Annual Report 2011

Regional Medical Research Centre

RMRC

100 Years of ICMR 1911-2011

# Annual Report 2011

## Regional Medical Research Centre
### (Indian Council of Medical Research)
### Bhubaneswar

Centenary
Year Celebration of
ICMR

1911-2011

# 100 YEARS OF BIOMEDICAL REASEARCH IN INDIA

# Annual Report 2011


## REGIONAL MEDICAL RESEARCH CENTRE
## BHUBANESWAR

# Contents

# From the Director's desk

The Centre continued its research endeavor in areas of vector borne diseases, diarrhoeal disorder, nutrition, viral diseases and MDR TB during the period (2011) along with translational research programs. Two new research field units in tribal areas of Odisha have been added to address health problems of the tribal population in close collaboration with Dept. of Health & Family Welfare, Govt. of Odisha.

During this period 22 scientific projects we were undertaken by this Centre , of them 17 were funded extramurally; sponsored by either Gates Foundation, DST, DBT, ICMR ,CSIR Task Force or NVBDCP. Of these 15 are ongoing and 7 got completed with logical conclusions. During the year 2010, the Centre has published 18 research papers and 13 during 2011. All the publications were published in indexed journals out of which 23 are in SCI Journals. The average impact factor for 2010 publications is 2.215 and 2.127 in year 2011 till date. The Center's library subscribed 32 foreign print journals and 30 Indian print journals for the year 2011 along with online subscription of 87 titles from Science Direct. Besides, the Centrer's library is a member of ICMR E-journal Consortia, NML's ERMED Consortia and JCCC@ICMR through which more than 4000 journals are accessible. The Institute is now connected with 100 MBPS NKN Leased line which is operational round the clock.

The Centre has generated Rs 4.5 crore through sponsored research in year 2010 & 2011. 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. During the period four scholars have submitted their thesis under Utkal University, Bhubaneswar and one has been awarded.*

*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 and Oral Cholera Vaccine was introduced in Public health setting for the first time in the country.*

*Collaboration with State Health Dept was strengthened in form of consultancy, undertaking evaluation of health programmes, diagnosis of referral cases in areas of Centre's expertise and investigation of epidemics and disaster management.*

*With total staff strength of 102 at present 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 to be filled up.*

*The Centre had organised several scientific meetings and symposia during the period to strengthen scientific interaction with local medical colleges and expertise from various other fields. During the centenary year of ICMR the Centre has organized the 8th joint Annual Conference of ISMOCD and IAE. Two workshops on Research Methodology were conducted inviting medical faculties and students from five states in the region.*

*During the year several developmental activities of the Centre were undertaken. Laboratory up gradation activities are ongoing including infrastructure modification by CPWD and procurement of modular LAB furniture from Godrej. Virology (Gr-I) Laboratory and TB culture Laboratory were completed and Inaugurated. 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 endeavour 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. The Centre will continue with its endeavour to achieve its goal.*

**DR. S. K. KAR**
**DIRECTOR**

# Highlights of Research Activities

This year the centre has addressed various clinical trials and applied research issues on filariasis, malaria, diarrhoeal disorders,nutritional disorders and viral diseases; besides translational research issues on Diarroheal disorder and Malaria. The centre is also continuing HRD programme and research programs to support the state public health system. In collaboration with the State Health department, Govt. of Odisha, two new field Units have been established at Raygada and Kalahandi to study the health problems of tribal populations and transfer of technology.

Mass drug (DEC) administration is being undertaken in the country with single annual dose of DEC (6 mg/kg) with albendazole targeting elimination of lymphatic filariasis by 2015 as per national health policy. But due to fear of side reaction and confusion in distribution of three dosages of DEC to different age groups at community level population compliance of the programme is limited. An open community based field trial conducted for last four years in three endemic villages has indicated comparative efficacy of 100mg of DEC to higher dosages like 200 or 300mg in reducing micrfilaremia prevalence. Xeno monitoring of vector populations showed > 75% reduction in infectivity rate with all the three dosages.The side reaction frequency and intensity was found to be significantly low ($p<0.05$) in lower dose (100mg) in all four rounds of MDA. The effect of fifth round of MDA is being assessed for the above parameters to check sustainability of the drug efficacy at three dosage levels.

Though circulating filarial antigen indicating established filarial infection is reported to be around 30% in pediatric age group in endemic areas overt clinical manifestation usually appears in late adolescence and adulthood. There is no evidence on lymphatic abnormality if any in *W bancrofti* infected children that can become forerunner for adult disease expression. There is need to generate evidence on such lymphatic pathology during this clinically silent phase in infected children and to look for opportunity of possible reversal with MDA. The results can be utilized in the current national programme as an advocacy tool for enhancing drug consumption in children. Continuing study with 57 endemic filarial infected children

between 5 to 18 years of age without overt clinical filarial manifestation subjected to ultrasonography and lymphoscintigraphy, indicated lymphatic pathology in 63.1% children visualized in form of lymphatic flow obstruction, lymphatic collateral channels and lymph node enlargement and presence of adult filarial worm in scrotal/ inguinal region in 14% children. All the enrolled children were given first dose of DEC plus Albendazole. Interestingly repeat lymphoscintigram after 6$^{th}$ and 12$^{th}$ month of treatment showed improvement in lymphatic flow in 68% (n=21) of those initially exhibited pathology.

The current Mass Drug annual administration (MDA) programme uses DEC (300mg) with Albendazole (400mg) to eliminate filariasis within 5-6 years. 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. A randomized open clinical trial was undertaken to find the comparative efficacy of the four arms((S1: DEC(300mg) +Alb(400) annual, S2: DEC(300mg) + Alb(400) biannual, H1: DEC(300mg)+Alb(800) Annual, H2: DEC(300mg)+ Alb(800) Biannual), recruiting 104 microfilarimics. Two years continuing trial indicated the group receiving 800mg of Albendazole with DEC (300mg), given biannually achieved higher clearance of Mf and disappearance of adult worm evidenced by absence of filarial dance sign in ultrasonogram at twelve month post drug. Out of 104 enrolled subjects 57.6% manifested side reactions (after first dose) like fever, headache, malaise, reeling head, drowsiness, nodule and testicular pain of mild to moderate grade. There was no significant difference in frequency of side reaction between Albendazole 400 & 800 mg group. The interim results indicated higher efficacy of high dose Albendazole.

The interim study on exploring possibility of filarial immunity acquired through maternal infection indicated influence of maternal infection on the development of subsequent anti filarial immunity in offspring. It was also investigated, the extent to which maternal filarial infection have sensitized or biased immune responsiveness in neonates towards a Th1 or Th2-like phenotype. Increased levels of IL-10 (Th-2) and down regulation of IFN-$\gamma$ (Th-1) have been detected in cord blood of children born to filarial infected mothers. High level of T- Regulatory cells and increased production of IL-10 in cord blood from infected mothers indicated that increased T-regulatory cells could down regulate inflammatory responses and may be associated with parasite survival.

In a separate study, the role of B-1 cells in human lymphatic filariasis is being evaluated.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) raised the possibility of poly reactive property of these antibodies. Demonstration of polyreactive property of ss-DNA antibodies in this study further indicated an important role for these antibodies to provide host protection against filarial infection.

A recent study comparing between uncomplicated and severe malaria indicated significantly diminished rosetting frequency in "O" blood group of patients compared to "A" and "B" blood group of patients. Type "O" blood group has been observed to be associated with protection against cerebral malaria (CM) (OR: 3.3, P < 0.0001), while type "A" and "B" had 2.5 and 5.0 fold risk of developing CM compared to "O" group respectively.

The State of Odisha 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 and Mohana block of Gajapati district during the period under report. The causative organism was El Tor variant of *V cholerae* which has shown resistance to Tetracycline, that was reported to state health department.

A phase four open trial in public health setting with oral cholera vaccine was undertaken for first time in the country , in a cholera endemic area of Odisha to determine the feasibility, acceptability and costs associated with the pilot introduction in collaroration with State Health department, International Vaccine Institute, South Korea and NICED, Kolkata.. Detailed census, GIS mapping, social mobilization and training to State Health staff was undertaken. Then vaccine was delivered in two doses with 14 days interval to 31562 populations (Children less than one year and pregnant women excluded). The mass vaccination of OCV within the public health setting was acceptable with coverage of 61%. The vaccine was safe with out any major side effect. The results of the efficacy of vaccination are being studied.

A randomized open trial has been designed to assess the effectiveness of 5 regimens using iron-folic acid, vitamin $B_{12}$, deworming supplementation, and nutrition education in controlling iron deficiency anemia among tribal adolescent girls in Gajapati district. Base line survey among 1025 adolescent girls revealed anemia in 90% (hemoglobin <12g/dl) of the samples, of which 36% had hypoferritinemia and 51% iron deficiency. Besides, other nutrition deficiencies like vitamin A by serum retinol (30%), folic acid (33%) and vitamin B12 (30%) was observed. The result of intervention indicated 5$^{th}$ regimens to be more effective as compared to other four regimens.

Epidemic of Dengue was reported from Odisha affecting 23 out of 30 districts. Dengue antigen (NS1) was detected in 45% of the cases and dengue IgM antibody in 15% of cases. Dengue serotype II virus was confirmed as the circulating strain. Chikungunya outbreaks have been continuing to occur in the coastal districts of Odisha since 2007. Eight districts were affected during the year which were investigated. *Aedes albopictus* was the most abundant vector with very high container and Breteau indices. Phylogenic analyses of partial E1 gene revealed circulation of ECSA genotype in the affected areas.

Investigation of viral infections from hospital based ( OPD and Indoor ) investigation and focal outbreak samples referred through state health system was undertaken in the

newly established virology Laboratory. It has shown presence of Rota Viruse in 20% of cases of diarrhoea which belonged to Genotype G ( G2, G4 and G9) and P (P4 and P9). Other enteric viruses were Noro G1 (3.7%), Noro G2 (1.5%), Astro(1.2%) and Adeno (22.3%)causing diarrhea and HAV(6.7%) and HEV(17.8%)causing hepatitis.HBV genotype D and HCV genotype 1b were the parenterally transmitted hepatic infections. Flu A (22%), H1N1 (18.6%), Rhino (13.8%), Para influenza (20%), Adeno (30.6%), Boca, HMPV and Parecho viruses were detected as the viruses causing respiratory infections especially in children. Viruses that cause encephalitis were also investigated. Herpes simplex virus was detected in 6%, Herpes Virus II in 2% and Japanese encephalitis was detected in only 1 subject.

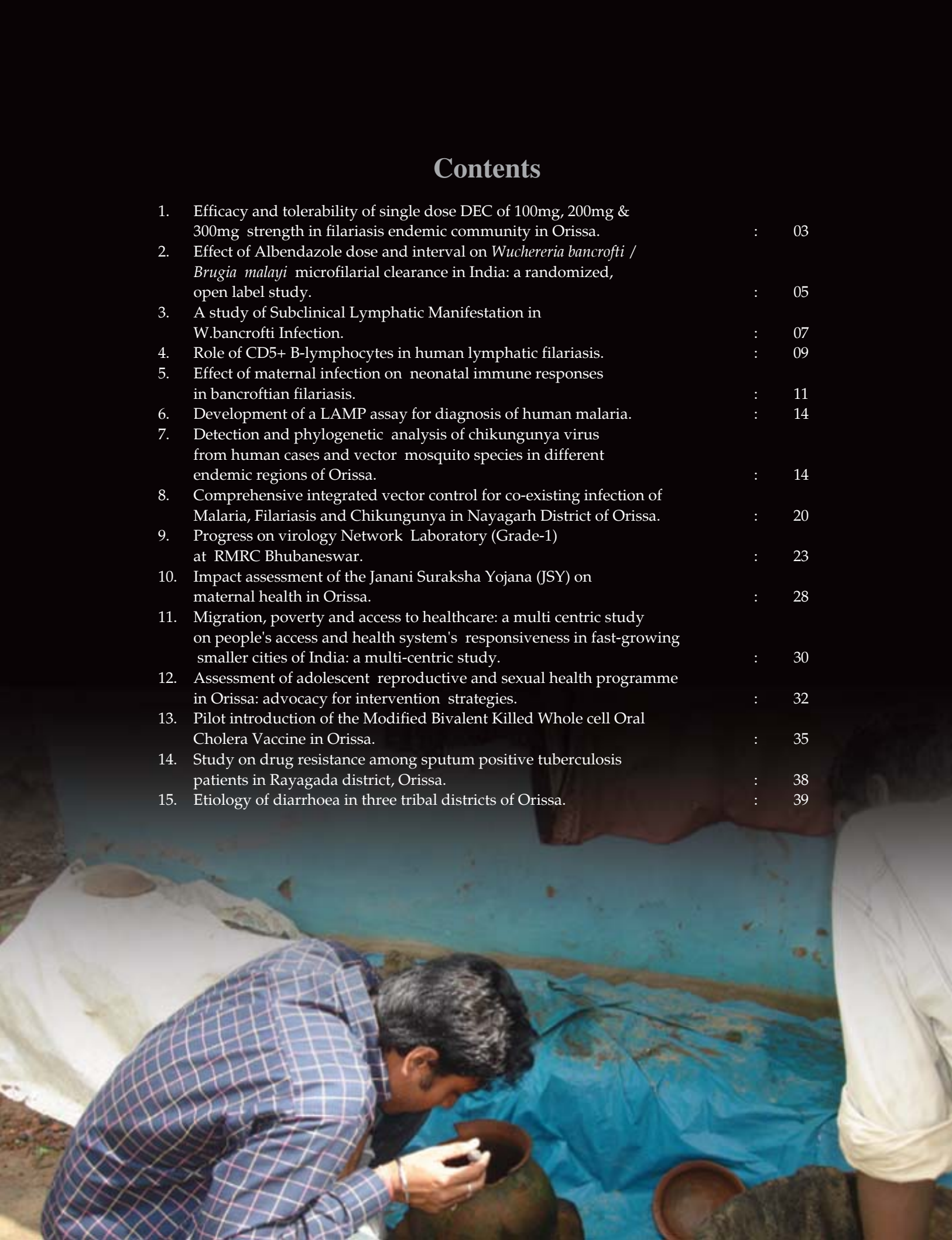
Under translational research the centre has developed two multiplex PCR based tools. One is to monitor the information of vector prevalence, incrimination of vector for malaria transmission, identification of the sibling species of vector and chloroquine (CQ) sensitivity of the parasite ingested by the 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 in this region. New areas of research like LAMP assay for field diagnosis of Malaria and development of cholera control strategy have been identified to have translational potential and being persued.


Dr. V.M. Katoch, Secretary DHR & DG, ICMR laying the foundation stone for establishment of BSL-3 lab. in the RMRC premises.

# 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 PIs | : | Dr. S. K. Kar |
| | | Dr. N. Mahapatra |
| Starting date | : | March 2006 |
| Duration | : | 5 years |
| Status | : | Intramural |

### Objectives:

1. To compare the efficacy of single dose DEC of 100mg strength in mf suppression with either 200mg or 300mg given uniformly in all age groups as annual doses of MDA in 3 different endemic communities.
2. To compare the frequency and intensity of side reaction of DEC observed with three different strengths given as annual dose of MDA.
3. To observe the effect on vector transmission in three different communities following annual uniform doses of DEC of varied strength.

This need to observe followings :

(a) Mf suppression effect of 100mg/200mg or 300mg DEC given as single annual dose of MDA.

(b) Effect on community load of microfilarimia

(c) Effect of low dose DEC of 100 mg compared to either 200 or 300mg in higher age groups 6-14 or above 14 yrs.

### Application of the research for National Health Policy:

Mass drug (DEC) administration is being undertaken in the country targeting elimination of lymphatic Filariasis by 2015 as per national health policy statement of 2002. The programme was based on mass administration of single annual dose of DEC in the recommended dosage of 6mg/kg body weight averaged for three age groups i.e. 2-5, 5-14 & >14 years. But the population compliance of the programme is limited largely due to fear of side reaction of the drug regimen and also by confusion in three strengths of the drug dosage distributed by volunteers to various age groups. The present study aims at generating evidence for use of low dose DEC given in uniform doses for all age groups to observe, whether it is equally efficacious and better tolerated. This result may help the national programme for elimination of filariasis in increasing the population compliance of the drug.

### Background & Study Outline

Three filarial endemic areas were selected and population census carried out. Baseline screening of mf and antigenimia undertaken on subjects providing consent. IEC activities organized in the villages about the purpose of the study, MDA and follow up evaluation. Then four rounds of annual MDA instituted in the three sites. Each site was randomly allocated for either of three 100mg, 200mg and 300 mg DEC regimen given uniformly to all ages. Population below 2 years, pregnant ladies and critically ill individuals were not covered with DEC. DEC administration was supervised and population was followed for a week for any reported side reaction for record and this was managed at house hold level. The population was followed up with annual investigation of Mf count and antigen detection after each sucessesive annual round of MDA. This was done by collection of night blood by finger prick collection. Thick blood smears made from 40µl blood for Mf count. The collected slides were coded, stained and mf count done by trained technicians. Vector survey was carried out at baseline and annually each year in three seasons for vector density, vector infection & vector infectivity after MDA and compared. *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.

All the information recorded in pre designed format, entered into computer using Excel and SPSS software and analyzed with help of statisticians of the centre and VCRC, Pondicherry.

It was planned to continue the assessment of effectiveness of the three dosages for five successive annual rounds of MDA. The study was recommended by ethical committee.

**Progress:**

Baseline parameters studied in 3 sites were comparable. The baseline investigation and the observations made following three annual rounds of MDA were detailed in the previous annual reports. Fourth annual round of MDA was instituted in May 2010 in the three sites and MDA coverage and side reaction were recorded. The study population was surveyed for microfilaremia by night blood survey at 48th month follow up and results are outlined below.

(a) DEC coverage and frequency of side reaction: Coverage of population for DEC intake was 74, 64 & 70% respectively for 100, 200 and 300 mg sites and frequency of side reaction was noted to be 0.87, 4.8 and 3.4% respectively. The side reaction frequency was found to be significantly low ($p<0.05$) in lower dose (100mg regimen) site in all four rounds of MDA (Fig.1).

(b) Change in community microfilariamia rate: With four rounds of MDA the mf rate was noted to be 0.12%, 0.15% and 0.11% in the three sites. There was comparable decline in the mf prevalence rate in the low dose (100mg) compared to 200 and 300 mg doses.

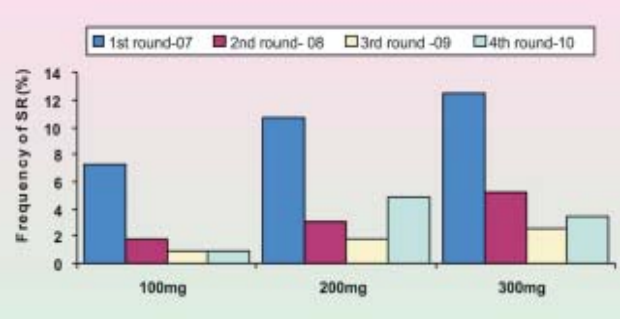
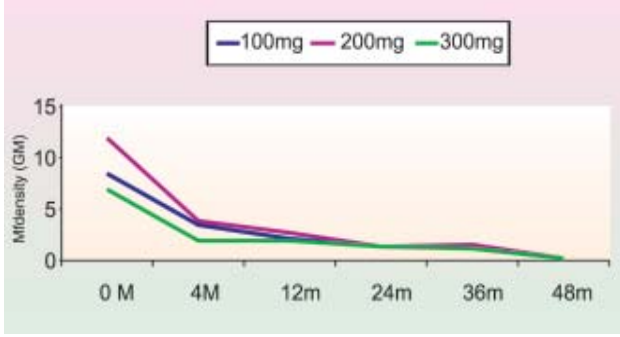



**Fig. 1.** Frequency of side reaction to DEC in three regimen groups in 4 successive annual rounds.

(c) Clearance of microfilarimia among subjects found mf positive at baseline: The individuals who were mf positive at baseline were followed at 48 months which shown mf clearance in 88.5% - 94.1% cases. ($p <0.01$). There was a similar reduction (84.28-86.4%) in the microfilarimia load expressed as geometric mean of mf density.

Mf count (geo mean) in the microfilarimic at 4th, 12th, 24th, 36th & 48th month.




(d) Change in vector infection parameters: The baseline vector survey showed the average per man hour density of *Culex quinquefasciatus* to be 34.8, 41.6 & 30.1 % in 100, 200 & 300 mg regimen. After 4th round of MDA, as the infective population of mosquito was reduced to low level using xenomonitoring tool. The DNA of the developmental stages *W. bancrfoti* parasites were detected or calculating infection and infective rate. The overall reduction after 4th round of MDA showed 81.4, 78.2 and 83.3% reduction in infectivity rate in 100, 200 and 300 mg regimen. Reduction in L3 load was also seen in all the three area after MDA.

**Subsequent plan of activity**

Since the 48th month result indicate comparable reduction of both mf frequency and density in all 3

sites with less side reaction, frequency in lowest dose of MDA continue the study for one more year. The study population will be subjected to night blood examination for microfilarimia and antigenimia at 60 months following fifth annual round of MDA. Vector survey will be conducted in the three regimen areas to look for the vector infection and transmission parameters. The effect of fifth 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 PIs | : | Dr. B. Dwibedi<br>Dr.A.S.Kerketta<br>Dr.S. S.Panda |
| (Dept. of Medicine, KIMS Hospital, BBSR) | | |
| Starting date | : | October 2008 |
| Closing date | : | April 2012 (Extended) |
| Funding | : | Extramural (GATES Foundation, USA) |

### Aims & Objectives:

1. To determine whether increase in dose or frequency of albendazole administration in MDA regimen is more effective in clearing microfilarimia in *Wucheraria bancrofti* microfilaria-positive cases.
2. To assess the effect on adult worm burden as assessed by Doppler sonography.

### Background

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 in combination with albendazole is used. While DEC is a powerful microfilaricidal agent, Albendazole possibly plays role in sterilizing adult parasite, hence suppressing resurgence of mf, when applied at 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. Hence the study was undertaken as per open level clinical trial with four treatment arms to look for a better efficacious regimen.

Clinical trial was registered. Ethical clearance was obtained by Institutional ethical committee. The study period was extended for 6 month (Up to April 2012) with approval from the funding agency, considering the follow up period as per baseline enrollment.

### Progress of work

#### Screening

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

#### Enrollment

All the 104 eligible subjects (88 male, 16 female) have been enrolled to the study at baseline. The patients were admitted to the hospital (KIMS, Bhubaneswar) in small batches of 3-5 persons for drug treatment after obtaining their consent. Each subject underwent pre drug 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 allocation to drug regimens (four)

The 104 microfilariaemic subjects were randomly assigned to one of the each drug regimens as given below.

**Table 1:** Treatment regimen and no. of subjects

| 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 |

### Baseline mf and FDS status

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. The study groups were comparable in terms of distribution of individuals with low and high mf density (Table 2). 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 were detected with norm nests showing Filaria dance sign (FDS) indicating presence of adult parasite in their lymphatics.

Table 2: Distributions of Microfilarimics in four arms

| Treatment arm | 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 |

### Follow Up

As per protocol the individuals were followed six monthly for investigation and treatment. Till date 104 subjects were completed follow up for 6 months, 103 subjects at 12 months, 93 subjects for 18 months and 38 subjects for 24 months. The Mf count & Og4C3 were repeated at $6^{th}$ , $12^{th}$ , $18^{th}$ & $24^{th}$ month. USG was repeated within 3 days of baseline drug intake and at 1 year and 2 years, only on the cases showing positive FDS in baseline assessment.

### Mf clearance

Mf clearance was observed to progress over time in all 4 regimen groups. The number of subjects with total mf clearance was noted to be 9, 19, 51 & 26 in all 4 regimens together at 6, 12, 18 & 24 months out of the subjects tested at each point. Significantly higher clearance was observed with H2 group (71.4% at 18 month) compared to other regimens.

### Mf density

There was sharp reduction in the microfilaria density (geo mean) at 6 month in all 4 regimen

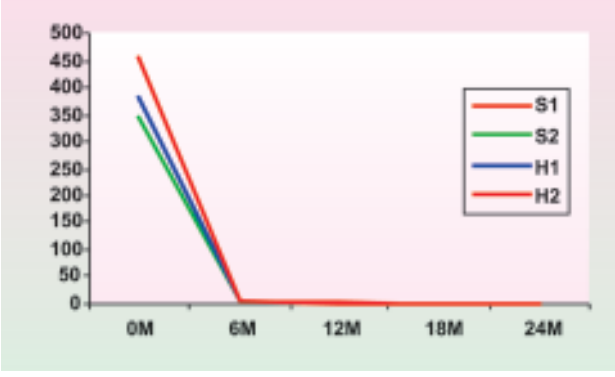



Fig. Change in mf density (GM) in 4 regimen

(98.3-99.3%) which was maintained with slow reduction in subsequent follow ups (12, 18 & 24 months) (Fig.1)

### Antigenimia

The mean antigen titre over 18 month period post drug dose not shows any subsequent reduction (51.7 - 82%).

### FDS in Ultrasonography

USG was done as third day following first drug dose with subjects showing FDS at baseline to see any immediate effect of DEC + Alb on adult parasite. It was repeated at 12 month & 24 months. At initial level 14, 15, 13 & 13 cases had visible FDS in the 4 arms. Percentage reduction of FDS observed on 3rd day was 7, 13.3, 23 and 38 in S1, S2, H1 & H2 doses respectively. At 1 yr percentage reduction was highest (92%) in H2 arm out of cases followed.

### 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. There was no significant difference in frequency of side reaction between Albendazole 400 & 800 mg groups.

52 subjects received the second dose at 6 month out of which six developed side reaction. 103 subjects received the annual dose at 12 month, out of which 9 had shown side reaction. At 18 month 44 subjects (S2: 24 & H: 20) received the drug and 4 had developed side reaction. All side reactions were mild, no severe adverse effect was reported.

### Plan for next year

All 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 Sub-clinical Lymphatic Manifestation in *W. bancrofti* Infection.

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

### Background

Several reports from filarial endemic regions globally including Odisha indicated that while most of the endemic children (25-30%) below 5 years of age get infected, overt clinical disease appears later in life, ie. late adolescence or adult hood. It is not clear about any pathology that develops following infection till the clinical signs appear. Lymphoscintigraphy evidences suggest sub-clinical lymphatic abnormality in mf carriers who does not show any clinical signs. Study on *B. malayi* infected children (3-15yrs) has shown evidence of sub clinical lymphatic pathology in form of lymphatic obstruction.

It was proposed to undertake an observational study to find out any 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.

### 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.

### Progress of work

### Screening and Enrollment

Screening was done in 13 endemic villages from Khurda district, Odisha for presence of mf and antigenemia covering 1343 children between 5 to 18 yrs of age. 66 subjects were identified as Mf positive and 36 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 (ALT, Hb%, serum creatine, urine pregnancy test) were conducted.

So far 57 subjects were enrolled after obtaining their parents consent who satisfied eligibility criteria, of which 31 subjects were assigned randomly to annual and 26 to biannual dose (DEC + Albendazole) group. Out of 57 subjects, 23 were symptomatic and rest of the children were asymptomatic, but with detectable mf and/or antigenimia.

Out of 34 asymptomatic children 16 were mf and antigen positive where 18 were positive only for Og4C3 antigen. In 23 symptomatic children clinical signs or symptoms of filariasis were observed in form of presence or history of lymphadenitis, lymphedema, testicular enlargement or hematuria. Out of these children 14 were positive only for Og4C3 antigen and rest were positive both for mf and antigen.

### Baseline investigation and follow up

All the subjects enrolled at baseline (n=57) had undergone lymphoscintigraphy and ultrasound examination. They were given the first dose of DEC + Albendazole in the dosages prescribed for their age and followed 6$^{th}$ monthly as per randomization. Till date 31 children completed 6 month follow up and 21 completed follow up for 12 month. All the investigations were repeated 6 monthly. The lymphatic abnormality noted at baseline was compared with the subsequent follow up results which is outlined below.

