 of them play major role in the transmission of malaria. Current knowledge on vectors and their precise role in malaria transmission is incomplete due to the fact that all major malaria vectors are complexes of more than one biological species which are morphologically indistinguishable species. Studies on their bionomics, distribution, role in malaria transmission have become important due to growing evidences that these species may differ significantly in biological characteristics especially those which are of importance from malaria control point of view, such as response to insecticides, vectorial competence, host preference and resting behavior. Therefore, proper identification of malaria vector is critical to the success of malaria control programme.

Limitations of traditional methods for species identification

Problems in classical *Anopheles* taxonomy include not only strong morphological similarity between species but also pronounced morphological variation within species. Accurate species identification usually requires rearing to correlate adult with immature morphology. This is a time consuming process. On the other hand cytotaxonomic methods based on ovarian polytene chromosome in semi-gravid individuals, which constitute a small proportion of the population and need expertise to perform the test.

Technology developed to overcome the traditional methods

In Orissa there is lack of trained entomologist for control programme. Molecular methods for species identification have received great attention in recent years. The methods have been applied to important groups of mosquito species complexes. Molecular tools are more field friendly because specimens can be dried and they require very little material like any part of mosquito body. DNA based methods for identification of cryptic species or closely related species have successfully been used with a numbers of Anophelines. We have recently developed a molecular tool for identification of main malaria vector of Orissa. The method was also developed for simultaneous detection of species complex, their human blood indices and presence of sporozoites from single mosquito. Basing on these techniques developed by our center the total screening of anopheline vectors can be undertaken in Orissa. Therefore the present study will be undertaken to screen the malaria vectors from different parts of Orissa and their vectorial attributes.

PHASE 1: Standardization of the methodologies for different parameters for vector mapping (first 12 months)

Work plan

(i) Mosquito sample collection sites: from all ecotype (one village each from plain, riverine, foot- hill, hill-top) of Keonjhar district.

(ii) Standardization of collecting devices for both adult and immature stages. Adult collection will be standardized by hand catches and trap using oral aspirators and light traps; collection of immature stages will be done by using different types of dipper and larval concentrators.

(iii) Type and habitat of collection: indoors (IR); human dwelling (HD) and cattle shed (CS).

(iv) Seasonal collection: Collection of mosquito will be done thrice in each season i.e. in starting, mid and end of the each season.

(v) Samples collections and transportation

a. Kits for sample collection: filter paper and dry mosquitoes (using electric bulb)

b. Adult will be collected by individual tubes.

(vi) Standardization of mosquito sample preservation and transportation to laboratory by adopting different methods.

(vii) Vector species composition: PCR assay in comparison with morphological identification. The blind coded samples collected by VCRC will be identified by the developed PCR methods to confirm the species identification and this will be the validation of our developed technique.

(viii) Optimization of the pool size for species composition, HBI, presence of sporozoite, drug resistance parasite in mosquitoes (*Pfcrt* and *Pfmdr1*) and insecticide resistance status of the species. Here different pools size will be taken for multiplex species identification with their vectorial attributes. The optimization of the pool size will be measured.

(ix) Standardization of the multiplex PCR reaction mix like concentration of primers $MgCl_2$ etc and designing new sets of primer for mass use.

Work Progress

Entomological work

1. Study area

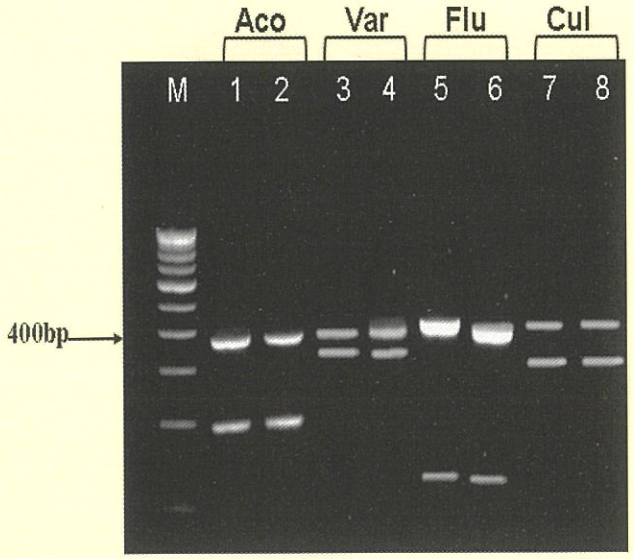
The samples used in the study originated from various localities of Orissa. The samples were collected from Nawarangpur. Adult female anopheline mosquitoes were collected with the help of a mechanical aspirator and light trap.The two process of collection were compared in the field condition. The sampling methods of mosquitoes were determined. Now we are standarising 0.5% of the houses of the villages for collection of mosquitoes. The mosquitoes were collected from human dwellings, cattle sheds and mixed dwelling. After field capture, all mosquitoes were first identified on the basis of their morphology (Christophers, 1933; Nagpal et al., 2005). After identification the blood-fed females were transported to the laboratory in individual tubes for egg laying.

Molecular work

2. Standarized the methods of the single step multiplex PCR for simultaneous detection of *Anopheles annularis* group, human host preference and *Plasmodium falciparum* sporozoite presence

i. The *Anopheles annularis* (*An. annularis*) group of subgenus Cellia Theobald (Diptera:Culicidae) includes five recognized species i.e. *An. annularis* Van der Wulp, *An. nivipes* Theobald, *An. pallidus* Theobald, *An. philippinensis* Ludlow and *An. schueffneri* Stanton. From the five, three most common species found in Orissa are considered for this study because of their remarkable vectorial and behavioral variation. To identify and know their role in malaria transmission we developed a single multiplex PCR based assay.

ii. The multiplex PCR assay, determining the *An. annularis* group species and the vectorial attributes. It is a rapid and efficient method that is applicable on a routine basis for identification and categorization of potential malaria vector from heterogeneous population in the group in a particular area. As the test is DNA based, it can be applied to all life stages of these mosquitoes for epidemiological investigations and vector incrimination studies.

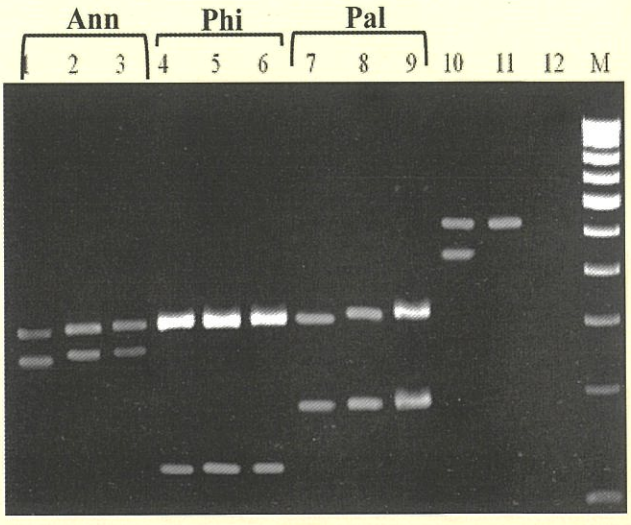


Lane 1, 2, 3, *An. annularis species* (285bp); Lane 4, 5, 6, *An. philippinensis* species (138bp); Lane 7,8,9, *An. pallidus* species (194bp); Lane 10, Presence of *P. falciparum* (429bp) as well as human (519bp) specific amplicons from the DNA isolated from blood of *P. falciparum*-infected persons; Lane 11, Human specific 519bp PCR product, DNA isolated from non-infected persons; Lanes 12, negative control without any DNA template; Lane M, 100-bp DNA ladder (NEB), Lanes 1–9 showed common 324-bp product from the D3 domain of 28S rDNA of *An. annularis* group.

2. Molecular approach for identification of members of the Myzomyia series of *Anopheles* subgenus Cellia (Diptera:Culicidae)

Myzomyia series of subgenus Cellia of genus *Anopheles* consists of 69 species found in Afro tropical, Mediterranean and Oriental region (Harbach 2004). In India, the series consists of 7 species namely *An. fluviatilis*, *An. culicifacies*, *An. minimus*, *An. varuna*, *An. aconitus*, *An. jeyporiensis*, *An. majidi* (Rao 1984). Among the species of the Myzomyia series, *An. culicifacies An. fluviatilis* are major vector of malaria in India. The *An. fluviatilis* is the most anthropophilic species and *An. fluviatilis* and *An. culicifacies* are endophilic members of the series and are the most efficient vectors of malaria. The other members of the series are mainly zoophilic and feed on human depending upon the avaibility and regards as secondary vectors of malaria in India. Vectorial and behavioral variations found among the species groups constitute the major reason for the need of accurate and precise identification.

We developed a single step multiplex PCR based assay on the basis of the D3 variation to efficiently distinguish the four members of the Myzomyia series namely *An. fluviatilis*, *An. culicifacies*, *An. varuna* and *An. aconitus*. The assay is useful because it rely on a single PCR to produce fragments that can be easily fractionated by electrophoresis on an agarose gel and that can clearly differentiate between the taxa, which are often misidentified or unsuitable for morphological taxonomy.

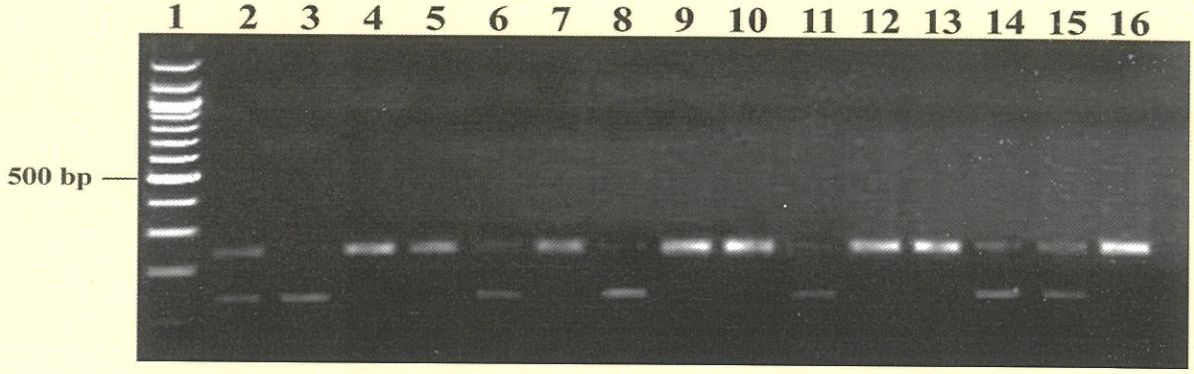


Ethidium bromide-stained gel electrophoresis of multiplex PCR products of the members of the Myzomyia series. Lane 1, 2 *An. aconitus species* (180bp); Lane 3, 4 *An. varuna* species (318bp); Lane 5, 6 *An. fluviatilis* species (120bp); Lane 7, 8 *An. culicifacies* species (290bp). Lane M, 100-bp DNA ladder, Lanes 1–8 showed common 368-bp product from the D3 domain of 28S rDNA of members of the Myzomyia Series.

The method was standarised to detect 7 anophline mosquitoes in a single PCR.

3. **Detecting the spread of chloroquine-resistant strains of *Plasmodium falciparum* by analyzing anophelines of malaria endemic zones of Orissa, India.**

- Developed an early warning system of spreading of drug resistance by identification of resistance strain from mosquito. To detect the spreading of drug resistant *P. falciparum* in a population, before any pathological symptoms are detected in humans is possible by analyzing the anophelines vectors, transmitting malaria.
- In the present work we have implemented a new strategy to detect the spreading of chloroquine-resistant (CQR) strains of *P. falciparum* by the major vectors prevalent in selected endemic regions of Orissa, India.
- Our study showed higher transmission rate of chloroquine-resistant strains of *P. falciparum* parasites by *An. culicifacies* and *An. fluviatilis*.



Lanes 2-16 are the Pfcrt gene (264 bp) Samples are subjected to *ApoI* digestion. Lanes 2, 3, 6, 8, 11, 14, and 15 are showing digested product of 136bp and 128 bp suggesting chloroquine sensitive strains.

Future Work: Standarisation of the mosquito sample collection and preservation and transportation will be done

ii. Validation of our technique will be done by other Institute like VCRC, NIMR. The blind coded samples collected by VCRC and NIMR will be identified by the developed PCR methods to confirm the species identification and this will be the validation of our developed technique.

2. Quadruplex PCR for diagnosis of *V. cholerae* O1 and/or O139 serogroups causing cholera: A novel technique

Principal investigator : Dr. H.K. Khuntia
Duration : 18 month

Background

Cholera is one of the major public health problems, causing large morbidity and mortality due to toxigenic *V cholerae*, all over the world. Amongst more than 200 serogrouops of *V cholerae,* only two serogroups O1 and O139 are known to cause epidemic and pandemic cholera. However non epidemic serogroups although not involved in cholera epidemics, can be pathogenic and are occasionally associated with small outbreaks of diarrhoeal diseases. Because untreated cholera leads to the unset of serious outbreaks and potentially great devastation, quick diagnosis and identification of the causative serogroups is necessary from public health point of view for appropriate antimicrobial therapy and to chase the spread of the outbreak. Conventional methods for diagnosis of cholera are not suitable for early detection and characterization of cholera causing Vibrios, since it needs a battery of biochemical tests, toxin assay, and slide agglutination with specific antisera. This takes long time. In order to overcome these problems we have developed a Quadruplex PCR based molecular diagnostic technique for simultaneous detection of serotype, biotype and toxigenic potential of *V. cholerae*. The sensitivity and specificity of this test is 100% and takes 2-3 hours for accurate diagnosis.

Objective:

1. To optimize and check the inter and intra observer variations of the newly developed Quadruplex PCR assay for detection *V cholerae*.
2. To map out the *V cholerae* strains found in Orissa by Quadruplex PCR by examining both hospital and outbreak samples
3. Transfer of the Quadruplex PCR technology from laboratory to the field

Progress

In response to the progress of Quadriplex PCR to carry out the objective-1 ie " to optimize and check the inter and intra variations of the newly developed PCR assay for detection of *V. cholerae*", another set of 20 lab stocked *V. cholerae* strains were subjected for Quadruplex PCR assay for validation during May and June, 2010. All the strains were found positive for *ctx A*, *tcp A*, *rfb* O1 and *tox* R genes.

In connection with this I am submitting three months budget plan of Rs. 36045/- to purchase primers, reagents and chemical for the progress of the objective-1.

Proposed Translational Research

1. Strategy for prevention of Hepatitis C virus (HCV) infection in endangered tribes

Principal Investigator : Dr.B.Dwibedi
Duration : 3 years

Objective

1. Identification and social evaluation of the risk factors for HCV infection for formulation of preventive strategy.
2. Implementation and evaluation of the intervention strategy in those tribal areas.

Summary of proposal: A recent study addressing Hepatitis virus infection among five primitive tribes indicated very high prevalence of HCV infection in two primitive tribes (Mankidia and Juanga). These tribes are primitive in their socio economic and cultural front and their population growth is either low or declining. Possible risk factors for spread of this infection has been studied in a cross sectional mode, which can be studied in depth pertaining to the socio cultural behavior and amenability to change. This can form the baseline for developing an intervention strategy to curtail transmission of infection in the addressed tribes. This strategy can be applied and translated into use by the national health programme that focuses tribal health.

Meeting of director with IVI scientist on oral cholera vaccine

Annual Report
2009 & 2010

Other Scientific Activities

Bhubaneswar

1. Establishment of ICMR Virology Network Laboratory (Grade-I)

Investigator	:	Dr. S.K. Kar
Co-Investigator(s)	:	Dr. B.Dwibedi; Miss S.Dixit
Starting date	:	March 2010
Closing date	:	March 2015
Funding	:	Extramural (ICMR)

Background

The proposal to establish a Grade-I Virology laboratory at the centre was approved by the Council during March 2010 and funds received in April 2010.It is one of the four centres approved under the Virology Network in the country, which aims at creating regional facilities to be involved in laboratory diagnosis, surveillance and research in viral diseases of importance.

Objective

To establish a grade I diagnostic virology laboratory with facilities for viral isolation, viral antigen detection, viral serology and molecular diagnostic virology including sequencing and to conduct outbreak investigation of viral diseases of regional and national importance including but not limited to

1. **Viruses transmitted by respiratory route**: Measles, Rubella, Mumps, Influenza viruses (A, B and C), Para influenza virus, Adenoviruses, Respiratory Syncytial Virus, Rhinoviruses, Coronaviruses.
2. **Viruses transmitted by intestinal route**: Poliovirus, Hepatitis A & E viruses, Rotavirus, Astroviruses, Calciviruses, Norwalk viruses, Enteroviruses.
3. **Vector Borne Disease Viruses**: Dengue, Chikungunya, Japanese encephalitis, West Nile, Kyasanur Forest Disease, Chandipura viruses.
4. **Zoonotic viruses**: Rabies virus, Nipah virus, Hanta virus.
5. **Viruses transmitted by body fluids**: HIV, Hepatitis B and C viruses.

Progress of work

The proposal involves construction of the laboratory, procurement of equipments, training of involved staff, establishment of laboratory techniques like serology, molecular diagnosis, sequence analysis, cell culture and isolation etc. in phased manner. Outbreak investigation, surveillance

during epidemic and interepidemic period and sporadic disease diagnosis of important viral diseases of the region and emerging infections would be carried out which will be strengthened by research subsequently.

For the above purpose, laboratory space has been identified in the second floor of the building and laboratory design made in consultation with Dr.D.A.Gadkari ex-Director NIV, Pune. Civil infrastructure modification has been planned accordingly and is in process with help of CPWD. Procurements of laboratory furnitures and equipments approved under the grant are in process and staff to be recruited shortly.

Besides these planned activities, a BSL-2 laboratory space has already been created as a part of the viral laboratory of the centre and equipments have been procured and installed to initiate outbreak investigation activities to support the state health system.

Hence with the available infrastructure and man power, outbreaks of influenza H1N1, Dengue, Chandipura and Hepatitis virus infections has been undertaken with immediate reporting to State health department with recommendations for timely prevention. Outline of these investigations are given in the Outbreak Investigation Chapter.

To identify and prioritise the viral disease of importance for the region, which needs to be investigated, a workshop was organized in the centre with experts from Medical Colleges, referral hospitals, public health organizations and State health department involving clinicians, public health experts and virologists.

Viral encephalitis which remains almost undiagnosed and an important spectrum causing sporadic and epidemics involving all ages with fatality was considered as the priority by the experts. So it is planned for laboratory investigation of the suspected cases of viral encephalitis admitted to different hospitals of the regions, besides the outbreak investigations activities.

Vector survey and virus isolation/ diagnosis in vectors has also been planned along with the clinicoepidemiological investigations, which is essential for public health prevention. A proposal has recently been approved by the Council on Chikungunya vectors and virus transmission which will be initiated after receiving the funds. This will be adjunet to and strengthen surveillance and research in the vector borne viral diseases, which are emerging in the region.

Outbreak Investigations of Viral diseases

Investigators

Dr. B.Dwibedi, Dr. R.K.Hazra, Dr. H.K.Khuntia, Dr. P.K.Sahoo, Miss. S.Dixit,

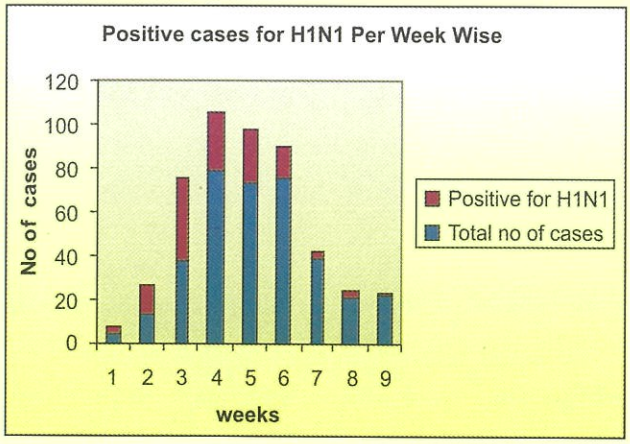
Susil K.Rathod, S.K.Mishra.

(i) Investigation of Pandemic Influenza H1N1in Orissa.

H1N1 infection was reported in Orissa for first time in August 2009. Keeping in view the state's need to test for swine flu(H1N1) infection BSL -2 laboratory has been setup at RMRC in September 2009 with the help of ICMR. For the laboratory setup and diagnosis of H1N1 short training was imparted to the RMRC staff in NICED, Kolkata and NIV, Pune and the centre continued laboratory investigation by Real Time PCR on referred samples from state health surveillance system.

In Orissa cases continued from August 2009 to January 2010; there after it came down. After March 2010 no more suspected swab samples were sent to the laboratory from state referral units. After a short quiescence suspected samples for swine flu started to be received from 7th July 2010 and first H1N1 positive case declared on 21st July 2010, during this season. Till date 370 number of samples from different Hospitals from different part of Orissa has been tested, out of which 95 came positive for H1N1 infection. The weekly epidemic curve (Fig- 1) indicated peak during August declining in September 2010. The positive cases were reported from 21 districts; initially starting from 6-7 coastal districts that later spread to western districts of the state. The results were communicated to state health department and concerned hospitals with immediate effect which facilitated treatment chemoprophylaxis to patients and contacts.

Referred samples and positive cases for H1N1 (week wise) July to Sept 2010



(ii) Outbreak of Dengue virus infection in Malkangiri district, Odisha:

A team from Regional Medical Research Centre (ICMR), Bhubaneswar consisting of clinician, entomologist, research fellows, insect collector and technician had visited Malkangiri district of Orissa for investigating in to report of Dengue infection in the district during second week of September 2010.

The team visited the district headquarter hospital (DHH), one rural village and three urban areas in Malkangiri town.

The team conducted house to house survey in the village / urban location to search for fever cases. Subjects having fever during the survey or within a week were examined and after examination the observations were recorded in a pre-designed format. Intravenous blood samples (4 ml) were collected and transported in cold chain to the RMRC laboratory for investigation. The samples were subjected to I_gM antibody capture ELISA specific to Dengue virus infection using the kit from National Institute of Virology (NIV) Pune).

Sixty one individuals were examined and samples collected for investigation. Twenty eight individuals were symptomatic and 11 of them shown positivity for Dengue IgM. Twenty three asymptomatic individuals (household contacts or neighbours) were also enrolled and three of them revealed Dengue IgM positivity. The clinical features observed in the individuals showing Dengue infection were fever, headache bodiache, photophobia , abdomen pain ,nausea, vomiting and rash.

Travel history to areas outside Malkangiri district, i.e, to Chhatisgarh or Andhra Pradesh were recorded in two individuals showing Dengue infection where most (12 out of 14) did not reveal movement beyond the district. The 14 individuals belonged to eleven house holds. Out of which nine households had isolated cases where as in one family there were 2 and in another family 3 cases which indicated family clustering also.

Vector survey was carried out in the village or urban locations in the houses and peridomestic areas to look for breeding places, adult mosquitoes and larvae. The main breeding spots observed in and around the houses were Earthen pots. Discarded tires, discarded plastic container, Jars, bottles large plastic jars, bucket, cemented tank and Steel or metal containers were the other breeding sites / sources found in the area. In both rural and urban locations surveyed the vector indices for *Aedes* musquitoes were higher than the critical indices for transmission (Table 1). These epidemiological evidences indicate indigenous, transmission of the virus through the available vector species i.e, *Aedes* mosquitoes.

This was reported to the district and state health officials and recommendations were made for breeding source reduction and anti vector measures, which was instituted to control further spread.

Table-1. Vector indices in the surveyed area.

	Rural	Urban				
	Padmagiri	Thana Sahi	Pradhanguda	Canal colony	Main road	Reclamation colony
No of Households surveyed	50	65	15	15	20	10
Houses +ve for Larvae	38	47	5	4	14	4
Container Index (CI)	54.4	66.6	38.5	52.5	61.2	27.2
House hold Index(HI)	76	72.3	33.3	26.6	70	40
Breteau Index(BI)	112	123	70	140	190	90

(iii) Hepatitis A and E Outbreak investigation

The centre received samples from two outbreaks of Jaundice from two districts of the state. The samples were subjected to serological tests which indicated Hepatitis A and E virus infection. It was reported to the Disease surveillance system to undertake control measures.

(iv) Chandipura outbreak Investigation and vector survey

Cases of sudden death mostly affecting children below 10 years were reported from three tribal areas of the state during September – October 2009. It was found to be due to Chandipura virus infection (Annual Report 2008-09).

Vector survey was undertaken following the outbreak in the affected village and one control village of Daringibadi area of Kandhamal district by entomologist of the centre and RMRI, Patna, to look for the possible vector i.e, Sand fly. During the survey Sand fly species; *Phleboromos argentipes* and sergentomyia was detected, from both human dwelling and cattle shed. Aedes vectors were also identified during the survey. This was reported to the state health department with recommendation for vector control. It is planned further for resurvey in the area to look for any resurgence and circulation of the virus.

(v) Hand foot and mouth disease: First report from Orissa

Cases with fever and vesicular rash of distal part of extremities and pharyngeal lesion were reported from Bhubaneswar, an urban setup. Cases were examined and clinically suspected to be hand, foot & mouth disease. Blood samples were collected and tested by PCR at NIV, Pune which has shown positivity for Entero virus.

2. Transmission of HBV infection from mother to new born & at early childhood

Principal Investigator	:	Dr. A.Mahapatra
Co- investigator	:	Dr. B.Dwibedi, Dr.S.K.Kar
Funding	:	Intramural
Starting Dage	:	November 2009
Closing Date	:	August 2010

Background

Hepatitis B virus infection is of serious concern because of long carrier state and fatal complication.It is established that the virus can be transmitted both vertically from mother to child and horizontally through parenteral modes. Infection acquired at the early age (infancy) has a greater chance of development into chronicity and clinical complications. Hence it is necessary

to know the age at infection and mode of transmission in the community. This information will provide inputs to the National immunization programme for consideration of universal immunization of the children.

In this light an attempt has been made by this centre to ascertain the magnitude of infection among the antenatal mothers and their new borns, by examination of mother's blood and cord blood during delivery.

In this study 247 pregnant mothers were enrolled from the antenatal clinic of Capital hospital, BBSR; out of them 143 had successfully completed their pregnancy and these many cord blood samples have been collected for detection of HBV infection. The written consent was obtained from the mothers at enrollment before the examination & sample collection. Blood from both mothers and cord were collected and subjected to HBs Ag test by ELISA following standard procedures.The results revealed HBs Ag positivity among the mothers to be 2.4% (6/247) and that of the cord blood to be 1.3 %(2/143). This result indicates that, there exists a risk of HBV transmission to the newborns from the infected mothers.

These findings can be utilized for further research in the population to know the magnitude and age at infection.

3. Urban Mosquito Control in Bhubaneswar Municipality

Principal Investigator	:	Dr. N. Mahapatra
Co Investigator	:	Dr. R.K Hazra
Collaborator	:	VCRC
Collaborator	:	Commissioner, Bhubaneswar Municipal Corporation (BMC)
Study period	:	Two years
Funding	:	Extramural (ICMR Translational Research)

Background

Mosquitoes are important insect pests from both a nuisance and a health point-of-view. At present there is an alarming situation due to the rising trend of mosquito density and mosquito-borne diseases in Bhubaneswar city. In the city, due to urban agglomeration, increase in both man-made and natural habitats have occurred, which are ideal conditions and conducive for the proliferation of mosquito vectors.

Hence, suitable, sustainable and effective mosquito control programs for Bhubaneswar city is highly essential for reducing the mosquito population to such an extent ,that neither they can cause biting nuisance nor transmit the disease. To achieve this, information regarding the

mosquitogenic condition is the first priority, after which suitable control programs can be developed. Therefore the study is proposed with the following objective.

Objectives

- To develop a geo reference map for two wards of Bhubaneswar municipal area through GIS incorporating all the information regarding the breeding spots like it's location, eco habitat, biochemical parameters, settlements, and mosquitogenic conditions for rainy, winter, and summer seasons..
- To identify the vectors, assess their larval and adult density in three seasons.
- To asses the susceptibility status of the vectors to different insecticide used by the BMC.
- To reduce mosquito density by implementing integrated vector control strategy (Source reduction by minor engineering, use of biolarvicide, using insecticides, education and training.)

Methodology

Phases	Activity	Duration
I	Preparatory phase (GIS map development,enumeration of breeding habitat etc, training to the BMC staff.)	6 months
II	Baseline data collection on larval, adult density of mosquitoes, susceptibility status towards different insecticide. Information regarding present status of the present programme.	6 months
III	Intervention phase	6 months
IV	Monitoring and Evaluation	3 months

Phase I

(i) Selection of ward:

Bhubaneswar city has been divided into 60 wards. Initially two wards will be selected which are highly mosquitogenic as per the report of the BMC.

(ii) Demographic data:

Collection of demographic data of the ward, housing pattern, water supply, animal population, socioeconomic status of the inhabitant.

(iii) **Development of GIS map.**

GIS map will be developed for the selected ward. From the map information regarding road connectivity, housing pattern, settlement, type of houses, vegetation, water bodies will be collected.

(iv) **Enumeration of major breeding habitats**

Initially, a systematic survey will be done to map all potential mosquito breeding sites within the ward. Breeding site not reflected in GIS map will be located using GPS, and then the spot will be incorporated in GIS map, so that the map will reflect all the breeding spot of the municipal area.

Breeding spots will be broadly classified as following

Cesspit (polluted water bodies with < 5meter of radius), Cesspool (polluted water bodies >5meter of radius), Pond, Tanks, Pucca drain, Kuchha drain, Septic Tank, Cemented tank, Canal system, Seepage of canal area, Irrigated field, natural drainage, Container breeding.

For each breeding site, a reference number will be assigned and information on. type of breeding spot, vegetation, water quality, surface area and height of the spot, distance of human settlement from the site will be collected.. The site information can then be entered into a database which will contain a detailed breeding history for each site, including:

A map reference location (e.g., street address or concession roads, sections or GPS coordinates).

- Description of sites that have produced larvae.
- The time of the season when the sites have mosquitoes.
- The larval density at each site.
- The species associated with each site.
- Type of equipment most appropriate to despatch to the location
- Any restrictions associated with the site (e.g., permission required, phone ahead, close gate, 24 hours notice required).

Phase II

(i) **Assessment of breeding status** (presence / absence of mosquito larvae)

Larval collection

All the breeding places will be searched for the presence of mosquito larvae by using a standard dipper (one meter stick attached to an enamel plastic bowl of 250 ml capacity). The standard method is to take from three to four dips from all the sides for one square meter area of a breeding

place. The no of dips to be taken will be calculated basing on the area of the site. The collected larvae will be identified up to genus level and larval count will be made to calculate the larval density as follows:

Larval Density: No of larvae/ dip

(ii) **Adult collection**

Adult mosquitoes will be collected in the morning from 6am to 9am from 10% of the households of the ward by sucking tube as well as using mosquito trap. The trap will be kept in some sampled household for overnight after seeing the mosquitogenic condition. The mosquitoes thus collected will be identified for different mosquito species and Per Man Hour Density (PMHD), and trap density will be calculated.

Per Man Hour Density: Number of mosquitoes collected /man/hour

Trap Density: number of mosquitoes / trap.

Location of each household, name of the house owner, type of house, mosquito density and other relevant information of the household will be recorded. (Form no 2). All these data will be entered in to a data base in a computer as baseline information.

(iii) **Detection of parasite:** The vectors of malaria and filariasis will be dissected to detect the parasite and infection rate and infectivity rate will be calculated.

(iv) **Susceptibility status**

The susceptibility of the target mosquito species (vector) to the pesticide been presently used by the BMC will be assessed by using WHO bioassay Kit.

(v) **Ward wise collection of mosquito borne disease data**

Prior to the implementation programme, the base line information on prevalence of the mosquito borne disease will be obtained from all the possible health infrastructure available in the city. The vector density and infection rate will be correlated with the disease prevalence. The measures of these parameters taken periodically can help assessing the status and trend of the vector transmission in Bhubaneswar

Progress of work

Detail of the breeding spot

Five selected breeding spots were surveyed during 2004 and these spot were mostly having clear, fresh water running without any obstructions. During that period the vector of malaria,

filariasis, chikunguniya though present but their density were much lower than the present situation and Armigeres mosquito which causes biting nuisance in evening hour has increased to almost 10 folds.

GIS mapping of the city and each ward was done in 2009. The larval and adult population (density) of some of the selected breeding place from ward no. 1, 2, 8, 11 & 36 were reassessed again in 2009 and compared with the data of 2004. But destruction to the drainage system was observed in all the five spot. The increase in both adult and larva density of mosquitoes are depicted below Figures 1 to 5. The details of the deterioration of these breeding spots of 5 wards favoring development of mosquitogenic condition are enumerated below.

Fig. 1: Comparison of Mosquito density (adult & larval) of breeding spot 1 (natural drainage flowing near Damana square) in 2004 and 2009.

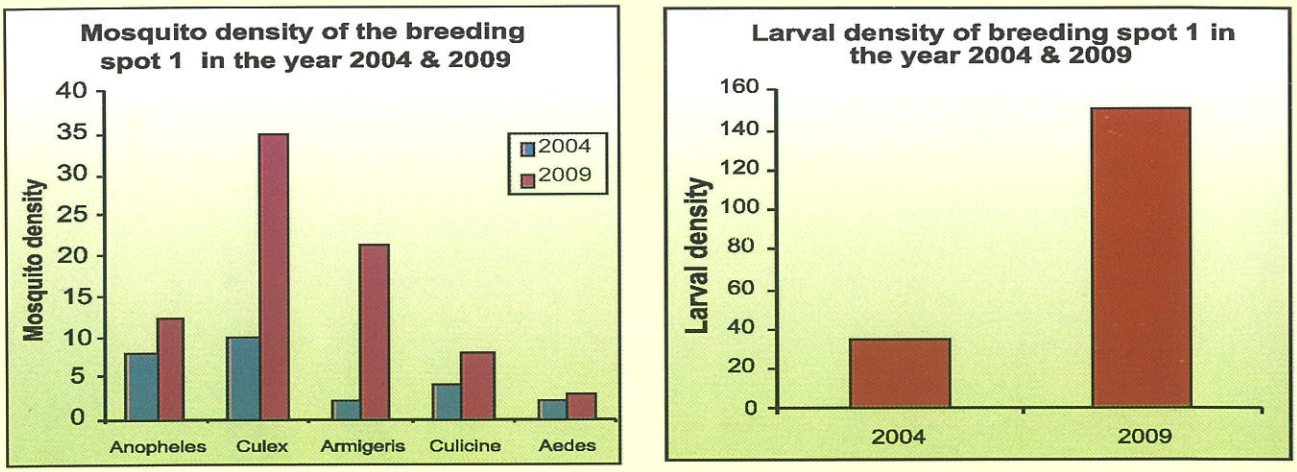


Fig. 2: Comparison of Mosquito density (adult & larval) of breeding spot 2 (natural drainage flowing Through Patia village) in 2004 and 2009.

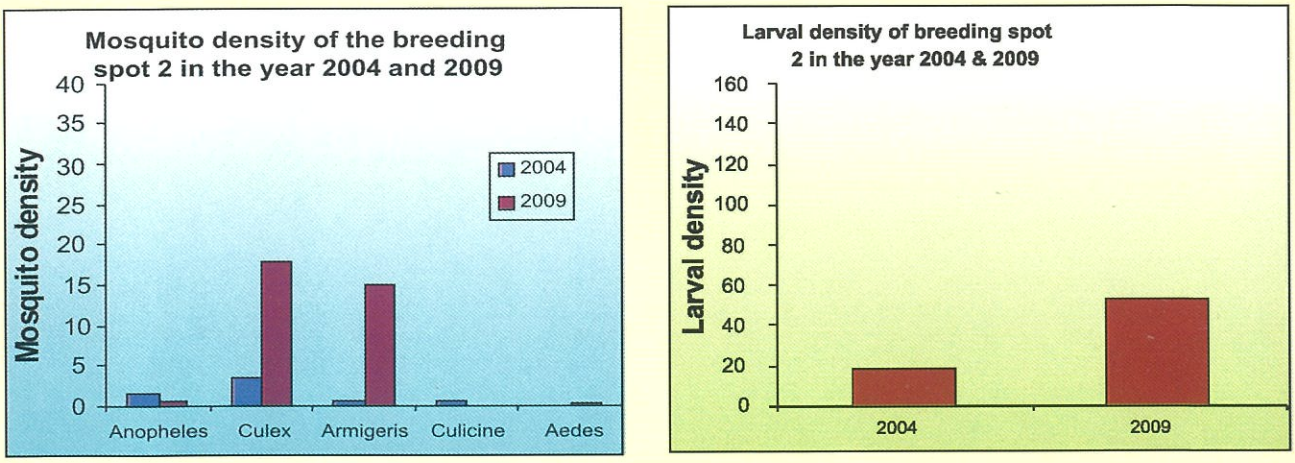
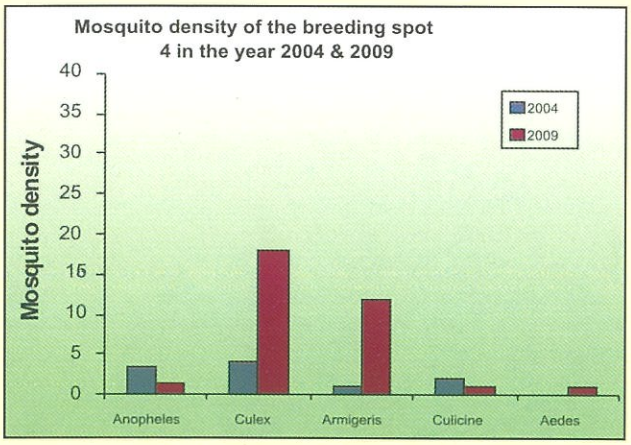


Fig. 3: Comparison of mosquito density (adult & larval) of breeding spot 3 (natural drainage flowing behind Chandrasekharpur housing board) in 2004 and 2009.