In the enrolled subjects, the initial microfilaria (mf) count ranged from 2 to 1540 mf/ ml (GM=208.75) 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 labeled sulphur colloid. The procedure was standardized before initiating the study. Effect on baseline pathology was evaluated by comparing the scintigraphic observation made at the follow up visit with the pretreatment (baseline) findings. The scintigraphic image showing visualization of lymph nodes and lymphatic channels on both the limbs and the tracer uptake ratio from the distal end of the limb was compared with the baseline observations in the same limb, to interprets on the lymphatic flow/ pathology and improvement if any.

Out of 57 subjects 36 (63.1%) had shown some abnormality in the lymphatic scan at baseline. Among them 36 had lymphatic flow obstruction, 8 had increased lymphatic collateral channels and 4 had lymph node enlargement. The earliest age showing lymphatic pathology was 6 years among the studied children. Ultrasonography has shown filarial dance sign (FDS) of adult worm in 8 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. Among them 10 (17.5%) children reported to have 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. During 6$^{th}$ month 9.6% children had side reaction, while at 12$^{th}$ month none reported of any adverse event.

Out of the 57 children (23 Symptomatic & 34 Asymptomatic) enrolled at baseline 31 (11

Symptomatic & 20 Asymptomatic) and 21 (10 Symptomatic & 11 Asymptomatic) have completed follow up investigation at 6 & 12 month respectively. Result of repeat lymphoscintigram at these time point compared with the baseline status has shown improvement in lymphatic pathology hence lymphatic flow in 68% (n=21) and 73% (n=12) of children who had baseline abnormality and followed at 6th and 12th month period respectively.

### Subsequent Plan

It is planned to enroll 100 subjects into the study. To achieve this screening for microfilarimia and antigenimia will continue for selecting 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 for 2 years.

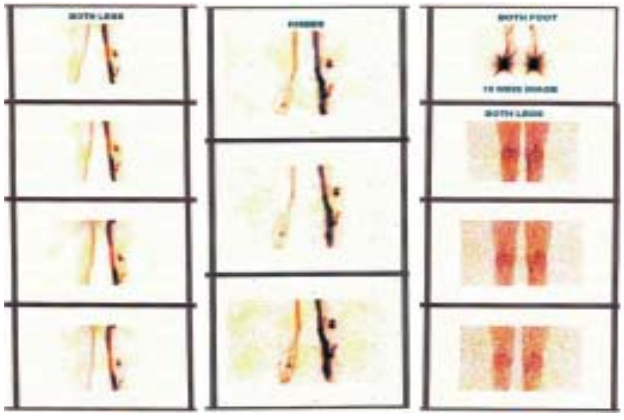



**Fig. 1**. Lymphoscintigraph of lower limb in a 12 Yr Male symptomatic child (MF & Og4C3 positive) showing lymphatic flow obstruction on left side at baseline and normal lymph flow at 12 month post Rx.

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

| | | |
|---|---|---|
| Principal Investigators | : | Dr A.K. Satapathy |
| Co-Investigators | : | Dr B. Dwibedi |
| | | Dr P.K.Sahoo |
| | | Dr S.K.Kar |
| Duration | : | Three years |
| Starting date | : | April 2010 |
| Closing Date | : | March 2013 |
| Status | : | 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 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. Several studies have shown that B1 subset of B cells play an important role in the outcome of infection in schistosomiasis, S. pneumoniae and experimental filariasis. However, whether the biological role played by B1 lymphocytes to provide host protection against filarial infection is largely unknown. In our previous year study we monitored the levels of B-1 cells ($CD5^+$ and $CD19^+$) in the clinical spectrum of lymphatic filariasis. B-1 cells were found to be low in microfilariamic patients. In normal humans and mice, B-1 cells produce antibodies that are mostly polyreactive nature and have low affinity. Most B-1 cells produce IgM, which bind to a variety of self-antigens. Therefore an attempt has been made to study the poly reactive antibodies in filarial infected population.

### Progress of work

In normal humans, B-1 cells are committed to the production of polyreactive natural antibodies. 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. Sera collected from different clinical manifestation such as chronic filariasis (CH), mf carriers (AS), cryptic (CR) and endemic normals (EN) were tested for their reactivity to a variety of antigens such as actin and ss-DNA and bacterial lipopolysaccaharides. We quantified IgG antibodies reacting to the SS-DNA in the spectrum of clinical manifestations. Ig G

antibodies to ss-DNA and lipopolysacharides are shown in Fig-1 and Fig-2. Significantly a low level of Ig G antibodies to ss-DNA was found in microfilarial carriers as compared to chronic patients. Patients with microfilariae seemed to have a lower response to actin than patients in the other categories of the disease as shown in fig-3.

Fig. 1

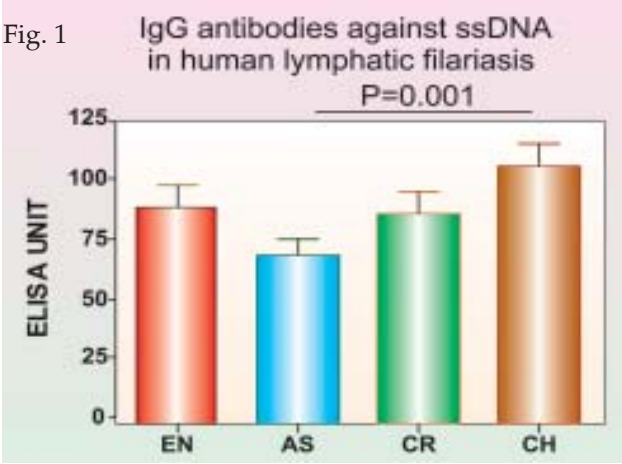



Fig. 2

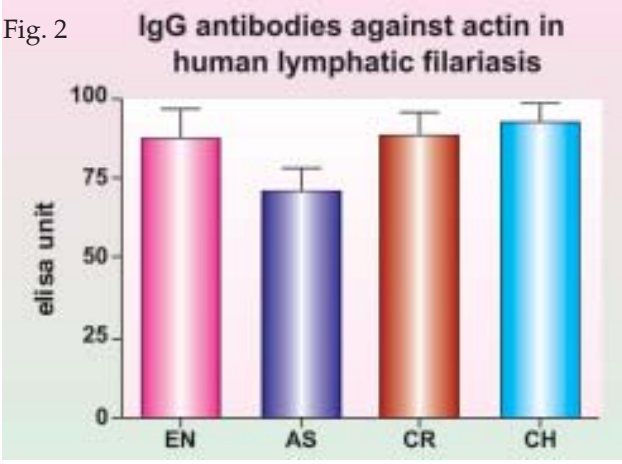
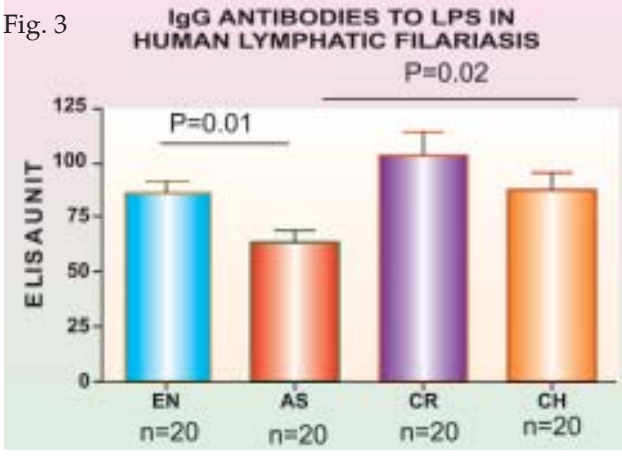


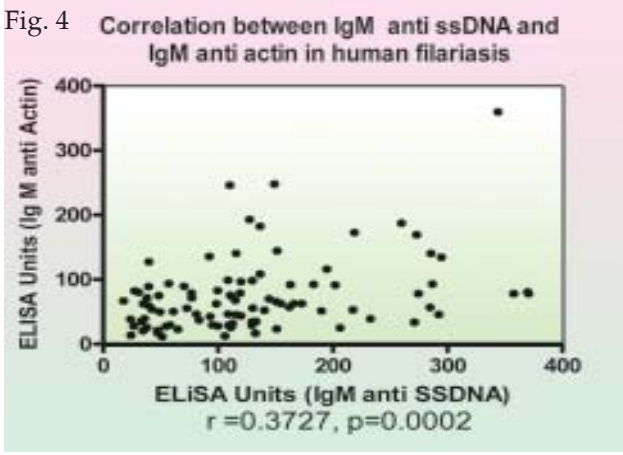

Fig. 3




B-1 cells are committed to the production of polyreactive natural antibodies mainly of IgM, In the previous annual report We have shown Ig M antibodies responses in human filarial sera reacting to lipopolysaccharide, SS-DNA and actin are significantly low in mf carriers cases in comparison to endemic normals and chronic patients.

Decreased levels of Ig G & Ig M antibodies response were observed to various antigens in mf carriers. Since reactivity to SS-DNA is the hallmark of polyreactive antibodies, 96 human sera were tested by ELISA for reactivity to ss-DNA and actin. A significant positive correlation was found between anti-actin and anti-DNA levels (r-0.372, P=0.0002) as shown in Fig.4. The absorbance values for anti-actin in 400-fold diluted sera were comparable to those of anti-DNA in 400-fold diluted sera indicating that most of the DNA-binding antibodies found in human sera are cross-reacting with actin.

Fig. 4




The cross-reactivity of anti-gal was further assessed by inhibition with soluble DNA. A single step absorption of sera with soluble myosin and ss-DNA reduced antibodies reacting with LPS (Fig 5)

similarly the reactivity to ss-DNA was effectively removed by absorption with actin (Fig-6)indicating that most of the DNA-binding antibodies found in human sera are primarily anti-gal, cross-reacting with DNA.

Fig. 5

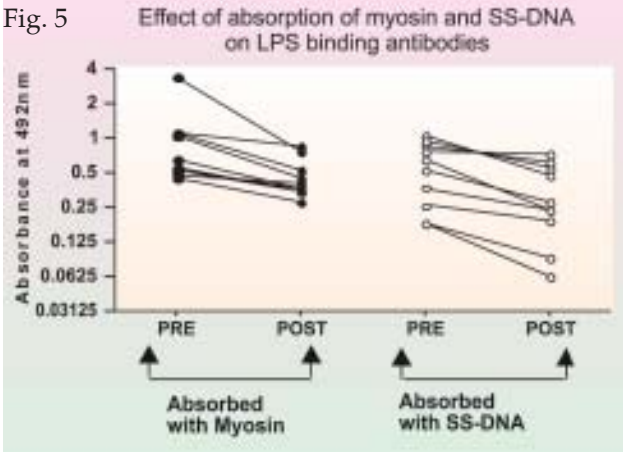
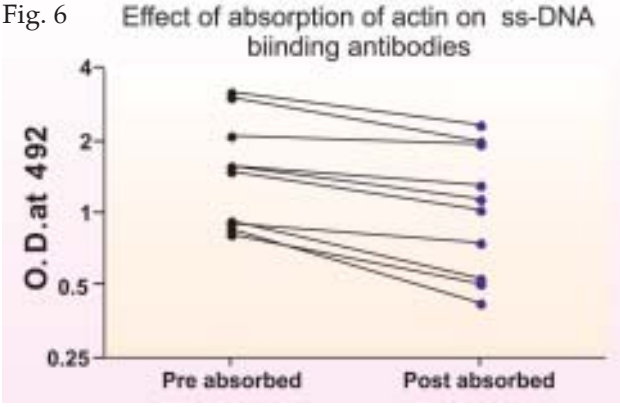
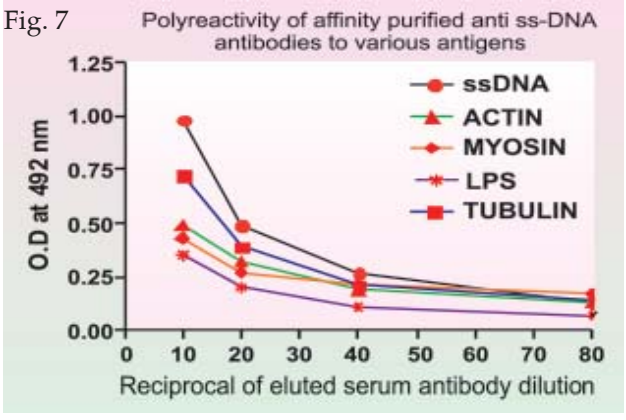



Fig. 6




Since naturally occurring poly reactive antibodies have been demonstrated to play a role in innate immunity as well as in immunoregulation, the polyreactive property of DNA binding antibodies has been evaluated by affinity purifying antibodies to SS-DNA from plasma collected from filarial patients by using a column of SS-DNA-Sepharose. Affinity purified antibodies to SS-DNA were purified by passing pooled human sera through a column of sepharouse linked to ss-DNA. Affinity purified antibodies were tested against a panel of antigens to evaluate the polyreactive nature of anti-ssDNA antibodies in human filarial sera. Affinity purified anti-ssDNA was found to react with ss-DNA, Actin, myosin, LPS when tested by ELISA as shown in fig.7 There was however no significant reactivity of affinity purified anti-ss-DNA to human serum albumin (data not shown) indicating the polyreactive properties of antibodies to SS-DNA observed in human filariasis. The demonstration of polyreactive property of SS-DNA antibodies in this study indicates an important role for true antibodies to provide hust protection against filarial infection.

Fig. 7




## 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 | : | Nov 2009 |
| Closing date | : | Dec 2012 |
| Status | : | 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.

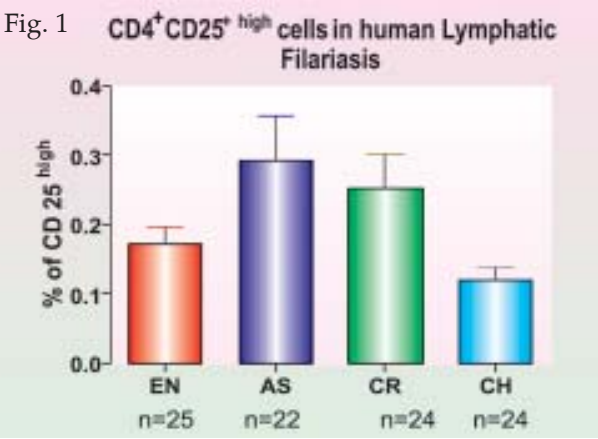
## Background

The impact of placental filarial infection on the immune competence of the neonate remains poorly understood. Filarial infection acquired through maternal origin has been considered a risk factor for increased susceptibility. A number of studies have shown that 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 while children of infection free mother have been shown to respond vigorously to filarial antigen with lymphocytes proliferation, production of IL-2 and IFN-γ. These observations are strongly suggestive of some form of immune modulation occurring in utero. We hypothesize that exposure of the fetal immune system to filarial antigens induces the generation of regulatory T cells which produce down regulatory cytokines to inhibit inflammatory responses and facilitate parasite survival.

## Progress of work

As mentioned in the last annual report we had observed that 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. This year we report further characterization of these findings. T regulatory 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

Fig. 1 $CD4^{+}CD25^{+\,high}$ cells in human Lymphatic Filariasis

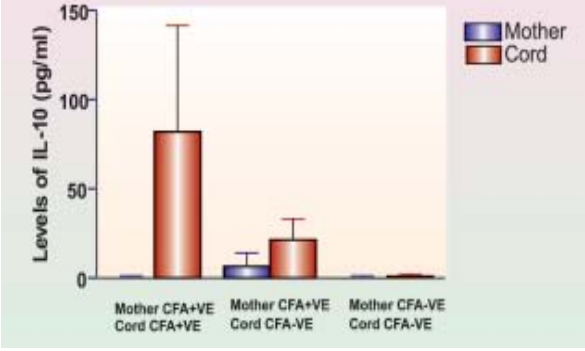



tumor necrosis factor receptor (GITR). These cells are anergic to proliferative responses in vitro. in response to stimulation. FOXP3+ cells are normally identified by virtue of high levels of expression of CD 25 on CD 4 T-cells. The CD 4 + cells with the highest level of CD25+ (CD4+CD25+ High) have been shown to appear as a tail to the right from major population when T-cells are double stained with anti-CD4 and anti-CD25 antibodies. Fig-1 shows the CD4+ T cells expressing highest levels of CD25+ (FOXP3cells) in human lymphatic filariasis. The functional T regulatory cells were found to be significantly high in microfilariae carriers compared to subjects with chronic filarial infection. The CD4+ T cells expressing highest levels of CD25+ (FOXP3cells) is being evaluated in cord blood of filarial infected and uninfected mother.

We investigated to which extent maternal filarial infection have sensitized or biased immune responsiveness in neonates toward a Th1 or Th2-like phenotype. Regulatory T cells produce down regulatory cytokines to inhibit inflammatory responses. IL-10 has been shown to down regulate Th1 type of immune response. Since T regulatory cells produce down regulatory cytokines such as IL-10, TGF-β to inhibit inflammatory response, we wanted to study whether transfer of filarial antigens influences the cytokines production in newborn. IL-

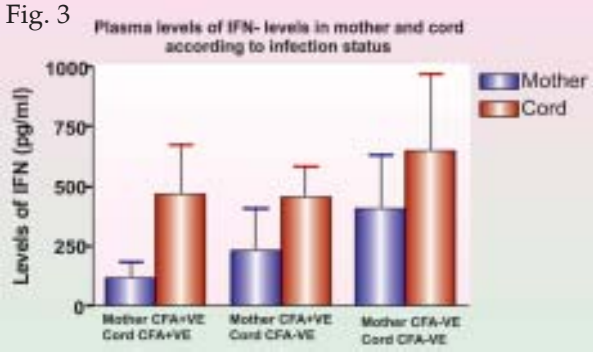
10 response was measured to assess regulatory responses. The samples were classified according to infection status (as shown by presence of CFA) of mother. Fig. 2 shows the plasma IL-10 levels in mother and children according to infection status. No significant difference was observed in plasma levels of IL-10 in infected and uninfected filarial mother. Children born from CFA +ve mother had significantly more IL-10 than in children born from uninfected mothers. IL-10 levels were significantly high in CFA +ve children born from infected mother compared to CFA–ve children born from filarial infected mother.

Fig. 2 Plasma levels of IL 10 levels in mother and cord according to infection status




A role for IL-10 has been attributed in down regulating effector immune responses. Since we observed increased plasma levels of IL-10 in cord blood of filarial infected mother, we quantified plasma levels of IFN-γ in paired maternal and cord samples to evaluate the influence of to evaluate the influence of maternal infection on neonatal cytokine production on Th1 type of immune response. Plasma levels of IFN-γ, in cord blood samples of uninfected and infected mother are shown in Fig.3. Plasma levels of IFN-γ were found to be low in cord blood of uninfected mother (Mother CFA-ve, Cord CFA-ve) moderate in CFA-ve cord blood born from CFA +ve mother (mother CFA+ve, cord CFA-ve) and high in CFA+ ve cord blood born from CFA +ve mother (mother CFA+ve, Cord CFA+ve). This differential production of IFN-γ is more prominent in children born from CFA +ve children born from CFA +ve mother than in children born from uninfected mothers indicating that transfer of filarial antigens at delivery was associated with increased levels of IL-10.

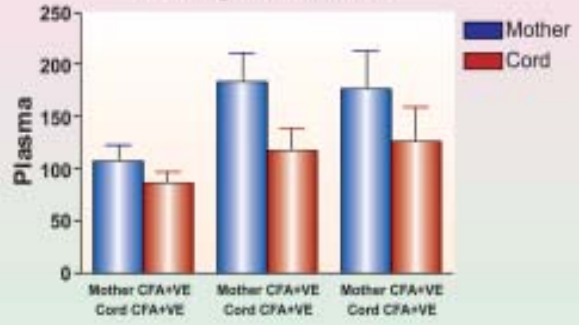
Fig. 3 Plasma levels of IFN- levels in mother and cord according to infection status




IL-5 was measured to assess type 2 responses as shown in Fig-4. Plasma levels of IL-5 was not significantly different between CFA-ve cord blood born from CFA +ve mother (mother CFA+ve, cord CFA-ve) and CFA+ ve cord blood born from CFA+ve mother indicating that transfer of filarial antigen from mother to cord blood does not influences IL-5. production.

An increased levels of IL-10 (Th-2) and down regulation of IFN-γ (Th-1) have been detected in cord blood of children born to filarial infected mothers. High level of T-regulatory cells increased production of IL-10 in cord blood from injected mothers indicate that increased T-regulatory cells could down regulate inflammatory responses and may be associated with parasite survival. The influence of maternal filarial infection on mitogoen and filarial antigen induced cellular responsiveness and cytokine production in cord blood of neonates is being evaluated.

Fig. 4 Plasma levels of IL-5 in mother and cord according to infection status



## 6. Development of a LAMP assay for diagnosis of human malaria.

| | | |
|---|---|---|
| Principal Investigator | : | Dr M R Ranjit |
| Co-Investigator | : | Dr S K Kar |
| Starting Date | : | July 2010 |
| Closing Date | : | June 2013 |
| Funding | : | EM (DBT) |

### Objectives

(i) To design species specific loop primers for detection of human malaria parasites

(ii) To optimize the reaction conditions for easy detection of the LAMP derived products

(iii) 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 of Work

Two sets of LAMP primers each for *P falciparum*, *P vivax* and *P malariae* have been designed this year excluding the set of primer reported in the previous year annual report (2009-2010) on the basis of the genus and species-specific nucleotide sequences of the 18S rRNA genes by using LAMP design software Primer Explorer V3. The primers designed last year did not work well. Therefore the LAMP assay is being standardized with new set of primers. Briefly parasite genomic DNA from archive blood was isolated following the standard chloroform isolation and phenol precipitation method. The LAMP assay is being carried out 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 MgSO4,20 mmol/L NH4SO4 , 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. 1ìl of commercially available fluorescent dye (Eiken Chemicals, Japan) is being used for detection of LAMP product. The reaction mixture is incubated in a hot water bath at 650 C for 30 minutes. The reactions conditions are being standardized to increase the specificity of the test. The study is in progress. Attempts are being made to design new primers from other regions of the 18S rRNA genes present in chromosome 11 of *P falciparum* in order to find out effective primers. Similarly LAMP primers for *P vivax* are being designed to test their diagnostic potential.

## 7. Detection and phylogenetic analysis of chikungunya virus from human cases and vector mosquito species in different endemic regions of Orissa.

| | | |
|---|---|---|
| Project Investigators | : | Dr. R.K.Hazra |
| Co-PI | : | Dr. B. Dwibedi |
| Starting date | : | Sept 2010 |
| Closing date | : | Aug 2013 |
| Status | : | EM (ICMR Task Force) |

### Objectives

1. Screening of human cases and selected mosquito species from defined areas of Odisha State for the detection of chikungunya virus infections by serological and molecular tests.

2. Nucleotide sequencing of the entire E1 genomic region for phylogenetic analysis.

### Progress of the project

### Chikungunya outbreak and study area surveys

The state Odisha is divided into four distinct physiographical regions i.e. northern plateau, central tableland, coastal areas and Eastern Ghats. The coastal areas have been the most endemic areas for chikungunya outbreak as per the Government of Odisha, Health Department. The study was carried out in nine districts which were affected by chikungunya i.e. Puri, Khurda, Cuttack, Kendrapara, Jagatsinghpur, Jajpur, Ganjam, Mayurbhanj and Gajapati district for chikungunya survey. Blood samples were collected from cases suspected to have CHIK infection after clinical examination. The clinical symptoms found to be self limiting without any neurological complication or death. The common symptoms are fever, Rashes and joint pain. Blood samples were collected from five districts viz., Khurda, Jagatsinghpur, Kendrapara, Gajapati and Ganjam whereas *Aedes* mosquito collections were done from all the nine districts as mentioned above and were brought to the laboratory for processing.

### Results

### Entomological Results

Mosquitoes both adult and larvae collection was carried out in rural areas of Khurda, Jagatsinghpur, Kendrapara, Bhadrak, Mayurbhanj, Jajpur, Cuttack, Puri, Ganjam, Gajapati district. From larvae and adult collection, five species of adult was confirmed by our team i.e. *Aedes aegypti*, *Aedes albopictus*, *Aedes vitatus*, *Aedes edwardsi*, and *Culex sps.* Out of these four *Aedes* species, *Ae. albopictus* was found to be dominant species in all the above surveyed districts. From the number of positive breeding spot surveyed, the Breteau Index of *Aedes albopictus* in all the blocks under each district was greater than 100 thereby

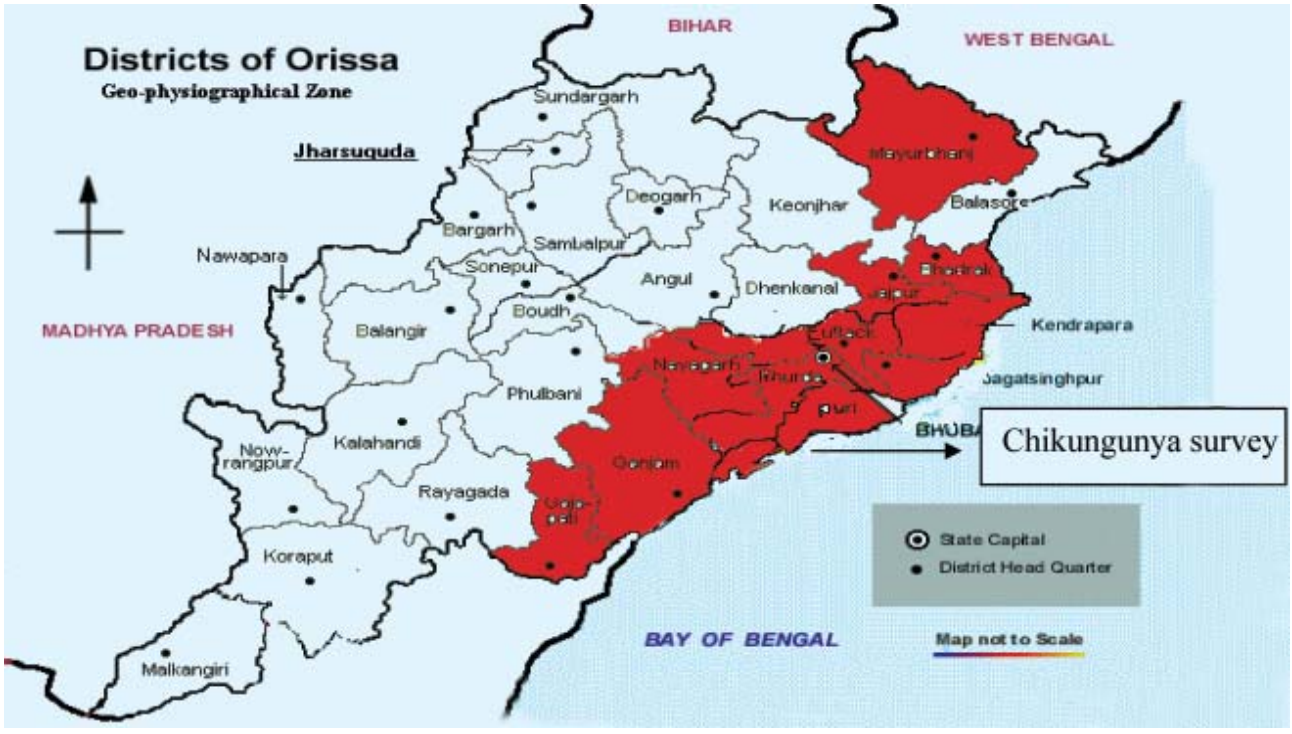



**Fig 1.** Map of Odisha showing affected areas of Chikungunya virus.

indicating high vector densities and hence being the main vector responsible for transmission of arbovirus in the affected areas. *Aedes albopictus* was found to be 42% followed by *Ae.aegypti* 23% and others are *Ae.vittatus* 11%, *Ae.edwarsi* 4%, remaining culicine species are 18%. ( Fig 2).

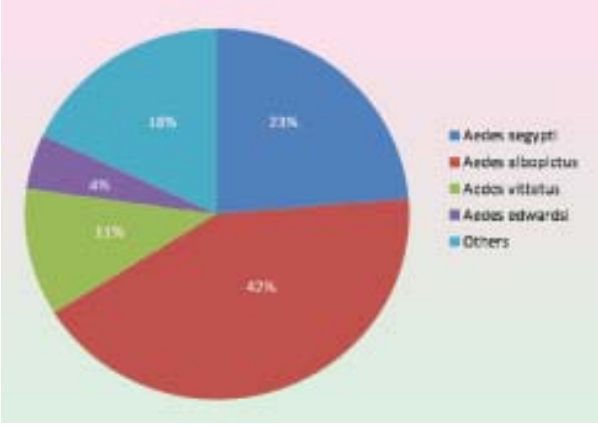



**Fig 2.** Distribution of Aedes species in different breeding habitats of Odisha.

**Multiplex PCR**: The multiplex PCR method detected maximum number of *Aedes albopictus* larvae and pupae in most breeding spots surveyed which is a prevalent mosquito species of *Aedes* in Odisha. Discarded tires were the most abundant breeding spots of *Aedes* mosquitoes that were obtained from the areas surveyed.

**Elisa:** 216 serum samples from suspected cases of chikungunya collected from affected areas were tested for CHIKV by antigen capture IgM ELISA. Out of 216 samples, 91 shown positive for CHIK IgM, thereby indicating acute epidemic outbreak in the affected areas (Table 1).

## Two step reverse transcriptase Polymerase chain reaction (RT-PCR)

RT-PCR detected the chikungunya viral E1 gene specific band at 294 bp from patients serum obtained from different epidemic areas. The gel photo is showing the presence of amplified viral E1 region.

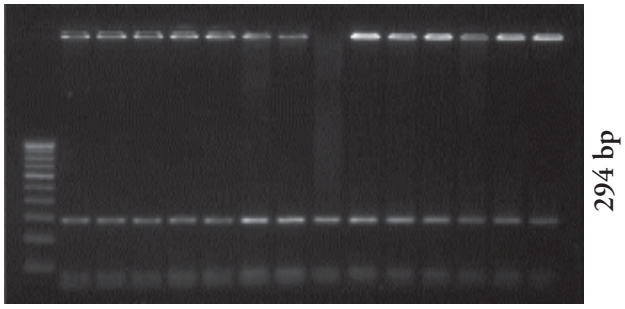



**Fig 3.** 1.5 % agarose gel photo showing the amplified E1 region of CHIKV.

## Molecular Detection of Chikv at E2 Region

Amplified products were checked in agarose gel electrophoresis to confirm their specific size.

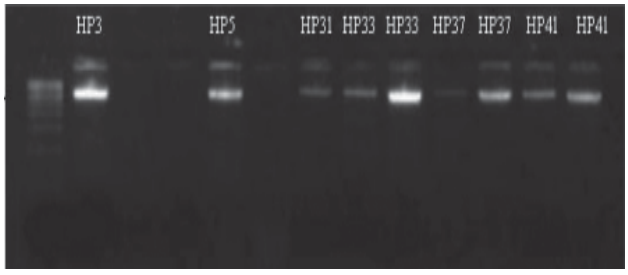



**Fig4.** Agarose Gel Electrophoresis of Hadapada (HP) isolates. Lane: 100 bp DNA Ladder, Lane2: HP3, Lane3: Negative Control (without DNA), Lane4: Negative Control (without RT product, Lane5: HP5, Lane6: Negative Control (without Primer Pairs), Lane7: HP31, Lane8: HP33, Lane9: HP33, Lane10: HP37, Lane11: Hp37, Lane12: Hp41, Lane13: Hp41.

## Genetic Analysis of Chikv at E2 Region:

The '**TPY**' domain which is known to be involved in binding to the capsid protein is well conserved in all the isolates. The **K252Q** mutation which is known to be the major determinant of neurovirulence within structural proteins was not observed.

## Phylogenetic Analysis:

From the isolated E1 gene, a phylogenetic tree was constructed which was given below. This phylogenetic tree indicates that the sequences (seq 4,

seq 5, seq 8, seq 11, seq 13) from Odisha was grouped along with sequences of CHIKV belonging to ECSA (East Central South African) genotype (Fig 5). Hence ECSA genotype of chikungunya virus can be attributed to recent outbreaks of chikungunya in Odisha. Further evidence of the ECSA genotype circulation in Odisha was due to the abundance of *Aedes albopictus* vector that efficiently transmits this genotype. This was supported by high larval indices of *Aedes albopictus* in different breeding spots surveyed.

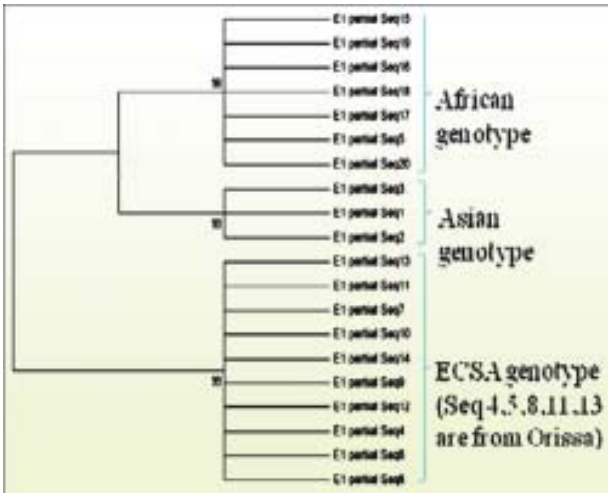



**Fig 5**: Phylogenetic tree of 294 bp sequence of E 1 gene of different genotypes of chikungunya virus showing all CHIKV sequences from Odisha belong to East Central South African genotype.

More number of positive cases were detected by RT-PCR from the samples collected during the

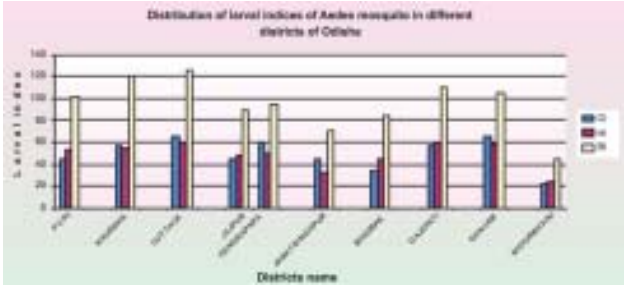


**Fig 6.** Graph showing distribution of larval indices of Aedes mosquito in different districts of Odisha. House Index (HI): Percentage of house infested with larvae or pupae. HI =Number of houses infested / Number of houses inspected X100Container Index (CI): Percentage of water holding containers infested with larvae or pupae.CI= Number of positive containers / Number of containers inspected X100, Breateau Index (BI): Number of positive containers per 100 houses inspected, BI=Number of positive containers / Number of houses inspected X100.

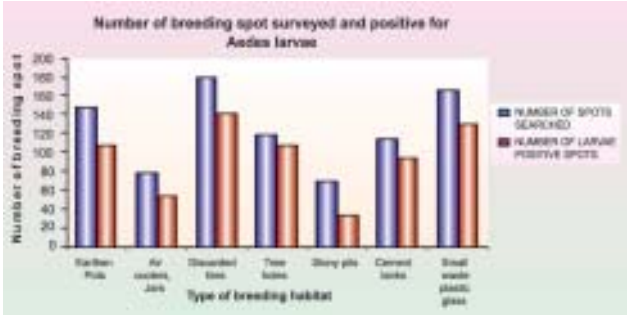



**Fig 7.** Graph showing the type of breeding spot surveyed positive for Aedes larvae.

**Table 1**: Number of blood samples collected and tested positive for CHIKV IgM ELISA and by RT PCR.

| Sl. No | Name of the District | Number of samples collected | % of +ve CHIK IgM (ELISA) | % of +ve CHIK (RT PCR) |
|---|---|---|---|---|
| 1. | Khurda | 45 | 51.1 | 71.1 |
| 2. | Jagatsinghpur | 41 | 70.7 | 85.36 |
| 3. | Kendrapada | 4 | 50 | 50 |
| 4. | Gajapati | 60 | 31.6 | 56.6 |
| 5. | Ganjam | 66 | 27.3 | 59.1 |
| | Total | 216 | | |

epidemic. The sensitivity depends on the days of collection after the occurrence of the symptoms.

*Aedes* mosquitoes were found in different breeding habitats of domestic and peridomestic areas. Main breeding sources inside the house are earthen pot, air coolers, unused utensils, cement tanks etc. In outside the houses the main breeding sources are discarded tires, cement tanks, jars, small waste plastic glass, flower pot etc.

**Discussion:**

Chikungunya outbreaks have been continuing to occur in the coastal districts of Odisha since 2008. Nine districts were surveyed based upon their high endemicity to chikungunya and recent epidemics that had occurred. Detailed entomological survey was done and detected that *Aedes albopictus* was the most abundant vector with very high container and Breteau indices, thus proving to be the main vector in this region. To assess the clinical and virologic parameters, we collected serum samples from patients in areas where outbreaks were continuing. The patients presented with intense clinical symptoms and morbidity was high. The cases were collected during the acute stage of illness which was found to be sensitive for both IgM ELISA and RT-PCR. Hence molecular methods of detecting CHIKV can detect the virus at an early stage of the epidemic. Phylogentic analyses of partial E1 gene revealed the circulation of ECSA genotype in the affected areas. Thus the more devastating outbreaks of chikungunya virus can be attributed to the circulation of ECSA genotype in these areas. More detailed work is required to be done for arriving at a well defined conclusion.

**Summary**

- Chikungunya outbreaks have been continuing to occur in the coastal districts of Odisha since 2008. Nine districts were surveyed based upon their high endemicity to chikungunya and recent epidemics that had occurred.
- Detailed entomological survey was done and detected that *Aedes albopictus* was the most abundant vector with very high container and Breteau indices, thus proving to be the main vector in this region.
- To assess the clinical and virologic parameters, we collected serum samples from patients in areas where outbreaks were continuing. The patients presented with intense clinical symptoms and morbidity was high.
- Phylogentic analyses of partial E1 gene revealed the circulation of ECSA genotype in the affected areas. Thus the more devastating outbreaks of chikungunya virus can be attributed to the circulation of ECSA genotype in these areas. More detailed work is required to be done for arriving at a well defined conclusion.

**Future work**

- Further screening of more areas during epidemics and intermittent periods needs to be done for entomological survey and vector identification.
- More number of patients' samples and mosquito species needs to be collected for more confirmatory results regarding identification of the chikungunya virus.
- Sequencing of the complete E1 and Nsp1 gene in more number of samples in order to establish phylogenetic analyses and confirm the genotype of CHIKV circulating in Odisha.
- To study the role of any secondary host that may act as reservoir of the CHIKV during non-epidemic period.
- The cell culture laboratory will be expected to be established in this year for culture of virus in mosquito cell line.

**Intradomestic breeding sites of Aedes**

**Peridomestic breeding sites of Aedes**

## 8. Comprehensive integrated vector control for co-existing infection of Malaria, Filariasis and Chikungunya in Nayagarh District of Orissa.

| | | |
|---|---|---|
| Principal Investigator | : | Dr. N. Mahapatra |
| Co-PI | : | Dr R K Hazra<br>Dr.S. K. Parida<br>Mr. N. S. Marai |
| Starting Date | : | April 2011 |
| Closing date | : | March 2014 |
| Funding | : | Started Intramurally and Apply for Extramural funds of ICMR Vector Task Force |

### Objectives

1. To develop comprehensive vector control strategy for control of malaria, filariasis and Chikungunya.
2. To implement the strategy to reduce disease transmission by reducing larval and adult density, vector longevity and man vector contact.

### Rationale

Several vector control programme targeting specific vector borne diseases like filariasis, malaria and Chikungunya are being operated in the country, while the vectors that transmit these diseases are prevalent in and around the households where population resides. There are lots of similarities in those causative vectors bionomics and habitats. Hence comprehensive strategy will help in controlling the vector population and transmission of the above three diseases which will be feasible, less laborious, and cost effective.

Therefore a study is being initiated in Odogaon PHC of Nayagarh district for implementing comprehensive vector control strategy.

### Progress

### Study site selection

Kural village of the Odagaon PHC of Nayagarh district (Fig.1) was selected as study site which showed co-prevalence of malaria, filariasis, chikungunya as per the Govt. data.

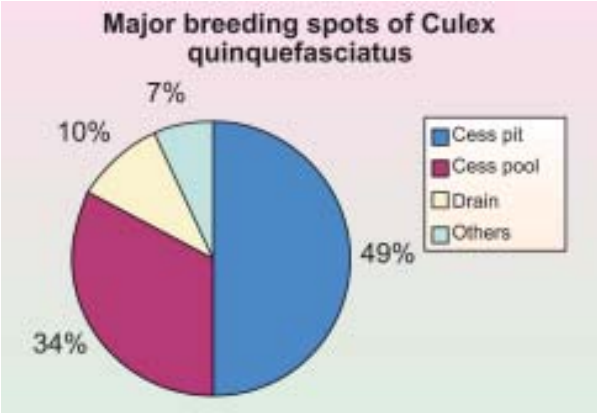
The initial vector survey was intensely carried out in the selected population reporting all the three diseases to assess their density and bionomics to generate data on transmission indicator which will be compared after intervention.

### Prevalence of diseases

Epidemiological data collected from the District Health Services, showed prevalence of Malaria: Slide Positivity Rate-7.8%, Filariasis: microfilaria rate- 6.8%, Chikungunya: attack rate-10.5% in Kural village.


**Fig. 1.** Odisha map showing the study area.

**Fig. 2**. Major breeding spots of *Cx. quinquefasciatus.*




Percentage of different breeding place showing presence of vector larvae

The above figure shows, cesspool and cesspit contributes >80% of the breeding of Cx.quinquefasciatus.

### Transmission indicators for:

### a. Filariasis:

**Vector density**- The filariasis vector *Culex quinquefasciatus* was found to be prevalent with an average man hour density of 21.34.

**Infection rate and Infectivity rate** - The developing stages of *Wuchereria bancrofti* infection (L1, L2, L3) were detected in the vector. The infection rate and infectivity rate were found to be 6.14% (28/456) and 4.17% (19/456) respectively.

**L3 load**- The L3 load was found to be 3.3.

**Larval density**- Total 773 numbers of breeding places were surveyed. Out of which 464 (60.02%) spots were positive for *Cx.quinquefasciatus.* (Fig 2). Larval density was found to be 65/dip.

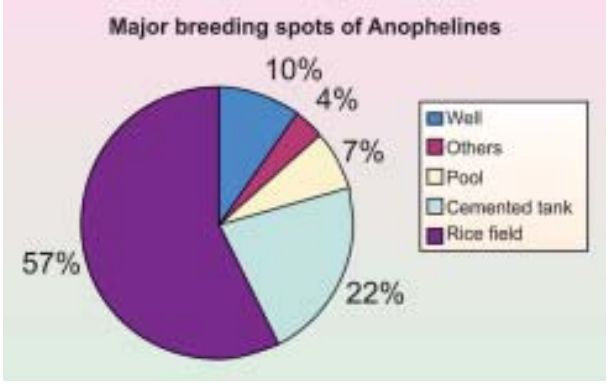
### b. Malaria:

**Vector density**- *Anopheles culicifacies* and *An.annularis* ,known vectors were present in the study village. The man hour density of *An. culicifacies* and *An.annularis* were 12.92 and 8.9 respectively.

**Sporozoite rate**-The sporozoite rate of *An. culicifacies* and *An.annularis* were 1.2% and 0.31% respectively conforming the indigenous transmission of malaria.

**Larval density**- Anopheline breeding was seen in 47.86% of breeding sites with larval density of 16/

**Fig. 3.** Major breeding spots of Anopheline vectors.




Rice field

As illustrated above Rice field contributed 57% of breeding of *An. culicifacies* and *An.annularis*.

dip.The breeding of the vector in different breeding habitats were given in fig 3.

### C. Chikungunya:

In Chikungunya transmission, *Aedes aegypti* and *Ae. albopictus* , larval breeding parameters like House index, Container index and Breteau index are measured as indicators for effectiveness of control programme.

**House Index:** Percentage of houses with one or more habitats positive for Aedes species.

**Container index**: Percentage of container infected with Larvae.

**Breteau index:** Percentage of infected containers per no of inspected house.

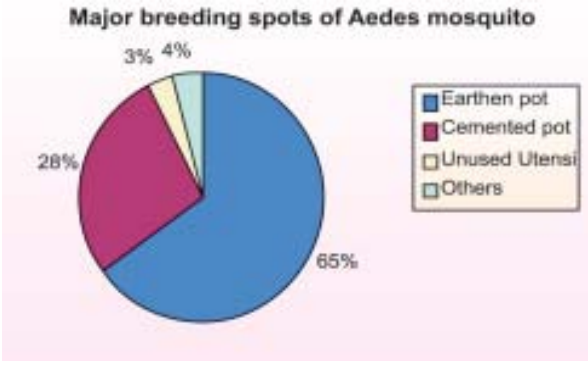
Hence all the above indices were calculated for our study area along with vector density and infection.

**Vector density**- Both the vectors of Chikungunya i. e. *Ae. aegypti* and *Ae. albopictus* were collected from the study village and the density were 5.61 and 8.06 respectively.

**Detection of Chikungunya virus**- *Ae. albopictus* mosquitoes are being processed for detection of Chikungunya virus. Out of 76 processed none found positive for the virus.

**Larval density**- Out of 773 breeding spots 21.09% was positive for *Ae. aegypti* and *Ae. albopictus* breeding with density of 25/dip.

**Fig. 4.** Major breeding spots of Aedes vector.

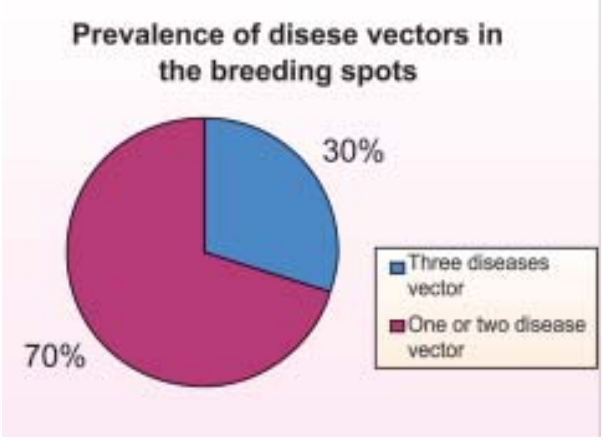



The figure.**4** shows the **earthen** and **cemented pots** are the most preferred breeding sites for Aedes.

The following Larval indices were calculated for *Ae. aegypti* and *Ae. albopictus*:

The **House index** is >8. **Container index** :**19** and **Breteau** index was 59%.

**Fig. 5.** Percentage of breeding spots positive for all the three vectors.



Out of the 475 positive larval breeding spots found, 30% breeding spots were positive for all the three diseases vector (malaria, filariasis and Chikungunya) (Fig.5). This indicates that control measures should be targeted towards these breeding habitats in a primary mode.

### Future plan

**A.** Entomological and parasitological survey will be continued for ito generate baseline data for one year and also in the intervention period..

**B.** Breeding spots will be stratified for implementing different larval control.

**C.** Basing on the baseline data of vector bionomics comprehensive control strategy will be developed using the following three intervention tool.

i. Source reduction.

ii. Use of Biolarvicides (*B.thuringiensis*, use of larvivorous fish)

iii. Long Lasting Insecticide Treated Nets (LLIN)

**iv.a:** Mobilization of the Community to participate in the control programme

**b:** Capacity building- Training will be given to identify Gram Kalyan Samiti (GKS) members on spraying larvicides, IEC activities will be done for community awareness and their active participation in the household as well as community level. Primary school teachers will be trained specifically for source reduction activities for Aedes mosquito control. However active members of all the different Govt. programme running in the village will be involved in carrying out activities at different steps of the control activities. So that improved resources at a local level could be done in cost effective manner.

The indicators which will be identified for the above activities will be developed as a tool for comprehensive vector control and this can be translated to the programme to be implemented on blocks as well as district level by the programme.

## 9. Progress of Virology Network Laboratory (Grade-I) at RMRC, Bhubaneswar.

| | | |
|---|---|---|
| Principal Investigator | : | Dr.B.Dwibedi |
| Co-PIs | : | Dr.R.K.Hazra, Miss S.Dixit |
| Co-ordinator | : | Dr.S.K. Kar |
| Starting date | : | March 2010 |
| Closing date | : | March 2015 |
| Funding | : | Extramural (ICMR) |

### Background

It was aimed at creating regional facilities to be involved in laboratory diagnosis, surveillance and research in viral diseases of importance.

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 inter epidemic 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.

### Objective

To establish a grade I diagnostic virology laboratory for 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), Parainfluenza 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

**I. Infrastructure development and man power training.**

The laboratory up gradation has been made according to the requirement of virology laboratory and it was inaugurated on 5th September 2011. Civil infrastructure modification and Procurements of laboratory furniture and equipments were undertaken.

In house training has been given to the recruited staff on serology and molecular diagnosis. One Scientist (Non-medical) and one SRF have undergone short laboratory training at KMC, Manipal and PGI, Chandigarh on molecular diagnostics and cell culture.

**II. Networking for information, Sample receipt, Investigation and reporting.**

Network has been established with the State Health Department, Medical Colleges and Hospitals of the region for referral investigation of sporadic cases and outbreak investigation. Outbreak investigations are being undertaken along with the state health team upon getting information through media or health system. Immediate report is being communicated to the concerned hospital within 3 days of sample receipt.

**III. Sample collection**

A. sporadic/ referred cases

Sporadic/referral cases were received by the centre from different hospitals from different districts. So far 3071 number of samples were collected from different Govt. and Private hospitals from odisha. The details of sample receipt from hospitals has been given in below mentioned tables (1 and 2).

**Table 1.** Sample Receipt from different hospitals and Medical colleges (Till 8.11.11).

| Source Hospital/Centre | No. of samples collected |
|---|---|
| Capital Hospital, BBSR | 387 |
| SCBMH, Cuttack | 340 |
| SVPPGIP, Cuttack | 275 |
| SuM Hospital, BBSR | 261 |
| Outbreak investigation | 933 |
| Other hospitals and PHC | 875 |
| Total | 3071 |

**Table 2.** Suspected viral diseases investigated.

| Sl. No. | Diseases investigated | No. of samples received |
|---|---|---|
| 1 | Chikungunya | 533 |
| 2 | Dengue | 251 |
| 3 | Respiratory infection | 180 |
| 4 | Influenza A (H1N1) | 444 |
| 5 | Measles | 197 |
| 6 | Chickenpox | 135 |
| 7 | Mumps | 5 |
| 8 | Hepatitis | 584 |
| 9 | Encephalitis | 346 |
| 10 | Viral diarrhea | 396 |

**B. Out break investigations**

Outbreaks of influenza H1N1, Dengue, Chikungunya, and Hepatitis virus infection has been investigated with immediate reporting to State Health Department with recommendations for timely prevention.

**(i) Dengue Out break(2010)**

Dengue outbreak was reported during September 2010 from two districts (Malkangiri and Gajapati). Survey was carried out in three urban locations and one tribal village of Malkangiri. 331 numbers of cases examined and samples were collected. In Gajapati district 2 villages were affected covering 158 cases. The epidemiological investigation indicated that house hold clustering of cases. Vector survey also showed abundance of *Aedes* vector breeding in the area.

**Observations**

Sero positivity varies from 4.5 to 23 %. PCR assay detected 20% of dengue type II virus from the site.

(ii) **Chikungunya Outbreak (2010)**

Epidemic survey was conducted in 2 villages on 1st and 2nd December 2010. Out of the total 45 cases, 23 positive cases were found out positive for Chik IgM.

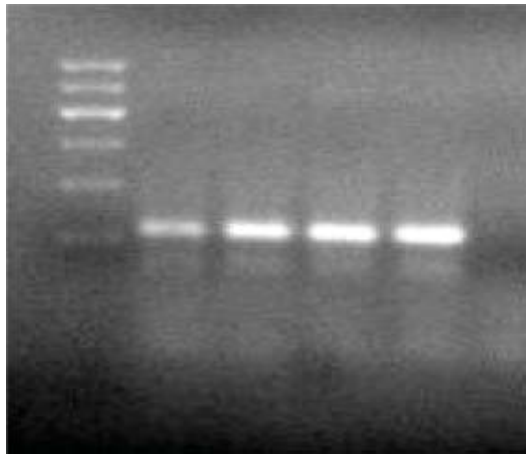
**(iii) Dengue Outbreak (2011)**

Outbreak was reported from Anugul district in middle of July 2011 (16th/18th July) with occurrence of few dengue cases in the Kalamchhuin and Gopal Prasad Sub-centres under the Godibandha CHC of Talcher Block, Angul district. RMRC along with the state Health Department conducted a survey in those affected areas. A total of 267 suspected dengue cases had been recorded from Angul district, of which 106 had been confirmed by laboratory testing.

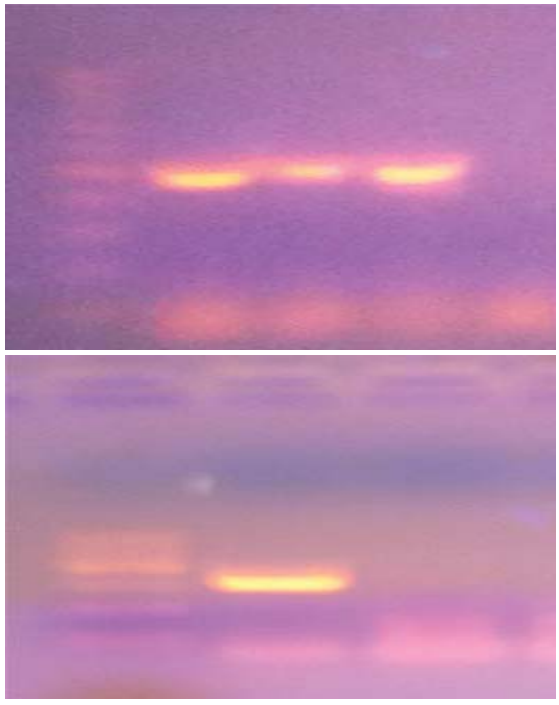
**IV. Laboratory Investigation:**

Laboratory investigation was done for the samples collected from outbreak areas as well as on the samples collected on sporadic hospital based cases by or referred to the centre.

Among vector borne diseases Dengue antigen (NS1) was detected in 45% of the cases where as in 15% cases dengue IgM was detected. On 50% of the positive samples PCR was performed and **dengue serotype II** was identified as the serotype. Chik IgM was detected in 28% of cases. 25% of the positive samples were subjected for genotype analysis and **Genotype-ECSA** was found in 25% of the cases.



Lanes 1, 2,3 and 4- type II dengue virus(119 bp) in human infected with Dengue virus infection



Chick E1(311bp) and E2(305bp) RT PCR in Human Serum

Among enteric viruses Rota antigen was detected in 29% of cases out of which 26.5% of cases were confirmed by Real Time PCR. **Genotype G2, G4 and G9 (G Type) and P4 and P9 (P Type)** were

detected in 20% of cases. Other enteric viruses detected were Noro G1 (3.7%), Noro G2 (1.5%), Astro (1.2%), Adeno (22.3%), HAV(6.7%), HEV(17.8%).

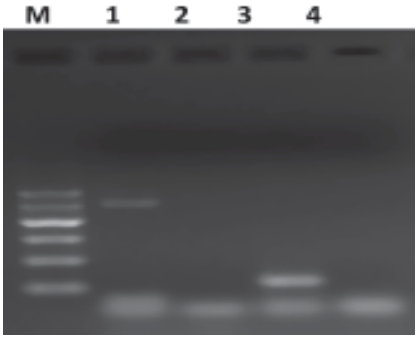
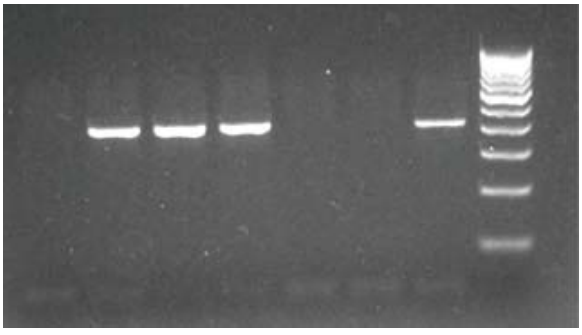
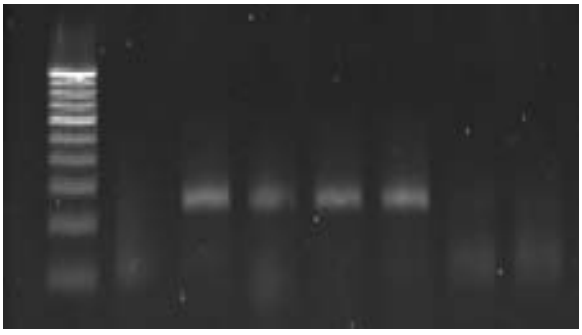



**Fig.** Rotavirus P and G genotype for Sample ID 1404 - P[4] G9.

Among the cases of jaundice screened for hepatitis virus infection, HBV and HCV were detected serologically in 9.3% and 0.9% respectively and genotyping was done in 16.5% and 3% of cases respectively where **HBV genotype D and HCV genotype 1b** were identified as the genotypes circulating in this region.