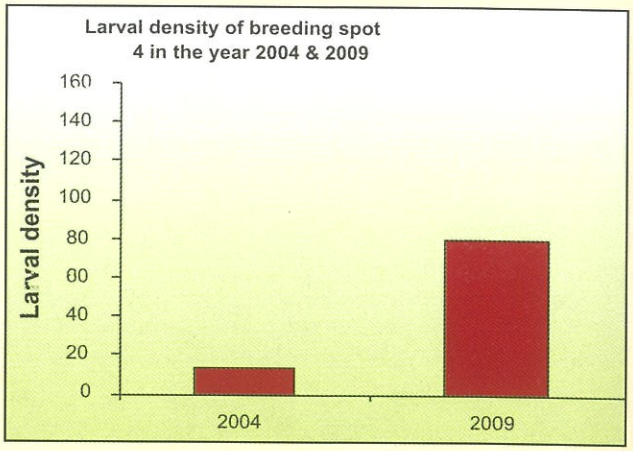
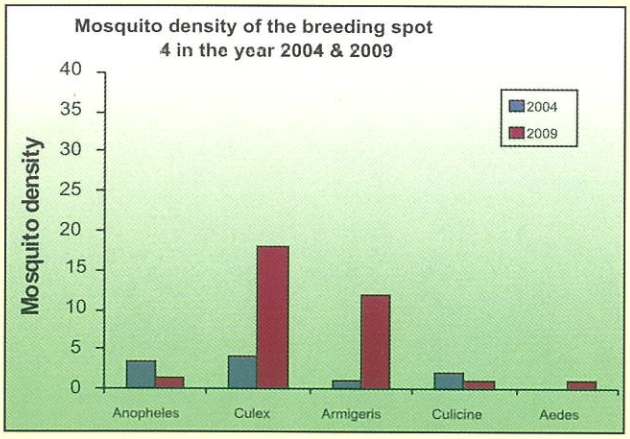


Fig. 4: Comparison of mosquito density (adult & larval) of breeding spot 4 (natural drainage flowing near Niladri vihar) in 2004 and 2009



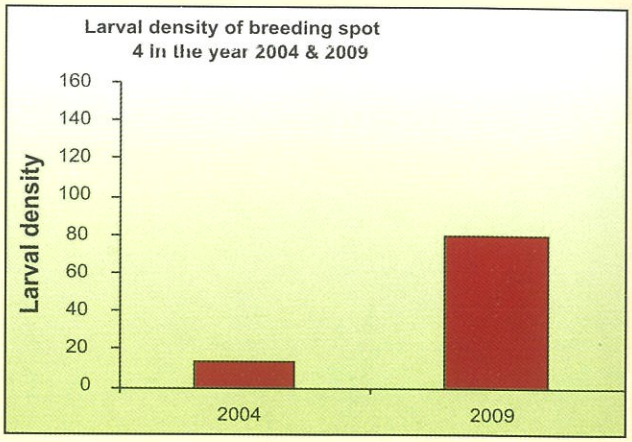
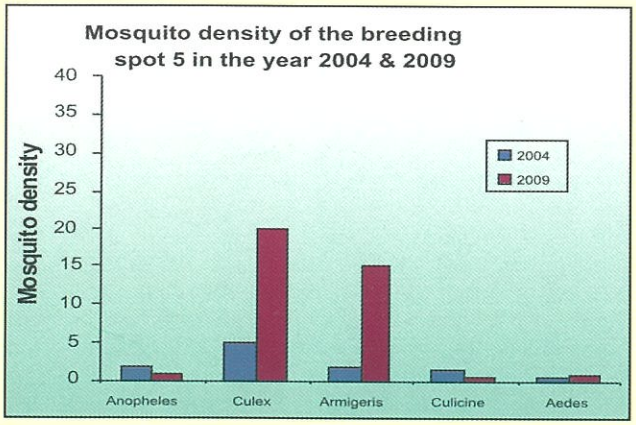
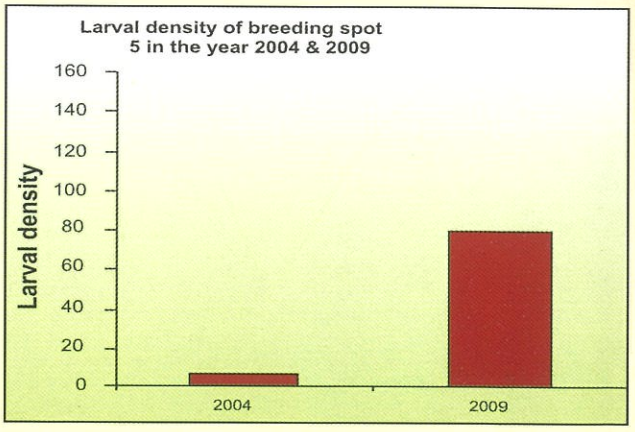


Fig. 5: Comparison of mosquito density (adult & larval) of breeding spot 5 (natural drainage flowing between Satyanagar and Saheed Nagar) in 2004 & 2009





4. Surveillance activity on Diarrhoeal disorders.

Investigator(s) : Dr. B. B. Pal, Dr H K Khuntia, Mr S. K. Samal

Large outbreak of cholera was reported in Rajnagar block ,Kendrapada district during April to July,2009 affecting 108 villages,715 people were affected with attack rate 0.74% and case fatality rate 0.98%.. The causative organism of this diarrhoeal outbreak was the hybrid strain of *V.cholerae* O1 Ogawa with ctxB gene of classical strain. The organism was isolated from the diarrhoea patients and also from the pond water. The spreading of the disease was due to person to person contact in the family having poor knowledge of sanitation, use of pond water for cleaning utensils and drinking purposes and migration factors etc. The molecular analysis on *V. cholerae* isolates by RAPD PCR exhibited that the strains were similar and they belonged to same clone as exhibited by PFGE analysis. Rectal swabs from severe diarrhoea patients were referred from 12 districts through the surveillance cell, Govt. of Orissa during this period. Out of 97 samples 40 were positive for *V. cholerae* O1 Ogawa reported from 7 districts and 32 were hybrid *V. cholerae* strains showing the dominance over normal El Tor strains. This is a warning that the future outbreaks/epidemics of cholera may happen due to this hybrid strain of *Vibrio cholerae*

(i) Surveillance activity at Puri and Bhubaneswar

During this period (April, 09- December, 2009) 142 rectal swabs were collected from Bhubaneswar and Puri areas for bacteriological analysis. Out of 59 (41.5%) culture positive samples, 25 (42.4%) were *E. coli*, 31 (52.5%) were *V. cholerae* O1 Ogawa and *Shigella* spp. was 1 (1.7%), *Aeromonas* spp. were 2 (3.4%) respectively. The resistance profile of *V. cholerae* O1 was Co Fr Na, for *Aeromonas* spp. it was A Fr Cf Na Nx Co and for *Shigella* spp. was Fr Na Co S respectively. Active surveillance should be continued among diarrhoea patients to look for the different enteropathogens and to define the

Table: Bacteriological analysis of enteropathogens from stool samples (April, 09 to December, 09).

	Number (%)
Total samples collected	142
Culture positive	59 (41.5)
E. coli	25 (42.4)
V. cholerae	31 (52.5)
V. cholerae O1	31 (52.5)
V. cholerae O1 Ogawa	31 (52.5)
V. cholerae O1 Inaba	0 (0.0)
V. cholerae O139	0 (0.0)
Salmonella spp.	0 (0.0)
Shigella spp.	1 (1.7)
Aeromonas spp.	2 (3.4)
Culture negative	83 (58.5)

shifting antibiogram patterns in this region. Out of 31 *V. cholerae* isolates;. 17 (54.8%) *V. cholerae* isolates showed ctxB gene of classical strain (El Tor variants) and known as hybrid strain, where as 14 (45.2%) of the isolates did not show ctxB gene of classical strain and hence known as normal El Tor strains.

(ii) Outbreak investigation at Rajnagar Block, Kendrapada District, Eastern Coast of Orissa, India (April, 09-July, 09)

Rajnagar block is situated at the eastern coast of Orissa, having a long saline track of Bay of Bengal. The block experiences cyclone/ flood almost every year which is followed by diarrhoeal disorders accounting for large morbidity and mortality in the state. Diarrhoeal disorders were reported in this block during the month of April 2009The detailed epidemiological investigation was conducted which is as follows:

Description of Block :

1	Total No of GP	=	18
2	Total diarrhoea affected GP	=	16
3	Total Block population	=	1,62,984
4	Total population affected	=	96878
5	No of villages	=	224
6	No of villages affected	=	108
7	Total No of cases	=	715
8	Total death	=	7
9	Date of Onset	=	14/04/09
10	Epidemic Over	=	09/07/09
11	Attack Rate	=	0.74%
12	Case fertility rate	=	0.98%

As per the information available from Rajnagar CHC, there was a great festival called Maha Bishuva Sankranti which was celebrated on 14.04.09 at Jarimula village near the temple, Matia

Mangala. There was a gathering of about 30,000 people on that day for the celebration of the festival which is a usual practice of the local people being celebrated every year. The diarrhoea cases were reported to Rajnagar CHC on 15.04.09 at midnight. The diarrhoea patients were reported from different villages who attended the festival on the above festival. 18 cases were treated and discharged on 16.04.09.

Index Case

While checking the hospital indoor diarrhoea records it was found that Banita Palei, 16 years/ female from Baghua village went to that festival on 14.04.09. She took sweets and drank water from the food stall at 10 am. She returned home at 4 pm and started loose motion on 15.04.09 at 7 pm. She was hospitalized, cured and returned on 16.04.09. Before onset of diarrhoea she did not visit any relative's house in the other village for the celebration of feast or ceremony before suffering from diarrhoea. No diarrhoea patients visited her house also. Gradually the number of cases increased in that village and gradually spread to other villages of the same block.

The diarrhoea cases were reported to Rajnagar CHC on 15.04.09 at midnight. The diarrhoea patients were reported to different villages who attended the festival.

Drinking water facility at the festival site

Only one functional tube well was available at the festival site. However drinking water was also provided to the people at 4-5 points by the local organizer.

Clinical History of patients

The diarrhoea patients were complaining of profuse rice watery stool, abdominal pain associated with vomiting and muscular cramping. After one passage of voluminous watery stool, the patients showed severe degree of dehydration. Averages 8-15 bottles of normal saline were given as IV fluid to maintain the rehydration of the severe diarrhoea patients.

Collection of Samples

Rectal swabs from indoor diarrhoea patients from Rajnagar CHC and also from the affected villages were collected for bacteriological analysis following standard techniques. Similarly water from different water bodies like pond, tube well, roadside water reservoirs were also collected from 12 villages to look for the presence of *V.cholerae*.

Table-1. Bacteriological analysis of enteropathogens isolated from diarrhoea patient

Total samples	41
Culture Positive	27 (65.9%)
Culture Negative	14 (34.1%)
E.coli	7 (25.9%)
V.cholerae 01	18 (66.7%)
Ogawa	18 (66.7%)
Salmonella	0
Shigella	0
Aeromonas	2 (7.4%)

Table-2. Analysis of water sample collected from Rajnagar Block

Sl. No.	Category	Total Samples	No positive for V.cholerae 01 ogawa (%)
1	Pond	29	2 (6.9%)
2	Road side small reservoir	4	0
3	River	1	0
4	Tube well	7	0
	Total	41	2 (4.9%)

The bacteriological and molecular analysis revealed that *Vibrio cholerae* O1 ogawa biotype Eltor with ctx B gene of classical strain was the causative agent for this outbreak which was also isolated from the pond water of different villages. The patients which have were culture positive for *V. cholerae* were reported from the villages like Rajnagar, Banabiharipur, Rajagarh, Nathapur, Kanhua, Deghi, Vodia, Khandipada, Kaitha.

Antibiogram

V.cholerae

Sensitive: tetracycline, norflaxacin, gentamicin, azithromycin, neomycin, erythromycin

Resistant: ciprofloxacin, cotrimaxazole, chloramphenicol, streptomycin, ampicillin, nalidixic acid, furazolidone.

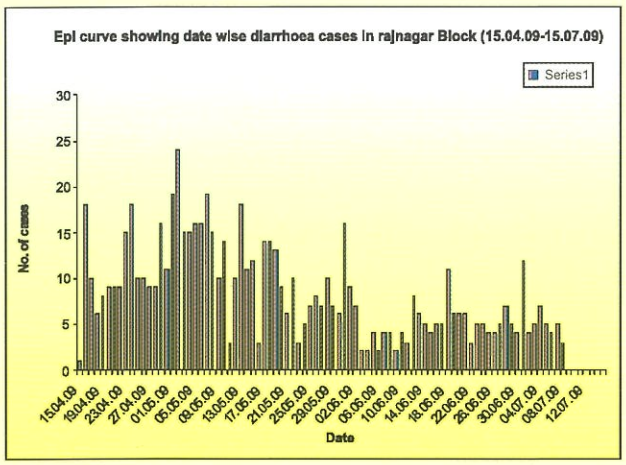
Out of 41 samples collected from diarrhoea patients (hospital and villages) 18 (66.7%) were *Vibrio cholerae* O1 ogawa biotype El Tor with ctx B gene of classical strain. Similarly, out of 41 water samples collected from different pond, roadside small water reservoir, river, tube well (12 villages) for the presence of *V.cholerae*. Only 2 out of 29 water samples (6.9%) were positive for *V.cholerae* O1 ogawa (Panchayat pond of Hatia and Kuruntia). People were using the pond water for bathing, cooking, cleaning utensils and sometimes use for drinking purposes also. The date-wise incidence of diarrhoea cases has been depicted in Fig 1 which started on 15.04.09 and the outbreak was subsided on 8.07.09. The highest numbers of cases were recorded on 2.05.09 and the total no. of cases was 715 reported from 108 villages with 96878 populations which were affected. The death case was reported from 7 different villages. The attack rate was 0.74% and the case fatality rate was 0.98%. Highest number of diarrhoea affected villages were recorded during the month of April (63) followed by May (41), June (03), July (01). The MAMA PCR assay on all *Vibrio cholerae* isolates indicated that all were Eltor variants of *V. cholerae* O1 ogawa with ctx B gene of classical strains. The RAPD analysis on 1281 and 1282 primers indicated that they are clonal in nature.

Month and year wise severe diarrhoea cases in Rajnagar CHC (2006-09) confirmed that this was a diarrhoea outbreak which was reported from April to July, 2009.

Spread of the disease

House to house survey on diarrhoea case and controls from different villages was conducted. This indicated that the people having low socioeconomic status were more affected. The spreading of the disease was due to contact from person to person in the family having poor knowledge of sanitation and hygienic practices, use of pond water for cleaning utensils and drinking purposes. Migration of the people from one village to other village attending the relatives who were suffering from diarrhoea was also another reason for spreading of the disease. The IEC activity on diarrhoeal disorders was undertaken through loudspeaker and local NGOs. Immediately instruction was given not to use the pond water for drinking, cleaning utensils and cooking rice with the pond water. Chlorination was done in different households and different drinking water sources. The people were aware for the proper disposal of excreta, vomits of diarrhoeal patients and by local health centre.

The present investigation indicated that this was a severe cholera outbreak lasting for 3 months affecting many villages and large population of Rajnagar block of Kendrapara districts (April, 09-July, 09). This cholera outbreak was due to the hybrid strain of *V.cholerae*. Active surveillance on diarrhoea patients and different water bodies are to be monitored in future to combat any future diarrhoeal outbreak in this area.



(iii) Isolation of *V.cholerae* from referral cases (January, 09-Dec, 09)

During this period out of 97 rectal swabs from diarrhoea patients from 12 districts were referred for bacteriological analysis from different districts through surveillance cell DHS, Govt. of Orissa. Out of 97 samples 40 were positive for *Vibrio cholerae* O1 ogawa biotype El Tor. Highest no. of cases was recorded from Mayurbhanja, Sundergarh districts. Out of 12 districts ,V.cholerae were isolated from 7 districts and they were mostly hybrid strains. Out of 40 *V. cholerae* O1 strains 32 were hybrid strains and 8 were normal Eltor strains which shows that hybrid *V. cholerae* strains are spreading to other districts of Orissa after the emergence in Kashipur area during 2007. This is a warning that future outbreaks of cholera may happen due to the El Tor variants of *V.cholerae* O1 with ctx B gene of classical strain.

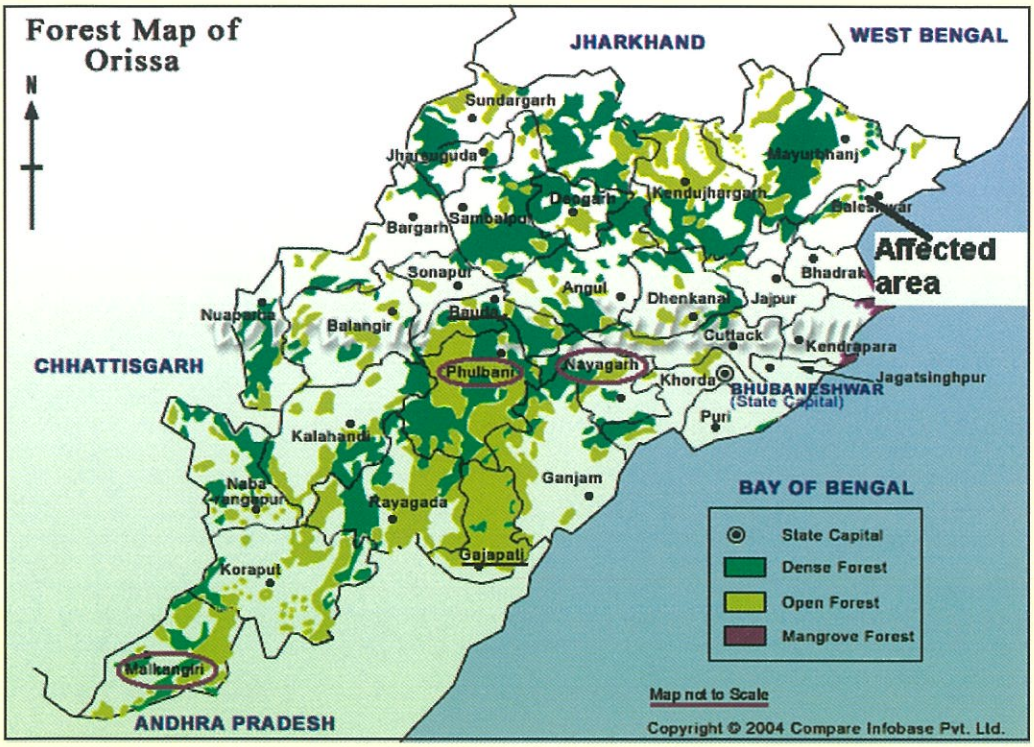
5. Investigation on Outbreak of malaria in Nilgiri, Balasore.

An outbreak of malaria was reported during the month of March in Kadumsul village of Siadimal Gram Panchayat of Nilgiri block of Balasore district. With request from Directorate of Health Services, Govt. of Orissa, a team from RMRC visited Kadumsul village of Siadimal Gram Panchayat for investigation during March-April 2010.

Ecology of the affected Village

Kadumsul is a foot hill village under the tribal dominated Nilgiri block(Fig-1). The village has more than 50 households with approx. population of 300. The households are separated from each other and most of the cattle sheds are 5-10 feet away from the household. The whole village was surrounded by mostly by Bamboos, Kendu and Mahula trees. There are four ponds in the periphery of the village which are used by the villagers for bathing, washing clothes etc,

Fig-1: Showing malaria affected area of Balasore district, Orissa.



Epidemiological Survey

Eleven blood slides were collected from fever cases on the spot .Out of which 3 were found positive on spot for Pf by RDT kits in the field. .The blood slides were brought to laboratory for the detection of the parasites. Out of 11 blood samples, six cases (54.5%) were found to be positive for *P.falciparum* by microscopy. Two cases had parasite density of 4000 and 16000 per 60 microlitre of blood. Rest four had parasite density ranges from 160 to400. Only asexual parasitaemic stages were observed in all the cases.It was found that three persons of the deceased family were also suffering from malaria. In some households all the family members were found to be suffering from malaria fever.

Entomological Survey

Seven species of mosquitoes were collected from the households i.e. *An.culicifacies, An.annularis, An.subpictus, An.vagus, Cx.quinquefaciatus, Cx.vishnui* and *Armigeres.* Among which two were known **vectors of malaria** i.e. ***An. culicifacies*** **and** ***An. annularis.*** Per Man Hour Density (PMHD) of these species are given below.

Mosquitoes Species prevalence in the affected village

Sl.	Species of mosquitoes	PMHD	Total nos Collected
1	*An.culicifacies* *	3	17
2	*An.annularis*	2	14
3	*An.subpictus*	10	60
4	*An.vagus*	7	34
5	*Cx.quinquefaciatus*	3	16
6	*Cx.vishnui*	2	12
7	*Armigeres*	1	5
	Total		161

- Critical density >3.3 PMHD

Clinical & Parasitological monitoring

A central camp was organised by RMRC team, local ASHA and female health worker for identification and treatment of the fever cases in the village besides the door to door survey by the investigators to understand the socio-economic, their behaviour and practice with regards to malaria. The finger blood collected from all the fever cases for RDT and microscopy. Those found positive for P.falciparum infection by RDT were given anti-malarial treatment as per the NVBDCP schedule. The thick smears were stained with JSB stain and examined microscopically.

The investigation revealed that three villages under Sialimal Gram Panchayat of the block Nilagiri were affected by malaria outbreak. The villages are located in the foot hill area and are inhabited by only one tribal group named "BHUMIZ". A total of 839 tribal populations reside in 157 households with average family size 5 persons in these villages. The lively hood was assessed and found that 101(64.3%) depends on daily wages, 52(33.1%) on farming and 4(2.4%) on forest gathering. There is no electrification in the village. All the houses are thatched type with a big veranda. The community is with a practice of outside sleeping after the whole day's tiresome activities. The habit of use of mosquito-net use was enquired from the house holds revealed in 75 (47.8%) house holds use mosquito net. Of which 35 (234.6%) have only one net, 27(17.2%)

have two, 8(6.0%) have three and (3.4%) have four mosquito-net in the families. Government supplied mosquito-net is available in only 5 (3.2%) families. These net have been received by the children those stay in residential school run by Tribal Welfare Department. The rest 96.8% house holds owned self procured mosquito-net.

During this outbreak a total of 172 cases affected by fever that includes 77(44.8%) male and 95 (55.2%) female. The median age of these patients is 22 years. Age distribution of the fever cases is given in (table -2). These cases presented with the clinical signs and symptoms like headache, chill rigor and fever. Very few had vomiting/diarrhoea. The cases received treatment from local health providers like ASHA 59(34.3%), SDH (23.3%), medical camp (30.0%), Quack or private practicener (5.2%) and traditional healers 2 (1.2%).

6. Referral Services for Haemoglobinopathies.

Investigator(s) : Dr. A Maharana, Mr B. Murmu and Miss Sujata Dixit

Referral services were provided by the center for diagnosis of suspected cases haemoglobinopathies from local and periphery PHCs, Hospitals and Medical colleges in Orissa. In the year 2009-10 .372 cases were evaluated.

Table1: Distribution of Different Hemoglobinopathy Disordes

Haemoglobinopathy	No of Cases (%)
Sickle cell trait cases	82 (22%)
Sickle cell disease	35 (9 %)
Beta Thalassemia trait	119 (32 %)
SBeta Thalassemia major	30 (8.10 %)
Hemoglobin E trait	11 (3 %)
Hb E/Beta thal	7 (1.90 %)
Hereditary Persistent Fetal hemoglobin	2 (0.5 %)
Sickle cell/Beta thal	8 (2.20 %)

Total number of Families investigated for Hemoglobinopathies was165,out of whom thalassemic disorders was noted in79 families and Sickle cell disorder in 58 families .The distribution of different Hemoglobinopathies disorders are given in Table1.

Table 2 and 3 shows Caste wise distribution Of Thalassemia and Sickle Cell Disorder.

Table-1 and 2. Caste wise distibution Of Thalassemia and Sickle Cell Direan ?

Caste		Beta thal Trait(%)	Beta thal Major(%)	Caste		Sickle cell trait (%)	Sickle cell major (%)
Sabar	ST	1.2	0.3	Kandha	ST	2	1.5
Kandha	ST	2.0	0.3	Kui	ST	0.9	
Khendu	SC	1.2		Kelibasi	ST	0.3	
Bhoi	SC	0.9	0.3	Saura	ST	0.3	
Kamar	SC	0.9	0.3				
Tanti	SC	0.6	0.3	Dhibara	SC	0.9	
Kondara	SC	0.6	0.3	Pana	SC	3.8	2.9
Pana	SC	3.8		Hadi	SC	1.2	0.3
Dhoba	SC	0.6		Mali	SC	1.2	
Keuta	SC	0.3	0.3	Tanti	SC	0.6	0.3
Katia	SC	0.3		Kotial	SC	0.3	0.3
Mochi	SC	0.3					
Thoria	SC	0.3		Chasa	General	4.1	1.5
Kalibrata	SC		1.2	Khandayat	General	1.7	0.9
Khandayat	General	1.7	2.9	Gauda	General	1.2	0.6
Karan	General	2.6	0.9	Agharia	General	0.6	0.6
Teli	General	0.3	0.9	Teli	General	0.3	
Brahmin	General	2.0	0.6	Barik	General		0.3
Chasa	General	4.1	0.6				
Gauda	General	1.2					
Gudia	General	1.5	0.6				
Kumbhar	General	0.9					

From the above tables it was seen that of thalassemia major is high in Khandayat groups followed by Kaibrata (1.2%), Teli(0.9%), Brahmin,Chasa,Gudia(0.6%),Sabar,Kandha,Bhoi,Kamara,Keuta,Tanti(0.3%). Proportion of thalassemia trait was noted to be higher among Chasa(4.7%),followed by Pana(3.8%),Karan(2.6%),Kandha(2%),Khandayat(1.7%). The prevalence of Sickle cell disorders is mostly found in Chasa(4.1%),Pana (3.8%),Kandha(2%), Khandayat (1.7%).It was found that Chasa group relatively higher affection of Hemoglobinopathies(Thalassemia(4.1%) and sickle cell(4.1%)).

(i) **Vitamin A Status in Haemoglobinopathy Patients**

Table-3. Vitamin A deficiency in Haemoglobinopathy

	Cases evaluated (N)	Hb %	Hematocrit (PCV) %	Cases found Vit A deficient N (%)	Plasma retinal (microgram/dl) in Vit A deficient cases
Sickle cell trait	7	11.3	32.8	4 (57%)	14.8
Sickle cell disease	5	7.4	25.1	2 (40 %)	12.4
Beta thal trait	11	11.1	39.8	2 (18 %)	10.9
Beta thal major	5	6.2	21	3 (60%)	10.8
Normal	11	11.3	38.1	4 (36.4%)	12.8

Out of these a subset of 39 cases were evaluated for vitamin A deficiencies. The result showed**that vitamin A deficiency is high in thalassemia major(60%) and Sickle cell disease(40%) cases than Normal (36.4%) counterparts.**

7. Services at Filaria OPD, Capital Hospital, Bhubaneswar.

RMRC,Bhubaneswar has been providing OPD Service at Capital Hospital,Bhubaneswar, for undertaking clinical activities on diagnosis and treatment of patients reporting to the Filaria OPD.

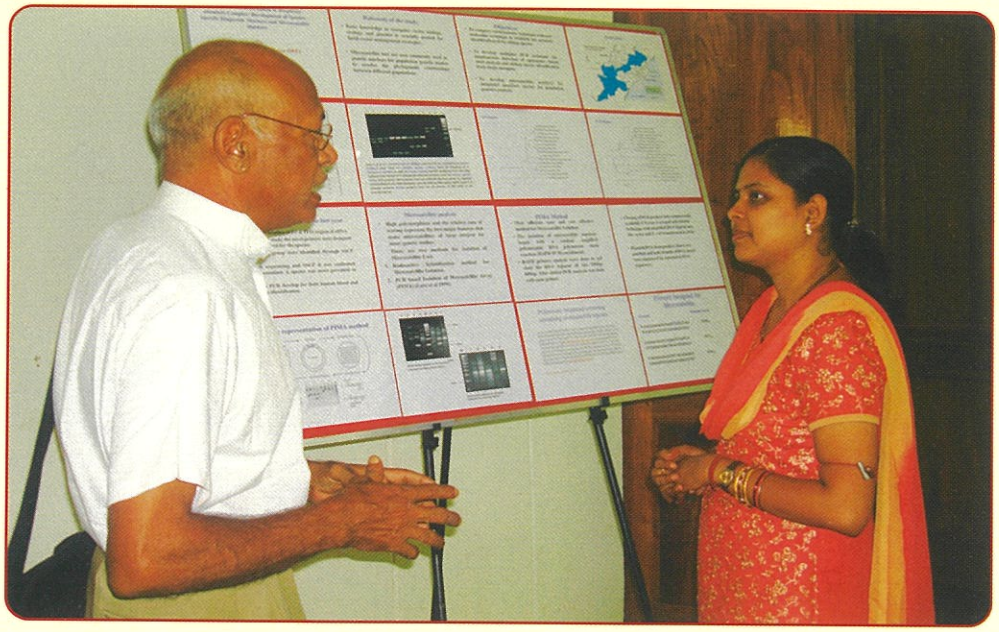
During this year 953 number of patients attended to the Filarial Out patient Department at Capital Hospital. Out of which 333(34.9%) cases reported for follow-up. Among the followed-up cases 188(56.45%) cases were male & 145(43.54%) were females. 620(65.06%) new cases reported

with different clinical presentations. Out of total new cases attended 386(62.26%) were male and 234(37.74%) were female. The commonest clinical presentation encountered was filarial Lymphoedema of different grades, which was marked in 284(45.8%) cases. The Lympoedema grade-1 was found in 84(29.57%) patients and was marked more among the patients of age group 16-30 for both male and females. Lymphoedema grade-2 was found in 45(15.84%), grade-3 in 43(15.14%) and Elephantiasis in 112(39.43%) cases and was marked more among the male and female patients of age group 46 and above years.

Lymphangitis(LNG) was reported by 3 cases only. Lymphadenitis (LND) was reported by 15 cases and was more among male patients. Cases with acute attack were found in around 63(10.16%) patients. Patients with Adeno dermato lymphongioadenitis(ADLA) was seen in 60 cases and was more prevalent among the patients aged above 30 years. A total of 4 patients reported with Hydrocele or Orchitis.

Apart from Lymphoedema,Lymphangitis,AdenoDermatolymphengiodenitis(ADLA) and Hydrocele cases, the other symptoms like inguinal lymph-adenitis, filarial nodule and tropical pulmonary eosinophilia was found in other reported patients in very small percentage.

All the lymphoedema patients were given advice on proper foot care management, limb elevation and bandaging. 27 cases were given Compression Therapy for lymphatic fluid drainage and reduction of edema size and improvement was noted.



Poster presentation by student during SAC meeting

Annual Report
2009 & 2010

Works of Ph.D Scholars

Bhubaneswar

1. Role of Nitric Oxide (NO) in the pathogenesis of severe *P falciparum* malaria

Name	:	Gunanidhi Dhangadamajhi
Status	:	SRF(CSIR)
Date of Joining	:	17th October 2006
Guide	:	Dr. M. R. Ranjit

Objectives

1. To investigate the genetic variants of nitric oxide synthase (NOS) genes (iNOS, eNOS and nNOS) & their association with clinical manifestation of *falciparum* malaria.
2. To estimate the level of TLR2 expression in the monocytes and its association with iNOS expression and Plasma NO production in patients with mild and severe malaria.

Background

Nitric oxide (NO) is produced during the enzymatic conversion of L-arginine to L-citrulline by 3 members of the Nitric oxide synthase (NOS) family of proteins, (iNOS, eNOS and nNOS) and has been proposed to have a crucial role in malaria pathogenesis. However, its mechanism of action during the disease is controversial.

Work Done

To assess the hypothesis that nitric oxide is critical in the pathogenesis of cerebral malaria, we analyzed genetic polymorphisms in iNOS, eNOS and nNOS gene and correlated with the clinical manifestation of severe *P. falciparum* malaria. The single nucleotide polymorphisms and a microsatellite locus in the promoter region of iNOS gene, which are known to enhance the NO production *in vivo* were analyzed in adult malaria patients living in the eastern part of India. The single nucleotide substitution -954G→C was found to be very rare and -1173C→T was not present in this population. But the genotypes with short form of CCTTT repeat (alleles of ≤13 repeats) were significantly associated with mild malaria (OR=2.89, 95% CI=1.955-4.295, P<0.0001). More interestingly the linear regression analysis revealed that increasing summed repeat number of both microsatellite alleles in an individual is a significant risk factor for severe malaria (OR=1.16, P= 0.0013) [**Dhangadamajhi et al, 2009: *Parasitology Research***]. Further, the genotyping of eNOS Glu^{298}→Asp, intron 4 VNTR (a/b) and T^{-786} →C revealed that the Glu^{298}→Asp substitution (P=0.005) and Asp-C-b haplotype (P<0.05/8) have protective association against severe *P. falciparum* malaria. Moreover, the median plasma level of nitrite-nitrate was found to be increased in individuals with the Glu^{298}→ Asp substitution [**Table 1**] and was significantly higher in the mild malaria group ($P < 0.0001$), but the increase was not significant in the severe malaria group ($P = 0.0528$). Comparison of allele and haplotype frequencies showed that the eNOS T^{-786} →C was strongly (D'=0.74) and significantly linked to the intron 4 a/b polymorphism with

the 4a allele being preferentially found in subgroup of individuals with at least one C^{-786} alleles (P<0.0001) [**Dhangadamajhi et al, 2009:** ***Infection and Immunity***]. Besides the two common alleles a/b, we have also observed two higher size variant rare alleles (c/e) in the intron 4 VNTR region of eNOS gene in mild group of malaria patients, with allele 'e' being unique to Orissa population [**Figure 1**] . Although, earlier studies have reported four different size variants a, b, c and d of eNOS intron 4 VNTR, the absence of smaller size variant '4d' in this hyperendemic region and the presence of four different size variants including two rare higher size variants with the finding of size variant 4e (7 X 27 bp) in mild malaria cases only, indicates that, the eNOS intron 4 might be under selection pressure. Further, the absence of significant association of eNOS intron 4 VNTR polymorphism with malaria reflects the possibility of this variants to be in linkage disequilibrium with other functional variants in regulatory regions of the eNOS gene as evidenced by the in vitro affects of eNOS intron 4 VNTR (a/b) polymorphism on the transcription efficiency in a haplotype specific fashion in linkage disequilibrium with the T-786C polymorphism in the promoter region [**Dhangadamajhi et al, 2010:** ***Nitric Oxide; Biology and Chemistry***]. Of the three isoforms of nitric oxide synthases (NOS), though iNOS expression is the major source of NO level *in vivo*, nNOS is the main isoform constitutively expressed in the neural tissues. However, there has been no investigation of the role of polymorphisms of the nNOS gene in the etiology of cerebral malaria. When we analyzed two single nucleotide polymorphisms (SNPs) of nNOS gene (-84G→A and 276 C'!T), responsible for decreased basal transcriptional activity in 200 patients with mild *P falciparum* malaria and 170 patients with cerebral malaria, Our results showed a significant association of AG genotype (OR = 1.83, 95%CI = 1.19-2.78, P = 0.007) and AA genotype (OR = 3.86, 95%CI = 1.42-10.5, P = 0.007) of nNOS -84G'!A substitution with cerebral malaria [**Table 2**]. Interestingly, when the nNOS variant genotypes were combined together for analysis, a significantly increased risk of cerebral malaria was associated with -84(AG+AA)/276(CT+TT) genotype (OR = 2.59, 95%CI = 1.46-4.60, P = 0.0016) and -84(AG+AA)/276(CC) genotype (OR = 1.89, 95%CI = 1.08-3.32, P = 0.0334). The effect of A allele seems to be dominant on the risk of cerebral malaria and low nitric oxide production might have contributed to the development of cerebral malaria [**Dhangadamajhi et al, 2009:** ***Infection Genetics and Evolution***]. The significant association of genetic variants of iNOS and eNOS which are known to enhance the NO production with mild malaria and the variation of nNOS which lowers NO production with cerebral malaria suggests that low nitric oxide production may be a contributing factor for the progression of cerebral malaria in Orissa, India.

The impairment in endothelial function, a key phenomenon of severe *falciparum* malaria has been shown to occur due to the homeostatic imbalance and the antagonistic interaction of nitric oxide (NO) and angiotensin II (Ang II) in several vascular diseases. Studies by most of the investigators in recent years reveal that, Endothelial dysfunction could be improved by nitric oxide and inhibitors of angiotensin converting enzyme (ACE) which converts AngI to Ang II or by the counter regulatory effect of ACE2 on ACE. To explore the hypothesis that Angiotensin II may play a role in the susceptibility to cerebral malaria, we performed genetic association study analyzing the ACE2 C→T SNP, and two I/

D polymorphisms (ACE I/D and IL-4 B1/B2). Our results showed that the 'D' allele of ACE I/D polymorphism, responsible for increased Ang II production had a significant association with mild malaria and the ACE2 C→T substitution had gender specific effect of possibly reduced expression of ACE2 in presence of 'T' allele in women leading to increased level of Ang II and hence protection against CM [**Table 3**]. Combined genotype analysis of eNOS Glu→Asp substitution responsible for increased NO production in *Plasmodium falciparum* infected individuals and ACE I/D polymorphism also showed stronger association of (Glu-Asp + Asp-Asp/ID + DD) genotypes with mild malaria ($P < 0.0001$) [**Table 4**]. Whether by its antiplasmodial activity and/or by some unknownmechanisms, Ang II protects from susceptibility to cerebral malaria remains to be investigated. These genetic findingsmay contribute to the understanding of malaria pathogenesis [**Dhangadamajhi et al, 2010: *Infection Genetics and Evolution***].

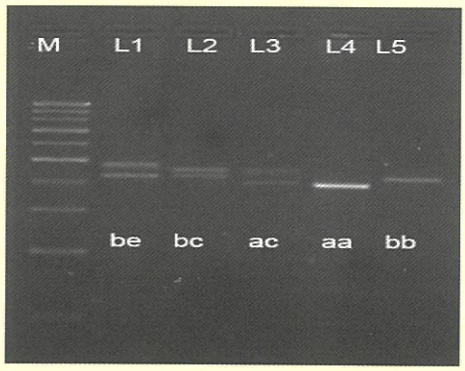


Fig1. Gel photo shows the presence of four different alleles (4a, 4b, 4c and 4e).Lane M: 100bp ladder; L1, b/e heterozygote with fragment size 420 and
474bp; L2, b/c heterozygote with fragment size 420 and 447bp; L3, a/c heterozygote with fragment size 393 and 420 bp; L4, a/a homozygote with fragment size 393 bp and L5, b/b homozygote with fragment size 420bp.