HBV Genotype D



HCV Genotype 1b

Viral respiratory infection was another important disease which was covered for laboratory diagnosis. Through Real Time PCR assay, many respiratory viruses were identified including some emerging viruses. The viruses those detected were Flu A 22%, H1N1 18.6%, Rhino 13.8%, Para influenza 20% and Adeno in 30.6% of cases. Emerging viruses like Boca, HMPV and Parecho viruses were detected with low prevalence.

Among air borne diseases Measles IgM was detected in 3.6% of the cases and Varicella IgM was detected in 40.3% of cases.

Viruses that cause encephalitis were also investigated. Herpes simplex virus was detected in 6%, Herpese Virus II in 2% and Japanese encephalitis was detected in only 1 subject. PCR amplification was attempted to detect Entero virus, HSV 1, HSV 2, JEV and WNV from CSF collected from cases of encephalitis. 40 samples were tested but none of the samples were found positive.

### Subsequent Plan

The above activities will continue for the next year. Cell culture will be established for Chik, Dengue, HSV and Measles Viruses, sequencing and typing will be established for Measles, Varicella and Influenza H1N1 viruses. Out break investigation will continue along with sporadic case investigation with collaborations of state hospitals. Network will be further strengthened to cover southern and western parts of Odisha.

Dr. V. M. Katoch DG, ICMR in the RMRC Virology Lab






Modernised virology & TB culture laboratory of RMRC, Bhubaneswar

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

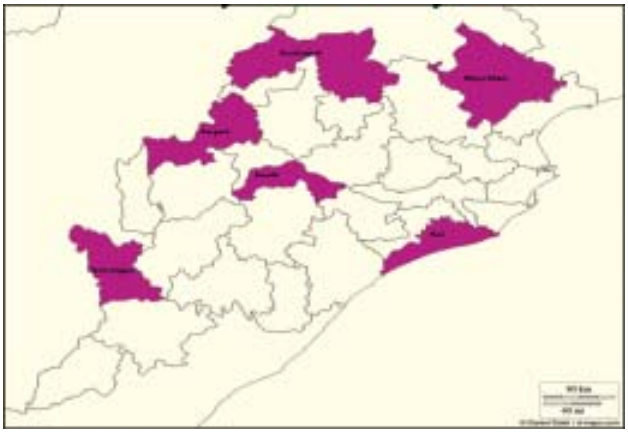
Principal Investigator : Dr. A S Kerketta
Co-Investigators : Dr. G Gulliyya
G Mallick
Starting Date : April 2010
Completion Date : January 2012
Funding : Extramural (ICMR, HSR)

### General objective

- To assess the impact of Janani Suraksha Yojana on maternal health in Orissa.

### Specific objectives

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



Study districts

### Work Progress

**Facility-based study**

The facility based survey was undertaken in six districts of Orissa namely Mayurbhanj, Sundargarh, Bargarh, Puri, Boudh and Nabarangpur. The study districts were selected on the basis of their geographical distribution in three different zones namely Northern plateau, Central table land & Coastal plains, Southern Eastern Ghats of the state with aim to cover both tribal and non-tribal population. In each district one district level FRU and two 24x7 facility CHCs or area hospital are included for facility based survey. The survey was undertaken amongst three groups of mothers i) pregnant mothers in their last trimester of pregnancy ii).mothers recently delivered iii).lactating or the mothers delivered in last 6 months were captured either from ante-natal clinic, maternity ward and post partum care centre.

So far a total of 1294 (300 ante natal care (ANC) + 994 post natal care (PNC) group were interviewed.

**Table-1.** age and other socio demographic data of Post Natal Care women.

| Age | | Literacy level | | Socio-economic | |
|---|---|---|---|---|---|
| 17-25 years | 68 | Illiterate | 320 | >10,000 to-12000 | 484 |
| 26-35 years | 866 | Primary | 202 | > 15000 to 36000 | 296 |
| 36-40 years | 58 | Secondary | 388 | > 37000 to 60000 | 150 |
| > 40 years | 2 | Higher secondary & above | 84 | > 60,000 | 64 |

The age and other socio economic data are given in table-1.

**Registration under JSY:** Out of total interviewed 97.2% had registered under JSY.

**Source of information on JSY:** 45% came to know about JSY during pregnancy by ASHA..

**Information given on JSY:** The information given about components JSY was on free institutional delivery (47.9%), cash benefit (39.6%).

### Antenatal care (ANC):

The data on ANC shows 97.81% of the mothers' availed antenatal cares check up from various public health providers.

Out of which 72.4% had three ANC check up, 8.9% had two and 18.7% had one check up.

Motivation for ANC was mostly from the husband of the women (82.1%) and ASHA 23.0% and rest from either relatives or friends.

**Micro birth planning:** During micro birth planning, date of next visit was discussed with 37.4%, place of next visit 9.3%, EDD 26.6%, place of delivery 23.3% and transport arrangement for delivery was discussed with a mere 2.6% of mothers.

**Transport arrangement:** Mode of transport used by the mothers to reach the institution was mostly auto ricksow-57.5% and Janani express was used by 25.4%.

**Person arranged transport:** By family members in 68.8% and by ASHA 57.5% and GKS 0.8%.

**Accompanying person:** In 60% cases ASHA accompanied the mothers to the institution and 29.4% were accompanied by the family members.

**Type of delivery & person conducted:** Out of total cases surveyed 77.3% had normal delivery while 22.7% had cesarean section.

Out of total normal delivery 52% deliveries conducted by the nurse and 39.2 % by the doctor.

**Outcome of pregnancy**: Out of total 95.8% yield live birth and 4.2% still birth.

**Duration of stay at the institution:** More than 48 hospital stay was found amongst 40% and < 48 hours was among 60% of the mothers.

**Expenses Incurred for ANC & PNC:** Out of those who attended ANC clinic 90% had to incur expenses for various purposes like pathological investigations, medicine, ultrasonography.

Amongst the women in post natal care 91.8% had to incur some expense for various purposes. Out of this 72.8% spent money on procurement of medicines.

### Post natal visit

Post natal visit by the health providers was reported by 23.3% mothers of which 76.5% had post natal visit was by ASHA.

**Cash benefit:** Out of the total 84.5% received the cash benefits for delivering at institutions.

**Quality of care assessment:** Out of the total women delivered at the institution 82.9% women are satisfied with the services provided at the institutions.

The reason stated for institutional delivery was cash benefit-32.1%, availability of doctors 39.7% and better care 28.2%.

The important thing liked by most of the women at the institution was availability of the doctor by 49.3%.

The things they did not like at the institution were payment for medicines and saline (32.0%), dealings and behavior of health personnel (20.7%) and cumbersome process of cash payment (9.9%).

**Inference:** The preliminary data shows that almost more than 95% mother registered under JSY and availed ANC. The early registration and cash beneficiary number indicates definite increase of institutional delivery.

### Future Plan

To assess the functioning of various processes indicators of JSY all the facility based and population based surveys will be conducted and impact of JSY in these study districts will be looked into.

## 11. Migration, poverty and access to healthcare: a multi centric study on people's access and health system's responsiveness in fast-growing smaller cities of India: a multi-centric study.

| | |
|---|---|
| Principal Investigator | : Dr. Anna S. Kerketta, |
| Co-Investigator(s) | : Dr. G Bulliyya<br>Dr. D Das, G Mallick |
| Starting Date | : May 2011 |
| Closing Date | : November 2013 |
| Status | : Extramural,<br>ICMR National<br>Task force. |

### Broad Objective

To assess the migrants' healthcare access in the vulnerability context of migration and livelihood insecurity, and to understand the factors (individual-/community-/system-level) affecting the migrants' access to healthcare services and to identify key points to develop an intervention to improve healthcare access to the socio-economically disadvantaged migrants.

### Specific Objectives

1. To study the demographic and socio-economic characteristics of the migrant communities.
   - (a) Migration history/duration of migration
   - (b) Age/gender/educational/occupational/religious/ethnic composition
2. To explore the community/organizational capacity of the migrants and migrant communities.
   - (a) The social networks/CBOs/NGOs of the migrants/migrant communities and their role in health/help seeking
   - (b) Formal and informal processes of decision making related to healthcare issues (at household level and community level)
   - (c) Organizational capacity of the migrant groups to negotiate for better services
   - (d) Existing communication channels available to these communities and their utilization pattern
3. To assess the availability, accessibility, adequacy, affordability and acceptability of the existing system of healthcare delivery to the migrants, in view of distinct features of migrants.
   - (a) To assess the felt healthcare needs, utilization and perceived relevance of available healthcare services by migrants
   - (b) To elicit the migrants' assessment of quality of healthcare
   - (c) To identify the perceived roles of the community and health system in improving the provision of healthcare services.
   - (d) To identify the demand-side barriers deterring the migrants' access to and utilization of healthcare services.
   - (e) To identify the difficulties and bottlenecks of government health services in delivering the healthcare service to the migrants
4. To explore the governmental processes of identifying new areas/settlements and processes of placing basic amenities like water supply and sanitation, health infrastructure and manpower (including outreach) to cater to those areas.
5. To assess the 'exclusion' of migrants from provision of healthcare services in the background of their migration status.
6. To review the existing modes of communication and IEC strategies by the health system and to identify the strengths and gaps in reaching the migrant communities.

7. To review the existing policies and regulations with regard to healthcare to migrants and slum population.
8. To develop an intervention model for improving the healthcare access to the migrants based on the results of the above formative research.

**Study Area:** Slums of Bhubaneswar (Capital city) of Odisha.

**Study population:** Population who have migrated to urban areas and currently been living in the urban slums/JJ clusters/slum like temporary settlements/camps, etc. during a period of 30 days to 10 years.

## Methodology of Formative Phase

### *Phase I: Formative Phase*

The formative study will be undertaken among the migrant communities and health system, specifically the public sector primary healthcare system. This phase of research will be used to assess the healthcare access to the migrant communities and to identify the obstacles, facilitators from both the migrants' and health system's perspective and to identify specific communication channels, and for identifying various stakeholders that can take part in the intervention for improving healthcare access to the migrants. This information will be useful in development and implementation of an intervention.

## Methodology

***Study design:*** Single-stage cluster random sampling will be used for selecting the migrant households. Households of eligible migrants (who have migrated and residing in the city at least since 10 years, but not lesser than 30 days) will be identified from various clusters in the city. Newer, denotified slums and camps will be id3entified. Snow-balling technique may be used during pilot survey for identifying this type of habitations.

***Data collection Techniques:*** Both quantitative and qualitative research methods will be used. Quantitative data pertaining to socio-economic, demographic details, healthcare seeking behaviour will be collected through pre-tested, interviewer-administered questionnaires. Qualitative methods like in-depth interviews, focus group discussions, key-informant interviews and case studies will be used to collect above data.

To assess the health system related factors in-depth interviews will be conducted with various health care providers with a focus on outreach of services, system's preparedness to provide services to the ever increasing migrant population, barriers and facilitators for the provision of the services to the migrant communities. Healthcare policy makers/ providers from the public sector primary healthcare services will be selected for conducting in-depth interviews.

***Statistical Analysis:*** The quantitative and qualitative data will be analysed by SPSS and Atlas/ ti, respectively.

*Quantitative Data*: The quantitative data will be computerized and analysed through SPSS. *Qualitative Data:* The qualitative data management and analysis will be done with the help of Atlas/ti (Scientific Software Development, Berlin, Germany), a software package for qualitative data analysis.

**Phase II: Intervention Phase (implementation of developed strategy and process and outcome/ impact evaluation):** Based on the formative research, an appropriate intervention with inclusive partnership strategy will be planned and implemented. An evaluation will be carried out at the end of the intervention.

## Sample size estimation

**For quantitative household survey**: The required sample size has been calculated using precision formula $n=z^2_{1-\alpha/2}(1-P)/\Sigma^2 P$ (Lwanga and Lemeshow, 1991). By considering prevalence of

utilization of government healthcare service (*P*) of 15% (This rate is based on an ongoing ICMR study in Delhi (Kusuma, personal communication) and it is considered as minimum rate and it will be considered for all study sites.), allowing 10% error and *P* with the 95% confidence interval, the sample size would be 2177. Since, it will be two-stage sampling by considering the design effect (DEFF) of 1.7, the sample size would be 3265, and this sample size with 5% of non-response rate, would be 3886.

**For qualitative household survey:** The sampling will be purposive and the selection of the participants will be continued till saturation of data is attained.

**Work Progress:** The base line data on the slums in Bhubaneswar the cities and health care system was collected from the municipal corporation. The slums have been identified through personal visit and discussing with the community members, leaders, corporators and the providers. After several visit and rapport building with the target population in the slums the data collection for formative research using pre-tested common questionnaire has been started. So far 55 numbers of slums have been identified and 470 questionnaire surveys have been undertaken in 10 numbers of slums.

### Future Plan

Complete quantitative questionnaire survey and initiate data collection by qualitative survey methods.

## 12. Assessment of adolescent reproductive and sexual health programme in Orissa: advocacy for intervention strategies.

| | | |
|---|---|---|
| Principal Investigator | : | Dr. G. Bulliyya |
| Co-Investigator | : | Dr. A. S. Kerketta |
| Starting date | : | May 2011 |
| Closing date | : | April 2014 |
| Status | : | Extramural (ICMR Adhoc project) |

### Introduction

Adolescents (10-19 years) comprise 22.8% (225 million) of the Indian population and their numbers are steadily rising. It is a period of transition from childhood to adulthood marked by rapid physical, physiological, sexual and behavioural changes. Adolescents are heterogeneous group, and their situation varies by age, sex, marital status, class, region and cultural context. A large proportion of them are out of school, malnourished, get married early, work in vulnerable situations, sexually active, and exposed to peer pressures. They poses a distinct array of health challenges including teeage pregnancy, unsafe abortions, excess risk of maternal and infant mortality, high-risk behavior, lack of awareness about contraception and reproductive tract infections and rapidly rising incidence of HIV / AIDS.

Adolescents are generally considered healthy by themselves, their families, even healthcare providers and society at large. Yet they are known to suffer significant morbidity caused by risk taking behaviour and inadequate access to health care. Yet, their access to health services remains poor. Improvement in health status of adolescents has inter-generational impact Thus it is important to influence the health-seeking behaviour of adolescents as their situation will be central in determining health, mortality and morbidity and the population growth scenario. To address this, Adolescent Reproductive and Sexual Health (ARSH) launched as a key strategy in the RCH-II under NRHM programme implementation plan to yield dividends in terms of delaying age at marriage, reducing incidence of teenage pregnancy, prevention and management of obstetric complications including access to early and safe abortion services and reduction of unsafe sexual behaviour. To achieve the goals, Adolescent Friendly Health Clinics (AFHC) services in line at institutional levels to provide quality healthcare, counseling services and to build a supportive environment.

## Objectives

The general objective is to evaluate the adolescent reproductive and sexual health (ARSH) program and adolescent friendly health clinic (AFHC) services through developing advocacy-based intervention in Orissa.

## Specific

- To assess the knowledge, attitude and behavior on reproductive health problems of adolescents
- To assess the quality of care at Adolescent Friendly Health Clinics;
- To assess the accessibility and utilization of health care services by adolescents; and
- To devise plausible ways and intervene with package of services to explore opportunities for improving utilization of adolescent health services.

## Methodology

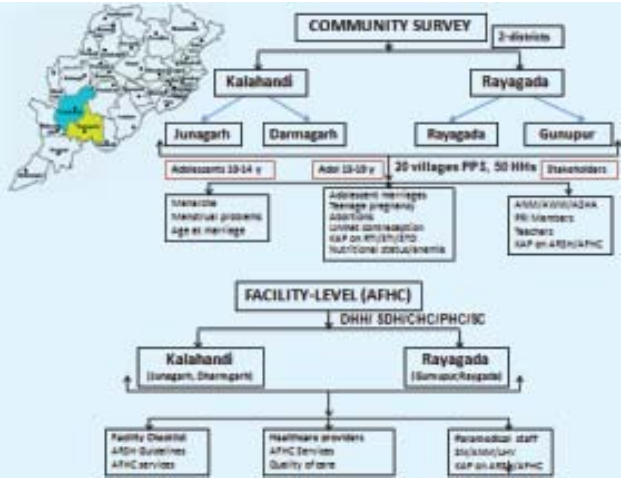
The study is conducted in two phases:

Phase-I comprise formative research on evaluation of baseline process indicators of ARSH program and quality of care at AFHS facility and at community levels. Situation analysis is the first step for systematic scaling up that involves collection of data and identifying gaps.

Phase II: The findings of phase-I will be used for strategy development and implementation of intervention programs based on baseline evaluation to improve ARSH services towards achieving the envisaged goals.

## Study area

The study is conducted in two districts that include Kalahandi and Rayagada, where 29% and 35 of women married before 18 years and adolescent health indicators are poor (DLHS-3, 2010). Multistage stratified random sampling procedure adopted for selection of blocks, villages households and respondents.




## Progress

Field surveys have been conducted in Junagarh and Damagarh blocks of Kalahandi district. The study population includes adolescents aged 10-19 years of both sexes (male, female) and marital status (unmarried/married) groups. A pre-tested questionnaire was used to collect data on adolescent reproductive and sexual health issues. The coverage of Household (HH) particulars and study population is provided in Tables 1 & 2. A majority of HH belong to Hindu religion. About 11% are scheduled castes and 23% are scheduled tribes, and 50% low socioeconomic category.

**Table 1.** Coverage of study sample.

| Households | Category | Percent |
|---|---|---|
| Religion | Hindu | 92.6 |
| | Muslim | 2.3 |
| | Christian | 5.1 |
| Community | SC | 10.6 |
| | ST | 22.8 |
| | OBC | 51.6 |
| | Others | 15.0 |
| Family size | ≤4 | 35.5 |
| | >4 | 64.5 |
| Socioeconomic status | Low | 49.4 |
| | Medium | 38.3 |
| | High | 12.3 |

**Table 2**. Characteristics of study population.

| Households | Male | Female | Pooled |
|---|---|---|---|
| Sex | 50.7 (214) | 49.3 (208) | 100.0 (422) |
| Adolescents 10-13 y | 35.5 (76) | 37.0 (77) | 36.3 (153) |
| 14-16 y | 37.4 (80) | 34.1 (71) | 35.8 (151) |
| 17-19 y | 27.1 (58) | 28.8 (60 ) | 28.0 (118) |
| Married | 8.4 (18) | 13.5 (28) | 10.9 (46) |
| Girls married <18y age | - | 7.2 (15) | - |
| Pregnant women | - | 2.4 (5) | - |
| Women with 1 child | - | 3.8 (8) | - |

A sample of 422 adolescents that include both sexes, irrespective of marital status and physiological status covered from household (270) and school surveys (Table 2). Almost equal number of participants were covered, 11% of them married, 7.2% of girls married before 18 years, 2.4% pregnant and 3.8% women with child. Early marriage followed by early pregnancy interrupts education of females exposes to higher reproductive health risks.

Majority of adolescents were aware of puberty and have prior knowledge on pubertal changes (Table 3). Age at menarche was 13.4 years. Considerable proportions of adolescent girls were aware about menstruation prior to menarche and facing premenstrual and post menstrual problems. About one fourth of girls were using sanitary pads

**Table 3.** Knowledge on adolescent reproductive health issues.

| Adolescents perception | Male (214) | Female (208) |
|---|---|---|
| **Puberty & marriages** | | |
| Knowledge of puberty changes | 52.6 | 64.2 |
| Aware of pubertal changes | 38.9 | 28.4 |
| Age at menarche (years) | - | 13.4 |
| Premenstrual problems | - | 58.4 |
| During menstruation | - | 48.6 |
| Post menstrual problems | - | 26.0 |
| Using disposable sanitary pads | - | 23.2 |
| Aware of legal age of marriage | 18.4 | 16.4 |
| Consequences of early marriages | 12.0 | 8.2 |
| Participated in family life education | 1.5 | - |
| **Contraception & RTI** | | |
| Aware about contraception | 32.0 | 21.6 |
| Aware about source of contraception | 22.0 | 8.5 |
| Aware about RTI/STD/HIV | 16.5 | 8.6 |
| Aware of routes of transmission | 2.1 | 2.1 |
| Aware about RTI/STI | 9.8 | 2.2 |
| Aware about HIV/AIDS | 59.5 | 42.4 |
| Source of getting information | | |
| TV/Radio | 26.0 | 15.6 |
| News paper/magazine | 7.2 | 4.2 |
| Healthcare worker | 5.91 | 6.4 |
| School teacher | 2.0 | 5.2 |
| Parents | 3.4 | 2.4 |
| Friends/peers | 5.0 | 8.7 |
| Nutritional status | | |
| Underweight (BMI-for-age <5$^{th}$ per) | 34.6 | 38.0 |
| Stunting (height-for-age <-2SD) | 33.2 | 34.1 |

during menstruation. Knowledge about legal age of marriage, consequences of early marriages, teenage pregnancies ranged 8-18% in both genders. Majority of adolescent not aware of RTI including HIV, routes of transmission and sources of getting contraception. Half of study population had aware of HIV/AID, getting the information from various media sources. Nutrition status revealed that one-third of adolescents had thinness (low BMI-for-age) and stunting or chronic undernutrition. Knowledge related adolescent health services including ARSH and AFHC are found to negligible.

### Plan for next year

The study is planned to evaluate process indicators in other selected blocks of the districts covering adequate sample as per proposal in identifying gaps in accessibility, availability of adolescent health services at community and facility levels. Strategy will be developed that include monitoring and impact assessment in improving adolescent health needs as per program goals.

## 13. Pilot introduction of the Modified Bivalent Killed Whole cell Oral Cholera Vaccine in Orissa.

| | | |
|---|---|---|
| Principal lnvestigator | : | Dr Shantanu K. Kar |
| Co-PIs | : | Dr. Anna S. Kerketta, Dr.H. K. Khuntia |
| Collaborators | : | State Health Dept, Govt. Of Orissa: Dr.Bikash Pattnaik, (Surveillance Officer) International Vaccine Institute, Korea: Dr. Sun heang Shin, Dr.Binod Shah, Dr.Anil Kumar Bhola, Dr. Alfred Pach, Dr.Brian Maskery, Dr.John Clemens, Dr. Md. Ali, Mr.Mahesh K Puri. |
| NICED | : | Dr. G. B. Nair |
| Starting Date | : | January 2011 |
| Completion date | : | February 2012 |
| Funding | : | Bill and Melinda Gates Foundation |

### Objective of the study

### Primary objective

To determine the feasibility, acceptability and costs associated with the pilot introduction of the modified killed whole cell oral cholera vaccine in India when given in a public health setting.

### Secondary objective

To identify obstacles to mass oral cholera vaccine implementation.

### Work Progress

**Preparatory activities:** The preparatory activity includes a multistage community outreach campaign to disseminate information about the step wise activities related to the mass vaccination programme. The local and potential stake holders, community leaders and volunteers were identified. Several meetings were organized along with the local health authority and the most appropriate means of communication for the information dissemination was identified.

### Socio-behavioral survey

The socio-beahavioural survey was conducted in-order to collect the informations related to diarrhoeal disorder and to use these informations for effective social mobilization campaign. Focus group discussions (FGDs) were held with the community members, leaders and health care providers.

### Social mobilization campaign

Since the oral cholera vaccine is a 'new' vaccine to both the providers and the community residents, extensive efforts were made on social mobilization activities, that started with sensitization of community leaders/members, stake holders like the local PRI members like MP and MLA of the district, the Block chairman, Block development officer, Panchayat samittee nominee, Sarpanch of the Gram Panchayat and grass-root level providers like ASHA, AWW and MPHW of the catchment area. Preparation of the campaign was entailed planning informational messages and media, and their dissemination through

the use of leaflets, stakeholder meetings, posters and other modes of communication.

The content of the sensitization meetings were derived from the Focused Group Discussions (FGDs). The suggestions on mode of social mobilization activities that came from the participants were consistent with the findings from the FGDs and they are:

(a) Distribution of leaflets and posters highlighting the importance and necessity of the campaign in the community.
(b) Putting of banners at the vaccination booths and in the community.
(c) Holding of meetings with various Self-Help Group (SHG) in the community.
(d) Miking in the community 2 days before and during the campaign.
(e) Inter-personal communication by the ASHA and AWW with the community residents.


Community mobilisation

## Census of the catchment population:

Census was undertaken using personal digital assistance (PDA) in order to obtain demographic characteristics of the study population for determination of target population for the mass vaccination programe and to support in evaluating feasibility, acceptability and effectiveness OCV. Census also acted as the first level of social mobilization as the researchers visited each and every household and through personal interaction made the community about the upcoming oral cholera mass vaccination.

The census revealed there are 51,872 populations in 9166 house holds. 94% house holds use community tap or hand pump as source of drinking water. Hand washing and open field defecation is a common practice by 80-84% population. Health facility is used by 89% people for care seeking for diarrhea disorder.

## GIS mapping of the catchment area:

The GIS mapping of the study area was done to facilitate to map spread of households, road networking, water bodies surrounding each household and to trace the vaccinated household later during cluster analysis of protective efficacy of vaccine in community.

## Micro-planning for the mass vaccination

A detailed micro-plan for vaccination was prepared in collaboration with the local health


GIS Map of Study Area

**Fig. 1.** GIS map of study area showing human settlement and household wise GPS coordinates.

authority. Given the logistic challenges, mainly in terms of human resources and cold chain capacity, mass vaccination was planned to be undertaken in 2 phases. The micro plan for Polio vaccination was used as the base for the mass vaccination with OCV.

### Mass vaccination

Mass vaccination was under taken by state health department in collaboration with RMRC for all population except less than one year and pregnant women after obtaining verbal consent/ascent. Two rounds of vaccination at least 14 days apart were scheduled for two dose of vaccine. Existing state government infrastructure and personnel were participated in this endeavor.

Registration of participants in vaccinatiion booth

OCV Vaccination

### Vaccine Coverage

In the two phases of vaccination programme a total of 31562(61.0%) people received vaccine out of total 51865 population covered in census. Of which 23755 (75.3%) received both the doses and 7807 (24.7%) received only one dose.

### Adverse Reactions

There were no major concerns regarding the safety of the vaccine. Few cases of vomiting and headache were reported immediate following vaccination that was investigated and found to be transient and reversible.

### Post vaccination survey

The post vaccination survey was conducted to evaluate the clinical acceptability of the vaccine and adverse events in a sample of 500 individuals. Random samples of up to 300 non-participants were also interviewed to identify reasons for non participation after obtaining informed consent for the interview.

**Inference:** The mass vaccination of OCV within the public health setting was successful. The vaccine was safe with out any major side effect. Though few cases of adverse effect reported those were transient and reversible.

### Plan for next steps

As per the suggestion of Scientific Advisory Committee of the Centre as well as State Health Department it is proposed to assess the effectiveness of the vaccine through a case-control study.

## 14. Study on drug resistance among sputum positive tuberculosis patients in Rayagada district, Orissa.

| | | |
|---|---|---|
| Principal Investigator | : | Dr Dasarathi Das |
| Investigators | : | Dr. B. Dwibedi |
| Collaborator | : | TRC, Chennai |
| Starting date | : | June 2011 |
| Closing date | : | June 2013. |

### Summary of the proposed research

Tuberculosis is one of the major public health problems of India and it carries more than 20% of the world's TB burden..The World Health Organization's has made it one of its Millennium Development Goals to reverse the incidence of TB by 2015. However the success of TB control world wide has been affected due to the emergence of drug resistant and extremely drug resistant tuberculosis. At present, only 2% of MDR-TB cases worldwide are being diagnosed, mainly because of inadequate laboratory services. India at present lacks adequate laboratory facilities for MDR-TB testing. According to WHO, there were more than 110,000 cases of MDR-TB in 2006, half of which occurred among new TB cases. This represents more than 20 percent of the global burden. It was estimated that 1-3% NSP cases and 12-17% re treatment cases are due to MDR-TB

The present study is planned in Rayagada district which is a tribal dominated district of Orissa.. The predominant tribes inhabiting in this district are Khond(71.09%), Saora(11.55%) and Sabar (7.17%) communities. According to the RNTCP data for Rayagada district in the year 2010 total 1637 patients have taken TB treatment, out of which new smear positive cases were 938. The annualized case detection rate per lakh population of Rayagada district for the year 2010 amounts to 163 with a failure rate of about 2%. Due to various environmental factors like climatic change, deforestation, industrialization the infection changes drastically and develops to a much higher level of resistance. The drug resistance primarily to Rifampicin and Isoniazid will be an indicator for MDR-TB prevalence in this area and will be helpful in planning TB control activities more efficiently.

### Methodology

I. Networking of District Tuberculosis Officer, DOTS providers, Lab Technicians with the research group in the district will be carried out.

II. The ongoing programme of TB care in Rayagada district has identified sputum positive cases on treatment, treatment failure cases in its 11 blocks. The sputum samples of patients under treatment and treatment failure cases will be collected in the respective microscopy centres and transported to the district headquarter. Both morning and spot samples will be collected from individual patient.

III. Two samples (morning and spot) from new smear positive patients attending OPD of District Hospital, Rayagada will be collected.

IV. Sputum samples will be collected in a sterile McCartney bottle with equal volume of 1% Cetyl Pyridinium Chloride in 2% Sodium Chloride.

V. The external surface of the vials carrying sputum samples will be decontaminated by dipping the vials in 5% Phenol for 30 minutes. The samples will be stored in room temperature for a maximum period of one week (Monday to Saturday) and in Sunday all the accumulated samples will be transported to RMRC, Bhubaneswar for culture processing and DST studies.

VI. The sputum samples will be processed at RMRC, TB Laboratory and will be inoculated to LJ medium, LJ medium with PNB. The drug susceptibility studies with Rifampicin and Isoniazid will be carried out by Proportion Sensitivity Test (PST) method.

## 15. Etiology of diarrhoea in three tribal districts of Orissa.

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

### Objectives:

(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

The project was initiated with the support of intramural funding in three tribal districts like Kashipur (Rayagada), Laxmipur & Dasamantapur (Koraput) and Mohana (Gajapati Dist blocks)Both the rectal swabs from diarrhoea patients (IPD and out break villages) and environmental water samples were collected for bacteriological analysis. In total 608 rectal swabs were collected from Kashipur (214), Dasamantapur (151) Laxmipur (142)and Mohana (101) blocks(Table-1). Out of total 330 culture positives *E. coli* were 249 (75.5%) followed by *V.cholerae* O1 Ogawa-60 and Inaba - 2 (18.8%), Shigella species 15(4.5%), Salmonella spp 4(1.2%). Among the Shigella Spp. isolated, *S. dysenterae* type-1 were 2, *S.flexnerae* - 10, *S. boydii* - 3. The *V.cholerae* O1 strains were sensitive to ciprofloxacin, norfloxacin, neomycin, azithromycin, gentamicin, chloramphenicol, Ofloxacin and resistant to tetracycline, erythromycine, co-trimoxazole, ampicillin, furazolidone, nalidixic acid. There was cholera epidemic reported in the Rayagada district from July to October,2010 accounting for high morbidiy and mortality affecting 8 out of 11 blocks. The cholera epidemic was affecting 96 grama panchayatas, 443 villages and 2087 diarrhoea cases and 41 death , with an attack rate 0.25. (Table 2) Similarly there was a cholera out break in two villages of Mohana block of Gajapati district during the month of August, 2010.The date wise incidence of diarrhoea cases in four blocks i.e Kashipur, K.singhpur, B. cuttack and Mohana has been described in Fig No-1. Out of eight diarrhoea affected blocks Kashipur, Kalyan Singhpur, B. Cuttack were worst affected. The present cholera epidemic in Rayagada district indicates that new blocks and new villages were affected in comparison to 2007 cholera epidemic. Consumption of contaminated water, un-hygienic condition, poor knowledge on diarrhoea and migration of people were responsible for acquiring and spread of the infection .The MAMA PCR results indicated that 60% strains were hybrid *V.cholerae* El for variant and rest 40% were normal El for strains. This indicates that the hybrid *V.cholerae* strains are dominating over the normal *V.cholerae* El for strains

**Table-1:** Bacteriological analysis of enteropathogens isolated from diarrhoea patients from four tribal blocks (May, 10 to July, 2011).

| | Kashipur | Dasmanthpur | Laxmipur | Mohana | Total(%) |
|---|---|---|---|---|---|
| Samples collected | 214 | 151 | 142 | 101 | 608 |
| Culture +ve | 127 | 63 | 80 | 60 | 330(54.3%) |
| E.coli | 72 | 56 | 77 | 44 | 249(75.5%) |
| *V.cholerae*(O) | 45 | 2 | 1 | 14 | 62(18.8%) |
| Shigella spp. | 7 | 5 | 1 | 2 | 15(4.5%) |
| Salmonella spp. | 3 | 0 | 1 | 0 | 4(1.2%) |
| Culture -ve | 87 | 88 | 62 | 41 | 278(45.7%) |

**Table-2:** Block and village wise diarrhoea cases and death in Rayagada district (15.7.10 to 4.10.2010).

| Sl.No | Name of the Block | No of affected GP | No of affected Village | Total no ofAttack |
|---|---|---|---|---|
| 1 | B.Cuttack | 19 | 87 | 347 |
| 2 | Gudari | 9 | 23 | 83 |
| 3 | Jagannathpur | 4 | 10 | 85 |
| 4 | Jemadeipentho | 22 | 74 | 291 |
| 5 | K.Singhpur | 13 | 109 | 751 |
| 6 | Kashipur | 16 | 96 | 383 |
| 7 | Kolanara | 7 | 18 | 62 |
| 8 | Muniguda | 6 | 26 | 85 |
| | **Total** | **96** | **443** | **2087** |

1. Total Blocks= 11
2. Total Blocks diarrhoea affected= 8
3. No diarrhoea outbreak block= 3 ( Padampur, Chandrapur & Ramanaguda)

**Table-3:** Analysis Water samples from four tribal blocks (May, 10 to July, 11).

| Block Name | Total samples | No. +ve for *V.cholerae* *Non O1 & Non O139 / O1 Ogawa* |
|---|---|---|
| Kashipur | 141 | 8 / 0 |
| Dashmanthpur | 46 | 1 / 0 |
| Laxmipur | 75 | 3 / 0 |
| Mohana | 99 | 0 / 4 (3-Open well, 1 - Tube well |
| Total | 361 | 12 / 4 |

and spreading to the diarrhoea unaffected areas in comparison to 2007 cholera epidemic.

Similarly, 361 environmental water samples were collected from four study blocks from different villages from different water sources like river, stream, nala, chua, dug well etc. for monitoring the presence of *V. cholerae* strains. Twelve water sources like river, chua, dug well and stream were positive for *V.cholerae* non O1 and non O139 strains, where as 4/13 water samples collected from open well, tube well from Mohana area during July, 2011 were positive for *V.cholerae* O1' Ogawa biotype El tor. (Table -3)

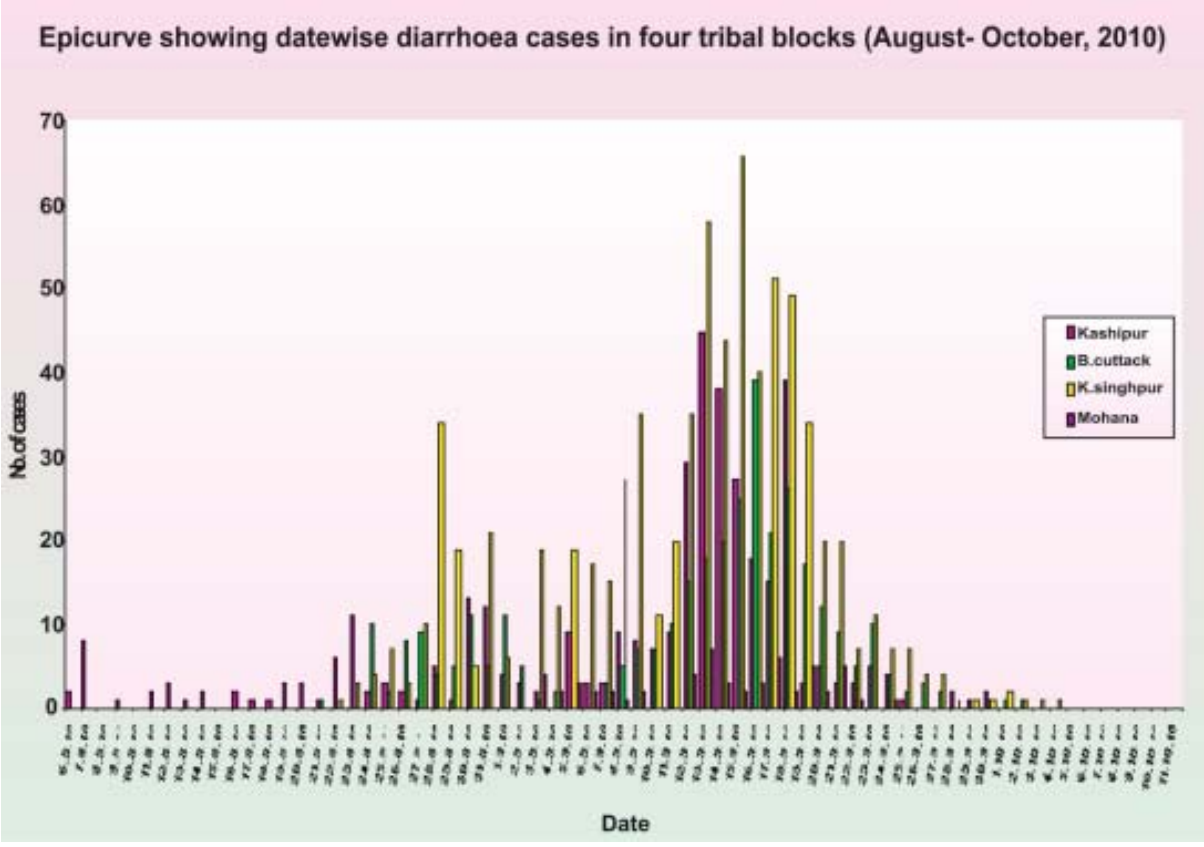



**Fig. 1.** Date wise incidence of diarrhoea cases in Kashipur,K. singhpur, B. cuttack and Mohana blocks.

Scientists & Students working in the modernised laboratory of RMRC, Bhubaneswar

# Translational Research

**Hon'ble Minister of Health & Family Welfare, Govt. of Odisha, Sri Prasana Acharya inaugurating RMRC field unit at Rayagada**

## 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

### 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.

### Work Progress

**Study area**

The samples used in the study originated from various localities of Orissa. The samples were collected from Mayurbhanj, Keonjhar Nawarangpur..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.

1. Molecular approach for identification of four malaria vectors of Odish

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

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

### Future Work

1. Conducting Work shop on the technique developed on the identification of the species and mosquito processing system with different category of staffs and scientists from state Govt., VCRC and NIMR before validation inter institutionally.

   (i) 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. Khuntia.

### 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.

### Work Progress

1 Successfully a kit has been prepared, which can be preserved up to 3 week at 00 C which gives

satisfactory results. This will continue for the experimental validity for longer period. As per the work plan, inter and intra validation of V. cholerae strains will be done. For this, 30 coded strains of V. cholerae have procured from NICED Kolkata. This validation work is on progress, the results of which will be sent in the next report.

### New Translational Research Identified

1. Establishment of Network for detection of MDR-TB in Raygada.
   Principle investigator : Dr. D. Das
   Duration : 3 years

2. Prevalance and pattern of reproductive tract infectios among tribal population of Rayagada.
   Principle investigator : Dr. M. R. Ranjit
   Duration : 3 years.

Prof. K.K. Talwar, Chairman, Medical Council of India, Dr. V.M. Katoch, Secy. DHR & DG ICMR & Dr. S.K. Kar, Director RMRC in the seminar on "Galvanization of Research in Medical college - Role of ICMR" held on 4th Jan 2012 at RMRC, BBSR

# Completed Studies

# Contents



## 1. 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 : March 2008
Closing date : March 2010 (fund received late / continued to 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.

### Work Done

During the period of investigation a total number of 374 P falciparum isolates from 8 districts (Fig. 1) [Sundergarh (Bisra:15, Rourkela:13), Mayurbhanj (Jashipur:15, Bisoi:5, Mananda:10, Badampahar:33), Keonjhar (Ghatagaon:10, Bansapal: 13), Anugul (Bantala:18, Godibandha:13), Nayagarh (7) Kandhamala (Daringbadi:50, Phiringia:4) Rayagada (Gunupur: 22) and Kalahandi (T. Rampur: 17), undivided Cuttack district:129 (Athagarh/ Jagatsingpur/ Kendrapara/ Jajpur) ] have been analyzed for CQ and S-P drug resistance markers.

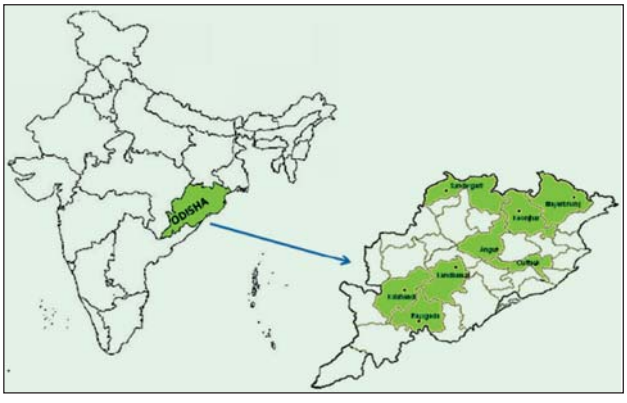



**Fig 1.** Map showing districts selected for the study.

The genomic DNA of P falciparum was isolated by phenol extraction and EtOH precipitation. The point mutations in PfCRT (K76T) / PfMDR1 (N86Y) and DHFR (N51 I, C59R, S108 N/T and V164L)/ DHPS (S/A436F, A437G, K/L540E and A581G) genes responsible for CQ and S-P drug resistance respectively were analyzed by PCR-RFLP method (Fig. 2). The microsatellite analysis in all samples and sequencing of the Pfcrt and Pfmdr1 gene have been done in around 40 DNA samples to investigate the origin of the mutations responsible for the CQ resistance in P falciparum isolates circulating in this part of the country.

## Conclusion

1. The over all frequency of Pfcrt(76T) allele is 82.7% and Pfmdr1(86Y) allele is 56.9% indicates that the CQ resistance P falciparum isolates are more prevalent in number than wild types and CQ may not be useful to the control programme in Orissa.
2. The high prevalence of both the mutations (76T and 86Y) is a great concern because, besides CQ resistance, 86Y has also been found to confer resistance to Artemesinin -based combination therapy (ACT) and lumefantrine.

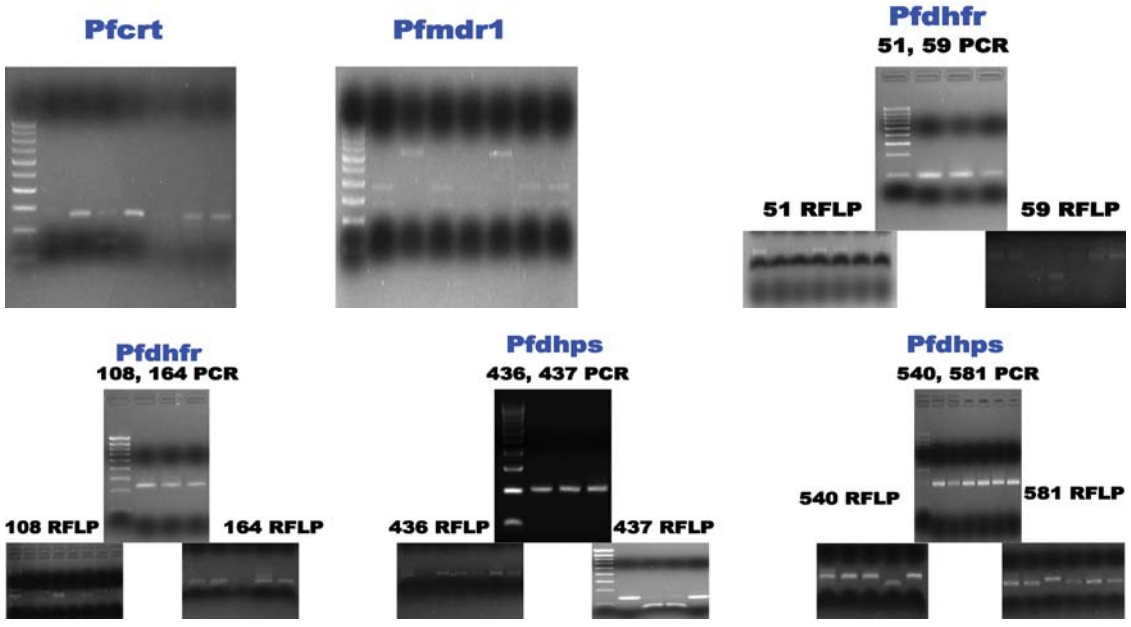



**Fig 2.** Gel photo showing PCR -RFLP fragments of the representative samples

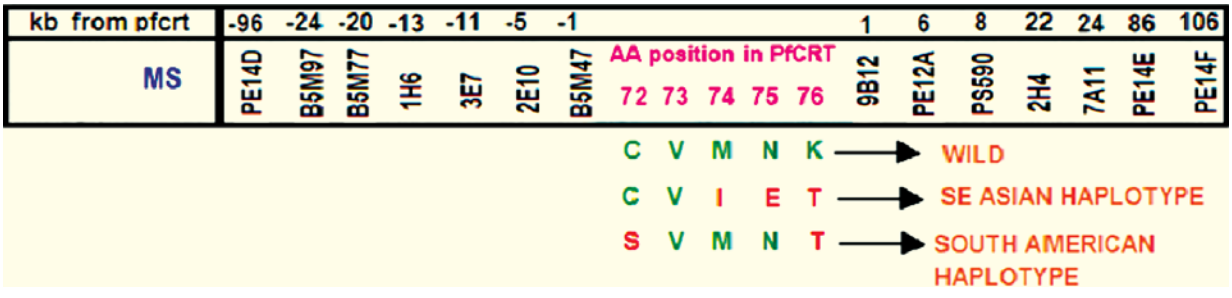
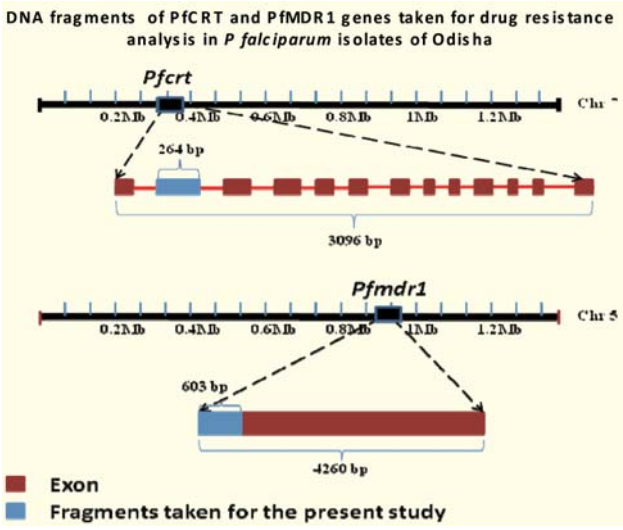



**Fig 3.** Schematic positions of the microsatellite positions of the Pfcrt gene

Fig. 4




3. The sequencing and microsatellite data reveals that both the genes are evolving under standard neutral model of molecular evolution in population sample of Odisha and quite different from Africa and South East Asia.

4. Strong LD between different SNPs in the Pfcrt gene indicates that many mutations in this gene possibly provide greater advantage to the CQR in P falciparum.

5. Observation of LD between two different SNPs of the Pfcrt and Pfmdr1 gene shows that the Pfcrt gene possibly acts synergistically for CQR phenotypes.

6. Analysis of point mutations in DHFR and DHPS genes responsible for S-P drug resistance indicates that quadruple mutation (responsible for RIII level of resistance) was conspicuously absent. Only combination of double mutations (RI level of resistance) has been observed in 4.4 % of the parasite population. This indicates that the parasite population of Orissa has not yet developed resistance to S-P drug combination. Therefore it can be used safely at this moment in combination with other potent antimalarials.

## 2. Development of intervention strategies to reduce iron deficiency anaemia among adolescent girls through iron-folic acid, deworming, vitaminB12 supplementation and nutrition education in a tribal block of Gajapati district, Orissa.

| | | |
|---|---|---|
| Principal Investigator | : | Dr.G.Bulliyya |
| Co-Investigator | : | Dr.B.Dwibedi |
| Starting date | : | May 2009 |
| Closing date | : | September 2011 (Completed). |
| Status | : | 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 B12 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,

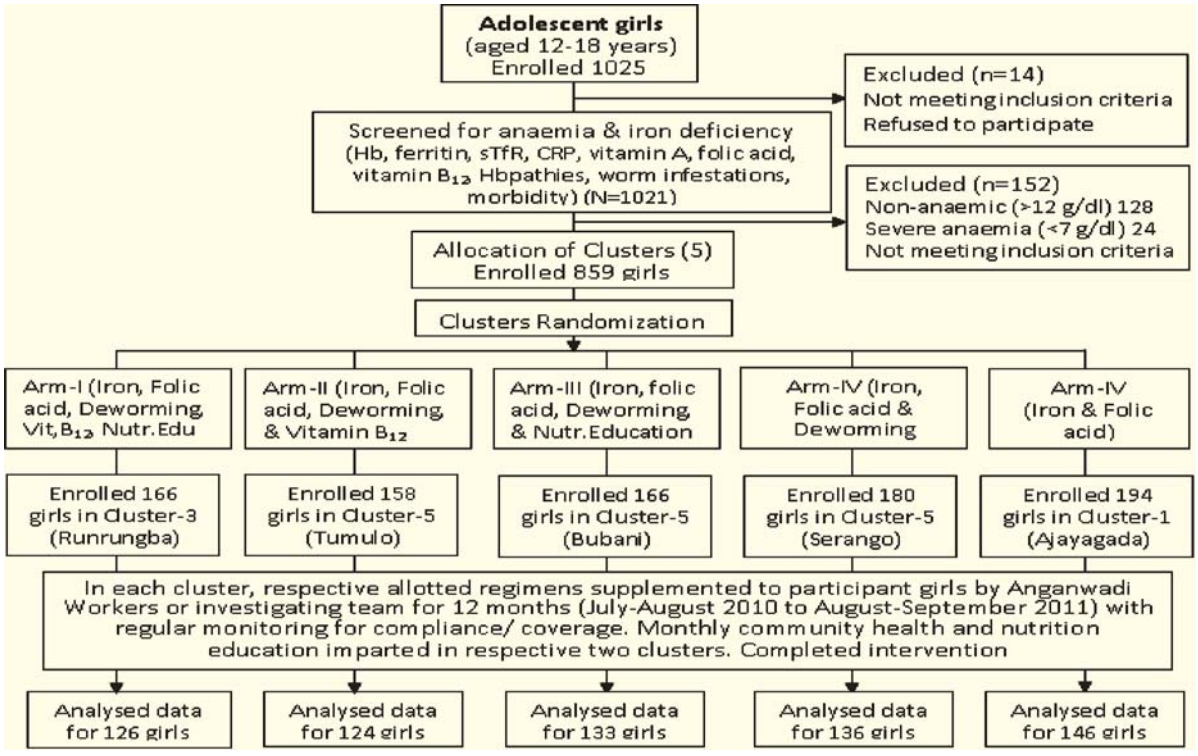



**Fig. 1.** Flowchart showing details at each stage of the study.

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 B12 and nutrition education through routine monitoring by the existing ICDS network.

## Methodology

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, 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 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

The study got the permission from the District Collector, who is the Chairman, of Integrated Tribal Development Agency (ITDA) of Gajapati district, for implementing project at Child Development Project Office (CDPO) of the Integrated Child development Services (ICDS). Gumma, one among the seven

revenue blocks in Gajapati district is selected for the study. Of 5 sectors in study block, Serango having highest tribal population is chosen for the study, where Lanjia Saura primitive tribe is the predominant inhabit.

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. Stool samples were collected and examined for intestinal worm infestations. 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 -200C till analysis. Serum ferritin, soluble transferrin receptor, c-reactive protein, folic acid and vitamin B12 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 iron stores and iron deficiency respectively.

### Observations

Baseline survey was conducted on a sample of 1025 adolescent girls aged 12-18 years 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. The baseline indicators include household socioeconomic status, demographic characteristics, morbidity status, estimations of iron deficiency (haemoglobin, serum ferritin, soluble transferring receptor, c-reactive protein, blood smear examination), confound factors of anaemia such as growth status (underweight, stunting), vitamin A (retinol), malaria, haemoglobinopathies (sickle cell, b-thalasemia) and stool examination for intestinal worm infestations.

### Study clusters

The study area is divided into 5-clusters considering distribution of target sample population of adolescent girls. This was done in the respect of geographical proximity, in order to avoid potential conflicts between neighboring villages bound together by ethnic and kin ties. In each cluster, four contiguous Anganwadi Centres were included to avoid cross contamination between regimen groups. These defined clusters were enlisted in alphabetical order ie, 1.Ajayagada, 2.Bhubani, 3.Rungrumba, 4.Serango and 5.Tumulo. In each study clusters, approximately 200 adolescent girls are available for study. Out of 1025 adolescent girls studied in baseline survey, only 859 girls are found eligible after following exclusion criteria i.e., non-anemic, severely anaemic girls and those suffering from severe illness.

### Randomization of study clusters

Random allocation of clusters 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 in alphabetical order sequentially to one of 5 arm-regimens in the order 1 to 5. Accordingly, regimen arms 1, 2, 3, 4 and 5 allocated to clusters 3, 5, 2, 4 and 1 respectively.

### Implementation of the intervention programme

The implementation period for the project was from August-October-2010 to July-September 2011. The study population continued to receive respective regimen arms of intervention for a period of one year with weekly supplementation. Simultaneously, a monitoring card supplied to each participants and Anganwadi Worker for compliance and coverage. A poster explaining preventive measures of anaemia are displayed locally at each Anganwadi Centre, Panchayat Raj Institutions and Schools.

### Study regimens

1. Iron and folic acid: Weekly supplementation of carbonyl iron equivalent to 100 mg of elemental iron and folic acid 1.5 mg (IRONIFOL capsules, Radicura Pharmaceuticals, New Delhi).
2. Iron-folic acid with vitamin B12: Weekly supplementation of carbonyl iron equivalent to 100 mg of elemental iron, folic acid 1.5 mg and vitamin B12 15ug (FEXID capsules, Overseas Health Care, Phillaur).
3. Albendazole: Half-yearly administration of albedazole 400 mg (ZENTEL tablets, Glaxo Smithkline Pharmaceuticals, Mumbai).
4. Health & Nutrition Education: Monthly IEC campaigns organized in the community targeting study population and health workers using banners, posters, leaflets, flip-charts etc developed in local language. Key messages communicated on health and nutrition through orientation trainings and posters at all community health statations.

### Compliance and coverage

A three-level monitoring system is being put in to the system, compliance to iron supplementation being self monitored by the girls and recorded using an independent monitoring card and a supplementation calendar. Community-level monitoring by the local Angawadi Worker recording in coverage and compliance charts. Coverage includes number of girls accessing the service, number of tablets received, adherence from the commune, adverse events. 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

Of the 859 adolescent girls enrolled for intervention, 666 girls completed the study of one year. A total 190 girls, from arms 1 (40) 2 (24), 3 (33), 4 (44) and 5 (48) could not be followed because of some girls got married and migration of their families. Endline impact assessment was carried out after 12 months intervention period. To assess the impact intervention, the changes in haemoglobin, iron deficiency, worm infestations, folic acid and vitamin B12 deficiencies.