Table-1. Comparision of median plasma NOx (interquartile range) between the various genotypes of eNOS polymorphisms in mild and severe group of malaria patients.

Polymorphism	Genotype	Overall (n=84) Plasma NOx, (μM)n		Mild (n=50) Plasma NOx, (μM) n		Severe (n=34) Plasma NOx, (μM) n	
4 VNTR	aa + ab bb	82.7 (69.4-101.4) 83.5 (63.8-92.0) P = 0.4524	38 46	81.7(75.4-97.4) 84.7(69.4-108.7) NS	20 30	76.05(43.6-83.4) 82.7(66.1-104.7) NS	16 18
T-786C	TT TC	83.4 (63.8-92.0) 85.3 (69.4-108.7) P = 0.1043	48 36	84.7(72.4-93.4) 91.0(70.9-113.2) NS	26 24	69.4(56.3-92.0) 82.7(69.4-94.04) NS	22 12
Glu^{298}→Asp	GG GT	75.4 (68.0-88.5) 99.4 (84.2-116.1) P = < 0.0001	58 26	75.4(69.4-89.8) 108.7(90.18-117.4) 18 P = 0.0001[a]	32	69.4(55.03-85.55) 88.4(72.7-110.5) P = 0.0528[b]	26 8

NS, not significant

a, In mild malaria patients, the Glu^{298}'!Asp substitution is associated with significant difference of median plasma NOx level.

b, In severe malaria patients of Glu^{298}'! Asp substitution, the median plasma NOx was increased in the genotype with T allele, but the increase was not statistically significant.

Table 2. Genotype frequency of nNOS polymorphisms in patients with mild and cerebral malaria.

Polymorphisms	Mild	Severe	OR (95%CI)	*P* value
nNOS 276C → T	*N* = 200	*N* = 170		
CC	90 (45%)	80 (47%)	1.00 (Ref.)	
CT	96 (48%)	80 (47%)	0.94 (0.61–1.43)	0.8293
TT	14 (7%)	10 (6%)	0.80 (0.34–1.91)	0.6672
nNOS −84G → A				
GG	116 (58%)	70 (41%)	1.00 (Ref.)	
AG	78 (39%)	86 (50.6%)	1.83 (1.19–2.80)	0.007[a]
AA	6 (3%)	14 (8.4%)	3.86 (1.4–10.5)	0.007[b]

The X2-test was used to determine diferences between genotype frequencies. Cl. confidence interval: OR. odds ratio. NS. not significant. a GG vs. AG., b GG vs. AA.

Genotype and allele frequency of studied gene polymorphisms in subjects with mild and severe *falciparum* malaria.

Genotype and allele frequency	Mild	Severe	OR (95% CI)	*P*-Value	$P_{corrected}$
ACE I/D	*N* = 228 (M = 154/F = 74)	*N* = 190 (M = 152/F = 38)			
I/I	20 (8.8)	40 (20.9)	Ref.		
I/D	152 (66.6)	122 (63.5)	2.5 (1.4–4.5)	0.0026	0.013
D/D	56 (24.6)	30 (15.6)	3.7 (1.8–7.5)	0.0002	0.001
ID + DD	208 (91.2)	152 (80.0)	2.7 (1.5–4.9)	0.0007	0.0035
D allele	0.579	0.479	1.5 (1.2–2.0)	0.0028	0.014
IL-4	*N* = 228 (M = 154/F = 74)	*N* = 190 (M = 152/F = 38)			
B2B2	122 (53.5)	104 (54.7)	Ref.		
B1B2	92 (40.3)	76 (40.0)	1.0 (0.7–1.5)	NS	–
B1B1	14 (6.2)	10 (5.3)	1.2 (0.5–2.5)	NS	–
B1B1 + B1B2	106 (46.5)	86 (45.3)	1.0 (0.7–1.5)	NS	–
B1 allele	0.263	0.252	1.0 (0.8–1.4)	NS	–
iNOS C → T	*N* = 226 (M = 153/F = 73)	*N* = 192 (M = 153/F = 39)			
CC	174 (77)	150 (78.1)	Ref.		
CT	48 (21.2)	38 (19.8)	1.0 (0.7–1.7)	NS	–
TT	4 (1.8)	4 (2.1)	0.86 (0.2–3.5)	NS	–
CT + TT	52 (23.0)	42 (21.8)	1.0 (0.7–1.7)	NS	–
T allele	0.124	0.120	1.0 (0.7–1.6)	NS	–
ACE2 C → T[a]					
Female	*N* = 74	*N* = 38			
CC	22 (29.7)	22 (57.9)	Ref.		
CT	40 (54.1)	14 (36.8)	2.9 (1.2–6.7)	0.0202	0.101
TT	12 (16.2)	2 (5.3)	6.0 (1.2–30)	0.028	0.14
CT + TT	52 (70.3)	16 (42.1)	3.2 (1.4–7.3)	0.005	0.025
C allele	84 (56.8)	58 (79)	–	–	
T allele	64 (43.2)	18 (21)	2.4 (1.3–4.6)	0.0052	0.026
Male	*N* = 150	*N* = 152			
C allele	106 (70.7)	90 (59.2)	–	–	
T allele	44 (29.3)	62 (40.8)	1.7 (1.02–2.7)	0.0409	0.204

The χ^2-test was used to determine the differences between genotype and allele frequencies. CI, confidence interval; OR, odds ratio; NS, not significant; data in parentheses are percentage value.
[a] Given the X-chromosomal localization of ACE2 C → T polymorphic locus, differences in genotype and allele frequency distribution between mild and severe malaria group of patients were performed in each sex groups.

Table 4.

Joint effect of ACE2 C → T, ACE I/D and eNOS Glu → Asp polymorphisms on the risk of cerebral malaria.

Combined genotype		Mild	Severe	OR (CI)	*P*-Value
ACE	*ACE2*[a]	*N* = 69	*N* = 38		
II	CC	1 (1.4)	4 (10.5)	Ref.	
II	CT + TT	6 (8.7)	2 (5.3)	12.0 (0.8–181.1)	NS
II + DD	CC	20 (29)	18 (47.4)	4.4 (0.4–43.6)	NS
II + DD	CT + TT	42 (60.9)	14 (36.8)	12 (1.2–116.6)	0.024
eNOS	*ACE2*[a]	*N* = 64	*N* = 38		
Glu-Glu	CC	12 (18.7)	24 (63.2)	Ref.	
Glu-Asp + Asp-Asp	CC	8 (12.5)	4 (10.5)	4.0 (1–16.0)	0.09
Glu-Glu	CT + TT	28 (43.8)	4 (10.5)	14.0 (3.98–49.2)	0.0001
Glu-Asp + Asp-Asp	CT + TT	16 (25)	6 (15.8)	5.3 (1.6–17.1)	0.0063
eNOS	*ACE*	*N* = 222	*N* = 188		
Glu-Glu	II	10 (4.5)	25 (13.3)	Ref.	
Glu-Asp + Asp-Asp	II	18 (8.1)	14 (7.4)	3.2 (1.2–8.8)	0.0274
Glu-Glu	ID + DD	132 (59.5)	124 (66)	2.7 (1.2–5.8)	0.0117
Glu-Asp + Asp-Asp	ID + DD	62 (27.9)	25 (13.3)	6.2 (2.6–14.8)	0.0001

The χ^2-test was used to determine the combined effect of different polymorphisms; NS, not significant.
[a] Combined genotype analysis involving ACE2 C → T polymorphisms were performed only for female.

Publications

1. **G Dhangadamajhi**, BN Mohapatra, SK Kar, MR Ranjit (2009).*Parasitol Res 104:1315-1320.*
2. **G Dhangadamajhi**, BN Mohapatra, SK Kar, MR Ranjit (2009). *Infect. Immun 77: 2943-2947.*
3. ***G Dhangadamajhi***, BN Mohapatra, SK Kar, MR Ranjit (2009). *Infect. Gen. Evol 9: 908-911.*
4. **G Dhangadamajhi**, SK Kar, MR Ranjit (2009). *Tropical Biomedicine 26 (3): 326-333.*
5. **G Dhangadamajhi**, BN Mohapatra, SK Kar, MR Ranjit (2010). *Nitric Oxide: 22: 58-59*
6. **G Dhangadamajhi**, BN Mohapatra, SK Kar, MR Ranjit (2010).*Infect. Gen. Evol 10: 337-341.*
7. **G Dhangadamajhi**, SK Kar, MR Ranjit. *Mal Res and Treat.* 2010, doi:10.4061/2010/973094.
8. **G Dhangadamajhi**, Rout B, SK Kar, MR Ranjit. *Trop Biomed.* 2010 **(in press).**
9. Rout R, **Dhangadamajhi G**, Mohapatra BN, Kar SK, Ranjit MR. *Infect Genet Evol* 2010 **(in press).**
10. Rout R, **Dhangadamajhi G**, Mohapatra BN, Kar SK, Ranjit MR. *As Pac J Trop Med* (2010) 412-420.

2. Molecular analysis of different anophelines and their vectorial attributes in different geographical region of Orissa.

Name	: Sunita Swain
Status	: SRF(ICMR)
Date of Joining	: 27th Jan 2009
Guide	: Dr. R.K.Hazra

Objectives

- Molecular identification of main malaria vectors of Orissa.
- To study the bionomics and vectorial attributes of major anophelines species of Orissa.
- To study the evolutionary relationship by molecular phylogenetic analysis of main malaria vectors.
- To study the susceptibility status of the main malaria vectors of Orissa.
- To study the genetic factors responsible for the mosquitoes vector competence to *Plasmodium falciparum.*

Background

Malaria is a leading cause of death worldwide and remains as a major health problem in many developing countries. There are 444 formally named species and 40 unnamed members of species

complexes recognized as distinct morphological or genetic species of *Anopheles*. The subgenus Cellia is divided into five series containing the important vectors and non-vectors of malaria. Both the potential vectors and the non-vectors of the genus *Anopheles* exhibit remarkably similar features and overlapping characteristics. Different molecular markers have been utilized to identify the different anophelines species group. The present study focuses on the use of molecular tools for species identification, along with their vectorial attributes of anophelines species group.

Work Progress

In the study we developed multiplex PCR assay to identify three of the most commonly found members of the *An. annularis* group of Neocellia series (Figure 1). Further we developed efficient multiplex PCR assay to distinguish the most commonly found members of Funestus group of Myzomyia and Annularis group of Neocellia (Figure 2). This is for the first instance that the seven species of the two series have been studied together.

To study the vectorial attributes i.e. human host preference and sporozoite presence help to develop a sensitive and effective assay that can clearly differentiate the malaria vectors from pool of non-vectors. We screened *P. falciparum* positive vectors by using Polymerase Chain Reaction based assay and thereafter detected K76T mutation in the Pfcrt gene, the chloroquine resistance marker, of parasites present within the vectors (Figure 3). This study showed higher transmission rate of chloroquine-resistant *P. falciparum* parasites by *An. culicifacies* and *An. fluviatilis*. To detect the spread of drug resistant *P. falciparum* in a population, before any pathological symptoms detected in humans is possible by analyzing the anophelines vectors.

In the study of evolutionary relationship, we examine different DNA sequences from multiple individuals of closely related species of *Anopheles* prevalent in the Indian subcontinent. It is necessary to have accurate phylogenetic reconstructions and species diagnostics for the study of malaria transmission and its relation to *Anopheles* evolution. The objectives of the present study are to examine phylogenetic relationships among members of the subgenus *Anopheles* prevalent in India by employing a number of genes, viz. D3 (28S rDNA), ITS2 (rDNA) (Figure 4 & 5), mitochondrial cytochrome oxidase I (COI), and mitochondrial cytochrome oxidase II (COII); and then use the phylogenetic tree to assign the position of the ambiguous vector species using the DNA sequences, which otherwise difficult when done with morphological markers.

In the study of susceptible status, the aim is to estimate the prevalence and assess the current distribution of the resistance mechanism in natural populations of anophelines species of Orissa. Standard WHO insecticide susceptibility tests were conducted on adults. Phenotypically characterized

mosquitoes were tested for the presence of knockdown resistance (*kdr*) alleles using the standard polymerase chain reaction assay. Over 75% and above of the malaria vector mosquitoes exposed was knocked down within an hour of exposure to the DDT. The genotype assay by PCR was done for the knock down and survivor' species of the susceptibility test. The *kdr* mutation was detected in frequency of 1%. This PCR assay heterozygous-resistant mosquitoes, which is an additional advantageous feature of molecular tools over conventional bioassay tests.

To study the genetic factor responsible for the mosquitos' vector competence to *P. falciparum,* immune response genes were screen for mutation in the *Anopheles*, which are positively selected in the pressure of *Plasmodium* transmission. For the preliminary study of the immune genes we consider the effectors system of the *Anopheles* i.e. Anti microbial peptides genes Defensin and Cecropin. Novel primers were designed from the sequences of *An. gambiae* from the GenBank. Standardization of the newly designed primer is going on for the field collected malaria vectors and non-vectors of malaria of Orissa. After that the polymorphism pattern in the genes in the vectors and non-vectors will be analyzed.

Fig. 1

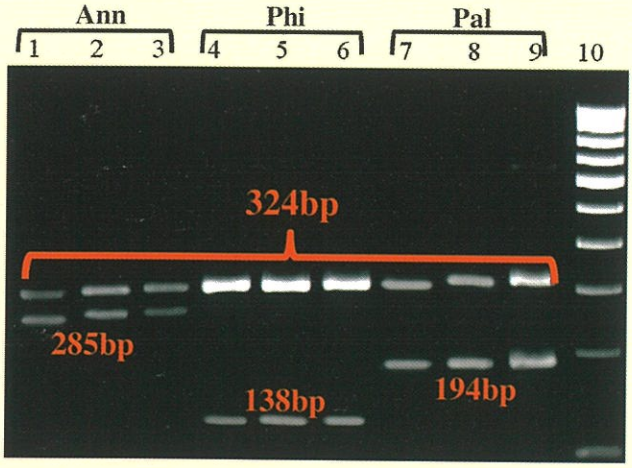


Fig. 2

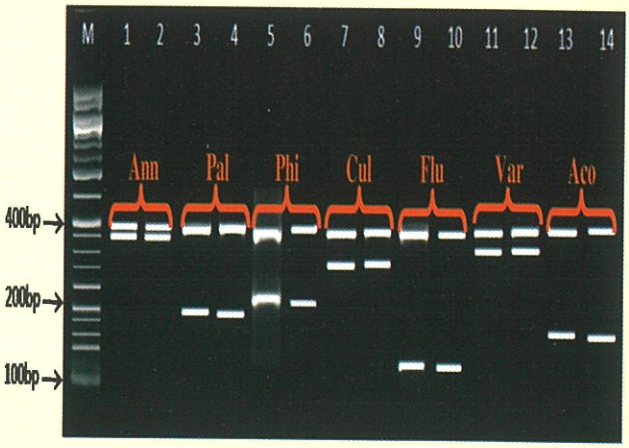
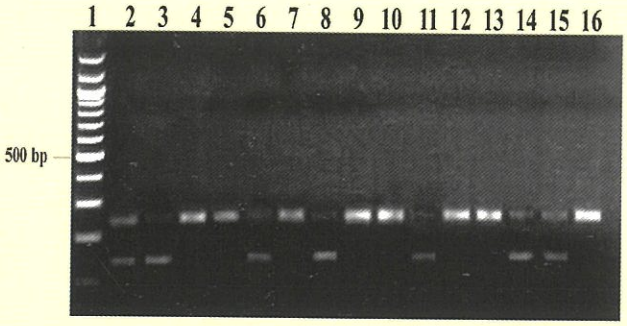


Fig. 3



Lanes 2, 3, 6, 8, 11, 14, and 15 are showing digested product of 136bp and 128bp suggesting chloroquine sensitive strains While rest lanes are chloroquine-resistant strains

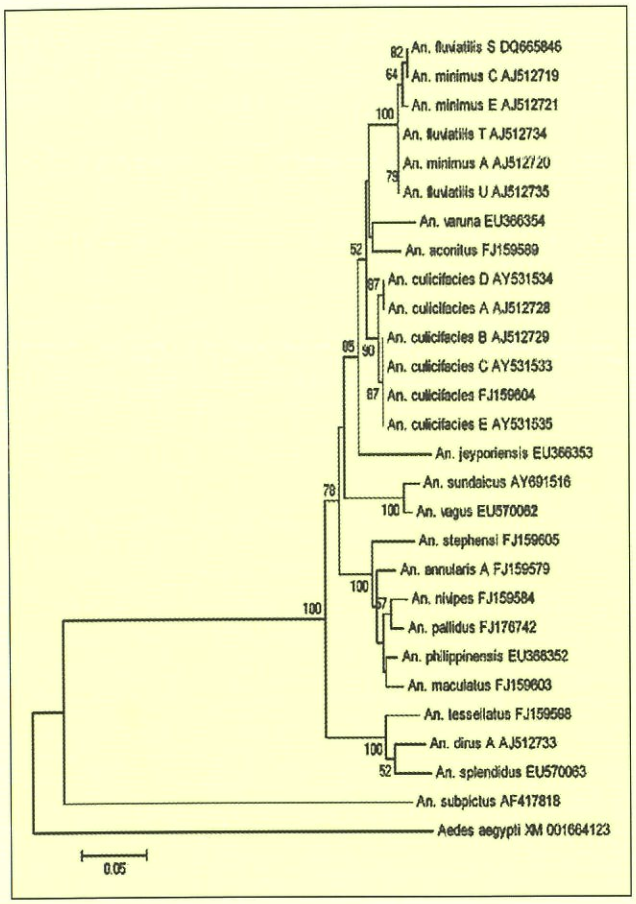


Fig. 4 Evolutionary relationships of 28 taxa using D3 rDNA sequence.

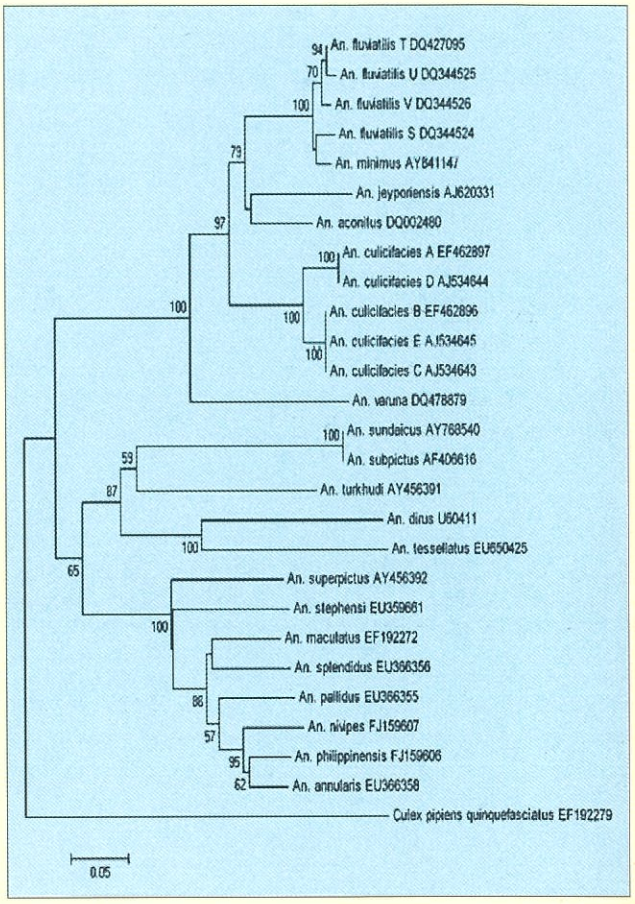


Fig. 5 Evolutionary relationships of 28 using ITS2 rDNA sequence.

Achievements:

- **Awarded as the Best Poster Presentation** at Xth International symposium on vectors & vector borne diseases at Goa, India from 4-6th November 2009.
- Total 42 DNA sequences submitted to the NCBI, GenBank with accession no. **EU366352-62, FJ159579-07** and **FJ176742-43.**
- **Swain S**, Mohanty A, Tripathy HK, Mahapatra N, Kar SK, and Hazra RK. Molecular identification and phylogeny of Myzomyia and Neocellia series of Anopheles subgenus Cellia (Diptera: Culicidae). ***Infection Genetics and Evolution*** **2010** Oct;10(7):931-9.
- **Swain S**, Mohanty A, Mahapatra N, Parida SK, Marai NS, Tripathy HK, Kar SK, and Hazra RK. The development and evaluation of a single step multiplex PCR for simultaneous detection of *Anopheles annularis* group, human host preference and *Plasmodium falciparum* sporozoite presence. ***Trans R Soc Trop Med Hyg.*** **2009** Nov;103(11):1146-52.

- Mohanty A, **Swain S**, Singh DV, Mahapatra N, Kar SK, and Hazra RK. A unique methodology for detecting the spread of chloroquine-resistant strains of *Plasmodium falciparum,* in previously unreported areas, by analyzing anophelines of malaria endemic zones of Orissa, India. ***Infection Genetics and Evolution*** **2009** Jul;9(4):462-7.
- Mohanty A, **Swain S**, Kar SK, and Hazra RK. Analysis of the phylogenetic relationship of *Anopheles* species, subgenus Cellia (Diptera:Culicidae) and using it in resolving the ambiguity of species position particularly in the morphologically similar species. ***Infection, Genetics and Evolution*** **2009** Dec;9(6):1204-24.

3. Risk Factors associated with the spread of malaria in the Rengali Left bank canal system of Orissa.

Name	:	Buli Kumari Panigrahi
Status	:	SRF(ICMR)
Date of Joining	:	10th Sept 2008
Guide	:	Dr. Namita Mahapatra

Objectives

1. To find out the malariogenic conditions, malaria situation in the command area of the dam and prevalence of the vector and other mosquito species.
2. Comparison of malaria incidence & vector fauna in the canal area before and after the construction of the canal.
3. Role of environmental factors associated with the vector-bionomics, such as vector density, sporozoite rate and insecticide susceptibility status etc.

Work Progress

Selection of Study villages

Taking into consideration, construction and networking of LBC-I and LBC-II, irrigation pattern, preliminary malaria data availability and approachability to the area, two villages from each PHCs have been selected for our study. The villages selected were from the Parjang PHC (where water has been released) and Analaberni PHC (where water is not released yet).

Epidemiological data analysis

The malaria incidence between pre (2001-04) and post release of water (2005-08) period indicates no significant changes in the Parjang PHC area (t=0.6387, df=3, P>0.05). However, there was significant decline in the malaria incidence in the Analabereni PHC area during 2005-08 in comparison to 2001-04 (t=35.5365, df=3, P<0.01) (Fig.1).

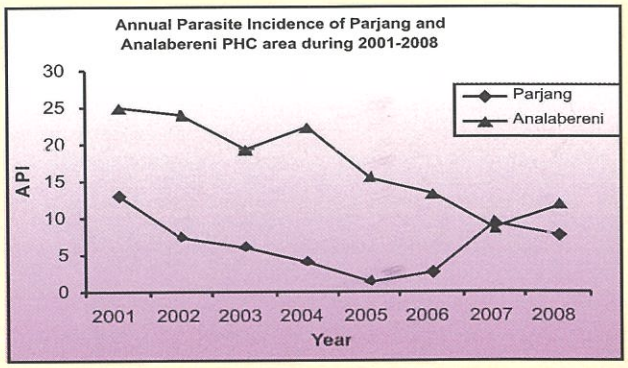
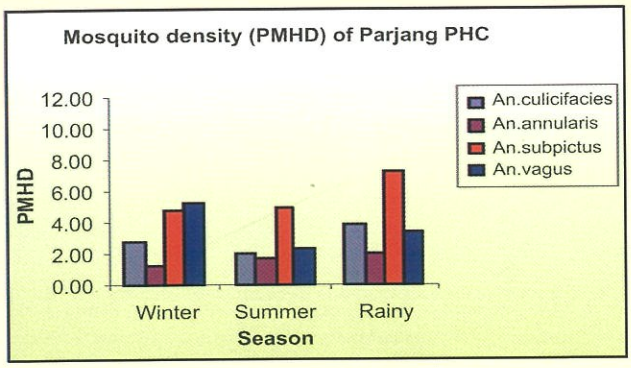


Fig.1.: Shows API of Parjang and Analabereni PHC respectively from 2001-2008.

Entomological Survey

Vector Density

The samplings for all the entomological studies were done as per the WHO procedure (WHO, 1975). The entomological survey revealed the presence of three efficient malaria vectors, i.e. *Anopheles culicifacies, An. annularis, An. fluviatilis along with An. subpictus and An. vagus. Four species of Culex* mosquitoes *viz., Culex quinquefasciatus, Cx. vishnui and Cx. whitmorie, Cx.gelidus* were also found in both the studied PHCs. *An. fluviatilis* is only found in the Analabereni PHC villages in rainy season. Total 1451 number of mosquitoes were collected (November 2008-June 2010) from both the studied PHCs. The season wise PMHD of one year mosquito collection is given in Fig. 2.



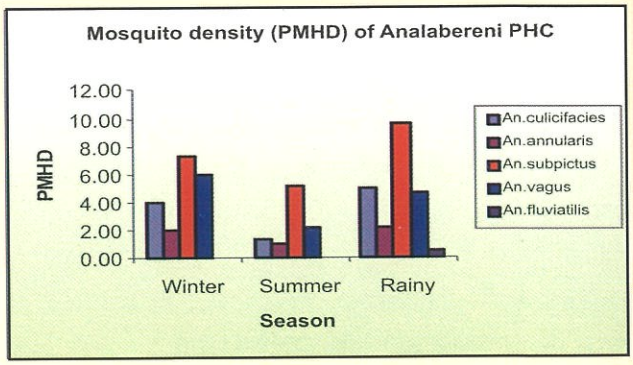


Fig.2. Distribution of different species of Anopheles mosquitoes collected from Parjang PHC and Analabereni PHC during November 2008-October 2009.

Identification of sibling species

An.culicifacies

Standardization for the identification of sibling species for *An. culicifacies* was done by Allele-specific polymerase chain assay (ASPCR) method as per Goswami *et al,* 2006 (fig.3). Four type of sibling species of *An. culicifacies* i. e. A, B, C and D were found in both the study areas. In total, 105 (Parjang) and 140 (Analabereni) samples (collected during November 2008 to June 2010) were processed for sibling species, prevalence of species B is more (64.76%- Parjang, 66.43%-Analabereni) followed by species C (26.66% & 25%), A (4.76% &6.43%) and D (3.81% 7 2.14%) in Parjang and Analabereni PHC respectively. (fig.4).

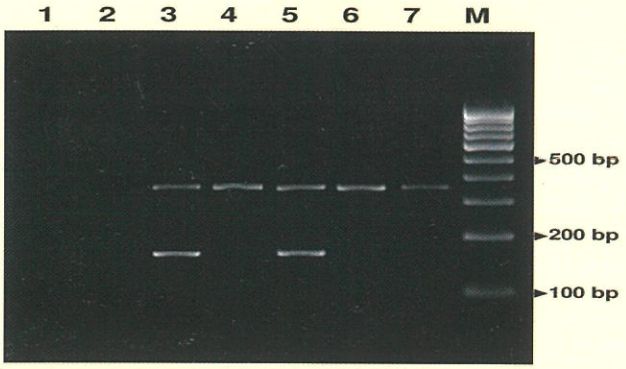


Fig.3. AD-PCR assay for the differentiation of species A and D of An. culicifacies Complex. Lane M,100 bp ladder, lane 4,6,7-species A showing amplification at 359 bp, lane 3,5-species D showing amplification at 166+359 bp.

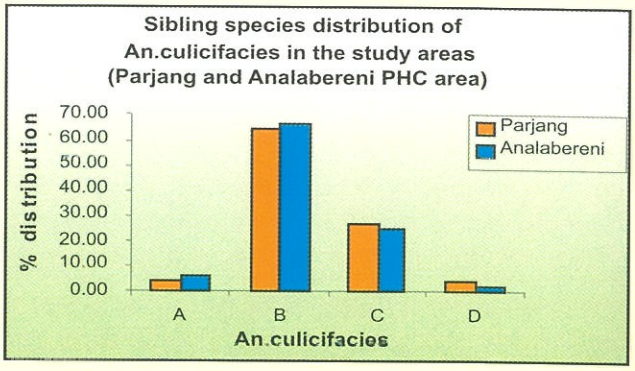


Fig. 4: Mosquito samples processed for sibling species of An. culicifacies by Allele-specific polymerase chain assay (ASPCR) (collected during November 2008-June 2010).

An. fluviatilis

Standardization for the identification of sibling species of *An. fluviatilis* was done as per the method of Mohanty *et al*, 2007. Identification of *An. fluviatilis* showed the presence of two sibling species of *An. fluviatilis* i. e. S and T in Analabereni PHC area. Out of four species processed, 3 were species S and one was species T.

Detection of Human blood meal (Host Specificity) and Sporozoite detection:

Mosquito samples were processed for the presence of human blood meal and the presence of sporozoites (table.1). Identification of human blood meals was carried out by the molecular method of Mohanty et al, 2007. Sporozoite detection was done by Nested PCR method following Mahapatra *et al*, 2006. From the processed mosquito samples AI was found to be 2.5-11.49%. Two number of *An. culicifacies*, one from each PHC was found positive for Pf sporozoite. Both the positive sample was *An. culicifacies* C. The primer pairs used for the detection of human blood meal and detection of sporozoite shows band at 519 bp (fig.5) and 205 bp (fig.6) respectively.

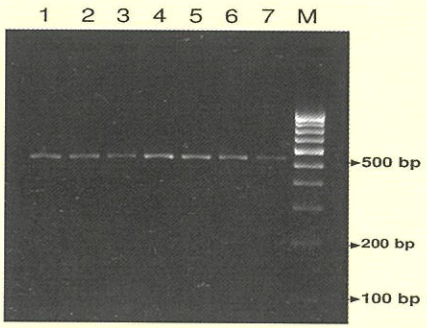


Fig.5: Ethidium Bromide-stained gel electrophoresis of PCR product of *An. culicifacies, An.annularis An. subpictus and An. stephensi* species. Lane 5, *An. stephensi* laboratory human blood fed sample, Lane 1-3, 6-7 test sample of *An. culicifacies, An.annularis An. subpictus*, positive for presence of human blood meal showing amplification in 519 bp, M lane-100bp DNA ladder.

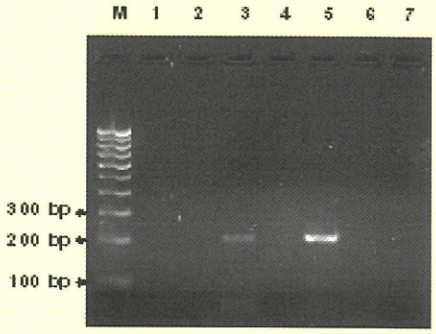


Fig.6: Ethidium Bromide-stained gel electrophoresis of PCR product. Lane 5, *Pf* positive sample, Lane 1-4 test samples of *An. culicifacies*, Lane 3, sample showing positive for *P. falciparum*, amplification in 205 bp, lane M-100bp DNA ladder.

Table. 1: Mosquito samples processed for presence of human blood meal and presence of sporozoites by PCR method (collected during November 2008-June 2010).

PHC	Species	No. mosquitoes processed	No. positive for human blood (AI)	No. positive for sporozoite
Parjang	*An. culicifacies*	105	3(2.86)	1(0.95)
	An.annularis	62	6(9.68)	0
	An. subpictus	140	11(8.57)	0
Analabereni	*An. culicifacies*	140	5(3.57)	1(0.71)
	An. annularis	78	9(11.54)	1(1.28)
	An. subpictus	140	15(10.71)	0
	An.fluviatilis	4	0	0

*Number in brackets shows percentage (AI- Anthropophilic Index)

Larval Survey

Mosquito larval sample were collected from experimental villages from different breeding sites (fig.7) such as River bed pools, ponds, seepages, rice fields, domestic containers, wells etc using dip method (WHO,1975). Larvae sample were brought to the laboratory for rearing till the adult emergence and then the mosquito identified. Detail analysis will be done after all the collections.

Fig.7 Breeding places around the study area. i. water logging in the irrigation during summer. ii. Seepage from the canal. iii. Formation of artificial pond near the study village due to construction work.

Knowledge, attitude, beliefs and practices (KABP) study:

Field surveys for this study were carried out in two study areas. The inhabitants of the villages are mainly belongs to Scheduled Caste (>80%). They are socially and culturally homogenous. These villages are divided by a number of hamlets (5-10). The literacy rate is extremely low and practically all the women are uneducated. Drinking water is mainly taken from wells.

A total of 115 persons in the age group of 15-50 yrs were included in this study. Only one person per household was included. They were selected by choosing a house at random in each hamlet of the village and then visiting every fourth or fifth house. The inhabitant who are readily available and who consented to the request of the researcher, was interviewed personally. A

questionnaire was prepared to cover the important aspects, i.e. knowledge, attitude and practices towards malaria and its control. Questions were asked by the interviewer and the responses were recorded.

There were 690 people in 115 study families; the ratio of male to female was 52:48. The number of people in each family ranged from 2-10 with an average of 6 persons per household. Literacy rate is 10-20 %, with a majority completing only their primary schooling (5th standard). Less than 2% had studied up to higher secondary. The people live in small mud houses with bamboo or straw roofs. The study revealed that major sources of income are through wage earning (50%), farm cultivation (40%) and working in small farms in the nearby city (within the district) (10%).

Out of 115 people interviewed, almost all the respondents were familiar with malaria (a fever). But only 6% really know that what caused malaria. Contribution of migratory population towards vector borne diseases is almost negligible (<2%). Only intra district migration is there in the study area.

4. Study of *Aedes* mosquitoes in various parts of Orissa with reference to transmission of arboviral disease.

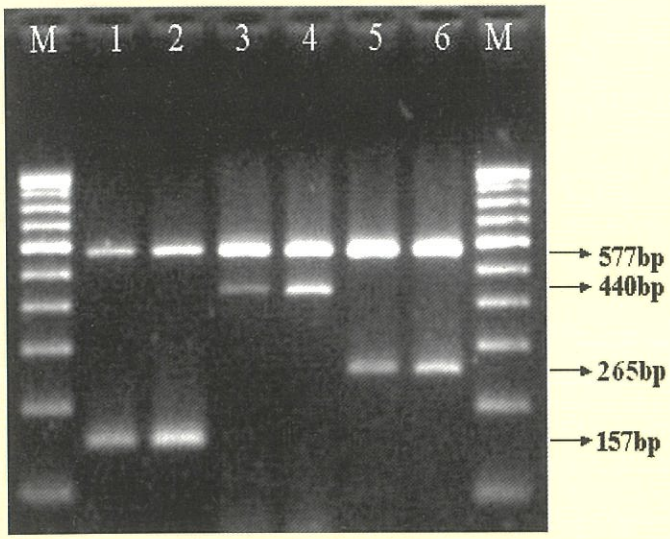
Name	:	Biswadeep Das
Status	:	JRF(ICMR)
Date of Joining	:	27th Jan 2010
Guide	:	Dr. R.K.Hazra

Objectives

1. To study the distribution and bionomics of *Aedes* mosquitoes involved in disease transmission in different parts of Orissa.
2. To develop a PCR based method for identifying the immature stages of different *Aedes* mosquitoes that will be collected from various parts of Orissa.
3. To identify Dengue virus and CHIKV in *Aedes* mosquitoes collected from different regions of Orissa.
4. To study the phylogenetic relationship in genes involved in inducing immunity in *Aedes* mosquitoes and to know the strain type of CHIKV virus operating in this region.

Work progress

Aedes mosquitoes, subgenus Stegomyia (Diptera: Culicidae) known to be major vectors of arboviral disease like yellow fever, dengue, chikungunya have recently emerged as important vectors of chikungunya in the coastal belt of Orissa state, India. *Aedes albopictus* Skuse and *Aedes aegypti* L, are commonly found here and play a major role in the transmission of chikungunya in the coastal areas of Orissa. *Aedes vittatus* Bigot, which is also found here, has the potential to transmit the chikungunya virus.



The state of Orissa is divided into four distinct physiogeographical regions, northern plateau, central tableland, coastal areas and Eastern Ghats (Fig1). The coastal areas have been the most endemic areas for chikungunya outbreak as per the Health Department report of Government of Orissa. The study was carried out in specific areas of six coastal districts of Orissa, India, i.e. Puri, Khurdha, Cuttack, Kendrapara, Jagatsinghpur and Jajpur. We collected larvae and pupae of the *Aedes* species from their natural and artificial water containing habitats. Collections were done from these areas after rainy season and samples were brought to the laboratory for processing. A total of 294 breeding spots were searched, out of which 106 were positive for the *Aedes* larvae. We developed a simple and rapid multiplex PCR method based on aligning the sequences of 18S rDNA region of the three *Aedes* (Stegomyia) species to clearly distinguish the immature stages from each other. DNA was extracted from the mosquitoes and larval samples that were collected and multiplex PCR was done to identify the Aedes species. *Aedes aegypti* showed band at 157 bp, *Aedes albopictus* showed band at 440 bp and *Aedes vittatus* showed band at 260 bp. *Aedes albopictus* was the most abundant species detected in different breeding spots of the regions surveyed. *Aedes aegypti* was detected in tree holes and *Aedes vittatus* was rare, detected in stony pits only. The multiplex PCR method will contribute greatly to the rapid identification of these three *Aedes* (Stegomyia) species during arboviral vector surveillance in this region.

Dengue is an arboviral disease caused by the positive sense RNA virus belonging to family flavivirus and is divided into 4 serotypes: Den 1,Den 2, Den 3, Den 4. The combination of any of the two or all serotypes results in the fatal dengue hemorrhagic fever leading to death of the patient. The dengue virus is transmitted by the *Aedes* (Diptera: Culicidae) vector mosquito belonging to subgenus: Stegomyia. The *Aedes* species lives near to human dwellings in clean water containing receptacles like tires, pots, buckets, plastic and glass containers, tree holes, stony pits etc.