### Impact on haemoglobin and iron deficiency

The mean haemoglobin concentrations among adolescent girls were compared between 5-arm regimen groups at baseline and after 12 months. There were significant differences in the change of haemoglobin for arm-1 (1.6 g/dl) followed by arm-2 (1.4 g/dl), arm-3 (1.2 g/dl), arm-4 (1.1 g/dl), and arm-2 (0.9 g/dl) respectively. There was considerable reduction in anaemia status (hb<12.0 g/dl) of participants in each arm ranging from 36 to 51%. The reduction is more pronounced in arm-1 than in other arms. There were reductions at the rate of 51%, 43%, 37%, 42% and 40% in arm-1, arm-2, arm-3, arm-4 and arm-5.

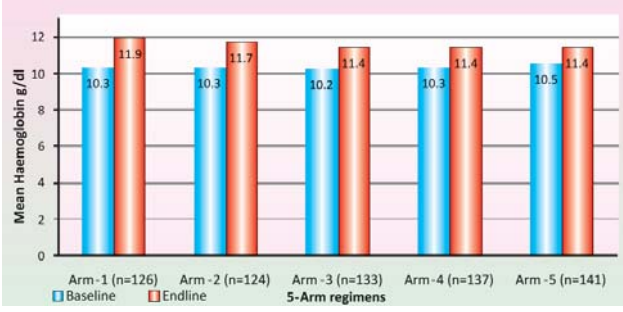



**Fig. 1.** Mean haemoblobin concentrations (g/dl) at baseline and interim assessment after 6 months of intervention.

The data for other indicators of study is being analysed for effectiveness of each regimen in terms of haemoglobin improvements and reduction of iron, folic acid and vitamin B12.

### Conclusion

The study revealed considerable improvements in mean haemoglobin and reduction in anaemia status

of adolescent girls in each arms of the intervention. The arm-1, where all the five regimens included shown to be more effective as compared to four regimens in arm-2 and arm-3, three regimens in arm-4 and two regimens in arm-5. The program needs to be expanded to state-wide and other adjacent states reaching a large-scale adolescent girls population. As well as improving the overall health of women, this will facilitate women entering pregnancy with adequate iron stores, thereby improving pregnancy outcomes, with enhanced potential for healthy growth and development of infants in the crucial early years.

### 3. Molecular characterization of *Anopheles* annularis complex. Development of species-specific diagnostic markers and microsatellite markers.

| | | |
|---|---|---|
| Principal Investigator | : | Dr. R.K.Hazra |
| Co P.I | : | Dr. N.Mahapatra<br>Ms.Aparna Priyadarshini Patra (SRF) |
| Starting Date | : | October 2007 |
| Closing Date | : | December, 2010 |
| Status | : | Extramura, 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 sporozoites identification, blood meal analysis and sibling species identification from single mosquito.
3. To develop microsatellite markers for Anopheles annularis species for population genetics analysis.

#### Background

For effective malaria control, proper identification of anophelines is very much essential. There are 10 main vectors transmit malaria in India. Anopheles annularis is one of the major vectors. Knowledge of vector biology and distribution of species has been limited by the absence of reliable diagnostic markers. In the past mosquito taxonomy depended mostly on using morphological characteristics, cytogenetic and isoenzyme markers. However, lack of expertise taxonomist, sometimes lack of collection of intact mosquitoes and other circumstances leads to difficulty in identification of closely related species. So molecular methods lead to the accuracy in species identification. This not only applies to sibling species, but also to member of closely related groups with overlapping morphological characters. Vectorial and behavioral variations found among these species groups or complexes constitute the major reason for need of accurate and precise identification. The Anopheles annularis group consists of potential malaria vector species classified in the Neocellia series of the sub genus Cellia. The group currently comprises five recognized species i.e. An. annularis (Van der Wulp), An. nivipes (Theobald), An. pallidus (Theobald), An. philippinensis (Ludlow) and An. schueffneri (Stanton). The last species is restricted to Java and Sumatra. The remaining four species An. annularis, An. nivipes, An. pallidus and An. philippinensis are wide spread and they occur in India also. The adults of the species are morphologically very similar and often difficult to distinguish,

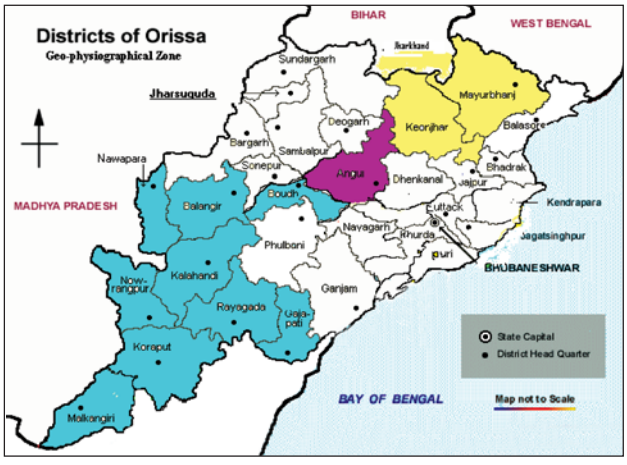



**Fig 1.** Map of Odisha showing different Geophysiographical regions.

especially those of An. philippinensis, An. nivipes and An. pallidus, which cannot always be identified reliably unless accompanied by larval and pupal exuviae.

### Study area

The study areas will be selected on the basis of different ecological conditions and basing on the malaria prone area of Odisha.

### Work Progress

Molecular markers like RFLP, SSCP and multiplex PCR assay will help to distinguish the members of the An. annularis group. Again population genetic structure and genetic diversity study of the An. annularis gives the distribution pattern of the species by RAPD, ISSR and microsatellite markers. Thus this work proposes to development of microsatellite markers for study of evolutionary and phylogenetic relationship of the species complex. Polymorphic microsatellite repeats array have become some of the most valuable DNA markers for a range of applications including genomic mapping, pedigree analysis and investigation of genetic structure of population. Development of new markers would eventually benefit gene mapping and quantitative trait loci analysis in the malaria vectors. This would be the first attempt to develop genetic markers which could be used in mapping genes of interest, to study population structure, gene flow etc. Development of microsatellite markers to study the evolutionary relationship in the vectors, population genetics and ecology including gene flow, dispersal, migration, relatedness and parentage of the species.

The DNA method used for identifying members of the An. annularis group was a PCR-RFLP that has been used in the discrimination of the members of the An. annularis group. The amplification and digestion of the D3 with Msp I, Hae III and I allowed the differentiation of the three species of the An. annularis group.

The multiplex PCR assay will help to determine the two essential factor of vectorial capacity i.e human host preference and sporozoite presence along with the species specific diagnosis of An. annularis group in the single PCR assay. For discriminating closely related members are designed novel universal primers that bind to the D3 region of the 28s r DNA of An. annularis group species where as species specific primers positioned along the D3 region of the respective species. Also for P. falciparum we have designed specific primers from r DNA of the P. falciparum locus.

In our study, genetic variability using RAPD and ISSR markers revealed little to high genetic variations in An. annularis populations with reference to their geographic existence in the state of Odisha and Jharkhand in eastern part of India. The RAPD and ISSR methods have been reported as an efficient tool to detect differentiation of geographically and genetically isolated populations.

### 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

## 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

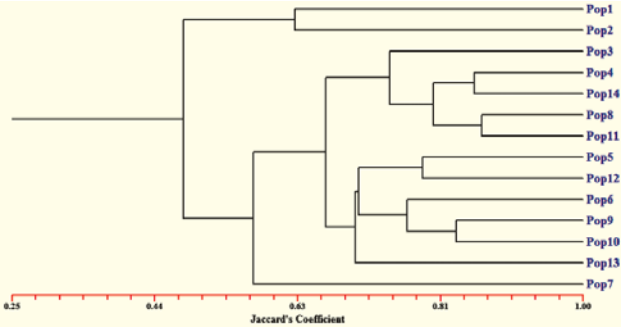



**Fig. 2.** Dendrogram for 14 An. annularis generated by UPGMA clustering using Jaccard's coefficient of similarity on microsatellite (SSR) markers analysis showing the genetic relatedness among the population. In horizontal axis scale represents genetic distance.

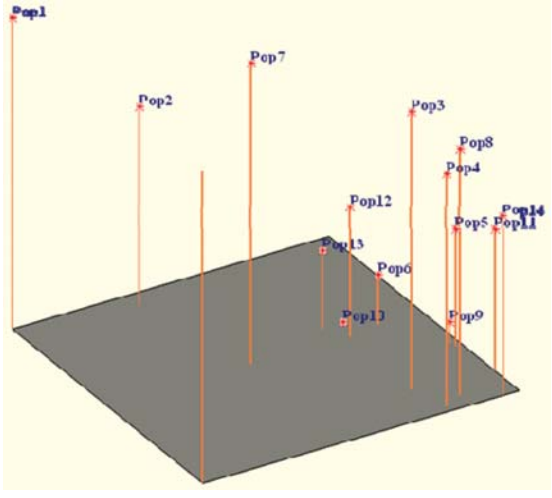



**Fig. 3.** Three dimensional graph based on Principal Component Analysis (PCA) showing three distinct clustering pattern of microsatellite (SSR) markers.

**Table 1.** Microsatellite repeats isolated from An. annularis.

| Microsatellite repeats found | | |
|---|---|---|
| AS1F | ATAGGGCCCGCACATGTAT | (CAA)5 |
| AS1R | GGTGGTAGCAGCGTGAAAGT | |
| AS2F | TGGATACATCCGTTGGTTGA | (CA)10 |
| AS2R | TGAGAGCTGCTAACACAGAGAGA | |
| AS3F | ATACGCACCCCTTTCCAAC | (GT)12 |
| AS3R | CTTGAGCGGGCACTGACT | |
| AS4F | CAGCCGGAAAATTGGTCA | (AGG)5 |
| AS4R | TCAGTTCGCTTCTTAGCCAGT | |
| AS5F | GATCTGTGTATCCTTGAGTGTAG | (GTG)4 |
| AS5R | GCAAAACCCTTCTCACATA | |
| AS6F | GATCACAGTAGTCGAAGTAAGAA | (A)10 |
| AS6R | AAATTCTTCTAATTCCTGCAT | |
| AS7F | ACCAGGGGTTCACAAATTCA | (CA)6 |
| AS7R | GAAGAAACAAAAGGCATAGGACA | |

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

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.

## Conclusion

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. The population genetic structure of a species is affected by a number of evolutionary factors including mating system, gene flow and its mode of reproduction as well as its natural selection. Population genetics studied by the microsatellite markers shows there is variation in

population structure with different geographical region of Odisha.

### 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. The genetic variation observed by RAPD and ISSR marker need to be further supported by using robust molecular markers such as microsatellite and mitochondrial DNA. Whether geographical populations consider genetically variable with respect to other parameter like vectorial capacities, vector competence and insecticide resistance etc. should be measured accordingly.

The Principal component Analysis and the Dendrogram obtained from microsatellite marker analysis showed all the species collected from same geographical region did not cluster in same group. The for this might be similar genetic variation occurred independently in different geographical regions or that migration of the species from one region to another resulted in false determination of geographic origin.

### Major Achievement

1. A novel technique was developed for mosquito processing method Multiplex PCR method was develop for sporozoite identification, blood meal analysis and sibling species identification from single mosquito of An. fluviatilis.
2. Single step multiplex PCR method was developed for simultaneous detection of Anopheles annularis group, human host preference and Plasmodium falciparum sporozoite presence.
3. 17 novel DNA sequences were submitted to Genbank, NCBI.
4. DNA fingerprinting and study of genetic diversity of An. annularis by RAPD and ISSR markers.
5. Development of microsatellite markers and population genetic study.

### Publications

1. Mohanty A, Swain S, Singh DV, Mahapatra N, Kar SK, Hazra RK (2009). 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 Odisha, India . Infect Genet Evol. 9(4):462-7.
2. Swain S, Mohanty A, Mahapatra N, Parida SK, Marai NS, Tripathy HK, Kar SK, Hazra RK (2009). 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. 103(11):1146-52.
3. Mohanty A, Swain S, Kar SK, Hazra RK(2009). 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. 9(6):1204-24.
4. Swain S, Mohanty A, Tripathy HK, Mahapatra N, Kar SK, Hazra RK (2010). Molecular identification and phylogeny of Myzomyia and Neocellia series of Anopheles subgenus Cellia (Diptera: Culicidae). Infect Genet Evol. 10(7):931-9.
5. Patra AP, Swain S, Das MK, Das B, Mahapatra N, Kar S, Hazra RK. Genetic Diversity of Anopheles annularis (Dipter:Culicidae) by

RAPD and ISSR markers in Odisha and Jharkhand, India. Communicated to Journal of Insect Science.

6. Patra AP, Das B, Mahapatra N, Kar S, Hazra RK. Isolation and Characterization of Microsatellite marker from Anopheles annularis (Dipter: Culicidae) from Odisha, India. (Manuscript under preparation.)

## 4. GIS Based Micro Planning For Malaria Control In High Endemic Areas: Orissa.

| | | |
|---|---|---|
| Principal Investigator | : | Dr. R. K. Hazra |
| Co-Investigators | : | Dr. N. Mahapatra<br>Ms. Mumani Das |
| Collaborator | : | Dr. M.M.Pradhan<br>Mrs. Mithun Karmakar, GIS consultant, NRHM,Govt.of Orissa. |
| Duration | : | One Year |
| 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

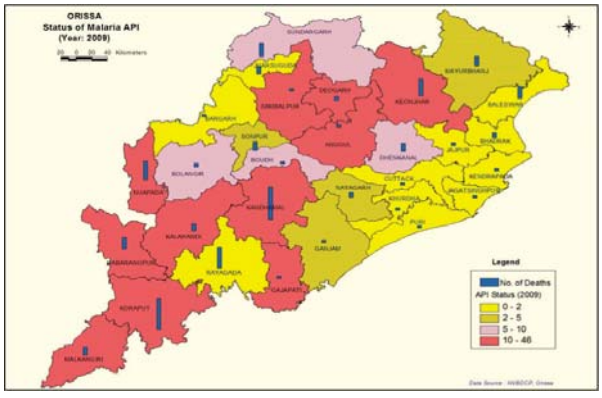
Malaria is maintained under the influence of diverse ranges of interacting conditions, many of which are not well understood. These conditions are closely related to the habits and lifestyle of different communities; the behaviour of the mosquitoes which transmit the disease; as well as climatic and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

As a tool for analysis and decision making, GIS and remote sensing opened a new horizon or 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. Orissa contributes maximum burden due to malaria to the nation. In 2006, nearly

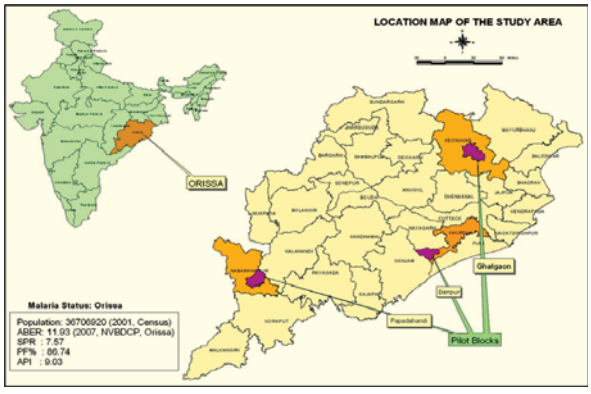
23 percent of cases and 17 percent of deaths due to malaria of the country have been reported from Orissa.

## Base line Survey

### (a) Selection of study area



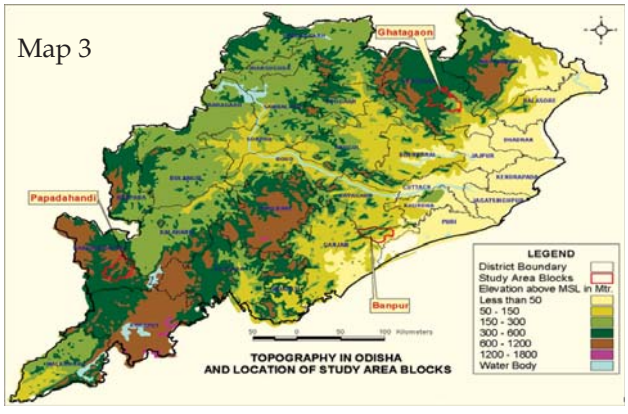

**Map 1:** Status of Malaria API in the year 2009

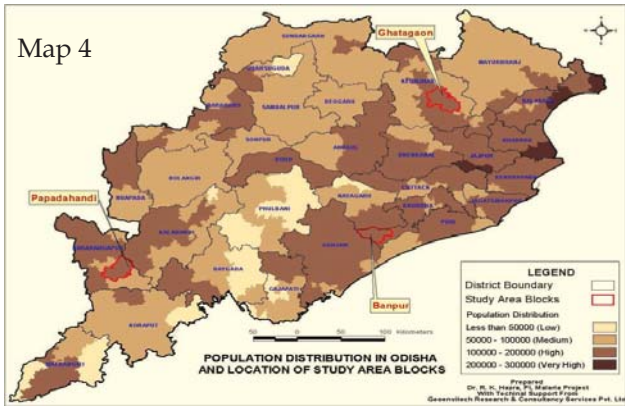



**Map 2:** Location Map of the selected study area

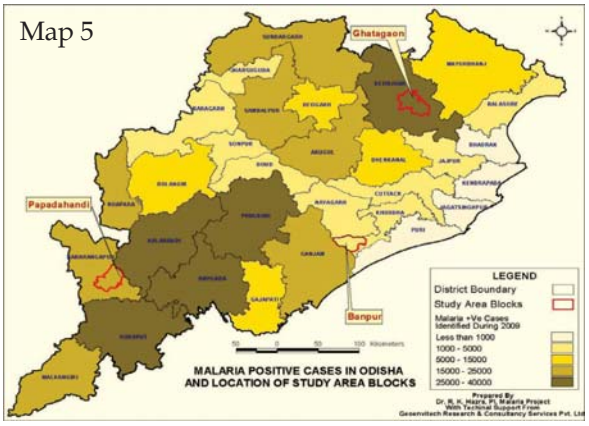
The study was conducted in three malaria endemic districts of Orissa. The State is having 118 tribal blocks spread across 12 districts out of which our study area includes two tribal blocks and one non-tribal. Orissa experiences different climatic conditions in its different parts and all six seasons are effective here. Three selected district are Nawarangapur, Khurda and Keonjhar. These three districts differ in their Physical condition, demographic pattern and socio-economic status.

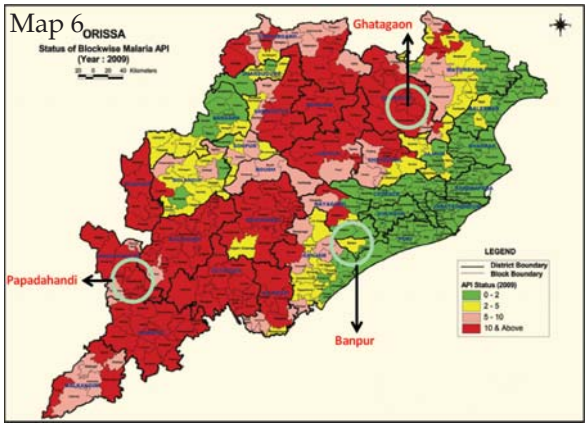
Three districts Keonjhar, Nabarangpur and Khurda were taken as pilot district and one PHC from each district was selected as study area. The ecology of three blocks taken is different from each other (Map-2). According to the agro-climatic division of Orissa Nawarangapur is a plateau coming under Eastern ghat (covers an area 5294.5 km²,20.3 to 17.50N latitude to 81.27 to84.10E longitude) is located about 2,000 ft (610 m) above sea level(Map 2).This District enjoys a generous rainfall every year. This plateau remains cool throughout the year. Keonjhar is hilly area coming under Northern plateau (covers an area of 8240 km² and lies between 21°1' N and 22°10' N latitude and 85°11' E to 86°22' E longitude, elevation of over 600m elevation from sea level) (Map 2)and it is covered with deep forest in some places. Khurda is under Coastal




**Map 3 & 4:** Topography and Population distribution of Orissa and location of selected study area




**Map 5 & 6 :** Map showing the Population and API status of the three selected blocks of the year 2009 for study of the three selected block.

tract (area of 2,887.50 km² and lies between 19 040' to 20 025' N latitude and 85 0 37' 30'' E longitude, 50 to 150 meter elevation from sea level) (Map 2) and it is a highly forested area covering 618.67 km² and annual rainfall is 1443 mm (Avg).

1. Base line Survey: As a base line study the mosquito samples were collected from Ghatgaon block of Keonjhar district, Papdahandi block of Nawarangapur and Banpur block of Khurda district.

2. Selection of villages: Based on high malaria endemicity.

3. Three time field visit for collection of three season (winter, Summer & Rainy) data for the study area was done.

4. Development of GIS maps:

4.1 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). The outputs were in raster image format, which were georeferenced in ERDAS Imagine image processing software. The georeferenced raster topomaps were then imported into GIS for vetcorisation and GIS layer development along with attributes.

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

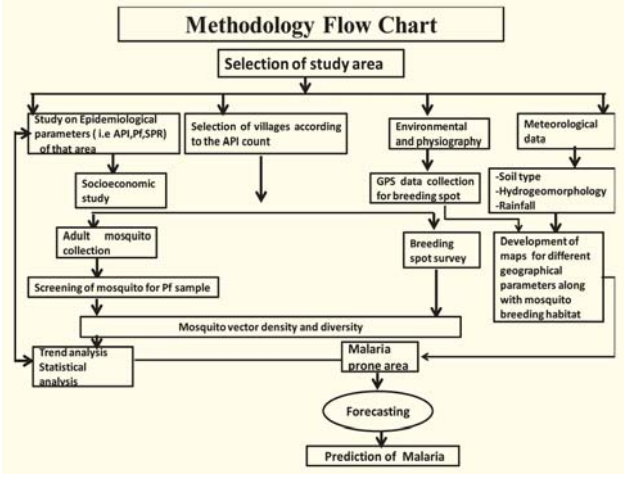
- The environmental parameters mapped in 1st phase included soil type, hydrogeomorphology, lithology.
- The satellite images of the concerned study area were georeferenced and the concerned parameters were interpreted through digital enhancement and visual interpretation technique with reference to secondary source maps collected earlier.
- The false color composites have been developed by taking band 2, 3 and 4 (visible and NIR bands).

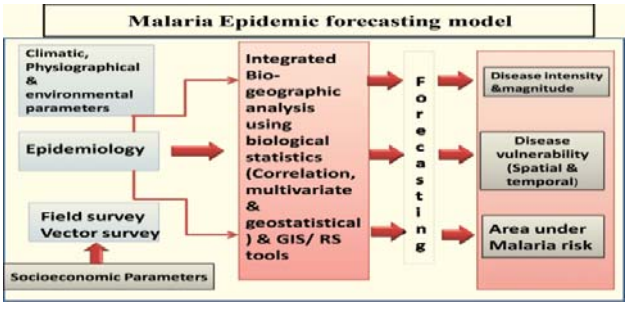
- The final vector polygon layers along with feature class attributes were developed in GIS.

4.3 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, Summer & Rainy seasons.

4.4 Trend analysis and identifying malaria risk areas by developing composite maps.



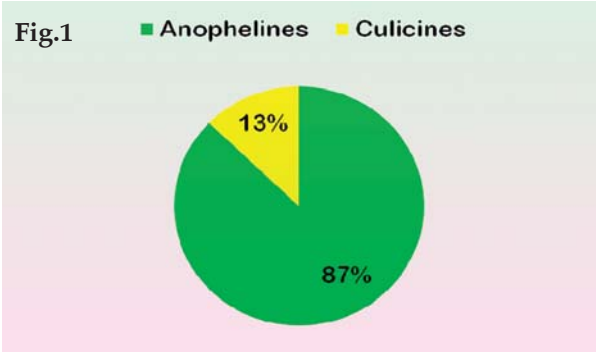

The three selected districts of Orissa differ very much in their population. Ghatagaon block of Keonjhar is highly populated where as Papdahandi of Nawarangapur is moderately populated and Banpur block of Khurda is less populated than the other two districts (Map 3). The selection of villages for the study was done of the three selected blocks by taking the previous three years API count (Map 5, 6, 7, 8). Keonjhar laying in the northern part of Orissa is one of the most endemic tribal districts having API of > 15 and PF% >90.* Whereas Nabarangpur lying in the southern part of Orissa is having > 98% PF and API>20*. Khurda lying in the eastern part of Orissa, though having very low API and PF but still Blocks like Banpur having vast forest cover experiences >5API and PF% >83*(Map 4). The study will help in understanding the malaria status under different physical, altitudinal, climatological and epidemiological condition.

## 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, Rainy. 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.



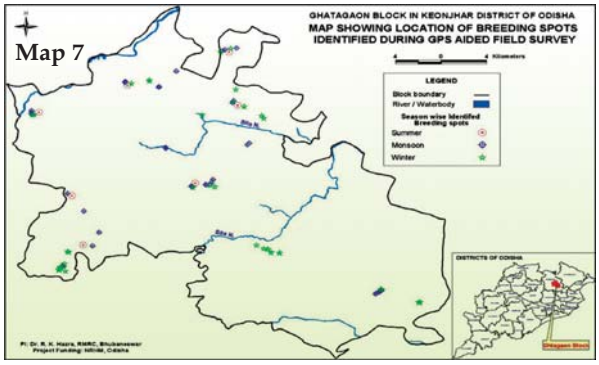
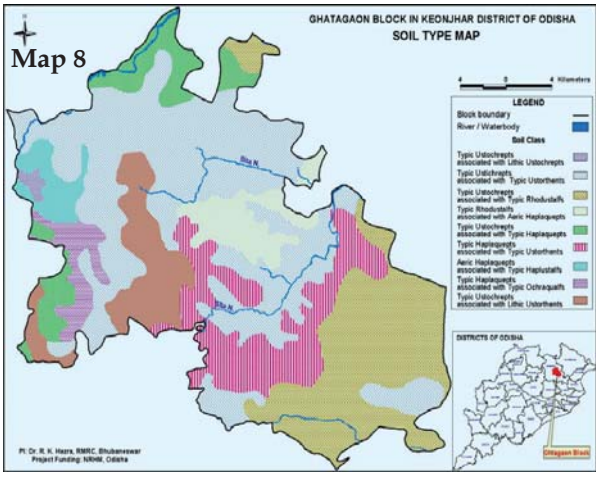
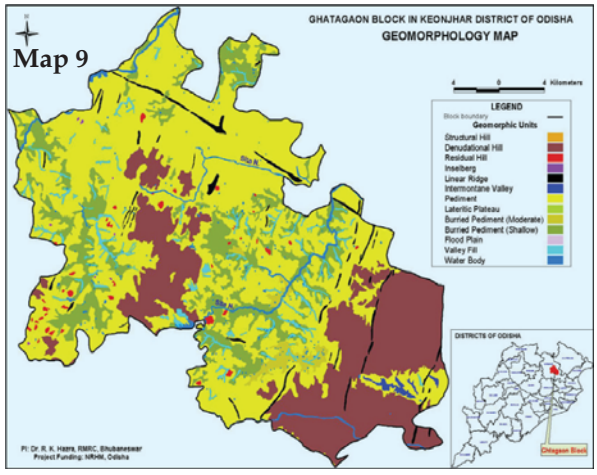




**Fig. 1& Map 7:** Showing percentage of species found in Ghatagaon block and the mapping of the breeding spots of the of Ghatagaon block.

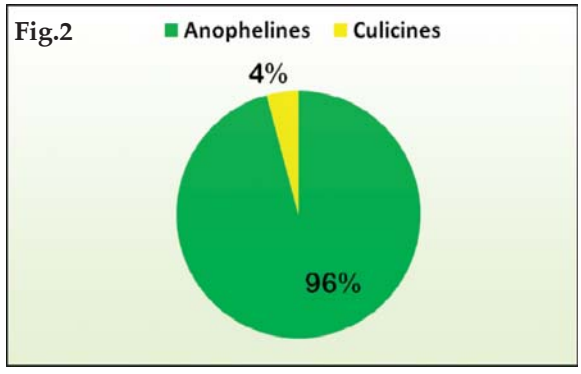



**Map 8 & Map 9:** Showing the soil and sampling location and Hydrogeomorphology map of Ghatagaon block.

**(b) Papdahandi Block of Nawarangapur district:**

Field study was undertaken in twenty selected villages of Papdahandi block. In this block An.



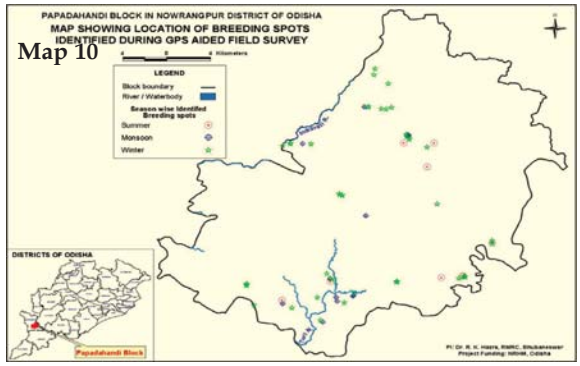
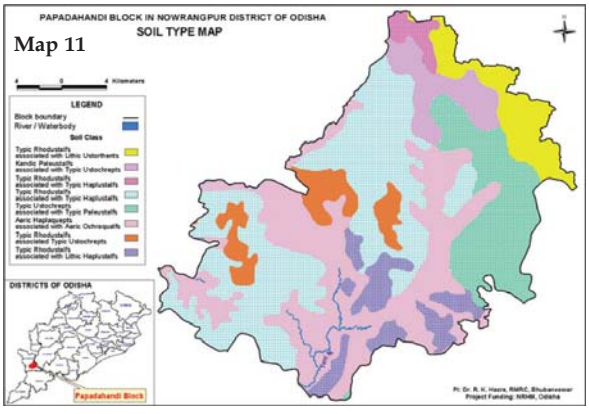
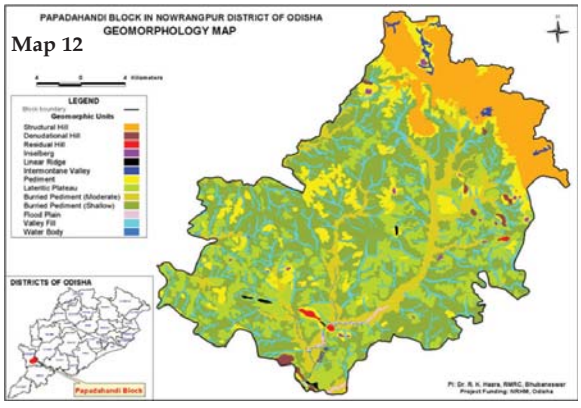




**Fig. 2 & Map 10:** Showing percentage of species found in Papdahandi block and mapping of the breeding spots of the Papdahandi block.

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.



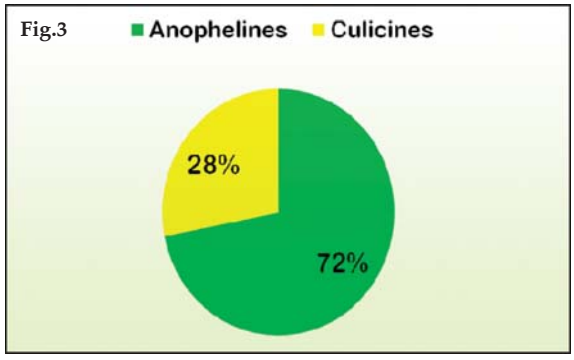

**Map 11 &Map 12:** Showing the soil and sampling location and the Hydrogeomorphology of Papdahandi block.

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.

**(c) Banpur Block of Khurda 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.



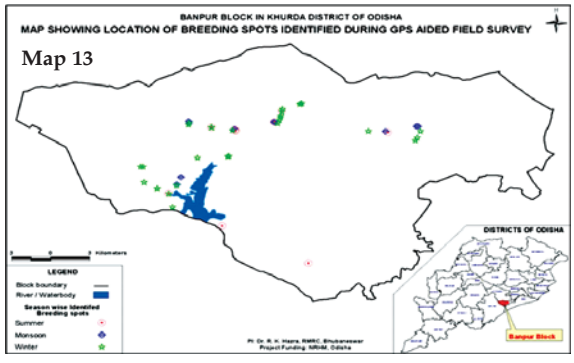
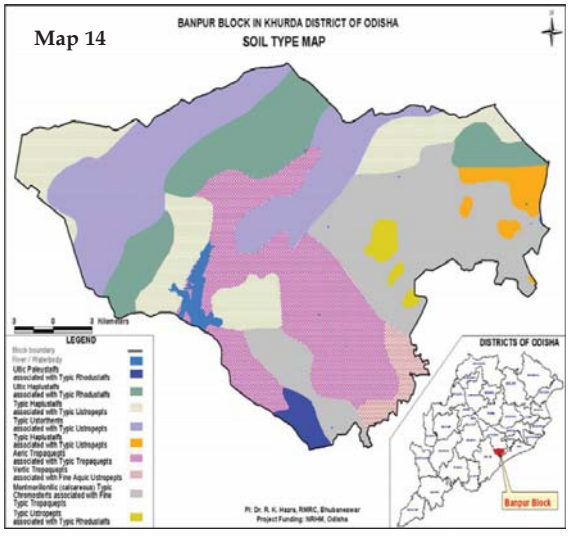
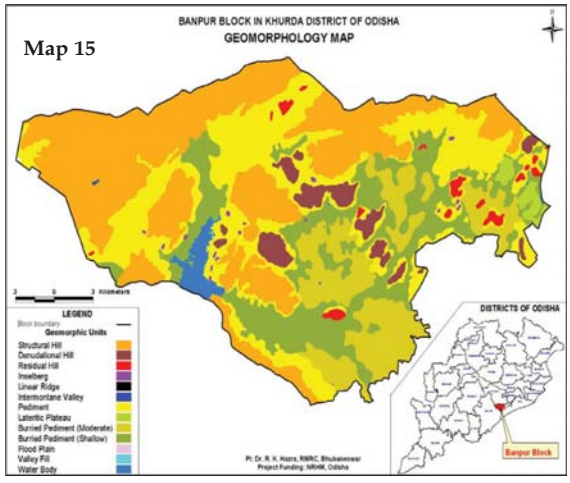




**Fig. 3 & Map 13:** Showing percentage of species found in Banpur block and breeding spots of the Banpur block.




**Map 14 & Map 15:** Showing the soil and sampling location and the Hydrogeomorphology Banpur block.

### Soil texture in study area

- It has been observed from the result that "Fine" and "Fine Loamy" classes are predominantly associated with malaria vector habitat.
- This may be due to the water holding capacity of this textural class as the clay & silt percentage in such classes is higher.

4. Vegetation index (ndvi derived from satellite image) in study area

- The Normalized Density of Vegetation Index (NDVI) has been developed for all the three blocks from the available satellite images using the formula: (Infra Red - Visible) / (Infra Red + Visible).
- It has been observed that the breeding spots are predominantly associated with NDVI values ranging from 0.15 to 0.45. These represent rice fields, agriculture and open forests.Pediment, Buried Pediment and Valley Fills are the predominant classes which are associated with breeding spots.
- In fact "Pediments" are gradually sloping bedrock surface located at the base of fluvial-eroded mountain range. These are also characterized by moderate or low slope.

### FCC satellite image of study area

- Satellite Images have been acquired from NRSC and other libraries.
- After getting digital image, they have been georectifed/georefrenced, digitally enhanced and other standard editing has been performed.
- The false color composites have been developed by taking band 2, 3 and 4 (visible and NIR bands).

### Entomological investigation with the help of GIS and RS with entomology

The geographical location of the identified larval breeding spot of each block was documented by using GPS instrument and they were mapped in their

| Number of Breeding Spots (Larvae +Ve Sites) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Banpur Block | | | Ghatagaon Block | | | Papadahandi Block | | |
| Breding Spots | Summer | Monsoon | Winter | Summer | Monsoon | Winter | Summer | Monsoon | Winter |
| Larvae +ve | 09 | 10 | 30 | 07 | 41 | 41 | 10 | 13 | 45 |

respective geographical map by using GIS software. The details of the geographical location of each breeding spot documented by the GPS instrument is shown in the map and details given in the table as annexure-1. In post monsoon i.e. in winter we got more number of breeding spot then the other two seasons.

- The results indicate that the "Winter" season (post monsoon) is the most vulnerable period for malaria vector followed by "monsoon".
- In Papdahandi block the main breeding spot we found is aquifers which are very common to Nawarangapur district and they also show positive site for breeding of Anopheles species.

However, no season has experienced absence of malaria breeding.

### Geology in study area

- The geology map have been developed using regional maps from secondary sources as well as satellite image and field visits.
- The results of overlaying breeding spot layer (as per GPS based field sampling) has been given below:
- The results indicate that there is no direct influence of geology on the malaria vector habitat.
- This is because geology is a regional phenomenon.
- However, indirectly through influencing ground water level (artesian aquifer) and geomorphology (it is controlled by bed rock), it has some impact on determining the habitat of malaria vector.

**Socioeconomic survey:** Review of the socioeconomic condition of people living in these blocks was done for getting an insight into the relation between living condition and malaria occurrence.The study was carried out in three blocks using pre tested pre designed questionnier. Data was collected interviewing head of the household. This survey was done in the year 2009 in the month of August and September.

A B

C D

E

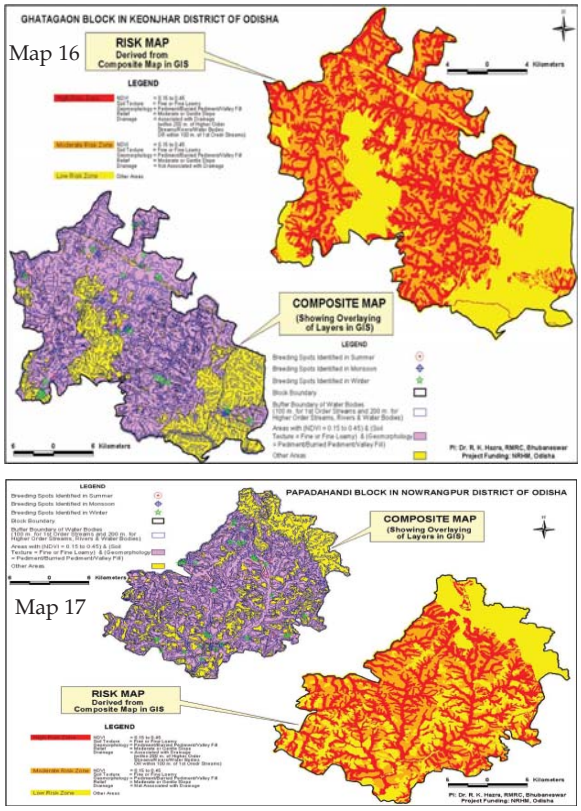
Photograph (A, B) Showing breeding spot of Ghatagaon Block, (C, D) breeding spot of Banpur Block, (E, F) breeding spot of Papdahandi Block.

### Risk Mapping

- Risk mapping has been done by performing various analytical functions in GIS, which included:

- Proximity analysis through developing buffer zones for natural drainage layer using attribute based multiple buffering technique. Thus 200 m. buffer zone has been developed for higher order streams and 100 m. buffer zone has been developed for 1st order streams.
- Overlaying of multiple layers like NDVI, Soil Texture, Drainage buffer, Geomorphology, Elevation and breeding Spots.
- Query Analysis for extracting rule based features like High Risk Areas, Moderate Risk Areas and Low Risk Areas.
- The Rules/Logics for query analysis has been developed through observations obtained from overlaying breeding spots upon various layers.

**Development of disease vulnerability model**




**Map 16 and Map 17** shows the risk map model of the malaria vulnerability of the selected ares.

## Conclusion

Some important information was obtained from the study on climatic and environmental factors over the mosquito breeding. The GIS maps were developed on NDVI, Soil Texture, Drainage buffer, Geomorphology, Elevation with compare to breeding Spots. All these map shows significant impact on mosquito breeding all around the year. The selected three blocks i.e Nawarangapur, Keonjhar and Khurda differ climatically and environmentally from each other as it is described earlier but the malaria situation is nearly similar in each district. On mapping the breeding spot and overlaying that with the soil and drainage map it was observed that the perennial breeding spots are mainly found in fine texture of soil with association of poor and imperfect drainage and high water retention capacity. Aquifers are also playing major role in mosquito breeding in some places where the water level is very close to the soil surface. These aquifers have water all around the year which is conducive for mosquito breeding. Low NDVI value and high vegetation index is also associated with mosquito breeding. It has been observed that the breeding spots are predominantly associated with NDVI values ranging from 0.15 to 0.45. These represent rice fields, agriculture and open forests. The dense forest at hill ridges, peaks and land with/ without scrub, barren land are not associated with breeding spots.

Remote sensing and GIS based mapping of environmental parameters like physical, climatic were explored. Factors responsible for malaria habitat were identified from the ground data collection and matching the same with the Remote sensing and GIS and taking all these geophysiographical and climatic factor through comparative assessment of environmental parameters of the study area selected, a physical vulnerability model for malaria epidemic was developed for three different sites.GIS based mapping of socioeconomic parameters of study area

at village level gives an overview to assess the socioeconomic vulnerability of the study area.An integrated vulnerability model develop to locate the risk area and parameters (Physical and climatic) which are responsible for perennial malaria transmission in three selected area and to ascertain malaria epidemic information through RS and GIS well in advance.

Monitoring environmental condition and overlaying the composite parameters in any area we can create an integrated early warning system where malaria may be a epidemic in future and early identification of potential epidemic situation.

### Achievement

The environmental parameters were mapped by the use of GIS tools as it was indicated in one of our objective. A vulnerable model was prepared for the three selected study blocks which fulfil one of the objectives. This model may be validated in other areas and it can be translated as vulnerable model for predicting the malaria epidemic and perreniality of malaria.

## 5. Diagnosis of pulmonary tuberculosis by culture and its drug sensitivity.

Investigators : Dr Dasarathi Das
Dr H K Khuntia
Collaborator : TRC, Chennai
Starting date : Feb.2009
Closing date : Dec.2011

**Brief about the project:** The current diagnosis of pulmonary tuberculosis under DOTS programme is based on clinical symptoms, radiological findings and sputum microscopy. However the sputum microscopy fails to detect the low or paubalillary infections and an estimated 5000 to 10000 balilli per ml is required for AFB staining. The AFB staining also can not differentiate between Mycobacterium tuberculosis and other mycobacterium. Though the culture of Mycobacterium tuberculosis in solid LJ media takes 6-8 weeks to grow, it provides definitive diagnosis and study of its drug sensitivity further helps in the control of the disease more effectively.

### Objective

To provide diagnosis for pulmonary tuberculosis based on culture in solid media (LJ) and carry out drug sensitivity pattern of the isolates.

### Methodology

Briefly sputum samples from tuberculosis OPD of Capital Hospital, 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 to two slants of LJ media and to one slant of LJ with PNB. The cultures were incubated at 370 C and growth (rough cauliflower like colonies) was observed at weekly intervals up to 8 weeks. The confirmation of M tuberculosis was made by growth on PNB, niacin and catalase tests. The drug sensitivity studies with Rifampicin and Isoniazid was carried out based on proportion sensitivity method.

### Findings

During this period sputum was collected from 1007 symptomatic individuals attending the OPD of Captal Hospital, Bhubaneswar. Out of which 15.8% samples were found positive by AFB staining. Culture was attempted on 146 sputum positive samples and 115 samples showed typical growth (rough, cauliflower like) on solid LJ media, while contamination was observed in 9 samples.

Out of the 115 isolates growth was observed only in two isolates with PNB. All the 113 isolates showed niacin positivity and lost catalase activity in 20 minutes at 680C / pH7.0. Out of 35 samples tested for drug sensitivity 3 and 13 samples showed mono resistance to rifampicin and isoniazid respectively while 2 samples showed resistance to rifampicin and isoniazid (MDR-TB). The DST for other samples are in process.

## 6. Mapping of P.falciparum susceptibility to Chloroquine in malaria endemic districts of Orissa.

Principal Investigator : Dr.A S Kerketta
Starting date : October 2008
Date of completion : April 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 anti malarial drug Chloroquine (CQ) has always played an important role in treatment and control of malaria. However; since early 60s the sensitivity of the parasite to CQ the best and most widely used drug for treating uncomplicated P.falciparum malaria has been on the decline. Over past five decades the drug resistance of Plasmodium falciparum has become an issue of utmost concern (Harald Noedl et al 2003). Resistance of parasite to CQ began from 2 Epicenters Columbia (South America) and Thailand (South East Asia). Since then resistance has been spreading world wide and reached Indian state of Assam in 1973. CQ has been the main stay of antimalarial drug treatment and used in the NMEP from 1960 and it is being extensively used in the modified plan operation at multiple levels as presumptive treatment by Drug Distribution Centre, fever treatment depots, Community Health Guides, ANM and PHCs as well as NMEP staff. But with the spreading of resistance by plasmodium falciparum to available treatment, drug sensitivity has become an issue of utmost importance for development of appropriate therapeutic guideline.

In Orissa state the CQ resistance was detected for first time in Gumagarh PHC of Phulbani district in December 1978 and then in Keonjhargarh town of Keojhar district in April 1979. In Koraput and Malkangiri district the RI resistance (15.8%) and RII resistance (2.8%) to CQ P.falciparum was reported in 1989. Since it is around four decades from the first report, it is expected that CQ resistance might have spread over entire state by this time due to increased population movements and a large-scale use of the drug. There is scarce report on P falciparum susceptibility to CQ in different high malaria endemic districts Orissa. Biswas et al reported 97% CQ sensitivity from Padampur and Basudebpur of District Keonjhar and only (3%) failure but the antifolate combination found to be fully effective. The Regional Institute of Health and Family Welfare have undertaken some patchy studies. But systematic data on CQ resistance at a single point of time is not available from the state. Therefore the present study is proposed with the aim for systematic mapping of the resistance status of CQ by P. falciparum over time and place.

### Study area

Out of the seven proposed study districts, four districts namely Keonjhar and Ganjam, Nawarangpur and Gajapati of Orissa were studied for P falciparum susceptibility status to CQ. One PHC area in each district was selected for the study. The selection was made basing on the past three years malariolocal data indicating the area as high malaria endemic area. Thus, the PHC Harichandanpur of Keonjhar, Badagada PHC of Ganjam, Tentulikhunti PHC of Nawarangpur and Gumma PHC of Gajapati districts were included in the study.

With the fund received in first instalment, the CQ efficacy could be studied in four districts only. There was a long gap between the release of first and second instalment. The interim report was submitted to NVBDCP. By the time of reception of second instalment CQ was withdrawn by the programme and Artemisinin Combination Therapy (ACT) was implemented as first line of antimalarial drug for treatment of uncomplicated P. falciparum malaria in Orissa. Therefore the protocol was modified to study

**Table 1:** Malariological surveillance data PHC studied for CQ& ACT efficacy.

| PHC/DISTRICTs | POPULATION | SPR | PF% | ABER | API |
|---|---|---|---|---|---|
| Harichandanpur/Keonjhar | 1697910 | 10.1 | 97. 3 | 26.8 | 27.1 |
| Badagada/Ganjam | 116116 | 9.11 | 88.0 | 11.1 | 10.0 |
| Tentulikhunti/ Nawarangpur | 89040 | 10.9 | 99.8 | 12.3 | 13.45 |
| Gumma/Gjapati | 74313 | 12.7 | 99.5 | 9.7 | 12.9 |
| Khajurikata/Dhenkanal | 172843 | 7.0 | 49.0 | 9.6 | 6.8 |
| Subdega/ Sundargah | 65288 | 3.1 | 95.7 | 43.6 | 13.5 |

the efficacy of ACT after obtaining due approval by the funding agency, in rest of the districts. Thus the efficacy of ACT was studied in Khajuriakata block of Dhenkanal and Subdega block of Sundargarh district where the CQ was replaced with ACT during 2008 due to high CQ resistance and reporting malarial death. The study areas are mostly hilly forest area and inhabited by tribal population. The average of 3 years malariogical data (2004-06) in given the study area Table-1.

## Material and Methods

The P.falciparum susceptibility to Chloroquine and ACT follow the guideline for assessment and monitoring of anti-malarial drug efficacy for the treatment of uncomplicated P.falciparum malaria (WHO/HTM/RBM/2001 & 2003).The study was undertaken in close collaboration with the state health facility available in the area. The study villages selected on the basis of experience of the health personnel on maximum number of fever cases reported to the health facility. In the village door-to-door survey was made for the detection the fever cases. Patients having fever or history of fever in 24 hours prior to the examination and not taken treatment were asked to come to a central place in the village for screening for their eligibility for the study. After recording the detail address of the patient, the clinical examination was done by the medical officer followed by measurement of body weight and axillary temperature by the team. The finger prick blood was collected for preparation of thick and thin smear for identification of parasite and density counting. The smears were transported to the temporary laboratory established by the research team in the field and was stained with Giemsa stain. Microscopy was done by two technicians independently. The patient found to have asexual parasite density more than 1000-10000 ml of blood and fulfilling the other eligible criteria were enrolled for the study.

## Dose schedule of CQ

- CQ dose schedule follows WHO recommendation (10mg/Kg Body weight) on days 0 and 1 and 5mg /kg on day 2 after or with food.
- A total 1500mg base of Chloroquine (adult dose) administered in three days.
- Day 0- First dose-600 mg base = 4tabs of 150 mg base each
- Day 1-second dose-600mg base = 4tabs of 150 mg base each
- Day 2-third dose-300 mg base =2tabs of 150mg base each
- Dose for children is 10mg/kg body weight on 1st dose, 10mg /kg body weight on 2nd dose and 5mg/kg on 3rd dose.

## Dose schedule of ACT

The drug dosing for the treatment for falciparum cases of different age group follows National Drug

Policy guidelines for diagnosis and treatment of malaria in India (NVBDCP 2009).

1. Sulfadoxine Pyrimethamine combination tablets (Sulfadoxine 500mg + 25mg Pyrimethamine)
2. Artemisinin derivative:Artesunate 4mg/ Kg once daily for 3 days
3. Other medication:Paracetamol was administered on Day 1-2 in case the patient's condition warrants.

(To keep the consistency in the drug availability in the tablets, the drug was obtained from state NVBDC. Thus the same drug used for the malaria control programme was used for the study).

### Drug administration and Follow up

The drug was given under the supervision of the team and after ensuring that the patient is not in empty stomach. Those who came without taking food were given biscuits to eat before administering the drug. The drug was given on 1st, 2nd and 3rd day thereafter followed on scheduled days like 7th, 14th, 21st and 28th day of enrolment. Each patient asked to stay in the camp at least for 30 minutes to ensure that she/he should not vomits out the drug. In-case of vomiting the same dose was repeated after ½ an hour. Any case found to have severe or complicated malaria during the follow up shifted to the nearest health facility for hospitalization and treatment accordingly.

### Results

In all the study districts a minimum of 6-8 number villages included in the study except in Nawarangpur district. Since in Nawarangpur district, the study was conducted in the month of May-June and the malaria incidence during that period was low. Around 100-150 fever cases had to be screened to get eligible study population. Thus the number of study population included in the study was 54, 55, 54, and 52 in Keonjhar, Ganjam, Nawarangpur and Gajapati district respectively. Similarly the study on efficacy of ACT was carried out in Sundargarh district amongst 51 and in Dhenkanala amongst 42 cases. The baseline information like mean age, mean weight and average parasite density on day 0 in both study area is given in Table.2 &3.

During follow up for CQ efficacy, in Keonjhar districts 50 out of 54, in Ganjam 50 out of 55, in Nawarrngpur 51 of 54 and in Gajapati 48 of 54 cases

**Table 2:** Baseline Information of the Population studied for CQ efficacy.

| Districts Weight (Kg) | No. of Cases studied | Mean Age (in Year) | Mean Weight Kg. | Average Body (Temp.$^0$C) | Average parasite |
|---|---|---|---|---|---|
| **Keonjhar** | 54 | 21.2 | 32.9 | 38.3 | 3962.4 |
| **Ganjam** | 55 | 16.38 | 29.0 | 38.1 | 18457.9 |
| **Nawarangpur** | 54 | 24.8 | 30.2 | 38.3 | 4714.0 |
| **Gajapati** | 52 | 16.3 | 29.9 | 38.4 | 10891.9 |

**Table 3:** Baseline Information of the study Population under ACT efficacy.

| Districts Weight (Kg) | No. of Cases studied | Mean Age (in Year) | Mean Weight Kg. | Average Body (Temp.$^0$C) | Average parasite |
|---|---|---|---|---|---|
| **Dhenkanala** | 42 | 21.2 | 30.7 | 38.3 | 3962.4 |
| **Sundargarh** | 46 | 16.38 | 32.0 | 39.1 | 18457.9 |

could be followed up for the clinical and parasitological response. Similarly for efficacy of ACT 42/45 and in Sundargarh 46/51 eligible case could be followed up till the end of follow up period.

The study revealed, Adequate Clinical and parasitological response (ACPR) of merely 13%, 7.5%, 24%, 4% in Keonjhar, Ganjam, Nawarangpur and Gajapati district respectively with CQ. The Early Treatment Failure (ETF) was marked in 64.8%, 64.2% 61.1% and 64% with Late Treatment Failure (LTF) including both clinical and parasitological failure marked in 22.2%, 28.3%, 14.9% and 32% of the population (graph–1). The efficacy of ACT studied in two district was also found to be declined in treatment of P.falciparum malaria in Sundargarh and Dhenkanala districts.

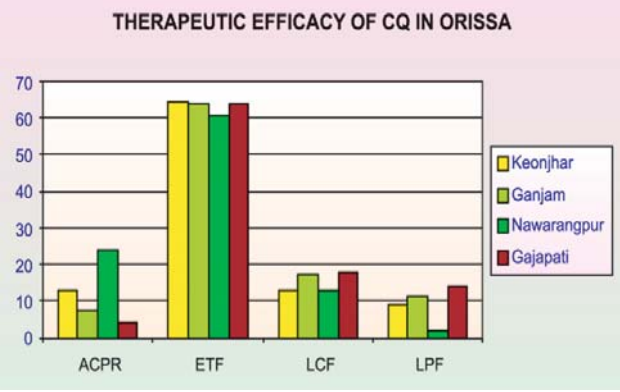



**Fig.1.** Therapeutic Response of CQ in Keonjhar, Ganjam, Nawarangpur and Gajapati districts of Orissa.

## Conclusion

Malaria is a major health problem in Orissa. The result of CQ efficacy study carried out at a single point of time, indicated reduction of sensitivity of CQ in treatment of P.falciparum malaria. The adequate clinical and parasitological response (ACPR) found to be as low as less than 25% with CQ. Based on the interim report and considering the wide spread CQ resistance in P.falciparum malaria infection in malaria endemic state, the National Drug Policy for treatment of uncomplicated malaria has been changed from CQ to ACT in the area/ block PHC reporting total treatment failure (ETF+LTF) of 10% or more. During 2006, ACT introduced in areas showing Chloroquine resistant falciparum malaria and since 2008 ACT extended to high risk Pf districts covering about 80-90% Pf infection. Thus in 117 districts of states like Andhra Pradesh, Jharkhand, Madhya Pradesh, Orissa and North Eastern states the steps was taken to change the anti-malarial drug treatment policy. In Orissa the ACT was implemented in 2007 that resulted in a drastic reduction in the morbidity and mortality due to malaria. But the anti-malarial drug resistance always has been the leading threat to ongoing malaria control effort. Therefore after replacement of ACT in all the earlier proposed study districts, ACT efficacy was studied in Dhenkanal and Sundargarh district where it was implemented during 2008. The preliminary evaluation of efficacy of current anti-malarial drug in two districts indicates declined efficacy in treatment of uncomplicated P.falciparum malaria. The people of the study villages in Sundargarh district are well off and prefer availing treatment at their door step even from less/un qualified persons (quack) unless until the case become serious and the usual antimalarial treatment is IM formulation of artemisinin compound. So after receiving one injection if the patient feels better they do not complete the course. Thus the irrational, incomplete use of Artemisinin as mono-therapy is prevalent in the study area of Sundargarh district. That might have caused the low response of P. falciparum parasite to ACT. But more studies should be undertaken consistently over time at reasonable representative site to detect the drug resistance at the earliest. Besides, continuous monitoring for rational and complete drug treatment should be given emphasis since it is one of the way to prevent it's spread besides other measures like strategy for easy access and expansion of use of combination therapy in the area.