In the month of September, an outbreak of Dengue was reported from the Padmagiri and Malkangiri block of Malkangiri district of Orissa. The RMRC team contacted the district hospital of Malkangiri and conducted a detailed survey of the two blocks to know the factors that triggered the outbreak All water-containing receptacles were searched for the presence of *Aedes* larvae. Discarded tires, pots, cement tanks, and plastic buckets, jars near to human dwellings were the main breeding spots for *Aedes* mosquitoes in the two blocks. Adult mosquitoes and larvae were collected from their habitats by using traps and brought to laboratory in plastic and glass bottles. Based upon their taxonomical

Malkangiri district						
	Padmagiri Block		Malkangiri block			
	Padmagiri		Thana Sahi		Pradhanguda	
Breeding sites	No of containers searched	No with Aedes Larvae	No of containers searched	No of with Aedes larvae	No of caontainers searched	No of with Aedes Larvae
Earthen pots	79	49	50	31	23	7
Discarded tires	11	5	16	14	13	8
Discarded small Plastics, jars	-	-	25	17	10	2
Discarded large Plastics, buckes	8	1	20	14	3	1
Cement tanks	5	1	9	4	3	2
Total	**103**	**56**	**120**	**80**	**52**	**20**
Total house holds with larval positive sites	**50**	**38**	**65**	**47**	**15**	**5**
Container Index (CI)	54.4		6636		38.5	
House hold Index (HI)	76		72.3		33.3	
Breteau Index (BI)	112		123		70	

and morphological characteristics, *Aedes* mosquitoes were identified. They were further confirmed by a multiplex PCR technique using the small subunit of ribosomal DNA of the *Aedes* species using a pair of universal primers and species-specific primers.

We found that both the blocks had very high container, household and breteau indices, with Thana Sahi village recording the highest larval indices. DNA was extracted from the mosquitoes and larval samples that were collected and multiplex PCR was done to identify the Aedes species. The mosquitoes which were collected were identified to be *Aedes albopictus* (60%), *Aedes aegypti* (30%), *Culex* (3%) *Aedes vittatus* (2 %) and *Armigeres* (2%). *Aedes albopictus* was detected in maximum number of breeding spots and hence is considered as the primary vector for the dengue outbreak in the Malkangiri of Orissa.

Achievements

Sequences submitted to Genbank : **HQ010436** (*Aedes vittatus*), **HM486433** (*Aedes aegypti*), **HQ010437** (*Aedes albopictus*).

Paper communicated entitled "Molecular identification of immature stages of *Aedes* (Stegomyia) mosquitoes using the 18S ribosomal DNA region by a single step multiplex PCR involved in transmission of chikungunya in the coastal areas of Orissa." to *Transaction of Royal Society of Medicine and Hygiene*

5. Investigation of the maternal infection on humoral and cellular immune response of neonates in lymphatic filariasis.

Name	: K. Gopinath Acharya
Status	: SRF(ICMR)
Date of Joining	: 30th Jan 2009
Guide	: Dr. A.K.Satapathy

Objectives

1. To study the humoral immunological responses to filarial antigens (Water soluble, detergent soluble and excretory secretory antigens) in cord blood samples of offspring and in corresponding mothers.
2. To evaluate the influence of maternal infection on filarial antigen induced cellular responsiveness and cytokine production in cord bloods of neonates.
3. To compare the expression profile of antigen-specific and non-specific cell mediated immune cells in cord blood samples of offspring and in corresponding mothers.
4. Characterization of the antigen responsible for in utero sensitization of fetal immune cells.

Work Done

Sample collection: 126 pairs of Mother and cord blood samples were collected in heparin vials and serum separation was done by centrifuging at 3000rpm for 30 mins.

CFA Assay: CFA assay was done by using Og_4C_3 ELISA kit of TropBio according to the manufacturer's user manual. Then all the samples were divided into three groups according to the CFA results the groups are as follows:

1. Both Postive: Both mother and its respective cords are CFA positive.
2. Mother positive: Only Mother is CFA positive and cord negative.
3. Both Negative: Both mother and its respective cords are CFA negative.

Parasite collection and antigen preparation: Live worms of *Setaria digitata* were collected from the bovine intestine from nearby slaughter house of Nandankana. Water soluble and detergent soluble antigens were prepared by grinding the worms in PBS and Tris-NP40 respectively. Excretory-Secretory antigens were prepared by culturing the parasites in DMEM media at 37p C for 48-72 hrs.

Antibody assessment by ELISA: IgG subclasses were assessed in the mother and cord pairs against all the three antigens.

Fig. 1 Percentage of samples in the 3 groups.

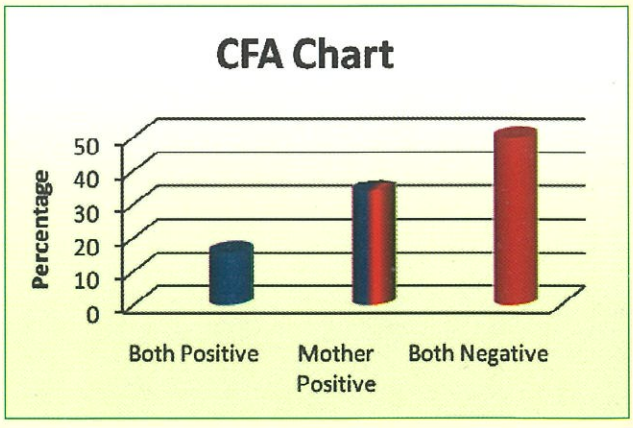


Fig. 2 Antibody units against different antigens in 3 groups of mother and cord blood.

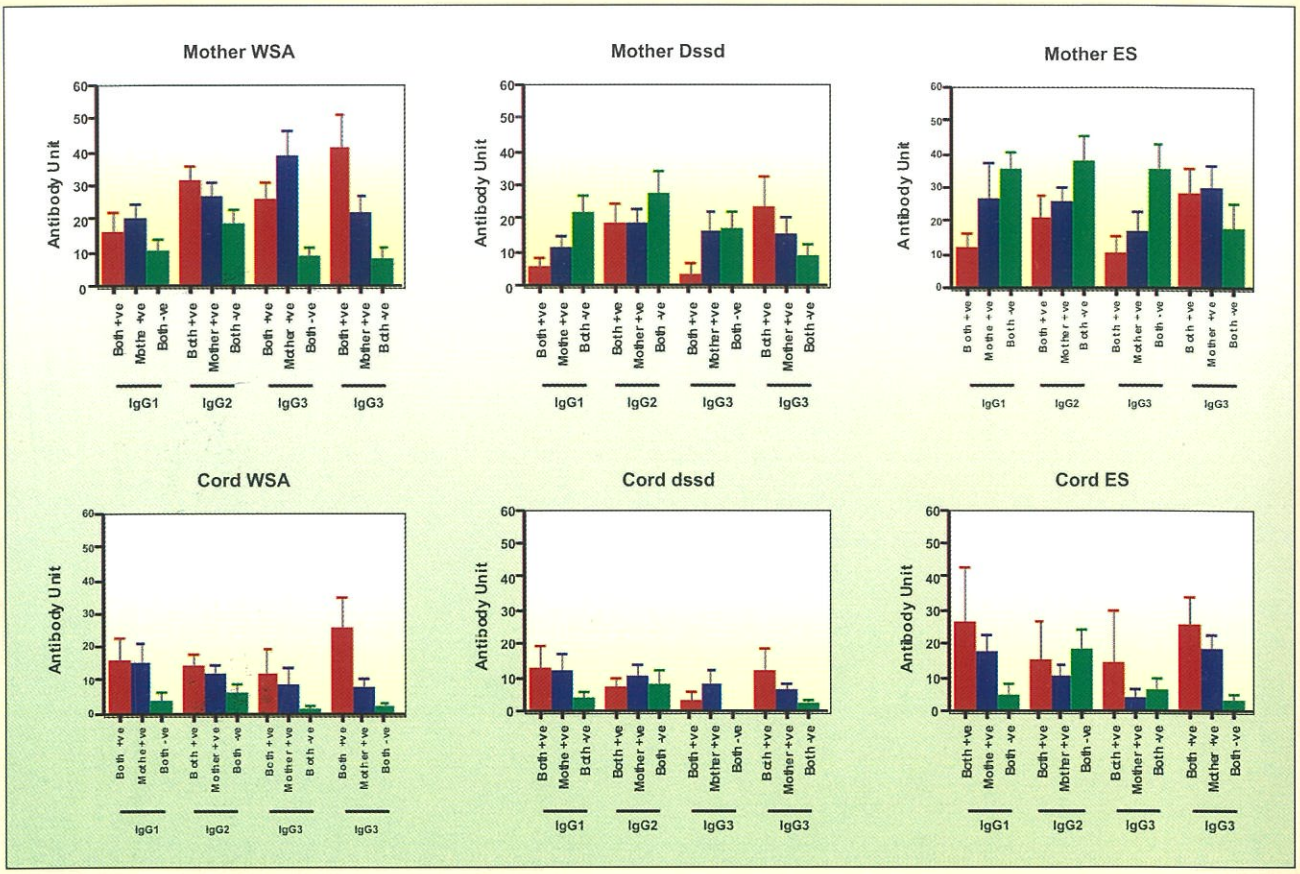


Table-1. Seropositivity percent in the three groups against the water soluble antigen

Maternal Infection Status	N	IgGI seropositive		IgG2seropositive		IgG2seropositive		IgG2seropositive	
		Maternal sera (%)	Cord sera (%)	Maternal sera (%)	Cord sera (%)	Maternal sera (%)	Cord (%)	Maternal sera (%)	Cord sera (%)
Both Positive	20	65	40	90	55	80	30	80	70
Mohter Positive	43	69	51	88	60	60	25	74	42
Both Negative	63	50	30	85	52	28	8	46	13

Table-2. Seropositivity percent in the three groups against the detergent soluble antigen

Maternal Infection Status	N	IgGI seropositive		IgG2seropositive		IgG2seropositive		IgG2seropositive	
		Maternal sera (%)	Cord sera (%)	Maternal sera (%)	Cord sera (%)	Maternal sera (%)	Cord (%)	Maternal sera (%)	Cord sera (%)
Both Positive	20	30	30	55	35	15	5	45	35
Mohter Positive	43	44	18	49	32	28	7	48	32
Both Negative	63	82	27	78	27	55	5	40	13

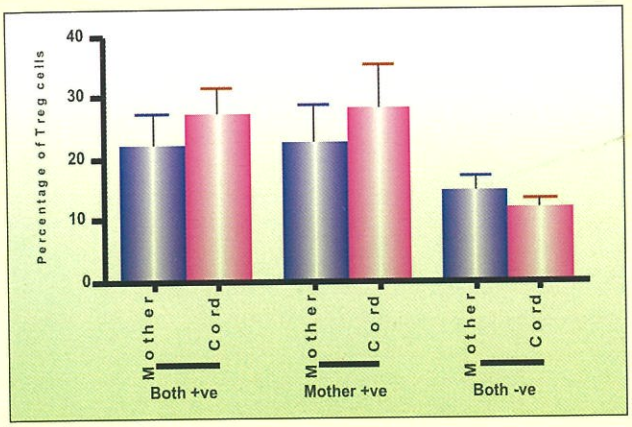
Table-3. Seropositivity percent in the three groups against the Excretory secretory antigen.

Maternal Infection Status	N	IgGI seropositive		IgG2seropositive		IgG2seropositive		IgG2seropositive	
		Maternal sera (%)	Cord sera (%)	Maternal sera (%)	Cord sera (%)	Maternal sera (%)	Cord (%)	Maternal sera (%)	Cord sera (%)
Both Positive	20	50	50	60	35	25	15	60	65
Mohter Positive	43	58	44	75	45	37	12	78	56
Both Negative	63	95	14	86	40	68	14	68	22

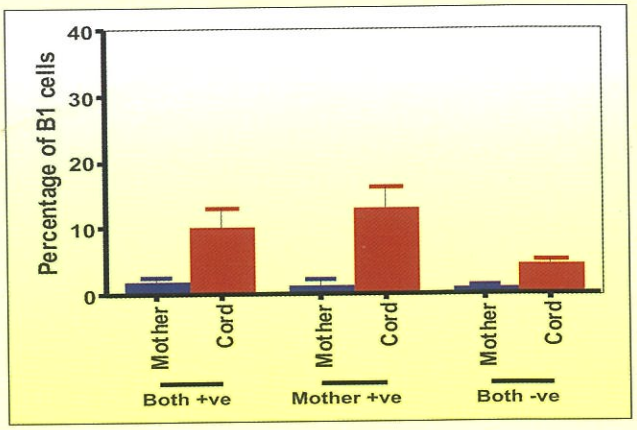
Quantification of T-regulatory cells and B1 cells: Expression of T-regulatory cells and B1 cell population were quantified in cord blood of infants born to infected and uninfected mothers and in corresponding maternal sample. These were quantified by Flowcytometry staining using cell surface markers. Cells that expressed CD5 and CD19 are B1 cells. Cells that express the surface marker CD4 together with CD25 are T regulatory cells.

Fig. 3 (A) T regulatory cells and (B) B1 cell profile of maternal and cord sera.

(A)



(B)



In maternal sera the seropositivity percent against water soluble antigens are higher in infected groups whereas the antibodies against detergent soluble antigens and excretory secretory antigens are higher in endemic normal population in comparison to infected group. In cord sera overall antibodies against all three antigens are higher in infected groups than endemic normal groups. IgG2 and IgG4 antibody responses against WSA are significantly higher in both positive group mothers than other two groups whereas IgG3 response is significantly higher in mother positive group than other two. IgG1 and IgG4 antibody responses are always higher in cord blood of infected mothers than the uninfected mothers. Preliminary study shows that the percentage of T regulatory cells are higher in cord blood samples than its respective mothers in CFA positive groups, where as in CFA negative group the percentage is lower in cord than its respective mothers. In all the three groups B1 cells are higher in cord bloods than its respective mothers but the degree of difference is

higher in Mother positive group. All these findings provide evidence for pre-natal sensitization occurs during filarial infection.

6. Studies on genetic aspects of essential hypertension in different population groups of Orissa.

Name	: Manisha Patnaik
Status	: JRF(CSIR)
Date of Joining	: 24th Sept 2009
Guide	: Dr. B. Dwibedi

Background

Hypertension, also referred to as high blood pressure, is the most common risk factor for cardiovascular and cerebrovascular diseases, in which the blood pressure is chronically and consistently elevated than the normal systolic and diastolic (120/80) pressure. It has affected over 1 billion individuals worldwide and in India, there were 65.5 million hypertensives in 2004 (Gupta R., 2004). Hypertension has been classified mainly into two types, essential, with no well defined, specific underlying cause and secondary with a known etiology. Human essential hypertension is a complex, multifactorial, and polygenic trait. 30-50% of the variation in blood pressure among individuals can likely be attributed to several causal genes which interact with environmental factors such as, for example, dietary salt to produce the final disease phenotype. A distinctive feature of Asian-Indian population is its genetic heterogeneity because of endogamy which has perpetuated local genetic diversity. Because of this, the genetic composition of the people of Orissa is unique in itself.

Angiotensin converting enzyme, Angiotensin converting enzyme 2, endothelial Nitric Oxide Synthase and 11 b-hydroxysteroid dehydrogenase are four of the candidate genes, the polymorphisms of which have been studied in different regions of India and relationships observed.(8-12) Since no report is available in this regard in the Orissa population, an attempt has been made to study the prevalence of essential hypertension in different population groups of Orissa and their association with clinical manifestation of essential hypertension. Therefore analysis of these polymorphisms was initiated in Orissa population.

Angiotensin Converting Enzyme (dipeptidyl carboxypeptidase), a zinc metallopeptidase, has an important role in circulatory homeostasis, which catalyses the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, and through protease activity, inactivates bradykinin, a potent vasodilator (13). Endothelial Nitric Oxide Synthase (eNOS or NOS3) is responsible for production of nitric oxide (NO) from L-arginine. NO has various physiologic regulatory roles and is involved in smooth muscle relaxation and blood pressure regulation. Angiotensin Converting Enzyme 2 is involved in the regulation of BP homeostasis and cardiac function. ACE2 converts angiotensin I to angiotensin1–9 and angiotensin II (Ang II) to angiotensin1–7 (Ang (1–7)) which has been reported to promote vasodilatation and counterbalance effects of Ang II. 11 b-hydroxysteroid dehydrogenase

converts cortisol to the receptor-inactive cortisone, protects the non-selecive mineralocorticoid receptor from occupation by cortisol. Mutations in the HSD11B2 gene generating a compromised 11BHSD2 enzyme activity, as occurs in the syndrome of Apparent Mineralocorticoid excess (AME) lead to overstimulation of the mineralocorticoid receptor by cortisol and sodium retention, hypokalemia and high BP.

Objectives: (i) To study the prevalence of essential hypertension in some selected populations of Orissa (ii) To investigate the candidate gene polymorphisms in associated with clinical expression of essential hypertension in the above study populations.

Work progress

Blood was collected from the subjects by veinipuncture and stored in EDTA vials. Plasma was separated stored at -20°C for biochemical analysis. Age, height, weight and waist measurements were recorded and BMI was calculated (weight in kilograms divided by the square height in meters).

DNA was isolated using phenol chloroform method. The DNA thus isolated was used to study polymorphisms by amplification of the regions containing mutations and protocols were standardized. The methods used have been summarized briefly as under.

Sl. No.	Gene	Polymor Phism	Method	Primers	Annealing Temp. (°C)	Enzy me
1.	ACE	I/D (intron16)	PCR	F:5'CTGGAGACCACTCCCATCCTTTCT3' R:5'GATGTGGCCATCACATTCGTCAC3'	66	-
2.	ACE 2	Intronl C>T	PCR-RFLP	F:5'GAAAGCCACATGCTTTAACAAG3' R:5'TTTTTCCATATCTCTATCTGATCG3'	55	TaqI
3.	eNOS	Intron 4a/4b	PCR	F:5'AGGCCCTATGGTAGTGCCTTT3' R:5'TCTCTTAGTCTGTGGTCAC3'	54	-
4.	eNOS	E298D (exon 7)	PCR-RFLP	F:5'CATGAGGCTCAGCCCCAGAAC3' R:5'AGTCAATCCCTTGGTGCTCAC3'	59	MboI
5.	eNOS	T-786C	PCR-RFLP	F:5'ATGCTCCCACCAGGGCATCA3' R:5'GTCCTTGAVTCTGACATTAGGG3'	61	NgO MIV
6.	11bH SD2	GAG> GAA (codon178 exon 3)	PCR-RFLP	F:5'AGGACACGGGGACTGGAAG3' R:5'GGGGGGCTCCTTTTTGCTCC3'	58	AluI

Further samples will be collected, polymorphisms and their relation to hypertension will be studied and biochemical analysis will be done.

The study will help to find out molecular markers for the diagnosis of essential hypertension. This may help in development of personalized medicine.

Topic: Study on micronutrients malnutrition with special reference to vitamin A and its associations with other major trace elements among children in orissa.

7. Study on micronutrients malnutrition with special reference to vitamin A and its associations with other major trace elements among children in orissa.

Name : Suchismita Behera
Status : SRF(ICMR)
Date of Joining : 25th June 2009
Guide : Dr. G. Bulliyya

Objectives

1. To assess the prevalence of vitamin A deficiency
2. To assess the prevalence of iron deficiency and anaemia
3. To establish the association of vitamin A with other micronutrients

The study is a PhD work. The synopsis has been already submitted to Berhampur University.

Background

Deficiencies of micronutrients are a global health problem. More than 2 billion people in the world are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc. The most vulnerable population groups to micronutrient deficiencies are pregnant women, lactating women and young children, mainly because they have a relatively greater need for vitamins and minerals and are more susceptible to the harmful consequences of deficiencies. For a young child, micronutrient deficiencies increase the risk of dying due to infectious diseases such as acute RTI, diarrhea, measles and contribute to impaired physical and mental development. Vitamin A deficiency is a major cause of morbidity and mortality in India and other developing countries. An estimated 5.7% children in India suffer from eye signs of vitamin A deficiency. In Orissa vitamin A deficiency is a significant public health problem. The median blood vitamin A levels among preschool children is 18.1ug/dl, which is less than the assigned value of 20ug/dl for children by WHO (NNMB 2006) and it is a sign of sub-clinical level of vitamin A deficiency. The percentage of children having deficient vitamin A levels (20ug/dl) is 57.7%.

Vitamin A level in the body is associated with infection, malaria, protein-energy malnutrition, anemia, iron, zinc, selenium, vitamin E and C, hemoglobinopathy, glucose-6-phosphate dehydrogenase activity. So it is necessary to evaluate all these parameters along with vitamin A in vulnerable sections of population.

Rationale

Despite high magnitude of subclinical VAD in preschool children of Orissa, data does not exist for different districts with considerable geographic and ethnic variation. The prevalence of VAD as well other micronutrient deficiencies is expected to be high among children, for which no data is available at district levels. This study can be helpful to understand the association of VAD and malnutrition in establishing effectiveness of other micronutrients and vitamins on this.

Study design

A cross-sectional study is being carried out covering children aged between 0-12 years that includes both preschool (0-5years) and school age (6-12 years) children.

Sample size

Sample size is determined based on sub-clinical vitamin A deficiency (low serum retinol) 56% in preschool children in the state, considering 10% relative precision of estimate and 95% confidence interval and a design effect of 2. The total sample size calculated for each group is 600 children and for two age groups (0-5 & 6-12y) comes to 1200.

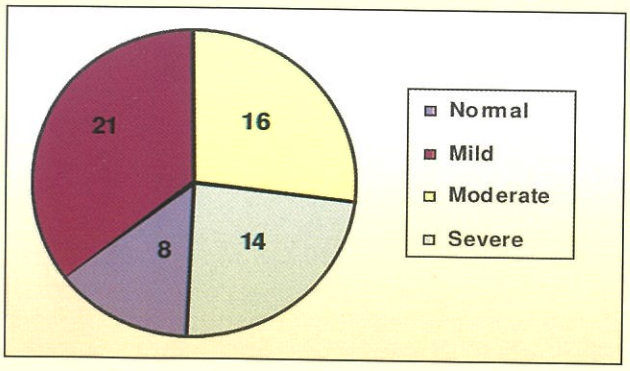
Work Progress

Sample collection - Anthropometric data (height, weight) of 120 children was collected using standard equipment and procedures. 61 blood samples (17male and 44females) were analyzed for vitamin A, 59 (17 male and 42 female) were analyzed for hemoglobin and 22 children (female) were analyzed for serum ferritin.

The mean hemoglobin and serum retinol levels were found to be 9.29±2.58 g/dl and 18.96±9.7µg/dl respectively.

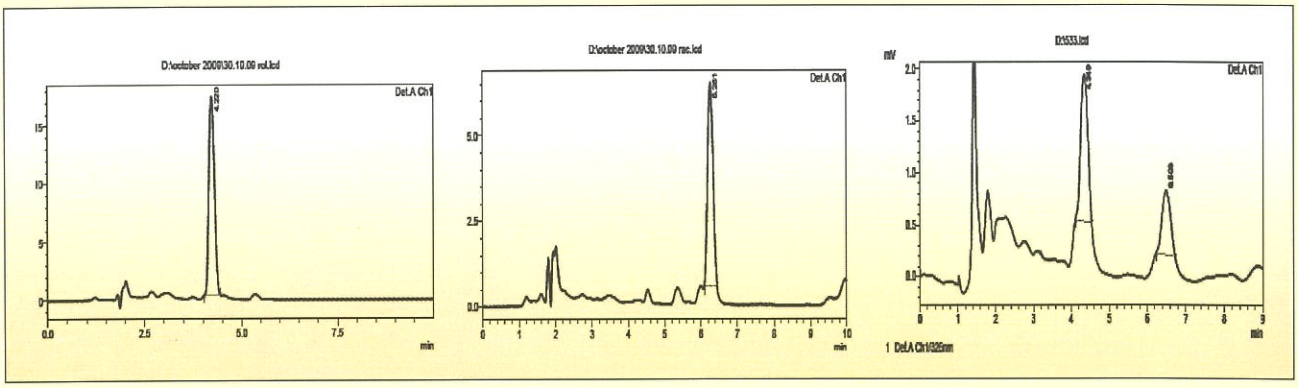
The prevalence of anemia is found to be 86.5% using WHO classification. Different grades of anemia were determined according to the criterion fixed by WHO/Unicef/UNO (2001).

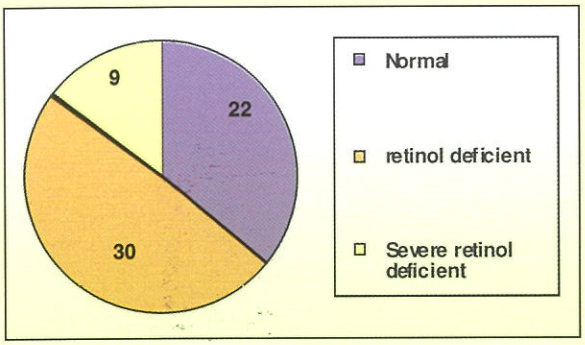
	Anemia measured by hemoglobin (g/dL)			
	Anemia	Mild	Moderate	Severe
Children 6-59 month	<11.0	10-10.9	7.0-9.9	<7.0
Children 5-11 years	<11.5	10-11.4	7.0-9.9.	<7.0
Children 12-14 years	<12.0	10 11.9	7.0 9.9	<7.0



Anemia grade (Hb)	Percentage (N)
Normal	13.5% (8)
Mild	35.6% (21)
Moderate	27.2% (16)
Severe	23.7% (14)

Serum retinol estimated by HPLC method was classified for children having subclinical vitamin A deficiency according to WHO (2009) reveals that 64% having retinol concentration less than 20ìg/dl.





VAD according to serum retinol level	Percentage (N)
Normal retinol level (>20µg/dl)	36.06% (22)
Retinol deficiency (<20µg/dl)	49.18% (30)
Severe retinol deficiency (<10µg/dl	14.75% (9)

Future Planning

Field work will be conducted for collecting data for covering the sample as per methodology. Nutritional status of children will be assessed and biochemical indicators such as vitamin A, E using HPLC, vitamin C using spectrophotometer, selenium & zinc using Proton induced X-ray emission; G6PD activity using spectrophotometer will be done. Data will be analysed to study prevalence of different micronutrient deficiencies in and the associations between various micronutrients.

8. Protein-energy and micronutrient malnutrition among preschool children in Bhubaneswar block, Orissa

Name : Priyadarsi Girija Sankar Sethy
Status : SRF(ICMR) completed
Guide : Dr. G. Bulliyya

Background

Malnutrition afflicts 60 million children in India, imposing social and economic costs that are hard to overstate. Protein-energy malnutrition (PEM) is the most widely prevalent form of malnutrition among children. It is an underlying cause of high child morbidity and mortality in underprivileged communities. It is impossible to give figures for the incidence of prevalence of severe PEM, as dinned by the welcome classification (Fuller and Elia, 1989). According to NNMB surveys, there has been a

decline in the prevalence of severe forms of PEM *ie*, marasmus from 1.3 to 0.6% and Kwashiorkor from 0.4 to 0.1% from 1975-79 to 1988-90 (NNMB, 1991-92). However, the moderate forms of PEM continue to be high. According to NFHS-2 survey report (1998-99), almost half of children under-three years of age (47%) are underweight, and a similar percentage (46%) is stunted. Wasting is less prevalent affecting 16% of children under three years of age. The overall scenario in the nutritional profile of preschool children in Orissa is much inferior as compared to other states (Bulliyya, 2003). A community based cross sectional studies show that the magnitude of undernutrition and PEM in preschool children is still a leading problem in Orissa (Mohapatra et al, 2000).

More than 75% of preschool children suffer from iron deficiency anemia (IDA) and 57% of preschool children have sub-clinical Vitamin A deficiency (VAD). Iodine deficiency is endemic in 85% of total districts. Over the last two decades, increasing attention has been directed towards specific deficiencies of trace elements such as iron, iodine, copper, zinc, manganese, cobalt, chromium, selenium, molybdenum, fluorine, tin, silicon, vanadium etc. The present work aims to evaluate the relation between the **some** trace elements in-patients with P.E.M, in an attempt to throw light on the possible role of these trace elements and that their deficiencies are associated with the manifestations of P.E.M.

HYPOTHESIS: Severity of protein energy malnutrition is associated with levels of micronutrients in preschool children. The current study will explore the correlation between micronutrients and magnitude of PEM.

OBJECTIVES: To evaluate the prevalence of protein energy malnutrition among preschool children; To assess the prevalence of micronutrient deficiencies (iron, vitamin A, iodine, zinc, copper and selenium etc.) and to establish association between PEM and various micronutrient deficiencies.

Work Plan

Study design: A cross-sectional study is undertaken in 8 GPs of Bhubaneswar block.

Study population: Study population is children aged under-6 years.

Study Area: Out of 19 GPs of Bhubaneswer block, 8 GPs were selected for the study.

Sample size: Sample size calculated based on the least prevalence variables of wasting. A calculated sample of 1046 children arrived based on expected prevalence of 27% wasting (in Kalahandi district), á error 1%, precision 5% and design effect of 2 allowing sex variation. A sample of 1100 preschool children aged under-6 years is included for the study.

Sampling method: A 30 cluster PPS sampling method is adopted.

Laboratory tests

- Haemoglobin by Cyanmethaemoglobin method (INACG, 1985).
- Serum ferritin by ELISA method.
- Vitamin A concentrations estimated by HPLC
- Urinary iodine by wet-digestion method (Dunn et al, 1993).
- Micro-nutrients (Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Bromine (Br), Selenium (Se) and Lead (Pb) analyzed using PIXE (Proton-induced X-ray emission) technique at Institute of Physics.

Observations

The nutritional status of children (n=900) as per standard deviation classification for height for age, weight for age and weight for height is summarized. Children were generally small for their comparison with the NCHS reference. In all 60.5% children were underweight/malnourished (<median-2SD weight for age of NCHS) while 3.2% severely undernourished among them. Using height for age criteria, the overall prevalence of stunting was 48.8 % (<-2SD) with 1.0% severely stunted (<-3SD) indicating the long duration malnutrition. Approximately 19.9% children were suffering from short duration malnutrition (wasting) with 1.7% severely wasted (<-3SD).

Analysis of blood sample for haemoglobin indicated a high prevalence of anaemia. According to cut-off points recommended by the World Health Organization about 80.1% of children had haemoglobin levels of less than 11g/dl. The mean haemoglobin levels (10.2 g/dl) among the children were below the cut of values. The proportion of mildly anaemia (56.0%) dominated over moderate (24.1%) grade of anaemia. Severe anaemia (<7 g/dl) among the children was not detected yet now. The value mean of trace elements of serum samples are like K, Ca, Mn, Fe, Co, Ni, Cu, Zn, Br, Se and Pb are 530.7±18.6, 275.3±13.6, 78.7±13.7, 613.4±21.7, 13.6±8.5, 12.7±3.2, 16.4±3.6, 15.1±2.6, 60.2±10.9, 1.1±0.9 and 0.4±0.9 µg/g respectively. The level of trace elements with

Fig.1. Estimation of Serum ferritin by ELISA method.

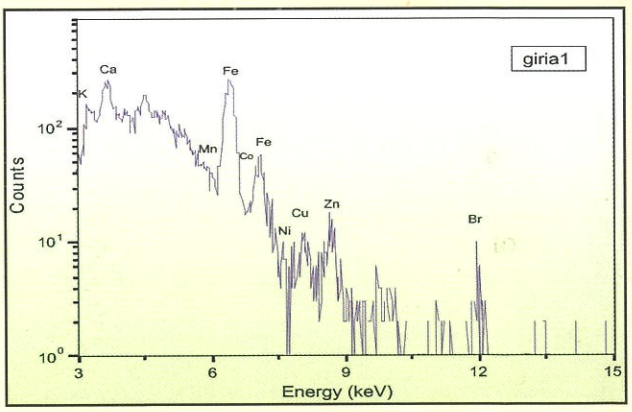


Fig.2. Analysis of Trace element using PIXE

reference to weight-for-age z-scores shows that levels of trace element like K, Ca, Fe, Co, Cu, Zn, Br and Se decrease from mild to severe grades. The values of K, Ca, Zn, are significant (P<0.05), where as Mn and Pb don't show any significant. The level of trace elements height-for-age shows that K, Ca, Fe, Co, Ni, Cu & Zn decrease from normal to severe grades. K, Ca, Co & Zn are significant, where as Mn, Fe, Ni, Cu, Br and Pb are not significant, Where as Se is nearly significant (P<0.06). The level of trace elements with respect to weight-for-height shows that K, Ca, Fe, Co, Cu, Zn and Br decrease from mild to severe grades of malnutrition, where as Mn, Ni & Pb had no effects. The values of K, Ca, Co & Zn are significant (P<0.05). The total no of sample analysis is yet to be complete.

09. A Study on Neurotropic Viruses Causing Encephalitis in Children and Adults in Odisha.

Name	:	Sushil Kumar Rathore
Status	:	JRF(ICMR)
Date of Joining	:	29th Dec 2009
Guide	:	Dr. B. Dwibedi

Background

Encephalitis is one of the life threatening diseases. It is the inflammation in the brain parenchyma resulting from the direct viral invasion or hypersensitivity initiated by virus or another foreign protein. Sudden fever,headache, stiff neck, photophobia, confusion and convulsions are some characteristic symptoms of viral encephalitis.It can occur in the individuals of all age group. Generally children are more affected than adults. But the adults who are affected include the person having compromised immune system and or belonging to elderly age group.The major causative agents are viruses but bacteria, parasites, protozoa and fungi have also been reported. Viruses causing sporadic and endemic encephalitis throughout the world are Herpesvirus, Enterovirus, Paramyxovirus, Togavirus and many Flaviviruses. In India the observed viruses are Flaviviruses, Nipahvirus, Enteroviruses Chandipuravirus, Myxo/paramyxoviruses, Chikungunya and Herpesviruses. Arthropods spread the disease as vector while birds and mammals act as amplifiers for the viruses causing encephalitis. Ticks, sandflies and mosquitoes are the main vectors for epidemics and outbreak.

Since the mid of 1950, Japanese encephalitis (JE) cases have been reported in various part of India.An outbreak of Japanese encephalitis in Orissa had also been reported and confirmed by 40% antibodies against JE virus. In 2005, an epidemic had occurred in Northern state of India and the possible pathogen was JEVGP78 with possibilities of spreading to new areas. A strong suspected outbreak of Nipah virus had also been first time reported in Eastern India with high fatality during 2001. Enteroviruses causing encephalitis had been reported from North India with 21% positivity and 3% positive for Japanese encephalitis virus. Encephalitis by Chandipura virus had only been reported in India.Death was reported from 78% cases of infection during a Chandipura virus outbreak that occurred in Gujarat.

There were several outbreaks of encephalitis with high mortality which remain unrecognized, with the first report as early as 1954 in Jamshedpur in central India, followed by those in Nagpur in 1958, in Raipur in 1965, and more recently in the Warangal District of Andhra Pradesh in 1997 and 2002. These outbreaks were attributed to Dengue, Chikungunya virus, Measles, JE, or Reye's syndrome because they were clinically indistinguishable and no definitive laboratory diagnosis was made.

Objectives

1. To identify the causative viral agents of encephalitis.
2. To assess the phylogeny of the viruses.
3. To study the clinical severity of the viruses causing encephalitis.

Work plane

1. Study area and subject enrollment

The study area will be the different Govt and private hospital in and around Cuttack and Bhubaneswar. Subjects will be enrolled after their consent.

2. Clinical Examination, Sample Collection and Sample storage

Clinical details and history will be described in predesigned format. Blood (2ml) or CSF (1ml) will be collected as per the consent. The sample will be stored at -70^0 C after transporting in cold chain.

3. Laboratory Diagnosis

Serology with ELISA

The sample will be subjected to IgM capture ELISA for different viruses The ELISA kit will be made available from NIV,Pune.

PCR and RT-PCR

The positive as well as false negative ELISA will be tested by PCR for the identification of specific viral genome. Genome of DNA and RNA viruses will be extracted by the help of respective extraction kits. RT-PCR will be performed for the RNA viruses like JEV, WNV, Dengue etc while PCR for HSV. Primers for each virus will be selected after analyzing the recently and commonly used primers in various related research work. Polymerase chain reaction will be started after loading the samples and reagents. The temperature for denaturation , renaturation and extension will be carefully adjusted as it may differ according to various primers.

Sequencing

The PCR product will be purified by using QIAquick PCR purification kit and sequenced by using Big Dye Terminator Cycle Sequencing Ready Reaction Kit and an automatic sequencer. Multiple alignments of nucleotide sequence will be carried out using Clustal X version 1.8. Phylogenetic analysis will be done using using MEGA. Reliability of different phylogenetic groupings will be evaluated by using bootstrap test with bootstrap replication available in MEGA. Percentage nucleotide identity (PNI) will be calculated by using P distance available in MEGA.

Work progress

Dengue is one of the neurotropic viruses causing encephalitis in human being. In search of encephalitis cases an outbreak investigation of Dengue wad made during the first week of September in Malkangiri district of Odisha. Out of 61 suspected cases 14 were found IgM positive for Dengue.Two positive cases manifested the sign of photophobia and retro orbital pain. . No patient did show symptoms of neurological deficit.

10. Molecular characteristics of rosetting in severe falciparum malaria

Ph.D.scholar	: Ronnaly Rout (SRF)
Guide	: Dr.M.R.Ranjit (Scientist D)
Department	: Molecular Biology
Institute	: Regional Medical Research Centre, Bhubaneswar
Registration	: Under Utkal University

Objectives

1. To investigate the frequency of rosetting of uninfected cells with infected RBCs in mild and severe *P falciparum* malaria cases.
2. To study the genotypes of *P falciparum* isolates associated with different clinical groups of malaria.
3. To find out the genetic polymorphism and level of expression of CR1 gene in mild and severe malaria patients belonging to different ethnic groups.

Work Progress

Rosetting is a *Plasmodium falciparum* virulence phenotype characterized by adhesion of infected erythrocytes (pRBC) to uninfected erythrocytes in the microvasculature causing obstruction and impaired tissue perfusion. It is mediated by parasite ligand DBL 1-á domain of *P falciparum* erythrocyte membrane protein (PfEMP1) on the surface of infected RBCs, binding to a variety of uninfected

RBCs receptors mainly complement receptor1 (CR1), an erythrocyte surface protein. The rosetting frequency was assessed between mild and severe cases and was found to be significantly high (Mann-Whitney U test, p = 0.0053) in severe malaria cases (median; 33.0%, interquartile range; 20.0% – 52.0%) compared to mild malaria (median; 15.0%, interquartile range; 10.0% – 32.0%) (Fig. 1A) and a moderately significant positive correlation of parasite density with rosetting frequency (spearman's correlation ñ = 0.613, P < 0.0001) (Fig. 1B) was observed. To explore the hypothesis that DBL 1-á polymorphisms could have some role in rosetting leading to severe malaria, three primer sets DBL 1-á - F1R2, F2R2 and FR were included for DBL 1-á polymorphisms which amplified overall two (a, b) three (a, b, c) and four (a, b, c, d) types of variants respectively sizes with 'a' fragment being the smallest and varied among mild and severe malaria cases. The results showed that parasite strains having the single fragment of F2R2 region ('a' and 'b') were associated with mild malaria whereas multiple fragments (ab and bc) of F2R2 (Fig. 2C) were associated with severe malaria (÷2 = 21.78; OR = 3.8, P < 0.0001). Although, no such relations were obtained for FR region, the percentage distribution of 'a' fragment appeared to be more in mild malaria cases, whether it was alone (18.4% in mild vs. 10.2% in severe malaria) or with other fragments (Fig. 2A). The DBL 1-á -F1R2 did not show any difference of variants in mild and severe malaria cases (Fig. 2B). Furthermore, when the rosette frequency were correlated with different size variants of DBL1-á region, the 'b' variant of FR region whose percentage distribution is more in severe malaria cases compared to mild and the multiple fragments (ab and bc) of F2R2 were observed to have high rosette frequency indicating strain specificity of higher rosetting leading to disease severity. The variants of DBL 1-á -F1R2 did not show any observed difference in rosetting frequency.

Moreover, CR1 level is genetically determined and associated with at least three SNPs in the CR1 gene in intron 27,(HindIIIA>T), exon22,(3650 A>G)and exon 33, (5507 C>G) comprising high (H) and low (L) expression haplotypes that are codominant. We observed an increased susceptibility of AA genotype of exon 22 3650 A>G (odds ratio =2.52, P=0.004) coding for higher expression of CR1 in cerebral malaria (CM) cases (Table 1). Haplotype frequency distribution of the three studied SNPs showed higher occurrence of AAC haplotype coding for high expression of CR1 in cerebral malaria cases whereas TGG haplotype coding for low expression of CR1 in severe malarial anemia cases (P<0.00625) (Fig 3). The CR1 gene expression level was determined for 27 samples freshly drawn by FACS scan (BD Bioscience) flow cytometry method and the results revealed significant differences, with HH genotype showing higher expression of CR1 for all these three SNPs (Fig. 4.). Thus the SNPs of CR1 gene correlates with its expression level in our studied population. Therefore, higher expression of CR1 may lead to the development of CM cases due to enhanced

Fig 1A. Rosetting frequency in mild and severe group of patient.

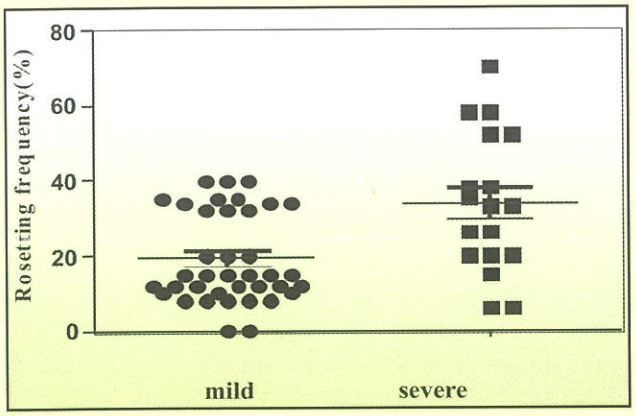
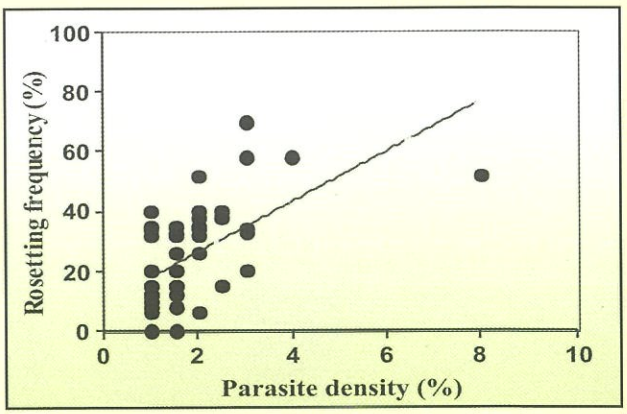
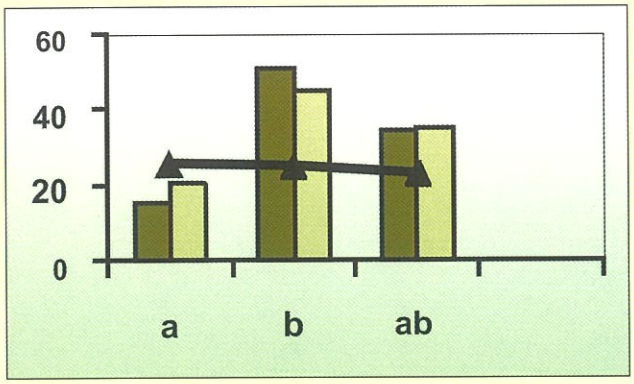


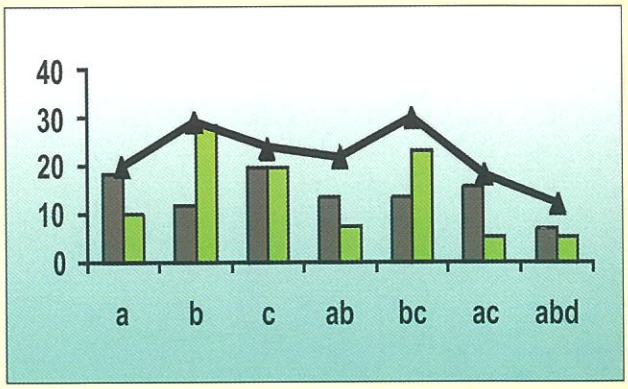
Fig 1B. Correlation of Rosetting frequency with parasite density



rosetting and or more IC binding causing sequestration in the microvasculature whereas deficiency of CR1 may lead to SMA by higher destruction of RBC due to complement mediated lysis or phagocytosis .

Fig 2. Mean rosetting frequency distribution amongst genetic variants of DBL 1-α and percentage distribution of these variants (A; DBL 1-α -FR variants: B; DBL 1-α -F1R2 variants and C; DBL 1-α -F2R2 variants) in mild and severe malaria cases.





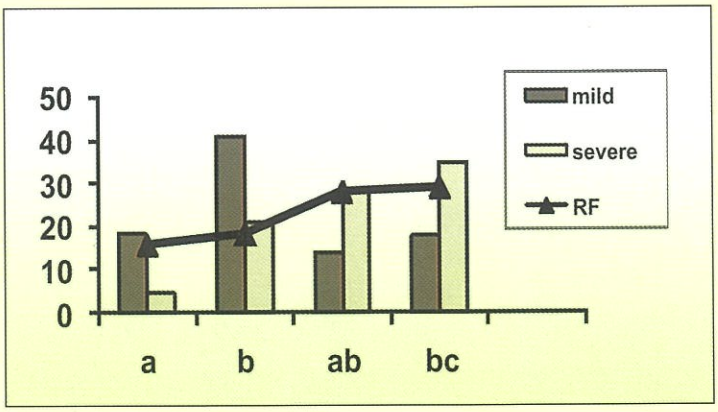


Table 1. Genotype and Allele frequency of CR1 gene for all the three SNPs in different clinical groups of malaria.

CR1 gene	Mild (210)	CM (41)	SMA (45)	CM+SMA (24)	MODS (36)	P value (Odds ratio) M/CM	M/SMA	M/CM+A	M/MODS	C/SMA	C/CM+A	C/MODS
Intron27 A>T												
AA	42	20	4	2	12							
AT	107	17	22	11	14	0.005, (2.9)	NS	NS	NS	0.003, (6.4)	0.02, (6.4)	NS
TT	61	4	19	10	10	0.0002,(7.2)	NS	NS	NS	<0.0001,(23.7)	0.0002, (25)	NS
A	0.45	0.70	0.33	0.32	0.52							
T	0.54	0.30	0.66	0.68	0.47	<0.0001,(2.7)	0.03,(1.7)	NS	NS	<0.0001, (4.5)	<0.0001,(4.7)	0.04
Exon22 A>G												
AA	23	14	3	-	12							
AG	85	21	18	15	12	0.04, (2.4)	NS	NS	0.008, (3.6)	NS	0.004, (20.9)	NS
GG	102	6	24	8	12	<0.0001,(10.3)	NS	NS	0.002, (4.4)	<0.0001,(18.6)	0.001, (37.9)	NS
A	0.31	0.59	0.26	0.33	0.50							
G	0.68	0.41	0.74	0.67	0.50	<0.0001, (3.5)	NS	NS	0.002,(0.45)	<0.0001, (4.1)	0.005, (3.0)	NS
Exon 33 C>G												
CC	37	9	5	-	12							
CG	103	23	17	16	16	NS	NS	NS	NS	NS	0.02, (13.3)	NS
GG	70	9	21	7	8	NS	NS	NS	NS	NS	0.02, (18.5)	NS
C	0.43	0.50	0.31	0.34	0.55							
G	0.57	0.50	0.69	0.66	0.45	NS	NS	NS	NS	NS	NS	NS

Note: NS; not significant, CM; cerebral malaria, SMA; severe malaria anemia, MODS; multi-organ dysfunction. M; mild. Odds ratio were derived from Fischer's exact test. P value < 0.05 was considered to be statistically significant.

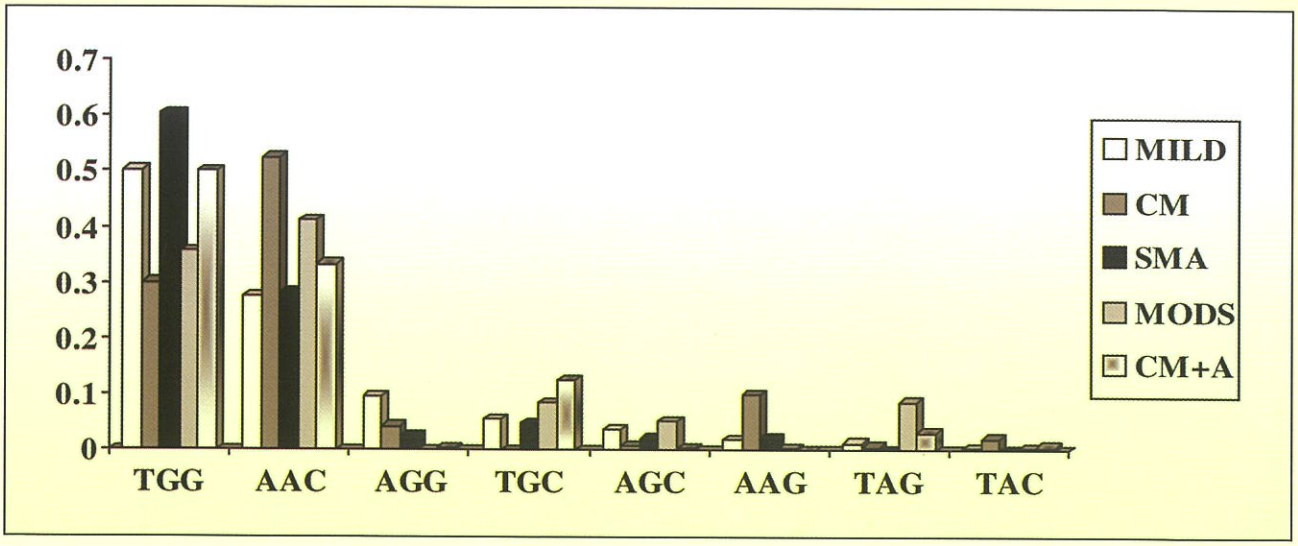
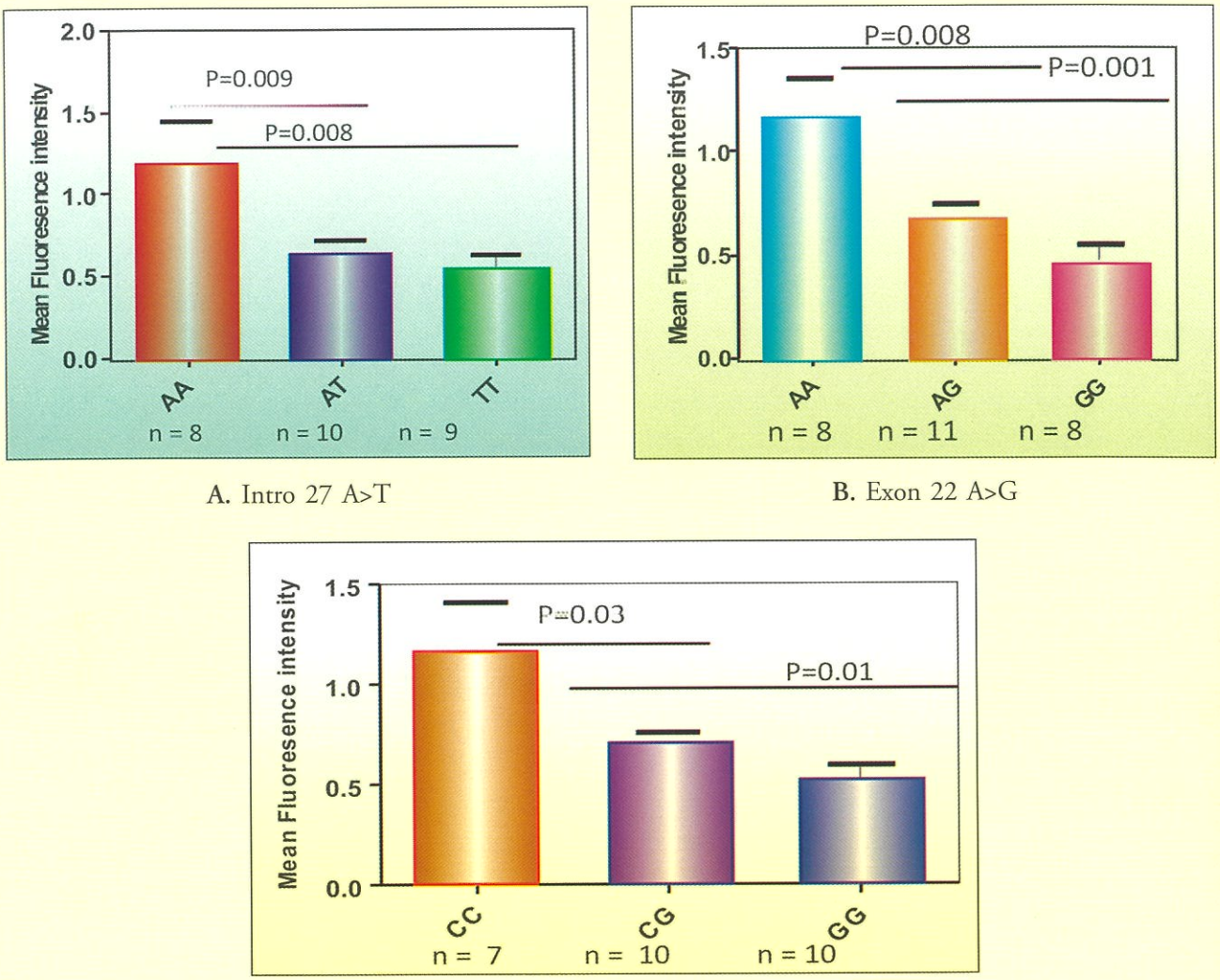


Fig 3 . Distribution of CR1 gene haplotype frequency for the three SNPs (intron27, exon22 and exon33) in different clinical categories of malaria



A. Intro 27 A>T

B. Exon 22 A>G

C. Exon 33 C>G

Fig. 4. Erythrocyte CR1 levels in relation to CR1 genotype for the three SNPs ((intron27, exon22 and exon33)

Publications

1. **Rout R**, Mohapatra BN, Kar SK, Ranjit MR. Genetic complexity and transmissibility of *Plasmodium falciparum* parasites causing severe malaria in central-east coast India. Tropical Biomedicine 26(2): 165–172 (2009).
2. **Rout R**, Dhangadamajhi G, Mohapatra BN, Kar SK, Ranjit MR. Genetic diversity of PfEMP1-DBL1 alpha and its association with severe malaria in a hyper endemic state of India. Asian Pacific Journal of Tropical Medicine. 3 (7): 505-509 (2010).
3. **Rout R**, Dhangadamajhi G, Mohapatra BN, Kar SK, Ranjit MR. High CR1 level and related polymorphic variants are associated with cerebral malaria in eastern-India. Infections and Genetics evolution journal, in press.

11. Development and spread of drug resistance in *Plasmodium falciparum* in Orissa.

PhD Registration	:	Utkal University (18th June 2007)
Name	:	Sasmita Kumari Das Sutar
Guide	:	Dr. M.R.Ranjit
Division	:	Department of Molecular Biology
Joining	:	October 2005
Completion	:	March 2010

Objectives

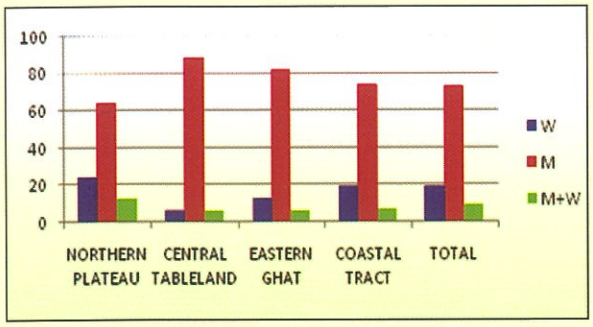
1. To observe the frequency of the genotypes of PfCRT / PfMDR1 associated with chloroquine resistance in natural *P.falciparum* parasite populations of Orissa.
2. To study the origin and spread of resistant alleles through the parasite population in this region.

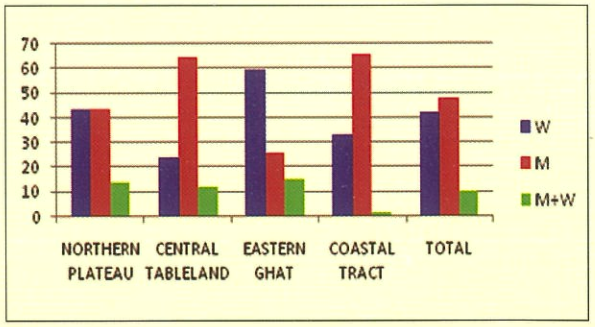
Work Done

The emergence of drug- resistant *Plasmodium falciparum* is a serious public health problem in India, especially Orissa where malaria is endemic. The development of resistance to antimalarial drug Chloroquine by *Plasmodium falciparum* is a major problem for the effective treatment of malaria because of its ease of administration, affordability and efficacy. Mutations in the *Plasmodium falciparum* PfCRT gene on chromosome 7 and possibly mutations in PfMDR1 gene on chromosome 5 have a role in conferring resistance against chloroquine. In this study, parasite isolates were obtained from 429 patients with malaria attending the malaria clinics at primary health centres (PHCs) of Sundergarh, Keonjhar, Mayurbhanj, Kandhamal, Rayagada, Angul, Kalahandi Nayagarh and Cuttack districts of Orissa. These districts were categorized into four physiographical regions. The parasite genomic DNA was extracted by phenol-chloroform and ethanol precipitation method. The frequency of the two important point mutations, 76T of PfCRT gene and 86Y of PfMDR1 gene, responsible for resistance to chloroquine were analysed by PCR-RFLP methods. Fourteen different microsatellite markers flanking PfCRT K76T mutations in the upstream [PE14D(-96kb), B5M97(-24kb), B5M77(-20kb), 1H6 (-13kb), 3E7(-11kb), 2E10(-5kb), B5M47 (-1kb)] and downstream [9B12(1kb), PE12A(6kb), PS590(8kb), 2H4(22kb), 7A11(24kb), PE14E(86kb), PE14F(106kb)] regions and four microsatellites markers flanking PfMDR1 N86Y mutations were also analysed by 8% non-denaturing polyacrylamide gel electrophoresis and ethidium bromide staining, to investigate the origin of this mutation in this region of the country. Further, DNA sequencing of second exon region of PfCRT gene (72-76th positions of amino acid sequence) and 5' end of PfMDR1 gene were performed on 40 isolates for determining the haplotypes prevalent in Orissa samples. 72.8% and 48% of the *Plasmodium falciparum* positive patients were found to harbor chloroquine resistance genes PfCRT 76T and PfMDR1 86Y respectively. Regionwise distribution of chloroquine resistance genes showed that 9 different genotypes are present in the parasite populations of Orissa and the frequency of the genotype PfCRT 76T + PfMDR1 86Y (50.5%) was more prevalent among all the genotypes. Dominance of PfCRT haplotype CVIET (67.5%) was observed from sequencing results

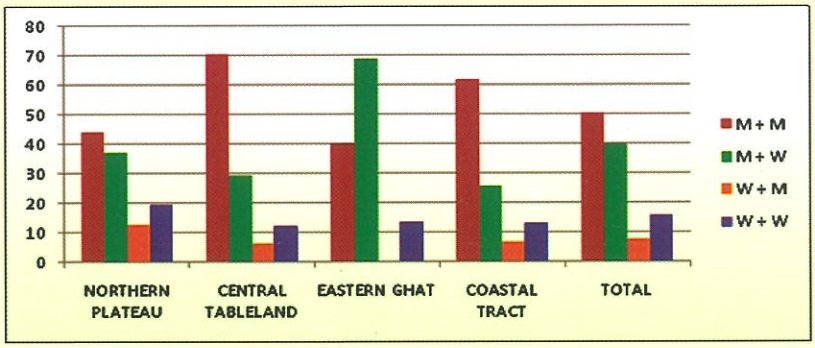
which is a Southeast Asian type of haplotype. The flanking microsatellite size polymorphism of chloroquine resistant genes showed reduced genetic diversity around the gene indicating selection pressure on the target gene.

Prevalence of Pfcrt and Pfmdr1 mutations respobsible for CQ resistance



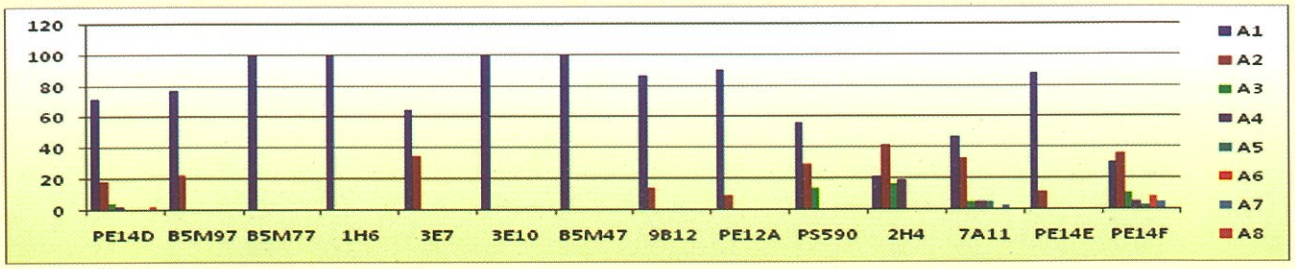


Prevelence of p.falciparum genotypes based on PfCRT and PfMDR1 point mutations

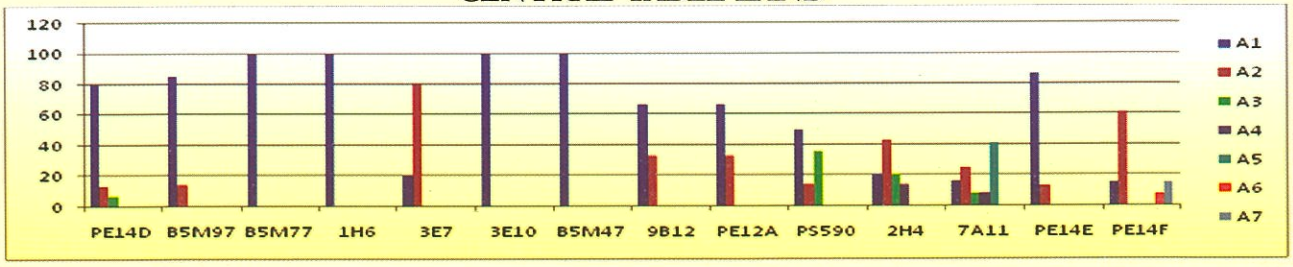


Microsatellite size polymorphism of CQR PfCRTGene

NORTHERN PLATEAU



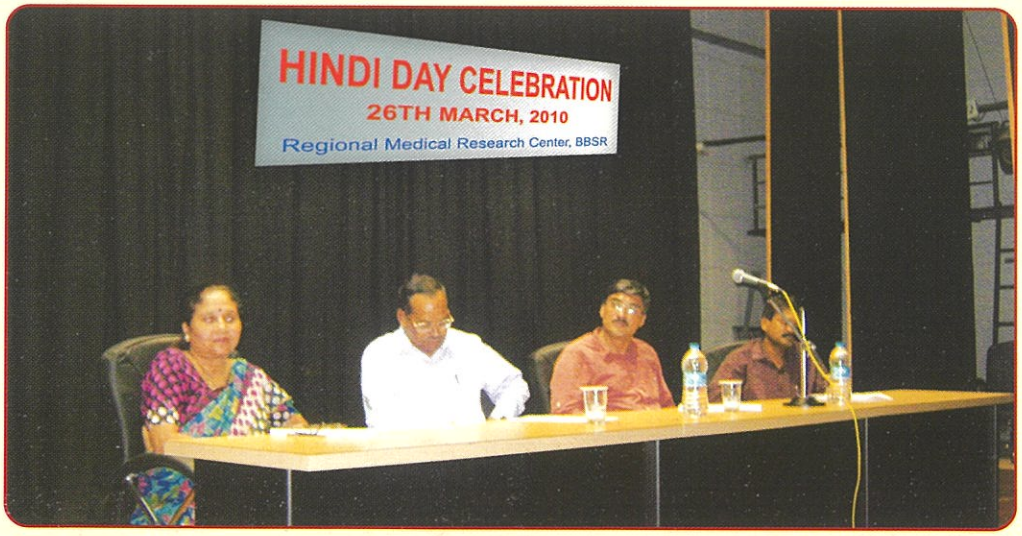
CENTRAL TABLE LAND



12. Role of B1 Lymphocytes and auto antibodies in Human Lymphatic Filariasis.

Name	:	Rashmi Mishra
Guide	:	Dr. A.K. Satapathy
Status	:	ICMR (SRF)

B-1 lymphocytes, a subset of B cells involved in innate immune response, express the surface pan-T marker CD5 together with the B-cell surface markers $B220^{lo}$,IgM^{hi}, IgD^{lo} and CD19 are committed to the production of polyreactive natural antibodies, mainly IgM then IgG and IgA, which bind a variety of self antigen (e.g ssDNA, actin, myosine, tubulin, myoglobin etc.). Several studies have shown that B1 subset of B cells play an important role in the outcome of infection in schistosomiasis, S. pneumonie and experimental filariasis. However, no information regarding status of B-1 cells in clinical manifestation of human bancroftian filariasis exists. Research on the role of B1 lymphocytes in human lymphatic filariasis is the central aim of this present study. Though B-1 cells produce auto antibodies (e.g IgM, IgG, IgA) against self antigens such as ssDNA, actin, myosin etc. we quantified profile of B-1 cells in different clinical spectrum of filariasis such as endemic normal, asymptomatic, cryptic and chronic manifestations. we have found that B-1 cells (CD5+CD19+) were found to be low in microfilariaemic carriers in comparison to chronic patients and endemic normal individuals indicating a biological role for B-1 cells in the out come of filarial infection. Since B-1 cells are committed to the production of polyreactive natural antibodies (e.g. SSDNA, actin, tubulin) mainly IgM, we quantified antibodies reacting to the SS-DNA in the spectrum of clinical manifestations. IgM antibodies to SS-DNA were significantly low in cryptic cases in comparison to endemic normals and chronic cases Also low levels of IgM antibodies to actin and LPS were demonstrable in microfilariae carriers (known to be immunologically hyporesponsive) in comparison to patients with chronic filarial disease and endemic normals (immunologically hyper responsive). The observation on decreased levels of Ig M antibody response to various antigens in mf carriers raise the possibility of polyreactive property of these antibodies.



Hindi Day Celebration at RMRC

Completed Studies

Contents

1. A study on immunoregulation and genotyping for cytokine polymorphism in human cerebral malaria

Co Investigator	:	Dr A. K. Satapathy
Principal . Investigator	:	Dr.B.Ravindran
Collaborators	:	Dr.Shobona Sharma, TIFR, Mumbai Dr B.K.Das, SCB Medical College, Cuttack
Starting date	:	Jan 2006
Closing date	:	June 2009
Status	:	Extramural (ICMR PRC)

Objectives

1. To study B-cells responses (IgG and IgE) to malarial phospoproteins, Viz. PfPO, Pf2, Pf9 and MSP1, MSP3, AMA 1 and GPI in cerebral and/or in multiorgan dysfunction in human P.falciparum malaria
2. To quantify T-regulatory cells a) CD4+ CD25+ b) CD4+ CTLA 4+ cells in circulation and CSF in human cerebral malaria
3. To type the following host gene polymorphism and to correlate predisposition to develop cerebral and/ or Multi-organ dysfunction in P.falciparum malaria: (a) IL-10; (b) TGF-b; c) TNF-a (d) TLR and (e) IFN-g

One of the severe pathological manifestations of P.falciparum infection is the cerebral malaria. Cerebral malaria is a severe clinical presentation of human Plasmodium falciparum infections. However, only a subset of P.falciparum infected patients suffer from such clinical symptoms. The factors contributing to the pathogenesis of cerebral complications are not understood completely. It is envisaged that cerebral malaria is caused due to genetic and immune responses of man towards P. falciparum. Various T-cells associated factors have been ascribed to precipitation of and/or protection from cerebral malaria. Development of cerebral malaria (in both humans and mice models) is attributed to inflammatory Th1 types of responses leading to production of high levels of TNF-?? Such hyperactivity of T-cell responses are now known to be down-regulated by regulatory T lymphocytes. Immunoregulatory T cells (CD4+/ CTLA-4+) have been implicated in immunoregulation of cerebral malaria. In this context the study of such T regulatory cells in human cerebral malaria is crucial. In the current study an attempt has also been made to study the host gene polymorphism involved in several arms of the immune response have been investigated for correlation with the clinical manifestation of P.falciparum malaria.

Observations

Patients reporting at the out–patient department and/or admitted to the department of Medicine at SCB medical college, Cuttack with short history of fever associated with unarousable coma were assessed clinically. Based on the status of clinical manifestation the patients were divided in to

complicated and uncomplicated malaria group. Blood samples of clinically non complicated *P.falciparum* malaria, complicated malaria with renal or hepatic involvement, multiorgan dysfunction were collected from SCB Medical college, Cuttack .$CD4^+$ and $CD25^+$ cells were found to be significantly low in complicated malaria in comparison to non-complicated malaria (Fig-1). Moreover, T regulatory cells were found to be significantly low in endemic control compared to both complicated and uncomplicated malaria.

Tregulatory cells (CD4+ CD25+) are known to exert their functions through a number of mediators such as fork head transcription factor (FOXP3), Cytotoxic T-lymphocyte antigen 4 (CTLA-4) and glucocorticoid-induced tumor necrosis factor receptor (GITR). The CD4+ T cells expressing highest levels of CD25+ (FOXP3cells) were also quantified in complicated and uncomplicated malaria. The CD4+ and CD25+ high cell population was significantly low in complicated malaria in comparison to uncomplicated malaria (Fig-2).

Fig-1

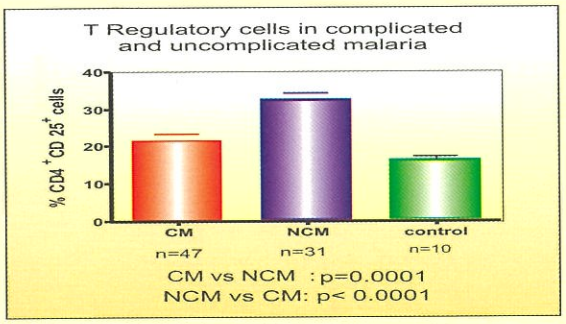
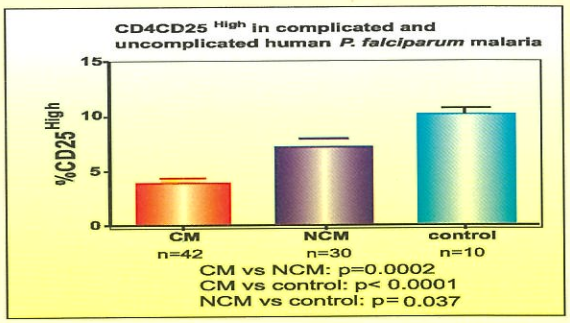
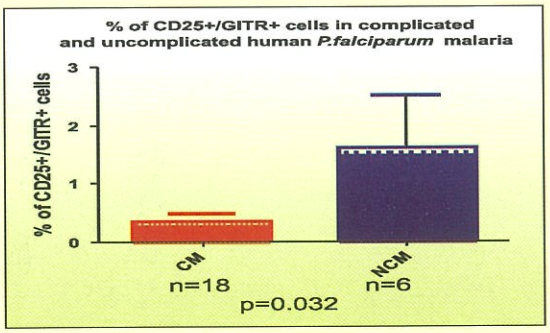


Fig-2



T regulatory cells were assessed further for expression of other activation markers. Additional markers to define regulatory T-cells included CTLA-4 (Cytotoxic T-lymphocytes antigen) and GITR (Glucocorticoid induced Tumor necrosis factor receptor). GITR is a surface receptor molecule that has been shown to be involved in inhibiting the suppressive activity of regulatory T-cells. Assessment of expression of GITR in CD25+ cells in complicated and uncomplicated malaria (Fig-3) reveals the increased expression of GITR in non-complicated malaria compared to complicated malaria. The observed low levels of CD4+CD25+, CD4+CD25hi+ and CD25+GITR+ in complicated malaria in comparison to non-complicated malaria indicating that reduced T-regulatory cells could be responsible for enhanced inflammatory responses observed in complicated malaria.

Fig-3



Genotyping: To type host gene polymorphism and to correlate predisposition to develop cerebral and/ or Multi-organ dysfunction in P. falciparum malaria, TGF-b, TLR-4 and TLR-2 gene genotyping have been standardized through PCR reactions, and samples have been analyzed using the gene specific primers.

The Toll-like Receptor (TLR) family is a group of pattern recognition receptors. TLR-4 is found on surface of mammalian monocytes, macrophages and neutrophils. It recognizes endotoxin (lipopolysaccharide present in cell wall of gram negative bacteria) as its ligand and results in induction of inflammatory molecules, which play a critical role in innate immunity against such bacterial infections. Two known mutations in TLR4 gene (Asp299Gly, Thr399Ile) have been known. Mutations of TLR-4 gene in human population results in quantitatively decreased LPS mediated signaling leading to enhance susceptibility to some of bacterial infections. Increased LPS mediated signaling could also be a contributing factor in some of the inflammatory conditions. Since, recently, P. falciparum GPI was reported to induce signaling via both TLR-2 and 4, we hypothesized that modified recognition or signaling via variants of TLR-4 could influence susceptibility to and manifestation of malaria.

To check the possibility of host genetic factors playing a role in the clinical outcome in the exposed individuals, the prevalence of TLR-4 Asp299Gly genotypes was assessed in 320 individuals including complicated malaria and un-complicated malaria. The prevalence of Asp299Gly polymorphism shows a significantly higher in complicated malaria than uncomplicated malaria (Fig-4). The homozygous mutant frequency was found to be higher in complicated malaria in comparison to uncomplicated malaria. This observation suggests that TLR4 mutation (Asp299Gly) may be a risk factor for developing complicacy in falciparum malaria.

TLR (Asp299Gly)mutation frequency in Co mplicated nad un-complicated malaria

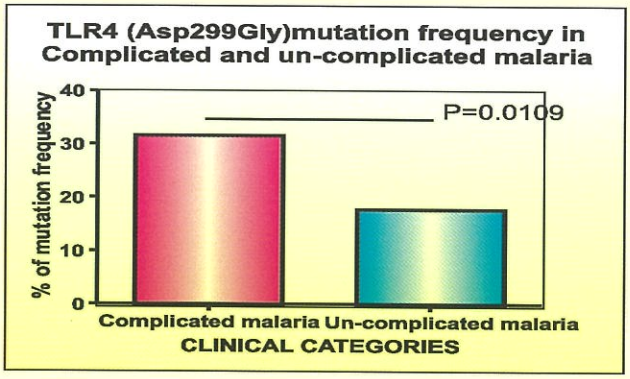


Fig- 4: TLR4 (Asp299Gly) mutation frequency in complicated and uncomplicated malaria.

The second major TLR4 (Thr399Ile) mutation is caused by a C to T substitution at position 1196 in the coding region. We assessed the association between TLR4 (Thr399Ile) polymorphism and clinical complication in P.falciparum malaria. The distribution of TLR-4 (Thr399Ile) polymorphism in different clinical manifestation was not significantly different. This result indicates that the TLR4 (399) polymorphism may have no role in complicated malaria.

Toll like receptor 2 is a member of Toll like receptors family, a Trans-membrane molecule whose extra cellular amino termini have leucine–rich repeat domains that are involved in recognition of peptidoglycan, lipoteichioc acid and bacterial lipoprotein. TLR2 is a surface receptor of macrophages and dendritic cells through which GPI molecule of malaria parasite induce signal to produce inflammatory molecules. Two common polymorphisms Arg753Gln and Arg677Trp which quantitatively decreased the inflammatory molecule productions were found to be associated with

susceptibility to many infectious diseases. We assumed that TLR-2 mutation could potentially influence clinical status in P.falciparum malaria.

We analyzed the distribution of genetic polymorphism of TLR-2 (Arg753Gln and Arg677Trp) genes in clinical outcome of falciparum malaria. A total of 206 samples were genotyped for TLR2 (Arg753Gln, Arg677Trp) polymorphisms by PCR-RFLP. Our results show three bands in all samples, which represented for wild type. All subjects were found to be wild type for TLR2 Arg753Gln and Arg677Trp polymorphism (Table-1). The prevalence of wild type alleles was 100 percent. To verify these findings we performed sequencing analysis of 40 samples randomly and we confirmed that there were no TLR-2 polymorphisms in this studied subjects. Hence these polymorphisms may not have any role in different clinical manifestation of falciparum malaria.

Table. Genotype and Allele frequencies for TLR2 (Arg753Gln and Arg677Trp) mutations in studied patients

Gene	SNP	Allele	Frequency (%)	Genotype	Frequency (%)
TLR2	Arg753Gln	Arg753	412/412 (100)	Arg753Arg	206/206 (100)
				Arg753Gln	0/206 (0)
		753Gln	0/412 (0)		
				Gln753Gln	0/206 (0)
	Arg677Trp	Arg677	412/412 (100)	Arg677Arg	206/206 (100)
		677Trp	0/412 (0)	Arg677Trp	0/206 (0)
				Trp677Trp	0/206 (0)

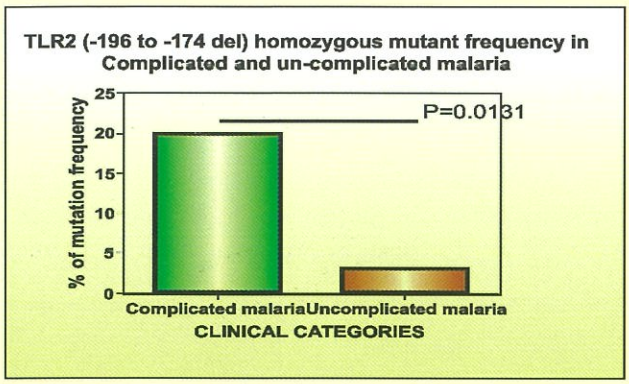
Another insertion/deletion polymorphism was reported which was due to a 22 base pair nucleotides deletion at 5'UTR region of TLR2, (–196 to –174 del). This polymorphism may significantly alter the function of TLR2 promoter, and thus may influence its activity. TLR2 (-196 to -174 del) polymorphism was accessed in different clinical outcome of *P.falciparum* infected individuals. The frequencies of genotypes were also found to be significantly differing in both clinical categories. As shown in figure-5 Homozygous mutant (del/del) was more prevalent in complicated malaria than un-complicated malaria (P=0.0131) on the other hand the frequency of wild type (ins/ins) individuals were more in un-complicated malaria than complicated malaria (P=0.0109). These finding indicates that TLR2 (-196 to -174 del) polymorphism is a risk factor for clinical complicacy in falciparum malaria.

TLR2 (-196 to -174 del) homozygous mutant freequency in Complicated and un-complicated malaria

TGF-β polymorphism:

Data from human malaria infections suggest that TGF-β inversely correlated with malaria severity. Although host genetic polymorphisms have been implicated in naturally acquired immunity and in

Fig-5



cerebral malaria, the status of polymorphism of TGF-β gene has not been examined and the current study was undertaken to fill this lacuna. The TGF-β (Leu 10 Pro) mutation is caused by a T to C substitution at codon 10 of exon-1 region. We assessed the association between TGF β (Leu 10 Pro) polymorphism in complicated and uncomplicated of *P.falciparum* malaria and the results are shown in **Fig**-6. There was no significant difference between complicated and non-complicated malaria cases suggesting that Leu 10 Pro mutation TGF-β gene does not play a major role in determining the clinical outcome in human malaria.

Conclusions

The severe pathological manifestations of Plasmodium falciparum infection consist of cerebral malaria (CM). Certain immune responses and host genetic polymorphisms have been implicated in such susceptibilities. This project has addressed some of these factors in patients from Orissa by genotyping for certain host immune response genes and cell phenotyping. Phenotyping the T cells from PBMCs showed presence of a larger proportion of T-regulatory cells amongst the uncomplicated malaria cases compared to cerebral malaria indicating a role for inflammation observed in complicated malaria. The genotype frequency for TLR-4 (299) mutation was significantly different between CM and UCM malaria. The prevalence of Asp299Gly polymorphism shows a significantly higher in complicated malaria than uncomplicated malaria. These observations suggest that TLR4 mutation (Asp299Gly) may be a risk factor for developing complicacy in falciparum malaria.

2. Role of *PfEMP1* subtypes in clinical manifestations of severe *falciparum* malaria

Principal Investigator	:	Dr M R Ranjit
Co-Investigator(s)	:	Dr A K Satpathy
		Dr S K Kar
Duration	:	2 years
Starting date	:	April 2008
Closing date	:	March 2010

Objectives

1. To study the expression profile and rosetting properties of *PfEMP1* subtypes in *P falciparum* isolates inducing different grades of clinical manifestations
2. To examine the antibody response to *PfEMP1*variants by different grades of clinical malaria cases.

Background

The pathogenecity and virulence of *P falciparum* has been linked to its expression of variant surface antigens (VSAs) that subvert acquisition of protective immunity and mediate infected RBC sequestration. Several studies have shown that parasites causing severe malaria in young children with little protective immunity tend to express VSA_{SM} which are serologically distinct from VSA_{UM} expressed by most parasites causing uncomplicated malaria and sub-clinical infection in older, and more immune, individuals. The PfEMP1expressed in the membrane of the late-stage-infected erythrocytes is a family of VSAs which binds to various receptors –such as CD36 or ICAM-1 on the vascular endothelium, CSA in the placenta, and CR on the red blood cells-that leads to microvascular obstructions in various organs. The PfEMP1 proteins are structured in to several semi conserved domains-namely an N terminal segment(NTS);various Duffy binding-like(DBL) domains, a cystein rich inter domain region (CIDR); in some instances ,a constant 2(C2) region; a trans membrane domain; and a conserved, C terminal acidic terminal segment(ATS), which represents the intra cellular part of PfEMP1 that anchors the protein to the cytoskeleton .In the *P falciparum* line3D7, PfEMP1 is encoded by approximately 59 *var* genes per haploid chromosome. Most var genes can be assigned to 1 of 3 types (var groups A, B and C) mainly according to their conserved 5' upstream sequences. In 3D7, the majority of *var* genes belong to the subtelomerically located *var* group B, whereas 13 *var* group C genes are arranged in chromosome internal clusters. Ten mostly larger, subtelomerically located *var* genes with a distinct domain structure belong to *var* group A. *Var* genes are exclusively expressed but undergo switching within parasite lineages .Recent molecular phenotypic studies conducted in Southern Tanzania and Papua New Guinea have found differential expression of *var* genes in severe (cerebral) and uncomplicated malaria cases. Since no studies has been done in this regard in India ,the present project proposal has been aimed to find out the association *var* gene sub groups with clinical manifestation of severe falciparum malaria expressed by the *P falciparum* isolates prevalent in Orissa.

Observations

This project proposal was initially submitted to DBT for extramural funding,after that it has been submitted again CSIR on August 2009. But, the project has been initiated with intramural fund since April 2008. During this period about 48 mild cases and 36 severe cases (cerebral malaria) has been selected for the study. About 2ml of venous blood was collected in EDTA and total RNA was isolated using TRI-reagent as recommended by the manufacturer (INVITROGEN).The RNA was purified and dissolved in DEPC water. The cDNA was synthesized using first strand cDNA synthesis kit (Fermentas) and random hexamer primers in a 20ul reaction mixture as recommended by the manufacturer. The quantitative estimation of the PfEMP1variant specific cDNA was done by Real Time PCR using QuantiTect SYBR Green PCR kit. Rox was taken as the internal reference dye and expression level was studied by relative quantification. The RT-PCR results indicate that the var group A of transcripts are more abundant in mild group of patients while var group B of transcripts are more abundant in severe group of patients in Orissa.

Conclusion: To validate this data similar studies has to be undertaken in other such endemic areas with increased number of samples.

3. Epidemiology of viral hepatitis in tribal populations of Orissa, Madhya Pradesh / Chhatisgarh and Jharkhand, India. – Multicentre study

Investigators	: Dr.S.K.Kar(PI)
Co-Investigator	: Dr.B.Dwibedi, Dr.A.S.Acharya,
Collaborator	: Dr. (Prof) S.P.Singh, (HOD, Gastroenterology), SCB Medical College, Cuttack
Status	: Extramural Funding ICMR Tribal Task Force
Date of commencement	: March, 2006
Duration	: 4 Years
Date of completion	: February, 2010

Aims & Objectives

The project aims at studying the epidemiology of viral hepatitis in tribal populations of Orissa. The objectives of the project were

a. To determine the prevalence of hepatitis A, B, C, D & E viruses along with the circulating genotypes of HBV & HCV.

b. To access the risk factors of transmission of hepatitis viruses

c. Prevalence of pre-core and basal core promoter mutants of HBV

Background

Viral hepatitis is caused by different viruses that belong to different taxonomical families and genera. Among them HAV & HEV are transmitted by faeco-oral route, HBV & HCV are blood borne viruses transmitted through contaminated blood, blood products and through improperly sterilised needles/ syringes. The prevalence of these viral infection in tribal areas of India mostly remains unknown. The present study would like to assess the prevalence of viral infection in three different tribal areas and to find out the contributory risk factors for transmission. It is assured that certain risk factors would be unique to practices followed in certain tribes. The primitive tribes are geographically isolated and quite shy of contact with community at large. Among all hepatitis viruses, HBV & HCV lead to chronic infections and super infections with other hepatitis viruses increases severity of the disease. Vaccines against HAV & HBV are now available but not yet been included in universal programme of immunisation due to its high cost. However, understanding the risk factors and prevalence rates might lead to inexpensive and appropriate intervention measures. Distribution of viral genotypes can also be useful in context of transmission within the tribes. It is known that the specific genotype is associated with severity in progression and then help in selecting appropriate therapeutic antiviral agent. The study will help comparing data at national level amongst primitive tribes and general population by comparing the data with available information at the national level.

Methodology

The study was conducted in five primitive tribes of Orissa covering all the six districts where these tribal populations inhabit. The study was undertaken following the protocol and ethical guidelines with necessary cooperation from the tribal welfare department, Govt. of Orissa. Laboratory quality and standardization of the procedures was assured during the study and data analysed following standard methodology. The methodology followed described below.

Sampling : At the initiation of the study no data was available in the country on prevalence of Hepatitis infection in primitive tribal population, except one or two reports showing very high prevalence of HBV in some specific tribe of Andaman. Thus at the outset, assuming the HBsAg prevalence among each tribe to be around 5% minimum sample size of 0.99% was considered to initiate the study. Those tribes having total population limited to few hundreds, 20% of population was decided to be included in the study.

To achieve the above target sample and to make the sample representative for each tribe, distribution of the five tribes in the study area was mapped and villages were selected randomly from each cluster inhabitations for each of the tribes to get the minimum sample. In the selected village, all the house holds and all the family members from all ages and both sexes were included for the study.

Fig-1.Map of Orissa showing Districts with inhabitation of studied tribes

Study area & Population

It was decided to include five primitive tribes namely Lodha, Saora, Mankidia, Khadia and Juanga in the present study covered a) Lodha, Khadia, Saora tribe of Mayurbhanj district b) Juanga tribe from Keonjhar and Dhenkanal. c) Mankidia tribe from Mayurbhanj, Balesore, Bhadrak, Deogarh and Jajpur district.

Figure-1 shows the study districts covered.

The population under study are primitive tribes and thus are very primitive in their social, cultural and other behaviours of day to day activity and livelihood. The population is totally scared of the developments in the socioeconomic, education, health awareness and health facilities of the present society.

Each of the tribes are distributed in more than one clusters formed by small villages or Village hamlets in the above mentioned areas. These villages are either located in forests or foot hill areas or as settlements established by the Government near by forest areas.

Practices relevant for parenteral transmission were looked in to during the study visits.

Making tattoo on the body parts was observed mostly in Khadia, Mankidia and Lodha tribes. During the process needles are used to make tattoo in a group of people .Sharing of razor & blade in the male subjects was recorded. Shaving is also done by village barbers. Body piercing practice and heat treatment /scarification for treatment of illnesses are also followed in the tribes. The possibility of sexual relationship with a many women other than the spouse of some tribe can not be excluded.

Field preparation and IEC activities

The community were approached in close liasoning with the local field label staffs of Govt. welfare department of the district and the block level official. Repeated meeting were conducted in presence of block officers and PHC medical officers in the villages.

Demonstration on the communicable diseases, especially viral hepatitis, diarrhoeal disorders and respiratory infections was made using audio-visual aids as well as direct interactive sessions with the community group. Cause & spread of viral hepatitis infection, presentation and complications of hepatitis was explained to the community using audio visuals (video shows) in the respective villages. It helped in getting community cooperation for the study procedures and subject enrolment, besides increasing the health awareness in the study population.

Subject enrolment, Examination and Investigation.

In the sampled villages, dejure census was made by door to door visit. The individuals were explained in detail about the purpose of study, methods, benefits & short comings before taking consent. The consent process was made in language understandable to them for providing informed written consent. All the willing individuals of both sexes from all ages were then enrolled in the study, after obtaining informed consent from adults and from parents for their children. During this process village heads were requested to remain present and assist in the process.

The designed questionnaire was filled in by the investigator after interrogating the individual or the guardian. Detailed clinical examination was done by the physician, which was recorded in the clinical proforma. The participants were given treatment for common ailments complained/ identified during the above examination by the team doctor. Subsequently venous blood samples (5 ml) were collected aseptically under direct supervision of the physician using disposable needles & syringes. The samples were coded & preserved and transported in cold chain.

Serological (ELISA) tests were done on all the samples for the markers of Hepatitis infection like HBsAg, Anti HBs, Anti HBc IgG for Hepatitis B virus and Anti HCV for Hepatitis C virus infection and HDV for Delta virus HAV and HEV infection was studied by the presence of IgG antibody in the serum. All the study sites used kits form same firm to maintain uniformity.

PCR test for HBV DNA was performed with the samples positive for HBsAg and the samples positive for HBV DNA were subjected to sequencing for identification of the genotype. The pre core region was also amplified and sequenced for identifying pre-core mutants if any.

Anti HCV positive samples were subjected to RT PCR for HCV RNA detection and subsequently processed for sequence analysis of the NCR and Core regions.

Result/ Observations

A total of 1765 individuals were covered during the study from the five tribes from Mayurbhanj, Keonjhar, Balasore, Deogarh, jajpur and Dhenkanal districts of the State which were the study area planned for the study..Table 1 shows the distribution of study population covered from different tribes.

Table-1: Tribewise population covered under this study

Name of the tribe	District	Census Population	Estimated Minimum sample size 0.9%	Population covered (%)*	Block covered
Lodha	Mayurbhanj	2405	22	242(10.06)	Morada, Suliapada,Udala
Saora		3740	34	212(5.66)	Morada, Suliapada
Khadia		15405	139	450(2.92)	Karanjia, Jasipur, Udala
Mankidia		713	6	262(36.74)	
Mankidia	Balasore	108	1	52(48.14)	Nilagiri
	Deogarh	134	2	64(47.76)	Deogarh
	Jajpur	36	1	23(63.88)	Sukinda
Juanga	Keonjhar	15719	141	260(1.65)	Gonasika
	Dhenkanal	16104	144	200(1.24)	Dhenkanal
Total	**6 districts**	54364	490	1765	**10 blocks**

*The sample covered was more than and sufficient for the sample size targeted for the study.

The clinical observations of the individuals enrolled into the study has shown that 140(7.93%) people had history of Jundice 7(0.39%) people had hepatomegaly and 11(0.62%) people had spleenomegaly. Icterus was observed in 19 (1.07%) individuals and pallor in 186(10.53%) individuals. Ascites was not present in any case.

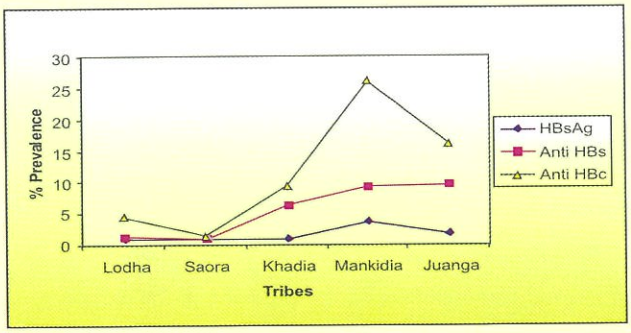


Fig. 2: Sero prevalence (%) of HBsAg, Anti HBc and Anti HBs in five tribes

Serological tests revealed that prevalence of HBsAg was 0.8%, 0.9%, 0.9%, 3.7% and 1.7% population in Lodha, Saora, Khadia, Mankidia and Juanga tribes respectively where as Anti HBc IgG positivity was 4.5%, 1.4%, 9.3%, 26.1% and 16% in those tribes. Anti HBs positivity was 1.2%,0.9%,6.2%, 9.2% & 9.5% in the respective tribes. Antibody to HCV was detected in 3.7%, 0%, 5.7%, 13.4% and 8.47% in Lodha,

Saora, Khadia, Mankidia and Juanga tribes respectively. It indicates that prevalence of HCV infection is much higher in 2 of the primitive tribes (Mankidia & Juanga) than the national average of <1% for HCV infection.

One HBs Ag positive sample was positive for Antibody to Delta virus infection (Anti HDV).

Evidence of exposure to Hepatitis A virus infection (i.e HAV IgG) was 75-87% and to that of HEV was 18-60% in the above tribes(Table 3) indicating endemic transmission through faeco oral route and there by development of antibody to Hepatitis A and E virus in the community. Table 2 and 3 indicated the tribe wise sero positivity for different Hepatitis viral infections.

25% of HBsAg positives are HBeAg positive

HBV DNA was detected by PCR in 53% of HBsAg positive samples and the HBV viral load ranged from< 250 to 2.62 x 108 copies/ml. All HBV DNA positive samples were subjected to genotyping and all were found to be Genotype D and all were wild type for pre-core DNA.

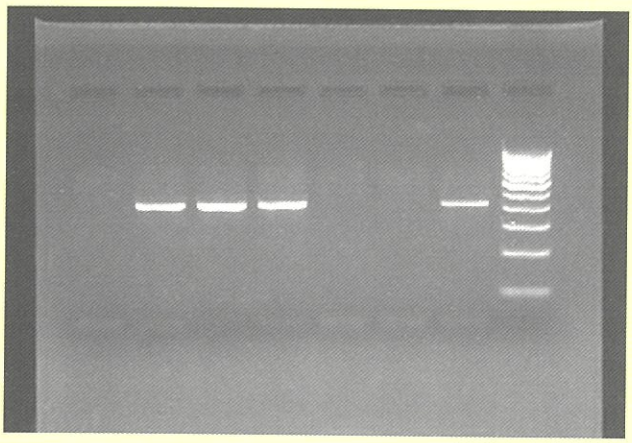


Fig- 2. HBV DNA('S' Region)

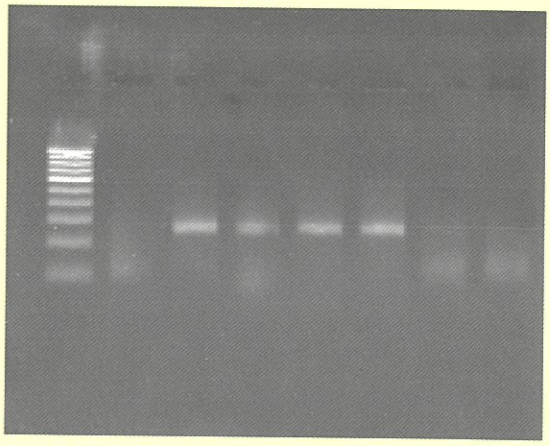


Fig- 3. HCV RNA(5' NCR)

To know the Genotypes present among these tribes all anti HCV positive samples were subjected to amplification by RT PCR. 36 samples were subjected for amplification of 5' NCR region, out of which 3 samples were found positive for HCV RNA but the core region could be amplified in one and sequenced for genotyping which revealed the genotype 1b.

Factors relating to both enteral & parenteral routes for transmission of hepatitis viruses were looked in. Possible parenteral routes like tattooing (7-32%), Sharing of razor(3-29%), Shaving at barber shop/village barber(18-34%), Body piercing(21-43%) and history of multiple injection(26-59%) were prevalent in varied frequencies in the studied tribes. Multiple regression analysis (Table 2) of the above indicated that Body piercing seems to be statistically significant in comparison to others in all tribes where as tattooing and H/O injection were found to be statistically significant in Mankidia and Juanga tribes too.

Table-2: Prevalent Risk Factors for Parenteral Transmission in five tribes

Tribe / Risk factors	Lodha (n=242)	Saora (n=212)	Khadia (n=450)	Juanga (n=460)	Mankidia (n=401)
Tattooing (%)	18 (7.4%)	29 (13.6%)	59 (13.1%)	61 (13.2%)	135 (33.6%)
Sharing of Razor (%)	18 (7.4%)	54 (25.4%)	139 (30.8%)	61 (13.2%)	86 (21.4%)
Body Piercing (%)	62 (25.6%)	66 (31.13%)	203 (45.1%)	215 (46.7%)	181 (45.13%)
H/O Multiple Injection (%)	89 (36.7%)	67 (31.6%)	79 (17.5%)	6 (1.3%)	18 (4.48%)
Shaving in Barber shop (%)	77 (31.8%)	61 (28.77%)	141 (31.3%)	99 (21.5%)	86 (21.4%)

Unsafe drinking water, unhygienic preservation of cooked food (Rice & Kanji), was observed as a common in all the tribes. Consumption of different meats like poultry, mutton, pork, jungle birds, reptiles (godhi) & rodents (wild rat) were noted which can be investigated subsequently in-depth. Unsafe drinking water and open field defaecation might have led to continuing exposure to HAV infection making about 80% of population exposed to it.

Prevalent risk factors among HCV positives were also analysed and subjected to Regression Analysis. Body piercing was found to be significant in all tribes except Juanga tribe.In Mankidia tribe, tattooing and H/O injection were also statistically significant along with Body piercing.

Conclusion

The study covered five primitive tribes from six districts of the state and addressed. hepatitis (A,B,C,D and E) virus infections in the study population.

The serological investigation of the above subjects has indicated thatHBV infection as HBs antigen positivity was prevalent in 0.8 to 3.7% cases in different tribes. HBV DNA was prevalent in 53% (17/32) of the HBsAg positives and 17% (23/132) of the HBsAg negative but anti HBc IgG positive samples (Occult HBV DNA), indicating potential for spread. Hepatitis B virus detected was of genotype D and non were with precore mutants.

Anti HCV infection was seen in 3.7, 0, 5.7, 13.4, & 8.47% of the subjects from Lodha, Saora, Khadia, Mankidia and Juanga tribes respectively. HCV RNA was detectable in 3 out of 36 cases tested by RTPCR and HCV was of sero type 1b.

The information will be dissceminated to the state health and tribal welfare department which will assist in taking public health measures.

Evidence of exposure to enterally transmitted Hepatitis A & E virus infection inform of IgG antibody was observed in 75.8 to 87.3% cases for HAV and 18.4 to 60.8% cases for HEV in different tribes which shows high transmission of the viruses by faeco-oral route, which was supported by improper hand washing practices, open field defecation and unsafe drinking water intake in more than 95% of study participants. Tattooing (7.4 to 33.6%) Sharing of Razor(7.4 to 30.8%) Body piercing (25.6 to 46.7%) History of multiple Injection (1.3 to 36.7%) & Shaving in Barber shop (21.4 to 31.8%) were the possible risk factors for HBV and HCV transmission which revealed that body piercing and H/O Injection were statistically significant for HBV/HCV infection.

This information will lead towards formulating a strategy for prevention of transmission of the infection in the endangered tribes.

During the study the laboratory procedure were standardize in the centre as a part of technology transfer and scientific and technical manpower could be trained in this area.

4. Gis Based Micro planning for Malaria control in high endemic areas: Orissa

Principal Investigator	:	Dr. R. K. Hazra
Co-Investigators	:	Dr. N. Mahapatra
		Ms. Mumani Das, Res.Asst.
Collaborator	:	Dr. M.M.Pradhan
		Deputy Director, Health, Govt.of Orissa.
		Mrs. Mithun Karmakar, GIS
		consultant, NRHM, Govt.of Orissa.
Starting date	:	September 2009
Closing Date	:	September 2010
Status	:	Extramural (NRHM)

Objectives

1. Remote sensing and GIS based mapping of environmental parameters (physical, climatic) to explore the factors responsible for malaria habitat development.
2. To develop a physical vulnerability model for malaria epidemic through comparative assessment of environmental parameters of study area selected at three different sites.
3. Mapping and analysis of the epidemiological parameters such as API, PF, SPR, etc on a time series basis to explore the spatio-temporal trend and pattern of malaria endemicity, drug resistance and impact of initiative for malaria control using GIS.

4. To develop an integrated vulnerability model for preparation of microplan for mitigation and management of malaria epidemic and to ascertain the efficacy of GIS and remote sensing in health care planning.

Rational of the study

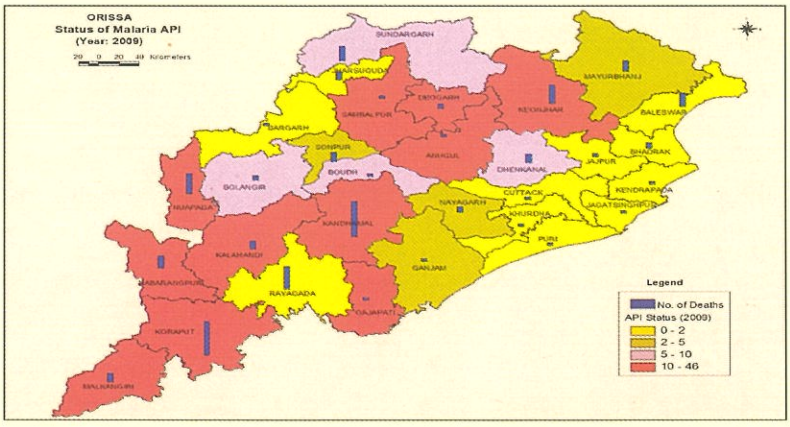
Small scale spatial variation and temporal heterogeneity in mosquito densities can have important consequences for disease transmission.In order to better understand the ecological aspects of important vectors and their influence on the epidemiology, the application of GIS, together with spatial statistical techniques, provide a means to quantify explicit malaria risks and to further identify environmental factors responsible for the re-emerged malaria risks, future public health planning and resource allocation.

Background

Malaria is maintained under the influence of diverse ranges of interacting conditions, many of which are not well understood. These conditions are closely related to the habits and lifestyle of different communities; the behaviour of the mosquitoes which transmit the disease; as well as climatic and other environmental factors attributes a lot.

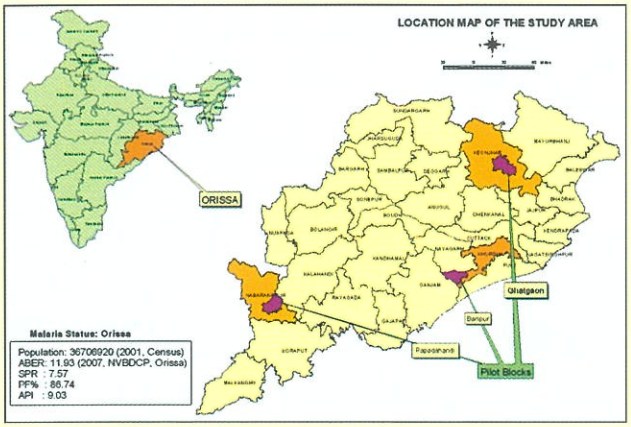
As a tool for analysis and decision making, GIS and remote sensing opened a new horizon for monitoring and control of disease. GIS offers new and expanding opportunities to look in to disease epidemiology. Even when used minimally, these systems allow a spatial perception on the disease. Control of malaria requires case detection; treatment of affected individuals and for curtailment of malaria transmission and vector control strategies. Vector control required knowledge of the ecology of breeding and resting habitat and behaviour. This required periodic survey but routine survey and implementation over vast geographical area are impractical as this will be time consuming and expensive. Therefore the questions need to be addressed through micro planning on control of malaria by using advanced technologies of Remote Sensing, GPS and GIS.

Status of Malaria API in the year 2009



Orissa contributes maximum burden due to malaria to the nation. In 2006, nearly 23 percent of cases and 17 percent of deaths due to malaria of the country have been reported from Orissa.

Location Map of the selected study area



Observations

1. Base line Survey: As a base line study the mosquito samples were collected from Keonjhar, Nawarangapur, and Khurda. Selection of villages based on high malaria endemicity. Field visit for collection of winter season data for the study area completed. Development of GIS maps.

1a. Generation of base line GIS layers from secondary sources.

- The base line GIS layer included Block boundary (polygon), Villages (polygon) as per Census, Transport network (line), Natural Drainage (line), Major Rivers & water bodies (polygon), Location of settlements (as per toposheet).
- These have been generated from 1: 50,000 scale topomaps.
- The methodology involved collection and scanning of topomaps of A1 size. The topomaps are (Papadahandi Block), (Ghatagaon Block) and (Banpur Block).

1b. Generation of GIS layers for environmental parameters through satellite image interpretation and limited field verification.

1c. Downloading, processing and mapping of GPS surveyed coordinates of sampled sites.

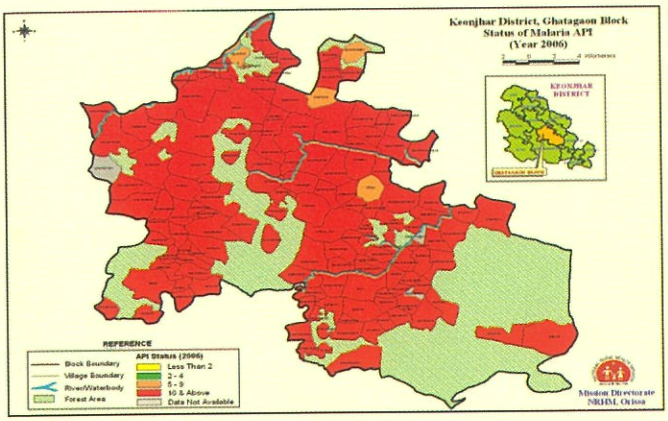
- The GPS used in the study were GARMIN make hand held GPS (Etrex Vista model). These were calibrated at known locations.
- So the GPS data were downloaded using Mapsource software and were then exported to dbase for editing and processing to make it compatible with GIS.
- Then these data were imported to GIS for mapping along with their attributes. The sampled locations have been mapped on the basis of larvae positive or negative for winter season.

2. **Selection of Villages:**

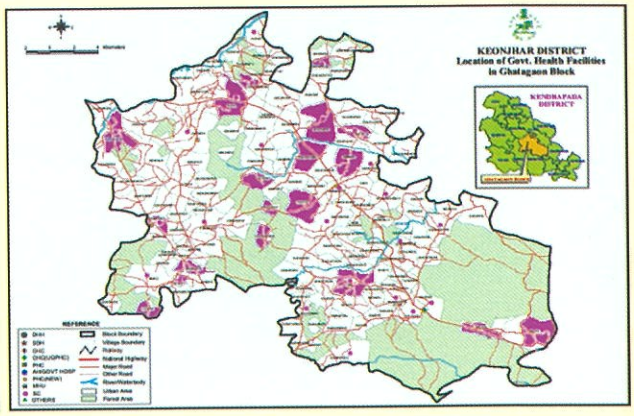
The state has total area of 15,5707 sq. km .The State of Orissa is located in the eastern coast of Indiaat 17 Degree 49' N - 22 Degree 34' N Latitude & 81 Degree 29' E-87 Degree 29' E Longitude. Orissa is mainly divided into four agro-climatic zones according to its demographic pattern. They are 1.Coastal tract, 2. Central table land, 3. Northern plateau and 4. Eastern ghat. From this four agro-climatic division we selected three districtsie. Keonjhar,Nawarangpur and Khurdha. Keonjhar is coming under Northern plateau, Nawarangapur is under Eastern ghat and Khurda is under Coastal tract. So these three district differ in their demographic pattern. The study was conducted in 20 villages of each district. The selection of villages was done by taking their API count in to consideration. Keonjhar

is one of the most endemic tribal districts having API of > 15 and PF% >90, Nawarangapur is having >98% PF and API>20 and Khurda very low API and PF but still Blocks like Banpur having vast forest cover experiences >5API and PF% >83. Selection of villages was done by taking high malaria endemicity in account i.e. considering the API count i.e. API> 10 of villages of the year 2006, 2007 and2008.

These three districts are very much different in their geographical location i.e. Land cover, river, soil type and also differ in climate Though they are very different in topography and climatic condition, but the previous year statistical analysis shows that they are quite close in malaria situation with each other.



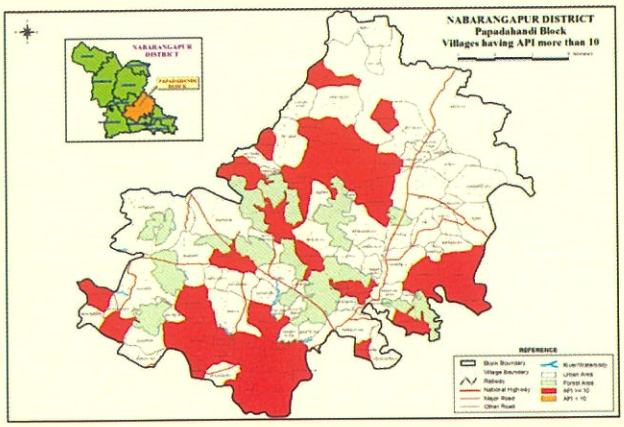
Map-1



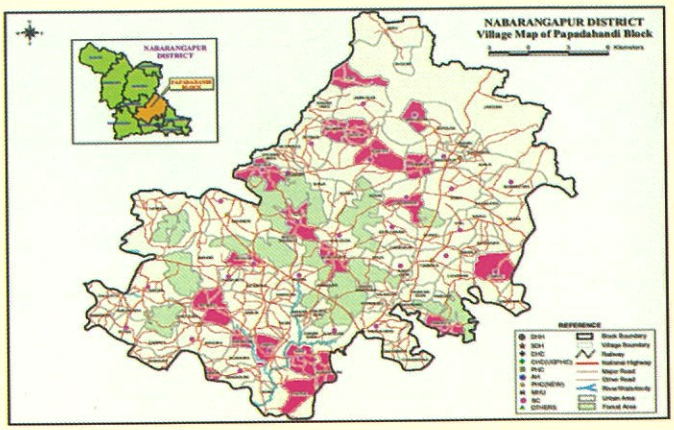
Map-2

Map 1 showing block map of Ghatagaon block of Keonjhar district with their API status more than 10 in red colour

Map 2 showing the selected villages of Ghatagaon block for the study in pink colour.



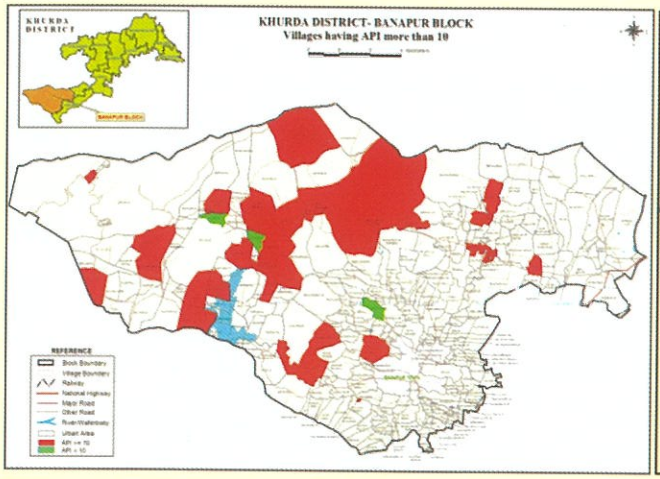
Map 3

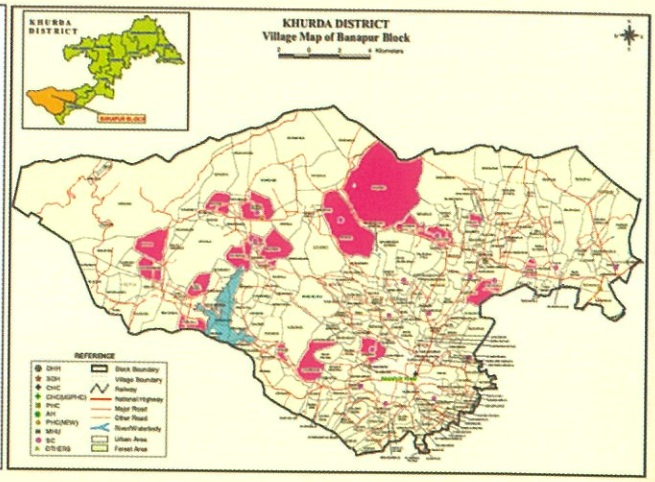


Map 4

Map 3 Showing block map of Papdahandi block of Nawarangapur district with their API status more than 10 in red colour

Map 4 showing the selected villages of Papdahandi block for the study in pink colour.





Map 5

Map 6

Map 5 showing block map of Banpur block of Khordha district with their API status more than 10 in red colour

Map6 showing the selected villages of Banpur block for the study in pink colour.

3. GIS survey

(a). Ghatagaon Block of Keonjhar district:

From the selected villages of Ghatagaon block during the one year study period three times field visit was done in three different seasons i.e. Winter, Summer, Rainey. During the field visit GIS data was generated using the GPS instrument and collection of adult mosquito and larvae positivity was tested during the field visit. The larval breeding spot were documented by the GPS instrument. During Ghatagaon block survey 79 breeding spot were detected in winter,40 in summer and 81 in rainy season and mapped by GIS soft ware. In adult mosquito collection An. pallidus An. varuna, An. splendidus, Cx. trytaerynchus, An. subpictus, An. vegus, An. fluviatilis, An. annularis, An. barbirostris, An. hyrcanus, and Cx. Vishnui and Cx. quinquefaciatus was mainly found.From these mosquitoes An. varuna ,An culicifacies , An. vagus, An. pallidus and An subpictus were found more in numbers from the collection of mosquito.

From the data obtained from winter season GIS survey one map was constructed according to the positivity of larval survey. Another two map on soil and hydro geomorphology of Ghatagaon block were developed by using GIS software.

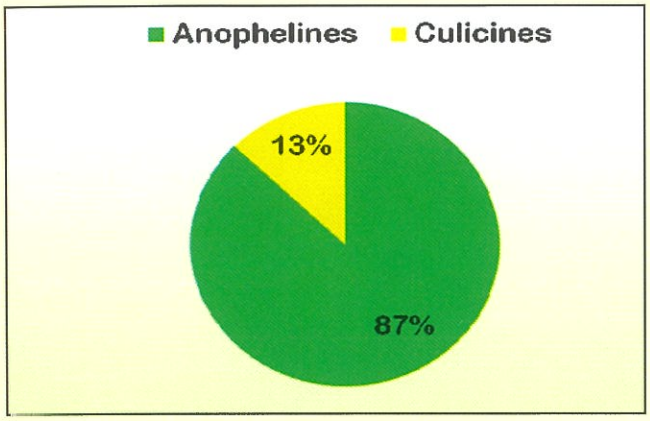
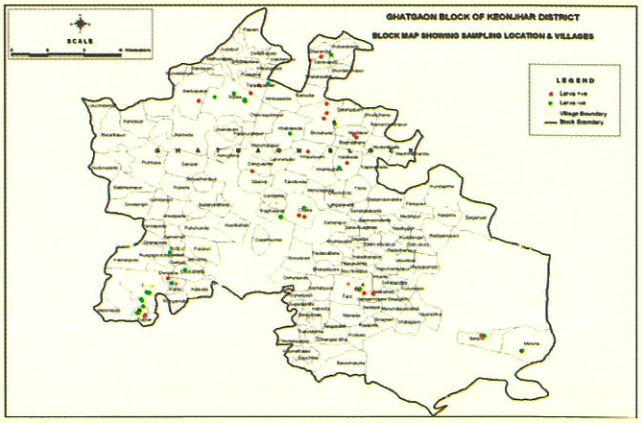


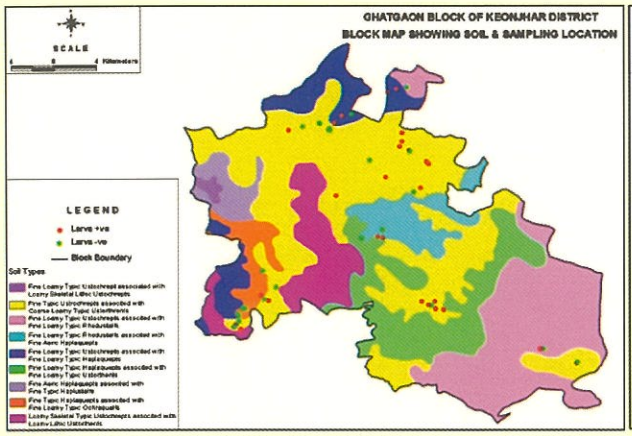
Figure 1



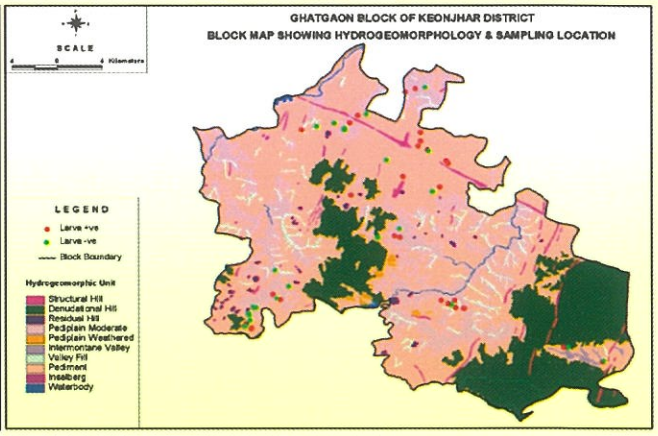
Map 7

Figure 1Showing percentage of species found in Ghatagaon block of Keonjhar districts

Map 7 showing the mapping of the breeding spots of the selected study area of Ghatagaon block.



Map 8



Map 9

Map 8 Showing the soil and sampling location of Ghatagaon block of Keonjhar districts

Map 9 showing the Hydrogeomorphology and sampling location of Ghatagaon block.

3b.Papdahandi Block of Nawarangapur district:

Field study was undertaken in twenty selected villages of Papdahandi block. In this block An. culicifacifacies, An. fluviatilis, An. annularis, An. vagus, An. pallidus, An. ramsayi, An. leucosphyrus, An. splendidus, An. hyrcanus, Cx. quinquifaciatus, and Cx vishnui are found. Among these species An. culicifacies and An. vagus was more abundantly found.The breeding spot were detected and data obtained from GIS survey in three seasons were ,35 in winter, 26 in summer and 68 in rainy season were mapped by GIS software.

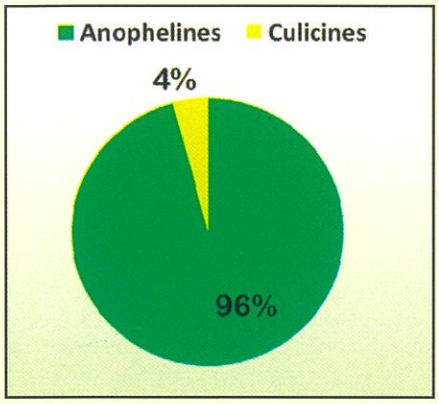
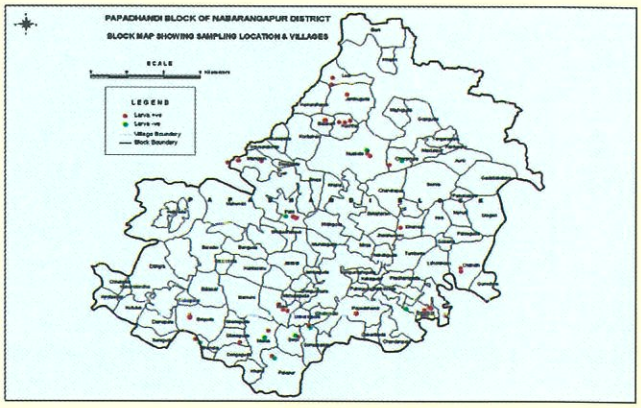


Figure 2

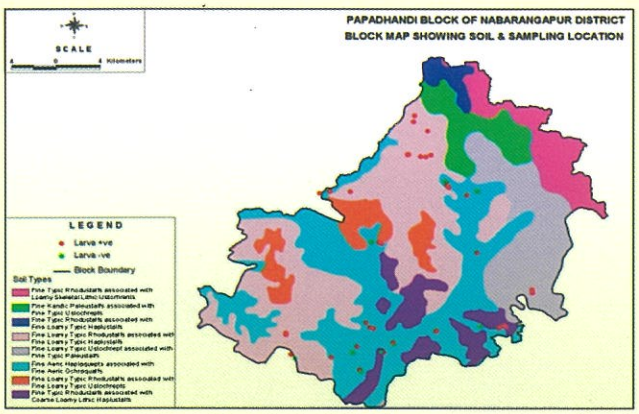


Map 10

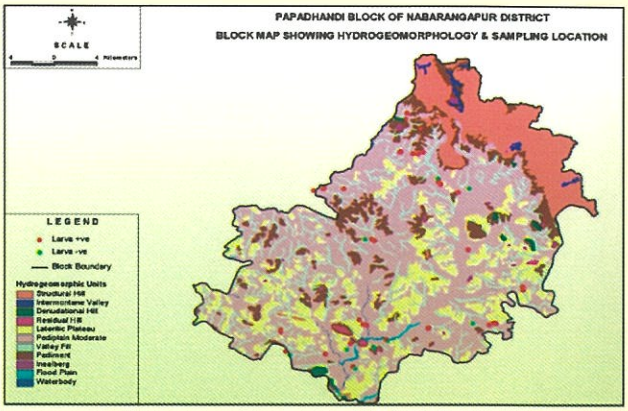
Figure 2showing percentage of species found in Papdahandi block of Nawarangapur districts

Map 10 Showing the mapping of the breeding spots of the selected study area of Papdahandi block.

From the above data and from GIS survey one map was constructed according to the positivity of larval survey. Another two map on soil and hydrogeomorphology of Papdahandi block was developed by using GIS software.



Map 11



Map 12

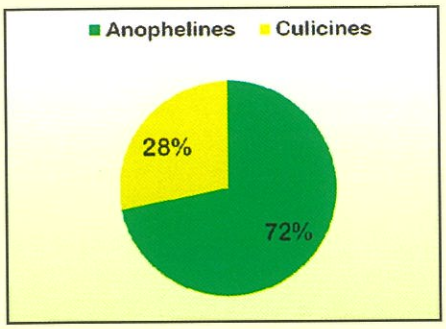
Map 11 showing the soil and sampling location of Papdahandi block of Nawarangapur districts

Map 12 showing the Hydrogeomorphology and sampling location of Papdahandi block.

3c. Banpur Block of Khordha district

Banpur is situated in 85 degree 10'E and 19 degree 47'N in the south-west of Khurda.To the west of Banpur the Salia Dam has been constructed .The dam has been constructed at the catchment area connecting two hills on both the sides and serves as a minor irrigation project. It is covered with thick vegetation. Old Teak plantations were available in this forest. Field study was undertaken in Badasula, Damiabarbara, Jhiripada Begunia Sahi, Niladriprasad, Begnaput, Bankiapali, Talabarei, Champadeipur, Kiagorada, Aranga, Dhunali, Regedisima, Kasipada, Nilpali and Patrapur of Banpur block.

Field study was undertaken in twenty selected villages of Banpur block. In this block An. culicifacifacies, An. fluviatilis, An. annularis, An. vagus, , An. karwari, , , Cx. quinquifaciatus, and Cx vishnui are found. Among these species An. varuna and An. vagus was more abundantly found.The breeding spot were detected and data obtained from GIS survey in three seasons were ,40 in winter, 23 in summer and 53 in rainy season were mapped by GIS soft ware.



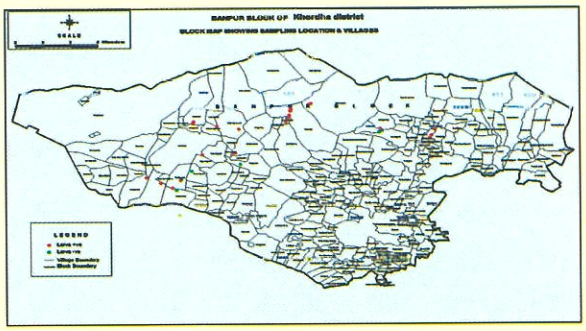
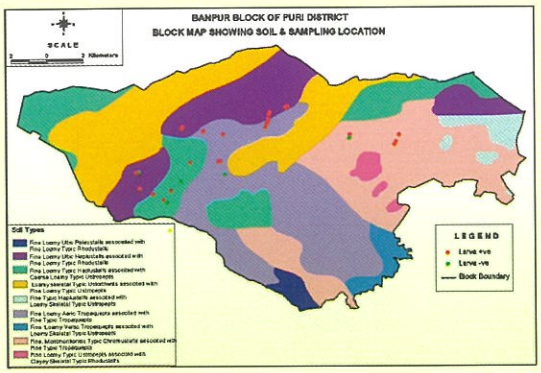
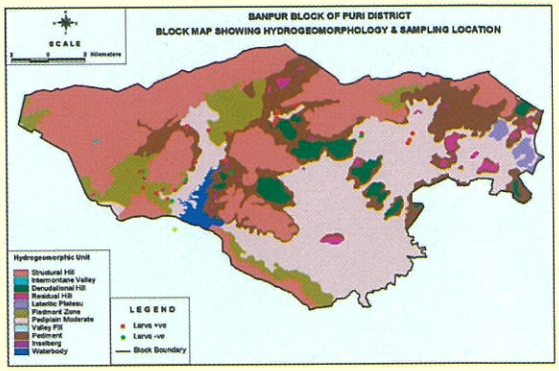


Figure 3 Map 13

Figure 3showing percentage of species found in Bankur block of Khordha districts

Map 13 showing the mapping of the breeding spots of the selected study area of Banpur block.





Map 14 Map 15

Map 14 showing the soil and sampling location of Banpur block of Khordha districts

Map 15 showing the Hydrogeomorphology and sampling location of Banpur block.

Sample collection and Sporozoit detection:

Mosquito collection was done from the three selected study area and they are further processed in the laboratory. At firstly each were identified by using standard key characters for identification of mosquito and Anopheles mosquito were separated and individual mosquito was dissected to head and abdomen. Then DNA was isolated from both the body parts and the isolated DNA was further screened for the presence of Plasmodium falciparum by giving specific primers for it. Total 1,400 house to house survey was done which include their socioeconomic status,use of bed net,ddistance from the nearest health unit as well as the medical facility was done in Ghatagaon, Papdahandi and Banpur block.

Generation of GIS layers for environmental parameters through satellite image interpretation and limited field verification.

- A socio economic map is being prepared using the GIS tools according to the data obtained from the House to house survey of the three blocks.
- The environmental parameters to be mapped in whichincludes include land cover/land use, NDVI, Rainfall, Elevation above MSL, Temperature and Humidity.
- The other resources and parameters to be mapped may include location of medical facilities, Population distribution (village wise), live stock distribution, any other provided by the client.
- The collection of data and mapping of these parameters have already been started and going on. The parameters like elevation, temperature, rainfall etc. may be mapped through digital surface modeling technique in GIS.

GIS based layer integration, overlaying and multi-criteria analysis

- It has been envisaged to utilize GIS to its optimum extent for various analysis through integration of various layers generated, which may include digital surface modelling, overlaying, multi criteria analysis, proximity analysis, nearest neighborhood analysis, descriptive statistical analysis etc.
- Data is being collected for 3 season i.e. winter, summer, rainy in order to compare the malaria prevalence in these 3 seasons for the concerned blocks located in different physical environment.

Picnic at Kapilash

5. Mapping of vector habitats for filariasis through remote sensing and geographical information system (GIS)

Principal Investigator	: Dr. N.Mahapatra.
Co Investigator(s)	: Dr. R.K.Hazra.
	: Dr. S.K.Parida
	: Mr. N. S. Marai
Collaborator	: Chief Executive, Orissa Remote Sensing Application Centre, Bhubaneswar
Starting date	: March 2007
Closing date	: February 2010
Funding	: Intramural

Background

Identification of landscape element that predisposed human to risk of filariasis transmission is important for understanding and controlling lymphatic filariasis. Features in landscape such as vegetation provided food and resources such as shelter, resting and developmental sites for the vector mosquitoes. Geographic Information System (GIS) technology, allows to study & examination of remotely sensed land elements that relate to vector abundance and therefore transmission risks. For mapping mosquito breeding habitats with associated health risk parameters, using Remote Sensing (RS) was not studied much. However, (Hassa & Onsi, (2004) used remote sensing as a tool for mapping mosquitoes breeding habitats and associated health risk to assist control effort and development plan. Therefore the present study was initiated to use GIS & RS as a tool for mapping filariasis vector breeding habitats in two filarial endemic districts & one non-endemic districts of Orissa.

Objectives

1. Mapping of vector habitats of filariasis in two endemic districts of Orissa through Remote Sensing and GIS.
2. Development of filariasis risk map

Observations

The microfilaria rate of the two endemic blocks namely, Jatani and Satyabadi were 12.4 and 10.2% respectively. Satyabadi block is endemic for both *Wuchereria bancrofti* and *Brugia malayi* infection whereas only *bancrftian* filariasis is seen in Jatani. Initially the land use parameters of endemic blocks when compared with non-endemic blocks revealed more water bodies, aquatic vegetation, rainfall and less forest coverage along with low elevation. Larval survey carried out in different breeding places in three blocks showed 58.4 and 61.7% breeding places to be positive for *Cx. quinquefasciatus* larvae in endemic blocks of Satyabadi and Jatani respectively while it was 22.4% in Angul a non-endemic block.

The distribution of *Cx quinquefasciatus* larvae in different breeding habitats are presented in Table 1. ***Ma.*** *annulifera* and *Ma. uniformis* larvae were only found in ponds of Satyabadi block.

Table 1: Distribution of vectors of filariasis in different breeding habitats.

	Breeding Habitats						
Places	Cesspit	Cesspool	Pond	Rice Field	Drain	Unused well	Others
Jatni (Wb)	C.q	C.q	C.q	C.q	C.q	C.q	C.q
Satyabadi (Wb +Bm)	C.q	C.q	Ma.a Ma.U C.q	C.q	C.q	C.q	C.q
Anugul (NE)	C.q	C.q	—-	C.q	C.q	—	C.q

C. q. = *Cx.quinquefasciatus* ,
Ma. a. = *Ma.annulifera.* Ma. u. = *Ma.uniformis.*

The larval distribution of *Cx.quinquefasciatus* was found more in drains followed by cesspit in both the endemic blocks but in non endemic block the presence of larvae was more in cesspool than in drain.

The adult mosquito collection revealed the presence of five genera i.e. *Anopheles, Aedes, Culex, Mansonioides, Armigeres. Cx.quinquefasciatus* was found to be the most dominant species in Jatani (56%) and Satyabadi block (44%) respectively. The M*ansonoides* contributed 21% of the total mosquitoes collected from Satyabadi Block. Anopheline mosquitoes were found more in numbers than *Cx.quinquefasciatus* (12%) in Anugul Block. The per man hour density (PMHD) of *Cx. quinquefasciatus* was high in Jatani (W.b) compared to Satyabadi (w.b+B.m) and very low in Anugul. The PMHD of *Ma.annulifera* and *Ma.uniformis* were 19.4 and 12.1 respectively. The infectivity rates of Cx.quinquefasciatus was 10.01% in Jatani and 5.4% in Satyabadi. Ma.annulifera *Ma.uniformis* showed infectivity rate of 12% and 6.8% in Satyabadi block.

Remote sensing survey & GIS Based Database Generation

The following spatial and nonspatial information layers were generated from satellite data after rectification with standard Survey of India Topo maps and masking the digital data by the district boundaries. The administrative boundaries like blocks & villages were collected from census data and were digitised using RzV software. The administrative databases were generated through ARC/ INFO GIS software package.

Remote sensing data helped in identification of breeding spots as mentioned above which are more than 6mt x 6mt spatial extent. The breeding spots which were not covered through satellite data were surveyed through G.P.S. The pre-field land use, drainage, vegetation and soil maps were checked on the ground level. Choked drains, dead wells and compost pits, ponds, swampy areas were surveyed using GPS tool; so that the exact geographical coordinates can be plotted on the base map easily.

Non-Spatial Data: The non-spatial data to be linked with spatial data i.e Demographic data, Climatic data, Water quality data of wells or water bodies were collected.

Climatic factor: Data on climatic factors such as temperature, relative humidity, rain fall and number of rainy days in a year were recorded.

A: Thematic layers were generated:

1. The climatological data e.g. rainfall, temperature and humidity were analysed and choropleths were generated.
2. Three dimensional terrain model of the study areas were generated using GPS derived altitude data and SOI toposheet derived spot heights.
3. All the remote sensing data derived themes like NDVI, drainage, Settlement, Soil moisture integrated with each other through GIS.

B: GIS Based Modeling

Linking of terrain parameters derived from R.S. with entomological, epidemiological & climatological parameters through GIS Software modeling. Buffering of different radial distances along rivers and settlement, were carried out. Correlation analysis between different R.S. derived terrain parameters, ground based vectors abundance information and entomological parameters and climatological parameters were carried out using statistical package like 'SPSS' and GIS package like ARC-GIS. This would highlight the correlation between terrains, climatological parameters. Spatial analysis of filariasis distribution in relation to environmental variables using GIS technology.

The field level data of entomological parameters collected by RMRC (ICMR) were converted into spatial (map) format using ARC/INFO 4.0 GIS package, by the ORSAC, the collaborator of the project. The point data (attributes) were linked with spatial database. Once the thematic layers and attribute database are generated, the filariasis risk maps have been generated through superimposition of the aforementioned layers and ground level information database.

All the spatial layers relating to terrain *i,e* Geomorphology, land use ,forest and soil PH have been intersected with each other , so that the complex terrain nature of the study area can be identified and analysed. Spatial environmental layer i,e. layer on rainfall ranges has also been intersected with terrain parameter layers. Prior to the intersection of all spatial layers ,the range characteristics of all themes have been identified and weightage factor has been assigned for each variables inducing filariasis in the study areas Table 6). Weightage for each filarial inducing factor (both terrain and environmental) is fixed on the variation between mean and standard deviation of observed values. In the initial stage, bivariate correlation between larval/adult density and dependent terrain and environmental variable has been carried out using SPSS 8.0 statistical software.

Thematic layer developed were block and village map, land use and land cover, soil type, river & canal system, water bodies, cropping pattern especially double crops. Satellite image was further used to derive thematic layers like forest cover, soil moisture index and Normalised difference vegetation index (NDVI).The analysis was done with Arc GIS 9.2 software to stratify the block. Thematic maps of environmental parameters were overlaid on filariasis prevalence map (mF rate) to identify the parameter responsible for different level of filarial incidence. Bivariate (Pearson' correlation) analysis explained a positive association of microfilaria rate with *Cx. quinquefasciatus* density ($r = 0.32$, $p<0.05$), *Cx. quinquefasciatus* density with soil moisture index ($r = 0.29$ $p< 0.05$) , with NDVI ($r = 0.11$) and with infectivity rate($r = 0.62$, $p<0.05$). The soil pH values were found to be highly significant ($P<0.01$) between endemic and non endemic blocks.

The current study clearly identified the risk factors to be the soil type, soil moisture index , normalised difference vegetation index, large network of canal and water bodies, and drainage which influence the breeding and proliferation of the vector mosquitoes which also directly influences the disease transmission. Taking into account the above factors, risk map of the two endemic and one non-endemic blocks are being developed. (Fig 1-3)

As a result, the combinations of all thematic layers, the filariasis risk zonation for all the three blocks on the study have been prepared. Three categories i,e low, moderate and high zones have been reflected on each map created through GIS. The values of mf rate ranging from 1-3 %, 3-6 % and 6-12 % were taken as low, moderate and high prevalence of bancroftian filariasis respectively. For Malayan filariasis these values were 2.1-2.15 %, 2.16-4.3 % and 4.4- 8.7 % for low, moderate and high prevalence respectively.

Fig: 1 filariasis risk map of Satyabadi block

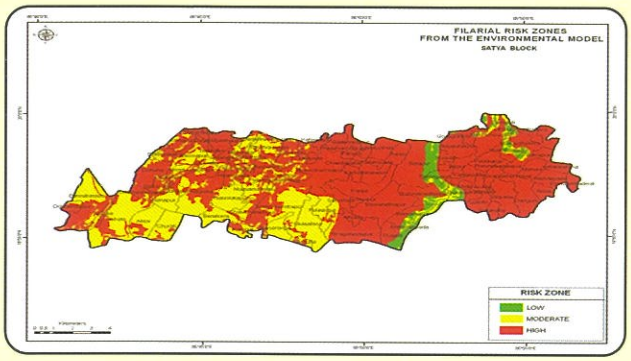
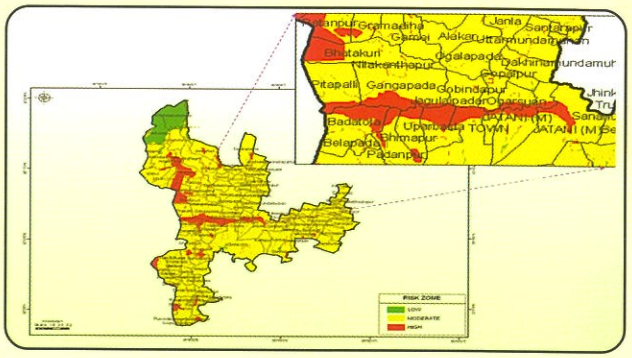
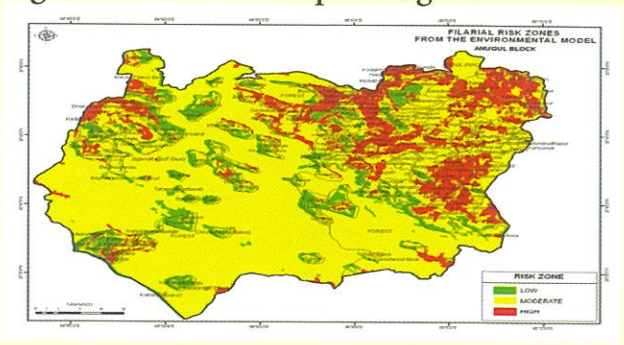


Fig:2 Filariasis risk map of Jatni block



Conclusion

Fig: 3 Filariasis risk map of Angul district



The filariasis risk maps for both endemic and non endemic blocks were developed. Significant differences were observed in soil pH, land use, land cover pattern and vector density, between endemic and non endemic blocks. The probable risk map for the non endemic block has also been developed. Due to migration of population from endemic area to non endemic area and changes in the land use and land cover pattern and ecological changes taking place the probability of spread of filariasis can not be ruled out. Continuous land cover modification is an important part of spatial epidemiology because it can help in identifying environmental factors for proliferation of vector mosquitoes which in turn transmit the disease and thus guide control intervention. Since this is a continuous phenomenon observed in both endemic and non endemic blocks of the state to continuous monitoring these maps will help in assessing the spread of filariasis to non endemic areas as well as impact of the intervention measure in endemic areas, which will help the government to take measures against transmission/ control of filariasis.

Annual Report
2009 & 2010

PUBLICATIONS & OTHER SCIENTIFIC INFORMATION

Bhubaneswar

Publications -2009

Sl No.	Publications	Impact Factor
1	Babu BV, Kar SK. Domestic violence against women in eastern India: a population-based study on prevalence and related issues. ***BMC Public Health*** 2009; 9(9):129.	2.223
2	Babu BV, Mishra S, Nayak AN. Marriage, sex, and hydrocele: an ethnographic study on the effect of filarial hydrocele on conjugal life and marriageability from orissa, India. ***PLoS Negl Trop Dis.*** 2009;3(4):414.	4.693
3	Bal MS, Beuria MK, Mandal NN and Das MK. Antigenaemia in young children living in Wuchereria bancrofti endemic areas of Orissa. ***Trans R Soc Trop Med Hyg 2009***; 103:262-265.	2.553
4	Bal MS, Mandal NN, Das MK, Kar SK, Sarangi SS, Beuria MK. Transplacental transfer of filarial antigens from Wuchereria bancrofti-infected mothers to their offspring. ***Parasitology.*** 2009 ; 23:1-5.	1.607
5	Das A, Manickam P, Yvan Hutin, , B. Pattnaik Pal BB, Chhotray GP, Kar SK and Gupte MD.Two sequential outbreaks in two villages illustrate modes of transmission of cholera. ***Epidemiology and infection;*** 2009 ;137(6):906-12 .	2.365
6	Das A, Manickam P, Hutin Y, Pal BB, Chhotray GP, Kar SK, Gupte MD. An outbreak of cholera associated with an unprotected well in Parbatia, Orissa, Eastern India. ***J Health Popul Nutr.*** 2009 ;27(5):646-51\	0.859
7	Das Anamika, Das TK, Sahu U, Das BP, Kar SK, Ranjit MR. CD36 T188G gene polymorphism and severe falciparum malaria in India. ***Trans R Soc Trop Med*** Hyg 2009 .203(7):687-90.	2.553
8	Dhangadamajhi G, Kar SK, Ranjit MR. High prevalence and gender bias in distribution of Plasmodium malariae infection in central east-coast India. ***Trop Biomed.*** 2009 ;26(3):326-33	0.649
9	Dhangadamajhi G, Mohapatra BN, Kar SK, Ranjit M.Genetic variation in neuronal nitric oxide synthase (nNOS) gene and susceptibility to cerebral malaria in Indian adults. ***Infect Genet Evol.*** 2009; 9(5):908-11.	3.223
10	Dhangadamajhi G, Mohapatra BN, Kar SK, Ranjit MR (2009).The CCTTT pentanucleotide microsatellite in iNOS promoter influences the clinical outcome in P. falciparum infection. ***Parasitology Research*** 2009.104:1315-20.	1.721
11	Dhangadamajhi G, Mohapatra BN, Kar SK, Ranjit MR. Endothelial nitric oxide synthase (eNOS) gene polymorphisms and P falciparum infection in Indian adults.***Infection & Immunity*** 2009; 77 (7):2943-7.	4.205

Sl No.	Publications	Impact Factor
12	Dwibedi B, Pramanik JM, Sahu P, Kar SK, Moharana T. Prevalence of genital Chlamydia infection in females attending an Obstetrics and Gynecology out patient department in Orissa. ***Indian J Dermatol Venereol Leprol.*** 2009 ;75(6):614-6.	0.976
13	Kerketta AS, Babu BV, Mohapatra S S S, Kar S K . Clinical profile of lymphatic Filariasis in children: a hospital based stucy from Orissa. ***Indian Paediatrics*** 2009 ; 46(3) :261.	0.962
14	Kerketta AS, Babu BV,. Clinicians' attitude on mass drug administration under the programme to eliminate lymphatic filariasis: a qualitative study from Orissa, India. ***Asia Pac J Public Health*** 2009; 21(1): 112-7.	0.763
15	Kerketta AS, Bulliyya G, Babu BV, Mohapatra SSS, Nayak RN. Health status of the elderly population among four primitive tribesof Orissa, India: A clinico epidemiological study. *Z Gerontol Geriatr* 2009. 42:53-59.	0.804
16	Mand S, Pfarr K, Sahoo PK, Satapathy AK ,Specht S, Klarmann U, Debrah AY, Ravindran B, and Hoerauf A .Macrofilaricidal Activity and Amelioration of Lymphatic Pathology in Bancroftian Filariasis after 3 Weeks of Doxycycline followed by Single-Dose Diethylcarbamazine. ***Am. J. Trop. Med. Hyg,*** 2009; 81:702-711.	2.795
17	Mandal NN, Bal MS, Das MK and Beuria MK. Protective efficacy of a filarial surface antigen in experimental filariasis. ***Journal of Helmnithology.*** 2009, 83, 47 - 50.	0.863
18	Mohanty A, Swain S, Kar SK, Hazra RK .Analysis of the Phylogenetic Relationship of Anopheles Species, Subgenus Cellia (Diptera: Culicidae) and Using It to Define the Relationship of Morphologically Similar Species. ***Infect Genet Evol.*** 2009; 9(6):1204-24.	3.223
19	Mohanty A, Swain S, Singh DV, Mahapatra N, Kar SK, Das AP, Hazra RK. A unique methodology for mapping the spread of chloroquine-resistant strains of Plasmodium falciparum, in previously unreported areas, by analyzing the anophelines of malaria endemic zones of Orissa, India. Inf. ***Gen.Evol.*** 2009 ; 9(4):462-7.	3.223
20	Pal BB, Khuntia HK, Samal SK, Kar SK, Patnaik B. Epidemics of severe cholera caused by El Tor Vibrio cholerae O1 Ogawa possessing the ctxB gene of the classical biotype in Orissa, India. I***nt J Infect Dis.*** 2009 ; 23.	2.167
21	Ranjit MR , Sahu U, Khatua CR, Mohapatra B N, Achraya A S, Kar S K(2009). Chloroquine-resistant p falciparum parasites and severe malaria in Orissa . ***Current Science*** 2009;96:1608-1611.	0.782

Sl No.	Publications	Impact Factor
22	Swain S, Mohanty A, Mahapatra N, Parida SK, Marai NS, Tripathy HK, Kar SK, Hazra RK. The development and evaluation of a single step multiplex PCR for simultaneous detection of Anopheles annularis group mosquitoes, human host preference and Plasmodium falciparum sporozoite presence. *Trans R Soc Trop Med Hyg* 2009. 103;11:1146-52.	2.553
23	Rout R, Mohapatra BN, Kar SK, Ranjit MR. Genetic complexity and transmissibility of Plasmodiumfalciparum parasites causing severe malaria in central-east coast India. *Trop Biomed* 2009, 26: 165-172 .	0.649
24	Khuntia HK, Samal SK, Kar SK and Pal BB . Emergence of Nalidixic Acid Resistant Vibrio cholerae O139 in Orissa, India and identification of its responsible protein component. *Journal of Pure and Applied Microbiology* 2009; 3(1): 371-374.	NA
25	Kumari, S., Parida, S.K., Marai, N.S., Hazra, R. K., Tripathy, A., Kar, S. K and Mahapatra, N. Vectorial role of An. Subpictus and An. Culicifacies in Angul district of Orissa, India. *Southeast Asian J. Trop. Med Public Health.* 2009;40(4):	NA
26	Mahapatra N, Panda SP, Acharya AS, Hazra RK, Parida SK, Marai N, Kar SK, Mahapatra A, Narasinghan K, Mohapatra S, Mahavadani UV. Toxicological response of Culex Quantifascitus and An. Stephensi to two different plant extracts. *Journal of Current Sciences.* 2009:14(1):291-296.	NA
27	Purohit B, Mahapatra A. A Review on High Burden of Malaria during Pregnancy: Need of Social Science Intervention. *Ethno Medicine.* 2009; 3 (1): pp- 33-38.	NA

Publications- 2010 (Jan- Sept)

1. Babu BV, Kar SK. Domestic violence in Eastern India: factors associated with victimization and perpetration. *Public Health.* 2010; 124(3): 136-48.
2. Dhangadamajhi G, Mohapatra BN, Kar SK, Ranjit M.Gene polymorphisms in angiotensin I converting enzyme (ACE I/D) and angiotensin II converting enzyme (ACE2 C-->T) protect against cerebral malaria in Indian adults. *Infect Genet Evol.* 2010 ;10(2):337-41.
3. Bal MS, Mandal NN, DAS MK, Kar SK, Sarangi SS, Beuria MK. Transplacental transfer of filarial antigens from Wuchereria bancrofti-infected mothers to their offspring. *Parasitology.* 2010; 137(4):669-73.
4. Dhangadamajhi G, Mohapatra BN, Kar SK, Ranjit MR. A new allele (eNOS4e) in the intron 4 (VNTR) of eNOS gene in malaria infected individuals of the population of Orissa (an eastern Indian state). *Nitric Oxide.* 2010; 22(1):58-9.

5. Pal BB, Khuntia HK, Samal SK, Kar SK, Patnaik B.Epidemics of severe cholera caused by El Tor Vibrio cholerae O1 Ogawa possessing the ctxB gene of the classical biotype in Orissa, India. *Int J Infect Dis.* 2010; 14(5):09.

6. Babu BV, Swain BK, Mishra S, Kar SK.Primary healthcare services among a migrant indigenous population living in an eastern Indian city. *J Immigr Minor Health.* 2010 ; 12(1):53-9.

7. Dwibedi B, Mohapatra N, Beuria MK, Kerketta AS, Sabat J, Kar SK, Rao EV, Hazra RK, Parida SK and Marai N. Emergence of chikungunya virus infection in Orissa, India. *Vector-Borne and Zoonotic Diseases.* 2010; 10(4): 347-54.

8. Khuntia HK, Samal SK, Kar SK, Pal BB.An Ogawa Cholera Outbreak 6 Months After the Inaba Cholera Outbreaks in India, 2006.*J Microbiol Immunol Infect.* 2010 ;43(2):133-137.

9. Mandal N N, Bal M S, Das M K, Achary G P and Kar S K. Lymphatic filariasis in children: Age dependent prevalence in an area of India endemic for Wuchereria bancrofti infection. *Tropical Biomedecine,* 2010 Apr. 27/1:41-46.

10. Sahu P, Kar S K, Mahapatra A. Seroepidemiological Study of Hepatitis B and C Viral Infection in Acute and Chronic Hepatitis Patients in a Hospital Setup in Orissa. *Journal of pure and applied Microbiology.* 2010; 4(1):251-255.

11. Khuntia HK, Samal Sk , Sahoo R K, Kar S K and Pal BB . Isolation and characterization of thermophilic bacteria from hot spring in Orissa. India. *Journal of Biosciences Biotechnology Research Asia.* (2010)-In Press.

12. Bulliyya G. Ethnographic and health profile of the Dongria Kondh: a primitive tribal group of niyamgiri hills in eastern ghats of Orissa. *Afro Asian Journal of Anthropology and Social Policy* 2010; 1(1): 11-25.

13. Rout R, Majhi GD, Mohapatra BN, Kar S.K, Ranjit MR..High CR1 level and related polymorphic variants are associated with cerebral malaria in eastern-India. *Infection, Genetics and Evolution,* 2010 (In Press).

14. Tripathy A , Samanta L, Das S , Parida SK, Marai NS, Hazra RK, Mallavdani Kar Sk and Mahapatra N. Mosquitocidal activity of methanolic extracts of Lantana cramera root and Anacardium occidentale leaf : Role of Glutathione-s-transferase in insecticide resistance" *J. Med. Entomol.* 2010 47(6):ME09122.

Chapter in Book

Bulliyya G, Kar SK. Diet and nutritional issues of scheduled tribes and primitive tribal communities. In: A.B.Hota Eds. *Critical Issues in Tribal development.* 2010; 248-262.

Meeting /Seminar/ Training program organised

1. Training program for Malaria Technical Supervisors (MTS) of Madhya Pradesh, Chhatisgarh, Jharkhand, Orissa and Andhra Pradesh.

The Centre has organized a series of training program of 2 weeks duration for Malaria Technical Supervisors of Madhya Pradesh, Chhatisgarh, Jharkhand and Orissa . To strengthen the on going malaria control programme in the country, the malaria technical supervisors has been appointed by NVBDCP in all the malaria endemic states of the country. On request of NVBDCP, New Delhi the training program for the MTS of above states was conducted by the experts of RMRC on different aspects of malaria like epidemiological, clinical diagnosis and case management, vector & its control and monitoring & evaluation. During this period 5 batches (25 trainees in one batch) of MTS have trained on various aspects of malaria control Program.

2. Opening of H1N1 Testing Laboratory in RMRC, Bhubaneswar

The BSL-2 Laboratory (H1N1 Testing Facility) was inaugurated by Sri Prasanna Acharya, Honourable Minister of Health & Family welfare, Govt. of Orissa on 23rd September 2009. This Centre is now catering the need of the state by giving diagnosis by RTPCR to all the referral cases.

3. Tuberculosis Meeting

A meeting was organized at RMRC, Bhubaneswar on Establishment of Mycobacterium tuberculosis culture and DST Laboratory on 6 th May 2010. The expert members present were Dr S K Kar, Director,RMRC, Bhubaneswar, Dr P R Nrayanan, Former Director, TRC, Chennai, Dr N Selvakumar, Scientist-F, TRC, Chennai, Dr Paresh Mohanty, IRL Microbiologist, Cuttack, Dr D Parija, WHO RNTCP State Level Consultant and Dr D Das, Scientist-D, RMRC, Bhubaneswar.

4. National Science Day Celebration

On eve of National Science day observation Dr. D Das, Sc-D and Dr A Maharana, Sc-B Conducted a series of lectures on 26-27th Feb. 2010 at Vanivihar High School, Bhubaneswar and Tribal School, Khandagiri on the topic "Infectious Diseases and Genetic Disorders" to the school students followed by blood grouping and sickling test for the students on the spot.

5. Hindi Day Observation

The Centre observed Hindi Day on 26th March 2010 in RMRC Auditorium. On this occasion hindi song and Hindi debate competation was held among staff and students of RMRC. Cash awards were distributed to the winners on 29th March 2010 on the occasion of RMRC Foundation day celebration.

6. Animal Ethical committee Meeting

The Animal ethical committee meeting was held on 5th June 2009 for review of the projects relating to ethical issues. S.K.Ray, Ex- principal, Orissa College of Animal Husbandry & Veterinary Science was the chairman of the committee. Prof. Sachidanda Das, Dept. of Zoology, Utkal University and Kasturika Patnaik, Social scientist and Dr. R.C.Patra, OUAT were present in the ethical committee meeting.

Scientific Conferences/Seminars attended

Dr S.K.Kar, Director

1. Invited as guest speaker in the National Seminar on "Status of Human Development of Fisheries in India" organized by Information International at Pantha Niwas, Bhubaneswar on 24th April 2009 and delivered a talk on "Health and Human development role on Nutrition".
2. Delivered a talk on "Vitamin A level and measles outbreak investigation at RMRC" at State Level Meeting on Measles mortality at SIHFW on 25th April 2009.
3. Delivered a talk on Situation Analysis on Malaria in Orissa at World Malaria Day meeting at NRHM Conference Hall , Bhubaneswar on 25th April 2009.
4. Participated in the meeting on Swine Flu H1N1 and delivered a talk on "H1N1 situation in India" organized by Commissioner cum Secretary, Govt. of Orissa at Conference Hall on 15th May 2009.
5. Participated in Brain Storming Session on "Data Repository & Clinical data management" at ICMR and presented a talk on Data Repository Situation in RMRC, Bhubaneswar" on 19th May 2009.
6. Participated in the meeting & delivered talk on Advise of National Technical Advisory Group on Immunization (Hib & Pneumococcal vaccine) at Conference Hall of DHS, Govt. of Orissa, Bhubaneswar on 25th July 2009 .
7. Participated in the meeting chaired by Hon'ble Chief Minister, Govt. of Orissa at Secretariat and talked on Diagnosis Status of H1N1 on 6th August 2009.
8. Participated in the GRAMSAT programme on Swine flue at ORSAC on 12th August 2009 and talked on Symptom, Signs and Diagnosis of H1N1.
9. Participated in Review meeting on Preparedness of H1N1 under Chairmanship of Hon'ble Chief Minister, Govt of Orissa at Conference Hall at Secretariat on 14th August 2009.
10. Invited as Pannel Member in the State Level Multi Stake Holder Forum on Millennium Development Goals (MDGS) at XIMB, Bhubaneswar on 30th October 2009 and delivered a talk on "Progress in Malaria control- future".
11. Delivered a talk on Health and Nutrition as an invited speaker at CIFA, Bhubaneswar on 27th Nov. 2009.
12. Participated as Chairman in "Hib Vaccine meeting" at ICMR HQ held on 2nd December 2009.
13. Participated in meeting on Global Environment Change & Health at ICMR Hqrs on 4th March 2010 and presented a paper on "Status on vector- Borne diseases and diarrhoeal diseases and climate effect".
14. Participated SAG meeting at ICMR HQrs on 18-19th March 2010 and presented centre's progress.
15. Participated in Hepatitis Investigators meeting at National Institute of virology, Pune on 25th March 2010 and presented data on Study of Prevalence of Hepatic viral infection in primitive tribes of Orissa.

16. Attended the ICMR Forum on Tribal Research at RMRCT, Jabalpur during 8-9th August 2010 and delivered a talk on "Research needs on the health of tribals in Orissa".
17. Attended "Meet the Press" meeting on H1N1 at State Secretariat along with Mr Prasanna Acharya, Minister Health & FW Orissa on 16th August 2010.
18. Inaugurated the "Regional Level CBSE Science Exhibition 2010" at DAV Public School, Chandrasekharpur and delivered a talk on "Climate Changes & Biology in Human Welfare" on 27th August 2010.
19. Attended H1N1 meeting with Secretary and Minister, Health, Govt of Orissa on 24th & 25th August 2010, and discussed on analysis of H1N1 death in Orissa.
20. Participated Vector Science Forum meeting at ICMR HQrs New Delhi on 13th Sept. 2010.
21. Participated Review Meeting on Malaria Control at NVBDCP, Bhubaneswar on 17th Sept. 2010.
22. Attended Technical Committee Meeting on Vitamin-A supplement programme at Dept. of Family Welfare, Bhubaneswar on 4th October, 2010.
23. Participated in Scientific Project Review Meeting of VCRC, Pondicherry on 22nd October, 2010.
24. Participated and delivered a guest lecture at TROPACON 2010 at KIIMS, Bhubaneswar on 29th October, 2010.
25. Participated in High Level Meeting on Long Term (Multi-sectional) Plan on diarrhoeal outbreak at State Secretariat, Bhubaneswar on 4th November, 2010.
26. Participated 30th APICON Orissa Branch at RMRC, Bhubaneswar and delivered talk on "Recent Epidemics in Orissa" on 13th November, 2010.
27. Participated in Director's meeting of ICMR at New Delhi on "Centenary Celebration of ICMR on 15th November 2010.
28. Participated in Malaria Review Meeting at NVBDCP, Bhubaneswar on 18th November 2010.
29. Participated in Annual Conference of Rheumatology Association (IRICON-2010) at Bhubaneswar on 25th November, 2010.
30. Participated in Raj Bhasha Hindi Conference at Goa from 26-27th November, 2010.
31. Participated in Expert Committee Meeting on Hypertension at ICMR New Delhi on 8th December 2010.
32. Participated in SAC Meeting of VCRC, Pondicherry on 15-16th December, 2010.
33. Participated in Core Committee of ICMR Assessment Board meeting from 20-23th December, 2010.

Dr. N. Mahapatra, Scientist-E

1 Participated Protocol development meeting of Vector Control Research Centre, Pondichery.
2 Attended meetings at ICMR Hq, New delhi on1 2nd August 2009 regarding additional post requirement in response to 11th plan at RMRC, Bhubaneswar.

3 Participated meetings at ICMR HQ, New Delhi on 6th Jan.2010 on operational research on vector borne diseases and feasibility of implementation in the NVBCPD Programme.

4 Attended the technical committee meeting at Cuttack Municipality Corporation on "Control of mosquito menace in Cuttack city" on 4th May, 2010.

5 Attended the technical committee meeting with BMC Commissioner along with his officials on "urban mosquito control of BMC area" on 25th June, 2010.

6 Attended the Scientific Review Committee (SRC) meeting of Department of Biotechnology on 15th September, 2010.

7 Attended a meeting of NVBDCP protocol Development for impact assessment of LLIN with State, VCRC, MRC Entomologist on 12th August, 2010.

Dr.M R Ranjit, Scientist-D

1. Attended the International Symposium on Vectors and Vector Borne Diseases held at Goa from 4-6 November 2009 and presented the paper entitled "High prevalence and gender bias in distribution of Plasmodium malariae in central east coast India".
2. Attended the National Seminar on "Climate change and Vector-Borne Diseases" organized by Asiatic Society, Kolkata on 6th February 2010 and delivered a guest lecture on "Epidemiology of malaria and its regional variations in Orissa".
3. Attended the workshop on BIOINFORMATICS organized by NARI,Pune in collaboration with Vanderbilt University, USA from 10th April 2010 to 23rd April 2010.

Dr. A.K.Satapathy, Scientist-D

1. Attended CPCSEA Regional Workshop on Role of CPCSEA in Education and Research held at Central Glass & Ceramic Research Institute, Kolkatta on 19th April 2010.

Dr. R.K.Hazra, Scientist-C

1. Attended International Symposium on Vector and Vector Borne Diseases from 4-6 November, 2009 in Goa and presented a paper.
2. Attended the National Seminar on "Climate change and Vector-Borne Diseases" organized by Asiatic Society, Kolkata on 6th February 2010 and delivered a guest lecture on "Climate and Vector Borne Diseases".
3. Attended the technical committee meeting with BMC Commissioner along with his officials on "Urban mosquito control of BMC area" on 25th June, 2010.
4. Attended a meeting of NVBDCP protocol Development for impact Assessment of LLIN with State, VCRC, MRC Entomologist on 12th August, 2010.

Dr A Mahapatra, Scientist-D

1. Attended National Conference on Hindi- Rajbhasha on 9-11 December 2009 at Puri organised by Bharatiya Rajbhasha Parisha, New Delhi.
2. Attended the work shop on Methods in Qualitative Research in Social Science by Samarth at NIV Pune from 29th to 31st Mar 2010.

Dr. G. Bulliyya, Scientist-D

1. Attended 'Maternal and Neonatal Health Coordination Meeting held on May 8, 2009 at UNFPA, State Office, Bhubaneswar and presented paper on Janani Suraksha Yojana: a scheme for improving maternal healthcare services and reducing maternal and neonatal deaths'.
2. Attended a 'State-level Meeting on Vitamin A Deficiency, organized by Indian Institute of Youth & Development, at IMAGE, OUAT, Bhubaneswar on May 9-10, 2009, and presented a paper entitled 'Micronutrient Deficiencies with particular reference to Vitamin A and strategies for prevention'.
3. Attended a State-level Panel Discussion on Millenium Development Goal-5 on Maternal Health held at Xavier Institute of management, Bhubaneswar on June 3, 2009
4. Attended "The Global IDD Prevention Day & Week 21-27 October 2009, Organised by Directorate of Health Services, Government of Orissa at Chetna, Bhubaneswar on 27th October 2009, and presented a paper on Strategies of Iodine Deficiency Disorders prevention: Current status in Orissa.
5. Attended 'International Seminar on Child Nutrition and Infectious diseases' held at Hotel Industhan International, Organised by Asian Institute of Public Health, Bhubaneswar on January 8, 2010.
6. Attended 'Consultation on Effective Community Management of Biodiversity in an Era of Climate Change and UN Millennium Development Goal Operation 2015 for KBK Region of Orissa', held at OUAT, Bhubaneswar on January 30-31, 2010.
7. Attended a 'Round Table Discussion Meeting on The Role of Education in Agrifood-based Approaches to Nutrition Improvement' held on May 10, 2010 at the Xavier Institute of Management, Bhubaneswar. Organised by International Potato Centre, New Delhi. Presented a paper on "Vitamin A: Clinical and Sub-clinical profile: Search for Sustainable Strategies'
8. Attended a State-level meeting on 'Malaria & malnutrition: The Mission Link?' organized by the WCD Department, Health and Family Welfare Department, NRHM, NVBDCP & TMST held on May 15, 2010, at NRHM Conference Hall, Bhubaneswar and presented a paper on 'Malaria and Malnutrition: Dual burden'
9. Attended an expert panel on 'Millennium Development Goals (MDG) Forum Meet on Achieving MDG-1: Eradicating Extreme Poverty and Hunger in Orissa held on July 14, 2010 at the Xavier Institute of Management, Bhubaneswar'
10. Attended 'ICMR Forum on Tribal Health Research on the eve of International Indigenous People's Day held on August 8-9, 2010 at Regional Medical Research Centre for Tribals, Jabalpur. Presented 'RMRC-Bhubaneswar Research Contribution on Tribal studies'

11. Attended 'Orientation Meeting and Training for Trainers for the Clinical, Anthropometric and Biochemical (CAB) component of the Annual Health Surveys (AHS), held on September 15-17, 2010 at National Institute of Health and Family Welfare, New Delhi'
12. Attended a meeting of the 'Technical Committee on Bundling of deworming with Biannual Vitamin A Supplementation Programme' held on October 4, 2010 at the Directorate, Director Family Welfare, Government of Orissa, Bhubaneswar'.
13. Attended a 'State level Child health Conclave on October 26, 2010 at Hotel Suryansh, organized by Directorate of State Institute of Health & Family Welfare, Bhubaneswar and presented a paper 'Undernutrition among under-5 children in Orissa'
14. Attended 'Orissa MDG Forum Meet on Neonatal Mortality to achieve MDG-4: Reduce Child Mortality held on October 29, 2010 at Xavier Institute of Management, Bhubaneswar.

Dr. D. Das, Scientist-D

1. Attended Training on Culture and Drug Susceptibility Testing in M tuberculosis at TRC, Chennai from 09.02.2009 to 20.03. 2009.
2. Attended Training course on Safety aspects in research applications of ionizing radiations from 7-15 Dec 2009 conducted by IARP, BARC, Mumbai.
3. Attended National Rajbhasha Sammelan from 25-27 Nov. 2010 at Goa.

Dr B Dwibedi Scientist-B

1. Participated in a meeting on 20th April 2010 held at Orissa secretariats organized by Commissioner cum Secretary WCD and participated in the discussion on Current Nutritional Programmes in the state and strengthening, RMRC participation.
2. Visited the laboratories during Sept. 2009 of NICED and NIV for short training on Swine flu Laboratory diagnosis procedures.
3. Participated in a meeting on August, Sept 2009 held at Orissa secretariats on state review of Laboratory testing and out break management of Swine flu with Chief Minister, Health Minister, Health Secretary.
4. Participated in a meeting on 6th January 2010 held at ICMR, New Delhi organized by ICMR on Review of Vector Borne Diseases in India.
5. Participated in an international seminar on 8.01.2010 held at Bhubaneswar organized by Asian institute of public health on Childhood nutrition & infectious disease.
6. Delivered a lecture on 9.2.2010 at RMRC, Bhubaneswar organized by RMRC-SIHFW collaboration on 9th Professional Development Course for Public health personnel of Jharkhand, Chhatishgarh, A & N & Orissa.
7. Delivered lectures on different sessions from 03.03.10 to 13.03.2010 held at RMRC, Bhubaneswar organized by RMRC, Bhubaneswar on Health structure of India to Malaria technical supervisors straining programme.

8. Participated in the press sensitization meeting on May 2010 held at Bhubaneswar organized by Regional Office of Health Services, DEC +ALB co administration during MDA for Filariasis in the state.
9. Participated in a meeting on May 2010 held at DHS, Bhubaneswar organized by state health department for preparedness and technical inputs on State & District health officers on introduction of DEC+ ALB in MDA.
10. Participated in a meeting on Sept. 2010 held at Orissa secretariats organized by State Govt. on preview on updates Swine flu laboratory investigation by Chief Minister.
11. Participated meeting in August & September 2010 held at Orissa secretariats organized by State Health Department on updates of vaccination policy and treatment during serve the pandemic.
12. Participated a seminar in on Sept 2010 held at SBVPGIP, Cuttack on Epidemiology, prevention and treatment of H1N1 infection in children.
13. Participated a review meeting in October 2009 held at Orissa secretariats organized by Department of Health on Swine flu, Chandipura & Encephalitis with Secretary Health, Orissa.
14. Participated a meeting on 29.08.2010 held at Regional Science Centre, Bhubaneswar and attained as Guest speaker on awareness programme on Swine flu at Regional Science Centre Bhubaneswar.
15. Participated in a session on 20.09.2010 held at RMRC, Bhubaneswar organized by SIHFW and delivered a lecture on 10th PDC course to district level health officers of Jharkhand, Chhatisgarh, Orissa and Andaman.
16. Participated in a review meeting on Sept. 2010 held at Orissa secretariats organized by Orissa Health Ministry on Severe Diarrhea outbreak and management in the state.
17. Participated in a meeting on May 2010 at RMRI, Bhubaneswar on priritisation of viral disease Investigation in Orissa (Medical colleges, Experts, Report on Hepatitis) - May 2010 like Encephalitis.

Dr. B. Sahoo, Lib & Inf. officer

1. Attended ICSSR National Seminar held at OUAT, Bhubaneswar and presented a paper on " E-Journal Consortia in ICMR Libraries in India" on 4-5 Sept. 2010.
2. Attended National Conference on Hindi- Rajbhasha from 25-27 November 20109 at Goa organised by Bharatiya Rajbhasha Parisha, New Delhi.

Dr. N N Mandal, Res. Asst.

Attended the training course on "THE CARE, BREEDING & EXPERIMENTAL TECHNIQUES OF LABORATORY ANIMALS" at National Centre for Laboratory Animal Sciences (NIN,ICMR), Hyderabad from 26th April to 7th May 2010. Mr. Jaladhar Naik and Mr. Pandav Sahoo were co-participants on Laboratory animal training program.

Dr. H.K. Khuntia, Res. Asst

Delivered a talk on "Strategy preparedness workshop on water borne disease scenario in Orissa" organized by Inter Agency Group (IAG), Orissa on 19th September 2009 at Redcross Bhawan, Bubaneswar, Orissa.

HRD Activities

Foreign Fellowship

Dr. B.B.Pal and Dr. H.K.Khuntia has undertaken WHO fellowship on "FETP Training on Epidemiology" at Thailand in June 2009.

Dr. A.S Kerketa has undertaken WHO Fellowship on Information, Education and communication for health, at Bereaue of Policy and Strategy, Ministry of Public Health, Nonthaburi, Thailand from 2nd -13th November 2009.

Ph.D Awarded

1. Clinical malaria: association of CD36 gene polymorphism and P.falciparum genotypes

   Investigator : Anamika Das
   Status : SRF (ICMR)- Completed
   Guide : Dr. M.R.Ranjit
   University : Kalyani University, W.B

Ph.D Submitted

1. Prevalence of HBV & HCV infection and their genotypes among acute/ chronic symptomatic hepatitis patients in hospital set up.

   Investigator : Prajoti Sahu
   Status : SRF (ICMR)- Completed
   Guide : Dr. S.K.Kar
   University : Utkal University

2. Molecular characterization of ? thalassaemia and its clinical significance in Orissa"

   Investigator : Sudhansu Sekhar Nishank
   Status : SRF (CSIR)-Completed
   Guide : Dr. M.R.Ranjit (Co-Guide)
   University : Utkal University

3. Malaria Preventive Intermittent Treatment of Choloroquine among the Pregnant Women- an Anthropological Perspective

   Investigator : Biswaranjan Purohit
   Status : SRF (ICMR)- Completed
   Guide : Dr. A. Mahapatra
   University : Utkal University

4. Factors affecting the vectorial competence of anopheles vectors in Orissa and its impact in Malaria.

Investigator : Asima Tripathy
Status : SRF (ICMR)
Guide : Dr. N. Mahapatra
University : Utkal University

Ph.D Registered

1. Role of microparticles and ABCA1 transporter in the pathogenesis of cerebral malaria"

Investigator : Ms. Upasana Sahu
Status : SRF (ICMR)- Completed
Guide : Dr. M.R.Ranjit
University : Utkal University

2. Molecular analysis of different anophelines and their vectorial attributes in different geographical regions of Orissa.

Investigator : Sunita Swain
Status : SRF (ICMR)
Guide : Dr. R.K.Hazra
University : Utkal University

3. Isolation characterization and diagnosis of A. hydrophilia isolated from freshwater fishes

Investigator : Suryakant Samal
Status : SRF (ICMR)- Completed
Guide : Dr. B.B.Pal
University : Utkal University

4. Molecular Identification of Anopheles subpictus and its role in malaria transmission in different eco-zones of Orissa, India

Investigator : Swati Kumari
Status : JRF (Lady Tata)
Guide : Dr. N. Mahapatra
University : Utkal University

5. Role of NO in the pathogenesis of severe Plasmodium falciparum malaria

Investigator : Gunanidhi Dhandamajhi
Status : SRF (CSIR)
Guide : Dr. M.R.Ranjit
University : Utkal University

6. Molecular mechanism of rosetting in severe falciaprum malaria

Investigator : Ronaly Rout
Status : SRF (UGC)
Guide : Dr. M.R.Ranjit
University : Utkal University

7. Role of B1-lymphocytes and auto antibodies in human lymphatic filariasis

Investigator : Ms. Rasmi Mishra
Status : SRF (ICMR)
Guide : Dr. A.K.Satapathy
University : Utkal University

8. Investigation on effect of maternal infection on cellular and humoral immune response of neonates in lymphatic filariasis

Investigator : K.Gopinath Acharya
Status : SRF (ICMR)
Guide : Dr. A.K.Satapathy
University : Utkal University

9. Risk Factors associated with the spread of Malaria in Rengali left bank Canal system of Orissa

Investigator : Buli Panigrahi
Status : SRF (ICMR)
Guide : Dr. N. Mahapatra
University : Utkal University

10. Study on micronutrients malnutrition with special reference to vitamin A and its associations with other major trace elements among children in Orissa

Investigator : Suchismita Behera
Status : SRF (ICMR)
Guide : Dr. G. Bulliyya
University : Berhampur University

11. Genetic polymorphism and its association with essential hypertension in different populations of Orissa"

Investigator : Manisa Patnaik
Guide : Dr. M.R.Ranjit
University : Utkal University

12. Bionomics and molecular studies of Aedes vectors prevalent in various parts of Orissa with reference to arboviral disease.

Investigator : Biswadeep Das
Guide : Dr. R.K.Hazra
University : Utkal University

13. Study of Bacterial pneumonia and meningitis in pediatric population.

Investigator : Ms. Chinmayee Priyadarshini Khuntia
Guide : Dr. S.K.Kar
University : Utkal University

14. Study of neurotrophic viruses causing encephalitis in adult and children.

Invesigator : Mr. Susil Kumar Rathore (ICMR-JRF)
Guide : Dr. B. Dwibedi
University : Utkal University

15. Origine and spread of drug resistance gene in P Phalcipaum population of Orissa

Investigator : Miss Sasmita Das Sutar
Guide : Dr. M.R.Ranjit
University : Utkal University

Dissertation Works for M.Sc. Students

1. Topic : DNA Polymorphism in Wucheria Bancrofti
Student : Sayeeda Banu, M.Sc (Appl. Microbiology), Utkal University
Guide : Dr. B. Dwibedi

2. Topic : Phenotypic characterization and detection of Toxic Genes of Laboratory stock of Vibrio Cholerae.
Student : Madhulita Patnaik, M.Sc.(Biotech) Ravenshaw University
Guide : Dr. B. B.Pal

3. Topic : Study on response of common antimalarial drug chloroquine in treatment of uncomplicated plasmodium falciparum malaria in tribal inhabited endemic areas of gajapati District of Orissa
Student : Pratyusha Kumar Dhal,M Pharm,Utkal University
Guide: Dr. A. S. Kerketta

4. Topic : Antibiogram study of uropathogens in a tertiary Care Hospital
Student : Arup Kumar Samal.M Pharm, Utkal University
Guide : Dr. A. Moharana

5. Topic : Genetic polymorphism of IFN- Gamma + 874 (T/A) and its association with different clinical categories of Filariasis.
   Student : Pragnya Paramita Jena, M.Sc.Biotech, Revenshaw University
   Guide : Dr. A. K. Satpathy

6. Topic : Status of VitamineA and Anemia among adolescent girls of Gajapati District of Orissa.
   Student : Isha Patnaik, M.Sc.Biotech, Revenshaw University
   Guide : Dr. G. Bulliyya

Training to School Students

Laboratory Visit by school Students: A team of 80 students from Jawahar Novodaya Vidyalaya, Munduli along with their teachers have visited RMRC, Bhubaneswar as part of the activities during Junior National Science Congress from 16-21 Nov. 2009. The students visited various departments and facilities in RMRC , Bhubaneswa the scientists and technical staff have trained the various techniques on laboratory procedure.

Facilities

Library, Information & Publications

The main aim of the Library & information centre is to provide relevant and latest biomedical information in the shortest possible time, to the researchers and biomedical scientists of the institute. Few years back information needs of the users were catered from MEDLINE CDROM (Off-line database). Now that trend has completely changed by providing online literature search through Internet and Online journals either through open access source or E-Journal consortia, The Library is fully WiFi enabled. This Wifi service at RMRC Library is great help to all Student and Researchers for preparation of manuscripts, projects and online literature search.

It provides both library and Information services not only to the scientists and researchers of this Centre but also to the researchers, doctors and academicians of this state. The foreign journal collection of this library is unique in orissa in the field of bio-medical sciences. For the calendar year 2009 & 2010 the library subscribes 41 foreign journals and 35 Indian journals in various fields of medical sciences and public health. The library possesses LAN printer- cum -digital Copier,in order to provide library, Information and reprographic support to its users. The library is computerized with Libsys-IV Library Management software. The library and information centre provides Local Area Networking (LAN) facilities to all scientists and researchers through dedicated LAN server from 9 A.M to 10.30 P.M. In publication activities, the library publishes Bi- Annually RMRC News Bulletin. Besides that, the library works as publication cell of the Centre which publishes periodically IEC materials on various diseases, Posters and pamplets on research findings. The Library and Information division publishes Annual Report of the Centre and monitor Publications of the scientists for scientometric Analysis by Council.

ICMR E- Resources

ICMR-EJC | JCCC@ICMR | ERMED | Science Direct

Online Journals

ICMR-EJC: ICMR E- journal Consortia : Through ICMR E -Journal consortia, all 26 ICMR Institutions of the country are able to access following five high impact weekly journals of the world where all latest research findings come out.

Journal	Web site
Science	http://www.sciencemag.org
NEJM	http://content.nejm.org
BMJ	http://www.bmj.com
Lancet	http://www.sciencedirect.com
Nature	http://www.nature.com

ERMED Consortia

In this consortium more than 2000 Medical journals are accessible at www.nmlermed.in. The participating libraries are National Medical Library (NML), all 26 ICMR libraries, AIIMS library, JIPMER library and other DGHS libraries. All journals are RMRC IP activated.

JCCC@ICMR: JCCC is J-Gate Custom Content (JCC) for a group of homogeneous consortia members developed by Informatics, Bangalore. JCCC@ICMR is an extension of JCC, for the Indian Council of Medical Research (ICMR). It covers 1941 journals received collectively at 29 institutions/centres of ICMR. It provides both abstract and full text articles and also facilitates for reprint request for all ICMR participating libraries.

Science Direct

For the calendar year 2009 and 2010 Library & Information division subscribes Elsevier's online package Science Direct (Immunology & Microbiology) bundle which carries 87 journals as per list enclosed. These journals are RMRC IP activated. The scientists can down load the full text of current issues and back files from 1995 Onwards.

Publication Cell

The library & Information Centre is doing publication activities of the Institute. RMRC News Bulletin and Library News Letter are being published from this division. IEC materials on various diseases are also made on local languages for distribution to the public. Besides, the division looks after the publication of Centre's Annual Report. Head of the library acts as editor of Library News Letter and Asst. Editor of RMRC News Bulletin.

The following are the publications from RMRC being published from library division.

RMRC News Bulletin

Library News Letter

IEC Materials on various diseases on Malaria, Filariasis, Sickle cell diseases, IDD, in regional language.

Posters on recent advances in Fiariasis, Malaria, Sickle cell diseases, and diarrhoea for children.

Library Trainee

The library & information division of the Centre have recruited two Library Trainees for the period of one year for Library automation purposes. The two trainees (Miss. Snigdharani Sahu and Miss. Ruchismita Prusty) are recruited as per Govt. of India apprentice scheme. During their practical training they have learn various facets of library and Information Science like, Classification in UDC, working on Library Automation software Libsys, News clipping activities, and day today job of the library.

Animal House

The center conducts experimental studies on animals. Animal facility in the center continues to be used for all research projects requiring animal experimentation. Currently Rabbits, M. Coucha, Balb/c mice, and Guinea pigs are available for experimentation. This animal facility has been registered with CPCSEA. All the projects concerning animal use/ experimentation are discussed in Animal ethical committee of the center. The facility is well maintained by animal house attendants. Staff has maintained periodic records of animal house. Pelleted feed procured from NIN, Hyderabad has been provided to the animals. Dr N.N.Mandal RA, Mr J Naik and Mr P. Sahoo have undergone an adhoc training course in The Care, Breeding & Experimental Techniques of Laboratory Animals in NIN, Hyderbad. Staff has maintained periodic records such as Form-C, Form-D etc of animal house as per provision of CPCSEA. This facility is maintained regularly with periodic inspection and health monitoring by veterinarian. Animal ethical Committee meeting was held to review the research work on animals.

Insectarium facility

At Present the centre has one insectorium which was developed before 19 years. Here cyclic colony of three genus of mosquitoes i.e. Aedes aegypti (LV strain), Anopheles stephensi and Culex quinquefasciatus maintained. The reared mosquito species were used in insecticide susceptibility status test, larvicidal bioassay plant extract bioassay test.The different plant extract having larviciding properties tested in our insectorium by our scientist and scientist from other Institute also send their material for testing. Cyclic development of Brugia malayi L3 developed and different aspects of and immunological studies were carried out by our scientist of the Institute. The insectorium was used for giving training to different persons time to time.

Now we are proposing for conducting virology work ie on Chikungunya, Dengue, West Nile and JE so proper maintenance of Aedes aegypti, Ae.albopictus and Culex vishnui group of mosquito will be

maintained so a special infected room will be maintained with utmost care so that a single mosquito can not be escaped.

To investigate the interaction between parasites and mosquito under natural conditions, An. stephensi will be fed on infected human blood, using the artificial membrane feeding technique. Gene expression will be monitored at 14, 24, 48 h and 10 days post infected blood meal, corresponding to the transformation of zygote into ookinetes, to the interaction of ookinetes with the peritrophic matrix and mid gut cells, and to the migration and early differentiation of ookinetes into oocysts, and sporozoites stages respectively.

We are now planning to modernization of the insectorium which is required for the centre for conducting future work. In our plan we divided the entire facility into three section i.e. larval rearing space, adult rearing room and infected mosquito room. Necessary required equipment s for each space is mentioned in the planning.

Budget and Resource Generation

The total sanction budget in respect of the Centre (Non-Plan & Plan) for the year 2009-2010 is 7.86 Crore and sanctioned budget for 2010-2011 is Rs. 4.76 Crore up to Sept. 2010 . The Head wise expenditure for 2009-10 of the budget is shown below. The resource generation during 2009-10 is 1.5 Crore and for 2010-2011 is 4.0 crore from the extramural grant and Ph.D program through UGC, CSIR and others.

BUDGET OF RMRC (09-10), SOURCE : ICMR

Establishment	Administrative Expenses	Contractual Service	Others	Equipment	Capital
450 Lakh	90 Lakh	86.82 Lakh	4.62 Lakh	45.57 Lakh	109.38 Lakh

24th SAC Metting at RMRC held on 19-20 oct 2010

RMRC Fundation Day Metting

24th Scientific Adivisory Committee

1	Dr.D.S. Agarwal B-24, Swasthya Vihar Delhi 110 092	Chairman
2	Prof.J.P. Muliyil Dept. of Community Health Christian Medical College Vellore 632 002	Member
3	Prof.R.K. Mutat Kar 64-Anand Park Aundh Pune 411 007	Member
4	Dr.Subrat K. Acharya Prof,. & HoD Dept of Gastroenterology AIIMS, New Delhi 110 029	Member
5	Dr.Satish Gupta Staff Scientist-VII and Chief Gamete Antigen Laboratory Chairman National Institute of Immunology Aurana Asaf Ali Marg New Delhi 110 067	Member
6	Dr.D.A. Gadkari Ex-Director National Institute of Virology 20-A, Dr.Ambedkar Road Pune 411 001	Member
7	Dr.B. Sesikeran Director National Institute of Nutrition P.O:Jamai Osmania Hyderabad 500 007	Member
8	Dr.P. Jambulingam Director, Vector Control Research Centre Indira Nagar Pondicherry 605 006	Member
9	Director of Health Services Directorate of Health Services Govt. of Orissa Heads of the Deptt. Building Bhubaneswar	Member
10	Dr. Lalit Rout Chief ECD, ICMR	Member
11	Dr.S.K. Kar Director, RMRC, Bhubaneswar	Member Secretary

Human Ethical Committee Members

Dr. Kabi Prasad Misra Sr. Consultant Cardiologist & 55, Ganesh Nagar Gandamunda, Khandagiri Bhubaneswar 751 030	Chairman
Prof. Aruna Mishra Laxmi Vihar PO: Sainik School Bhubaneswar	Co-chairperson
Dr. P.K. Dash Director, Medical Education & Training Heads of the Dept Building Govt. of Orissa Bhubaneswar 751 001	Member
Mrs Kasturika Pattanayak Ex-Chair Person, Social Welfare Board Govt. of Orissa, 1, Lewis Road Bhubaneswar.	Member
Dr. P.K.Acharya N-1 A/10 IRC Village Near CRP Square, Bhubaneswar 751 015	Member
Dr. Sisir Kumar Mahapatra Sr. Consultant Physician Surya Nivas, Plot No:B-1/91 Lingaraj Vihar, Pokhariput Bhubaneswar 751 002	Member
Sri. Himadri Mohapatra Toshali Plaza, 2nd floor Satyanagar, Bhubaneswar	Member
Prof. Rita Ray HoD Sociology Utkal University Vani Vihar, Bhubaneswar 751 004	Member
Dr.S.K. Kar Director R.M.R.C., Bhubaneswar	Member Secretary

Animal Experimentation Ethical Committee

1.	Biological Scientist	:	Dr. M.R. Ranjit, M.Sc., Ph.D Regional Medical Research Centre Bhubaneswar.
2.	Two scientists from different Biological disciplines	:	Dr. R.C.Patra, Prof. & Head Dept. of Veterinary Medicine OUAT, Bhubaneswar - 751 003 Dr R.K. Hazra, Ph.D. SRO Regional Medical Research Centre Bhubaneswar.
3.	A veterinarian involved in the care of animals	:	Dr. S.K. Ray, Ex-Principal Orissa Coll. of Anim. Husb. & Vet. Sc. Qr.No.M-109 Baramunda H.B. Colony Bhubaneswar 751 003
4.	Scientist In-charge of the Animal facility	:	Dr. A.K.Satapathy, Scientist-D Regional Medical Research Centre Bhubaneswar.
5.	A biological scientist from outside the Institute	:	Prof. Sachidananda Das, Ph.D., PG Dept. of Zoology, Utkal University Bhubaneswar.
6.	A non-scientific socially aware member	:	Mrs Kasturika Pattanayak Ex-Chair Person, Social Welfare Board Govt. of Orissa, 1, Lewis Road Bhubaneswar.
7.	Main Nominee of the CPCSEA	:	Dr. Kishore Chandra Mohapatra Plot No:17, Gautam Nagar PO:BJB Nagar, BBSR 751014
8.	Link Nominee of the CPCSEA	:	Dr. Dwarikanath Mohanty Plot No:1215/1654, Khandagiri Bari Bhubaneswar 751 030
9.	Member - Convener	:	Dr. S.K.Kar, M.D., Director, Regional Medical Research Centre Bhubaneswar.

Technical Equipment Purchase Committee

1.	Dr. A.K. Sahoo Principal Scientist CIFA, Kausalya gang Bhubaneswar- 751 002	Chairman
2.	Dr. P.Das Sr. Scientist CIFA, Kausalya gang Bhubaneswar- 751 002	External Member
3.	Dr. N.K. Debata Prof. Microbiology SUM-I Iospital, Bhuabnesware	External Member
4.	Dr. M. R. Ranjit Scientist-D RMRC, BBSR Member (Sub. Specialist)	Member (Sub. Specialist)
5.	Dr. B. Dwibedi Scientist-B RMRC, Bhuabneswar	Member
6.	Mr. G.Behera Accounts officer RMRC, BBSR	Member
7.	Dr. A.K.Satapathy Scientist-D RMRC, BBSR	Member Secy

Technical Building Maintenance Committee

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Er. P.K. Pattanik Retd. Sup. Eng. (Elect.), CPWD	Member
Er. P.K.Mohanty Retd. Asst. Engineer (Civil)	Member
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Dr. B. Dwibedi Scientist-B	Member
Mr. G. Behera Accounts Officer	Member

Staff

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Dr. M.R. Ranjit, M.Sc., Ph.D.	Scientist-D
Dr. A. Mahapatra, M.Sc., M.Phil., Ph.D.	Scientist-D
Dr. A.K. Satapathy, M.Sc., Ph.D.	Scientist-D
Dr. G. Bulliyya, M.Sc., Ph.D.	Scientist-D
Dr. B.B. Pal, M.Sc., Ph.D.	Scientist-D
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Dr. R.K. Hazra, M.Sc., Ph.D.	Scientist-C
Dr. Bhagirathi Dwibedi, M.B.B.S, M.D	Scientist-B
Dr. A. Maharana, MBBS, M.D	Scientist-B

Technical Staff

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Mr. H.K.Jena	Attendant (Services)
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Mr. Baburam Behera	Attendant (Services)
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Students

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Rasmi Mishra, M.Sc	SRF (ICMR)
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Mr. Rajendra Mohan Kissan	Apprentice Library Trainee
Mr. Chakradhar Naik	Attendant (Services)

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Mr. P.K. Behera	Driver

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Mr. R.K. Sahoo	Driver
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Printed at : Textbook Bureau, BBSR, Ph : (0674) 2541162