## 7. 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
Starting date : August 2007
Closing Date : Dec 2011
Funding : Intramural

### Objectives of formative reasearch

### 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 objective

- Assess people's knowledge and perception on malaria and available treatment
- Assess the treatment seeking behaviour of the community
- Assess the knowledge and perception of providers on malaria and its treatment
- Develop strategy for improvement of accessibility and utilisation

### Study Progress

**Formative research (Using both Quantitative & Qualitative method)**

**Community perception**

In-depth Interview with head of house hold (142 households covering 1200 population) was undertaken by door to door visit.

The findings of the community perception by in-depth interview was validated by illness narrative by the adult cases or parents of children suffered from malaria in last 15 days. The result shows, 26.0% contacted ASHA for malaria treatment on first day against none expressed availing the facility of ASHA in in-depth interview. Rest sought treatment from quack and only 12.0% from the government hospital. 16.0% had availed the service of the multiple providers depending on the severity of the disease. Those who went to Govt Hospital were mainly for the treatment of children. The reason for obtaining different facility indicated in the table below. (Table-1)

**Community knowledge and Perception (using Focus Group Discussion (FGD)**

***Cause of malaria*** **-**The community perceives malaria as an important health problem. It is caused by exposure to cold & heat, by drinking unsafe dirty water or mosquito bite as they stay in foothill and riverbank area they get exposed to mosquitoes.

***Transmission-*** The transmission of the disease occurs through eating/sleeping together with the malaria affected person having fever. Only few could say that it is get transmitted through mosquito bite but only one woman could say the actual mode of transmission.

***Signs and symptom-*** The signs and symptom of malaria is well recognized by the people as it is endemic for malaria.

**Table-1:** Reasons for obtaining different facilities by IDI.

| Providers | ASHA | Quack | Govt Doctors |
|---|---|---|---|
| Distance | 0.5-1KM | 1-2KM | 20KM |
| Faith | Less | Strong faith | Strong 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 |

**Table-2 :** Communiperception & treatment practice by IDI.

| Perception & knowledge | Percentage |
|---|---|
| Experience on malaria | 95% families |
| Cause of malaria | Mosquito bite-71.1% Other causes like hard labour, exposure to cold & heat -28.9% |
| Treatment Initiated | Day 1- 28.4% , Day 2-64.8%, Day 3-4 - 16.6% |
| Knowledge on availability of | 47.2% individuals |
| anti- malarial drug with ASHA | |
| Treatment seeking | Quack -100% |
| Knowledge on the duties of ASHA by the villagers | No idea on duties of ASHA- , 55.0% slide collection- 24.6%, hygiene maintenance-14.1%, meetings- 6.3% |

### Treatment seeking for malaria

- Regarding treatment seeking, the entire group opined that the first person to approach for malaria treatment is the quack who resides in the nearby village.
- Second person to approach: more experienced (malaria expert) in case first quack's medicine does not work.
- Third person to approach- Medical doctor at Govt health facility. Incase the quacks treatment does not work every body prefer to visit a doctor as they know the difference between a qualified doctor and a quack.

***Reason for opting the quack-*** The reason for opting the quack service is easy access, less cost, credit facility their availability at any moment, less time consuming contrarily to the government doctors who have limited OPD times and the people have to spend a full day to go to a medical facility & more importantly their faith on quacks, payment of fees and costly medicines.

***Service from grass root level provider ASHA:*** Only three women out of 40 opined to have visited ASHA and got CQ tablets. Others do not know the role of ASHA in malaria control programme. One woman said "we know that ASHA accompanies the pregnant women for institutional delivery". Some have heard that ASHA keeps the anti-malarial drugs but they do not have faith on ASHA so they do not approach her.

***Malaria control programme:*** None got any facility neither as drug nor insecticidal spray or mosquito net. One woman said "Since last 20 years no one has visited their village with regards to malaria". Long back (20 years) one malaria worker was visiting the houses regularly and taking blood slides and giving the drugs. Their house has never been sprayed.

### Lacunae identified

Community level: Low awareness on disease, it's cause, mode of transmission and available services under control programme

Providers' level: Low confidence level to perform rapid diagnostic test by ASHA, Non performance of duties by health workers

Facility level: No monitoring system at community level, Non maintenance of timeliness of reporting back the microscopic result.

Non- availability of required quantity of logistics the drugs, RDTs, mosquito net, insecticides etc.

**Community strength:** Existing of CBOs, success stories of prohibition of alcohol consumption in the village, available qualified youth.

**Inference:** The formative study indicates the poor awareness of community on disease as well as the control programme. Use of Government health facility is very low.

The community tuned innovative strategy will be developed basing on finding of formative research and implemented through community participation for which separate study as extramulral funding will be undertaken.

# Other Scientific Activities

**Dr. V.M. Katoch, Secretary DHR & DG, ICMR addressing in ISMOCD & IAE Annual conference organized by RMRC Bhubaneswar on 15th April 2011**

### A. District wise isolation of V. cholerae from Orissa (referred from IDSP unit, Govt of Orissa (period: June 2010 - December, 2010).

During this period under report (June 2010 to December 2010) 75 rectal swabs from diarrhoea patients from different districts were referred for bacteriological analysis. Out of 75 samples 28 were positive for Vibrio cholerae O1 El Tor biotype. Out of 28 V. cholerae O1 strains 26 belongs to Ogawa serotype and two were Inaba. Out of these 28 strains 60% belongs to hybrid V .cholerae strains and rest 40% are normal El Tor strains. This indicates that hybrid V.cholerae strains are spreading to other districts of Orissa after its emergence in Kashipur area during 2007. Subsequently a localized outbreak occurred in the coastal areas of Rajnagar block of Kendrapada district. This is an early warning that the future outbreaks of cholera may happen due to the hybrid strain of V.cholerae O1 sero group. The antibiogram profile of V.cholerae strains isolated from different districts shows variable results. The strains isolated from Ganjam, Nuapada, Kashipur and Rayagada districts were showing 100% tetracycline resistance; where as it was not observed in the strains isolated from Kalahandi ,Nabarangapur, Sundargarh and, Malakanagiri districts. So judicious use of antibiotics is very much essential so far trhe drug resistance is concerned.

### B. Surveillance activities on diarrhoeal disorders in Puri District ( From January -April 2011):

A total of 81 rectal swab samples were collected from indoor diarrhoea cases from Satyabadi and ID hospital, Puri for bacteriological analysis. Out of the total samples analyzed 39 were V.cholerae O1 Ogawa, 1 Shigalla spp and 11 were E. coli. MAMA PCR on representative strains of V. cholerae indicates that most were hybrid V. cholerae strains. V. cholerae stains were

**Isolation of V. cholerae from diarrhoea patients from different districts (DHS-June to Feb'11).**

| Sl. No | District Name | Total No of samples | No of +ve V.ch 01(0/I) | MAMA PCR RESULT | | |
|---|---|---|---|---|---|---|
| | | | | Normal ElTor | Hybrid | Notdone |
| 1 | Kalahandi | 16 | 8 | 4 | 4 | - |
| 2 | Sundergarh | 3 | 2 | 1 | - | 1 |
| 3 | Nuapada | 9 | 3 | - | 1 | 1 |
| 4 | Ganjam | 12 | 2 | - | 2 | 3 |
| 5 | Malkangiri | 6 | 1 | - | - | 1 |
| 6 | Koraput | 3 | 0 | - | - | - |
| 7 | Rayagada | 6 | 4 | 1 | 3 | - |
| 8 | Kandhamala | 2 | 0 | - | - | - |
| 9 | Nabrangpur | 13 | 2 | - | - | 2 |
| 10 | Nayagarh | 2 | 0 | - | - | - |
| 11 | Khurdha | 5 | 1 | | - | - |
| | **Total** | 75 | 28(26-O,2-I) | 6 | 10 | 8 |

sensitive to: azithromycin, norfloxacin, ciprofloxacin, gentamicin, ofloxacin, doxycycline, chloramphenicol, streptomycin and tetracycline and were resistant to:aAmpicillin, nalidixicacid, furazolidone, erythromycin, neomycin and co-trimoxazole.

### C. Surveillance work on Meningitis

During this period 50 samples were processed. Out of 48 blood samples and 2 CSF samples 8 samples were cultured positive for Staphylococcus aureus.

### OPD facility of the centre at Capital Hospital, Bhubaneswar

(Dr B. Dwibedi, Dr A.S. Kerketta, Mr B. N. Murmu, Mr B.N. Sethi & Mr H.S. Nayak)

The centre is providing out patient facility to patients of lymphatic filariasis and haemoglobinopathy. The facility is being utilized for referral investigation & diagnosis of suspected cases of filariasis and haemoglobinopahy from different parts of the state. Besides, the facility is providing treatment to acute and chronic filarial disease including decompression therapy for lymphedema reduction. The facility is also being utilized for collection of clinical information and biological samples for diagnosis and research including viral diagnosis, bacterial meningitis and hypertension related to research projects of the centre as well as PhD programmes.

During the year 470 cases of lymphatic filarial diseases attended the set up, Out of them two third of cases have chronic filarial disease as grade II - IV lymphedema. Rest were having acute episodes of adenolymphangitis. The cases were examined and ADLA attacks were identified and treatments provided. Lymphedema management was provided with preventive chemotherapy, foot hygiene and intermitted de compression therapy. 78 cases of lymphedema (grade II & III) received intermittent decompression and there was 20-30 % reduction in the edema in the subjects.

192 cases were referred with suspected haemoglobinopathy disorder and laboratory investigation undertaken. 33 Cases of thalasemia and 24 cases of sickle cell and 5 cases of E thal were detected. Parent's blood samples were collected and lab test report provided to help them in planning subsequent pregnancies. 387 samples were collected from capital hospital, Bhubaneswar for viral infection diagnosis and 51 no. of samples were collected for bacterial meningitis diagnosis using the facility by the project staff. 200 cases of hypertension were enrolled from the hospital set up, on which genetic markers studied by our research scholar and the bio chemical test report provided to the patients.

The services offered at the above facility have benefited the patient and the state health department in diagnosis and treatment of the cases. This also supported the research activity of the centre which required clinical facility and clinical information that supplemented the laboratory and epidemiological expertise of the centre.

### Activities of NNMB Unit, Odisha

### Assessment of Diet and Nutritional Status of Rural Population, 3rd repeat Survey

The National Nutrition Monitoring Bureau (NNMB) since its inception in 1972 under the Indian Council of Medical Research (ICMR) in the states of Kerala, Tamilnadu, Karnataka, Andhra Pradesh, Odisha, West Bengal and Uttar Pradesh has been carrying out diet and nutrition survey in the rural areas.

In the annual steering committee meeting of N.N.M.B. during the year 2007-08 had recommended that the N.N.M.B. should take up 3rd repeat survey on rural population to study the change in the nutrition situation with respect to 1975-79, 1988-90, 1996-97 surveys. In view of these recommendations the Central References Laboratories CRL of NNMB proposed to carry out the 3rd rural repeat survey during 2009-10.

## Objectives

### General Objective

To assess the current status as well as time trends of rural population in all 10 NNMB states. In addition, it is also proposed to assess the prevalence of obesity, diabetes and hypertension among adult rural population of ( 18 years).

### Specific Objective

(i) To assess the food and nutrient intake among different age, sex and physiological groups of rural population in NNMB states.

(ii) To assess nutritional status in terms of anthropometry, clinical examination and study the time trends.

(iii) To assess the history of morbidity among all the individuals covered for anthropometry.

(iv) To assess the prevalence of obesity, diabetes and hypertension among adult men and women ( 18 years).

(v) To assess knowledge and practices about hypertension and diabetes among adult men and women ( 18 years).

(vi) To assess infant and young child feeding practices of mother index.

The NNMB Odisha unit carried out surveys in the rural communities of 105 villages from 11 districts viz. Bolangir, Balasore, Boudh, Ganjam, Jagatsinghpur, Cuttack, Jajpur, Kalahandi, Keonjhar, Mayurbhanj and Sundergarh. The team collected the data in addition to routine diet and nutrition assessment prevalence of obesity according to waist circumference and waist hip ratio, hypertension and diabetes mellitus card.

The investigation included collection of data on

- Households demographic
- Socio-economic particulars
- Nutritional anthropometry
- Clinical examination for nutritional deficiencies
- 24 hours recall method of diet survey (to assess food and nutrient intake of HHs individuals)
- History of morbidity (during preceding 15 days)
- Fasting blood glucose levels.
- Infant and young child feeding practices of mother index.
- Collection of dried blood spots for DNA extraction etc.

In the year 2011 NIN suggested to collect 3-4 drops of blood for carrying out Genetic Epidemiological studies in India in near future by creating DNA Bio repository.

The NNMB Odisha unit started 3rd repeat survey from Aug. 2009 till 2011.The unit has covered 105 villages in 11 districts where 2100 households are covered and 1050 diet survey has done and 1022 DBS samples has collected and data are sent for analysis to NIN Hyderabad.

## Workshop/Training Attended

- Dr. A. R. Mohanta, ARS (Medical) participated "ICMR Research Methodology Workshop" conducted by Division of Health Systems Research (HSR) of ICMR and Regional Medical Research Centre (R.M.R.C.), Bhubaneswar held at R.M.R.C., Bhubaneswar from 5th to 7th January 2011.
- Dr. A. R. Mohanta, Asst. Research Scientist attended the orientation training programme on collection of DBS from 14th to 18th February 2011 at NIN.
- Mr. Guru Charan Mantri, Lab Technician has undergone the training on collection of DBS from 14th to 18th February at NIN, Hyderabad.


Dr. S.K. Kar, Director addressing to the Scientists in ISMOCD & IAE conference organized by RMRC Bhubaneswar.


Book release by Hon'ble Minister Health & FW Govt. of Odisha Sri Prasana Acharya
occasion of RMRC field unit inauguration at Rayagada.

# Works of the Ph.D Scholars

**SAC chairman Dr. D.S. Agarwal, Dr. Rasmi Arora, Chief, ECD, ICMR & Prof. Sarman Singh, AIIMS, New Delhi interacting with Ph.D Scholar during 25th SAC meeting.**

## 1. Role of B1 Lymphocytes and autoantibodies in human lymphatic filariasis.

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

### Objectives

1. To study the profile of B1 cell in filarial infected human population.
2. To study the association of poly-reactive antibodies with B1 cells in different clinical groups of filarial patients.
3. To study the role of B1 cells in cytokine response by filarial carbohydrate and protein antigens in human filariasis.
4. To study the Fas-FasL expression profile of B1 lymphocytes and its immune regulatory role in mediating CD4+ T-cell apoptosis in human filariasis.

B lymphocytes plays a pivotal role in adaptive immune response by patrolling the arm of humoral (antibody mediated) immunity is present in two distinct subsets viz; B1 and B2 cells. B1/B2 cells are characterized by expression of distinct surface markers and by secretion of different profile of antibodies. Surface expression of CD5, CD45R$^{low}$, B220 $^{low}$, IgM high and CD19 are hallmark of B1 cells while B-2 cells are conventional B-cells. B2 cells produce diverse repertoir of antigen specific antibodies. B-1 cells have been shown to produce polyspecific autoantibodies (antibodies against LPS, actin, myosin, ssDNA) mostly of IgM isotype. B1 subset of Bcells play an important role in outcome of infection in Schistosomiasis, S.pneumonie and experimental filariasis but the status of B1 cells in clinical manifestation of human bancroftian filariasis is still not clear. Since b-1 cells are committed to the production of polyreactive natural antibodies (e.g ssDNA, actin, myosin etc) mainly IgM, We quantified antibodies reacting to the ssDNA, actin, LPS and myosin. We also quantified other antibody isotypes such as IgG reacting to ssDNA, actin, LPS and myosin. It was observed that plasma of microfilariaemic carriers have significantly low levels of IgG antibodies to ssDNA, myosin and LPS as compared to other clinical categories of filariasis (Fig.1, b-d) where as in case of actin , no significant difference in IgG titres was found in different clinical categories of filariasis (Fig.1a).

The cross-reactivity of anti-ss DNA was further assessed by inhibition with soluble DNA. Absorption of sera with soluble myosin and ss-DNA reduced antibodies reacting with LPS similarly the reactivity to ss-DNA was effectively removed by absorption with myosin. There is a significant difference between pre and post absorbed plasma samples in case of all the graphs shown in Fig.2 (a-d) indicating that most of the DNA-binding antibodies found in human sera are cross-reacting with DNA.

Fig.1a

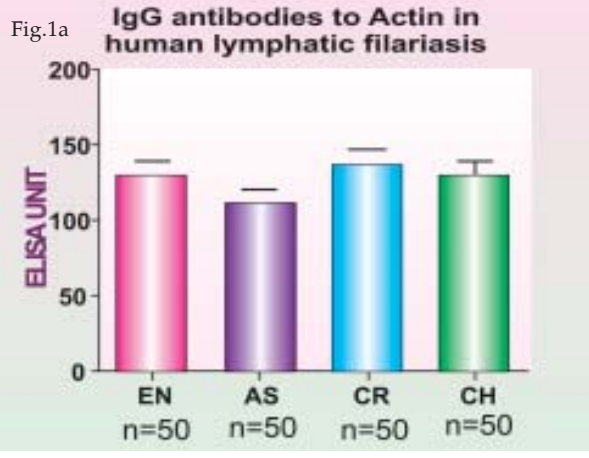



Fig.1b

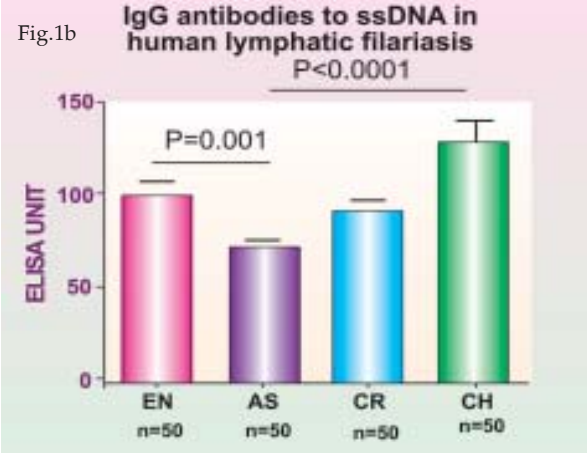


Fig.1c

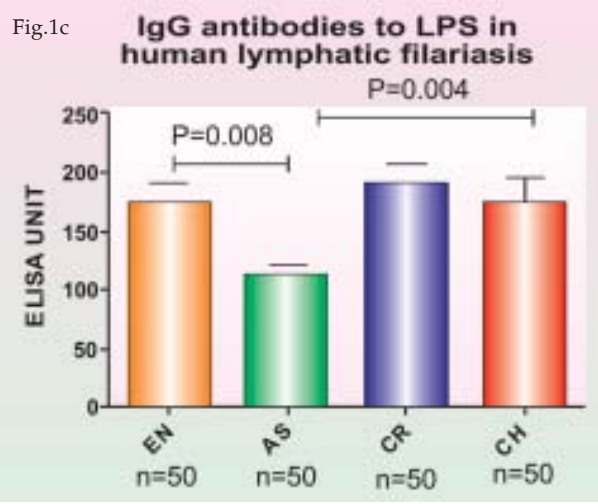



Fig.1d

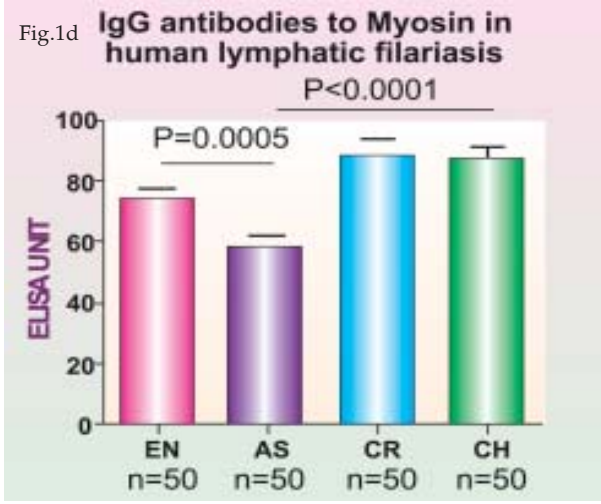



Fig.2a

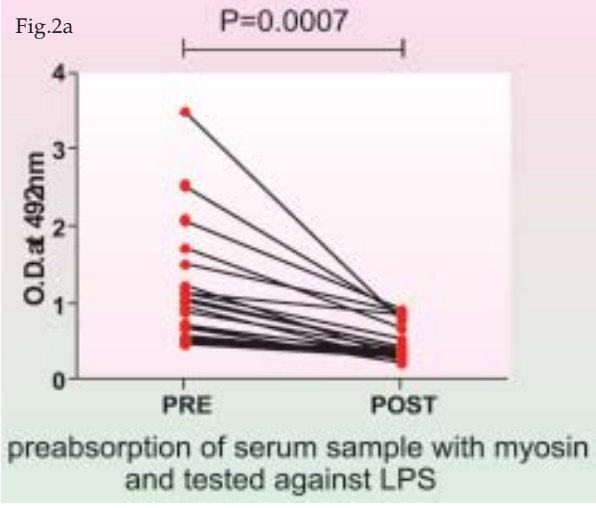



Fig.2b

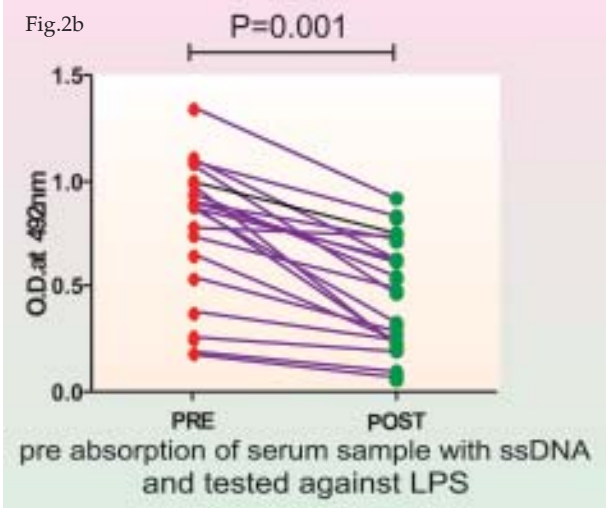



Fig.2c

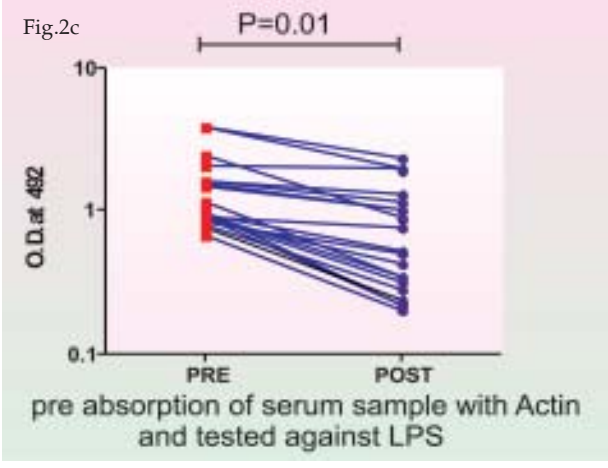
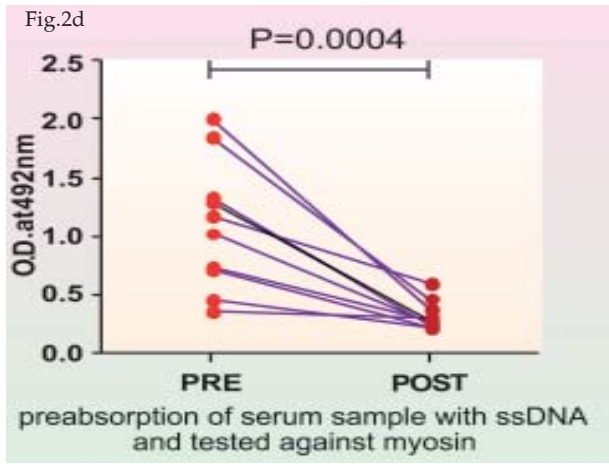



Fig.2d



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

Name : K Gopinath Achary
Status : SRF(ICMR)
Date of joining : 30th Jan 2009
Guide : Dr. Ashok Kumar Satapathy

### Introduction

Epidemiological studies have shown that children born to microfilaraemic mothers are more likely to be microfilaraemic compared to children whose mothers are amicrofilaraemic during gestation and have been shown to impair filarial Ag-specific T cell responses (Steel et al, 1994). Women commonly harbor filarial infections during their child bearing years, raising the possibility that the developing fetus may be exposed to filarial antigens in utero and thereby have altered immunity and susceptibility to infection during early childhood (Malhotra et al, 2003). Placental transfer of filarial antigens and prenatal exposure develop a state of tolerant in the offspring diminishing the anti filarial immune reactivity and making them more susceptible to infection than offspring of uninfected mothers (Haque et al, 1982) and affects development of subsequent immune responses.

### 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 Progress

#### Study population

The study subjects are inhabitants of different villages in Khurda district of Orissa state, which is located approximately 40-50 km away from the state capital Bhubaneswar and about 40 km away from Bay of Bengal. The study area is documented as highly endemic area for *W. bancrofti* with a microfilaria rate of about 12% (Beuria et al. 2003). It was reported that 45.4% of the children below 15 years of age from this area were either infected or had clinical manifestations of the disease and IgG antibody positivity 75.4%, 84% and 95.8% were observed in 1-5 yr, 6-10 yr and 11-15 years age group respectively (Mandal et al.2010). Paired maternal and cord blood samples (n=145) were collected from O & G Department of Khurda Hospital. All the mothers included in the study, had no symptoms of clinical filariasis at the time of admission. Complicated delivery cases were excluded from the study. Informed consent for participation was obtained before subjects were included in the study. Age of the mothers ranged from 18 to 37 years with a mean age of 25 years. Venous blood samples were collected from mothers before delivery and venous umbilical cord blood samples from neonates were collected immediately after birth..

#### Circulating filarial antigen (CFA) assay

CFA in the serum of mother cord blood samples were detected by using an Og4C3 ELISA test kit (JCU Tropical Biotechnology, Queensland, Australia) according to the manufacturer's instruction.

A total of 145 pairs of mother and cord blood samples were collected, examined for the CFA status by using an Og4C3 enzyme linked immunosorbent assay. Out of 145 pair samples 46% mothers were CFA positive and rest of the samples were CFA negative (Table-1). Among the CFA positive samples 34% samples were found to be positive for both mother

**Table 1:** Prevalence of circulating filarial antigen in maternal and cord blood samples.

| Maternal Infection Status | N | % | Cord blood infection status | N | % |
|---|---|---|---|---|---|
| CFA +ve | 66 | 45.6 | CFA +ve | 23 | 34.8 |
| | | | CFA -ve | 43 | 65.2 |
| CFA -ve | 79 | 54.4 | CFA -ve | 79 | 100.0 |

and its corresponding cord blood indicating transfer of CFA from mother to cord and 66% samples were found to be positive only for mothers and not cord blood which means there is no transfer of CFA from mother to cord.

All the samples were separated into 3 groups according to the CFA status of the mother and its respective cord blood i.e. Both positive group: Mother and its respective cord blood were CFA positive (Both Positive: M+ C+); Mother positive group: Mother was CFA positive and its respective cord blood was CFA negative (M+ C-); Both Negative group: Mother and its respective cord blood were CFA negative (M- C-).

The mean age of mothers in all 3 groups were statistically significant and geometric mean of CFA units of mothers of M+C+ group was significantly higher than other two groups (Table-2).

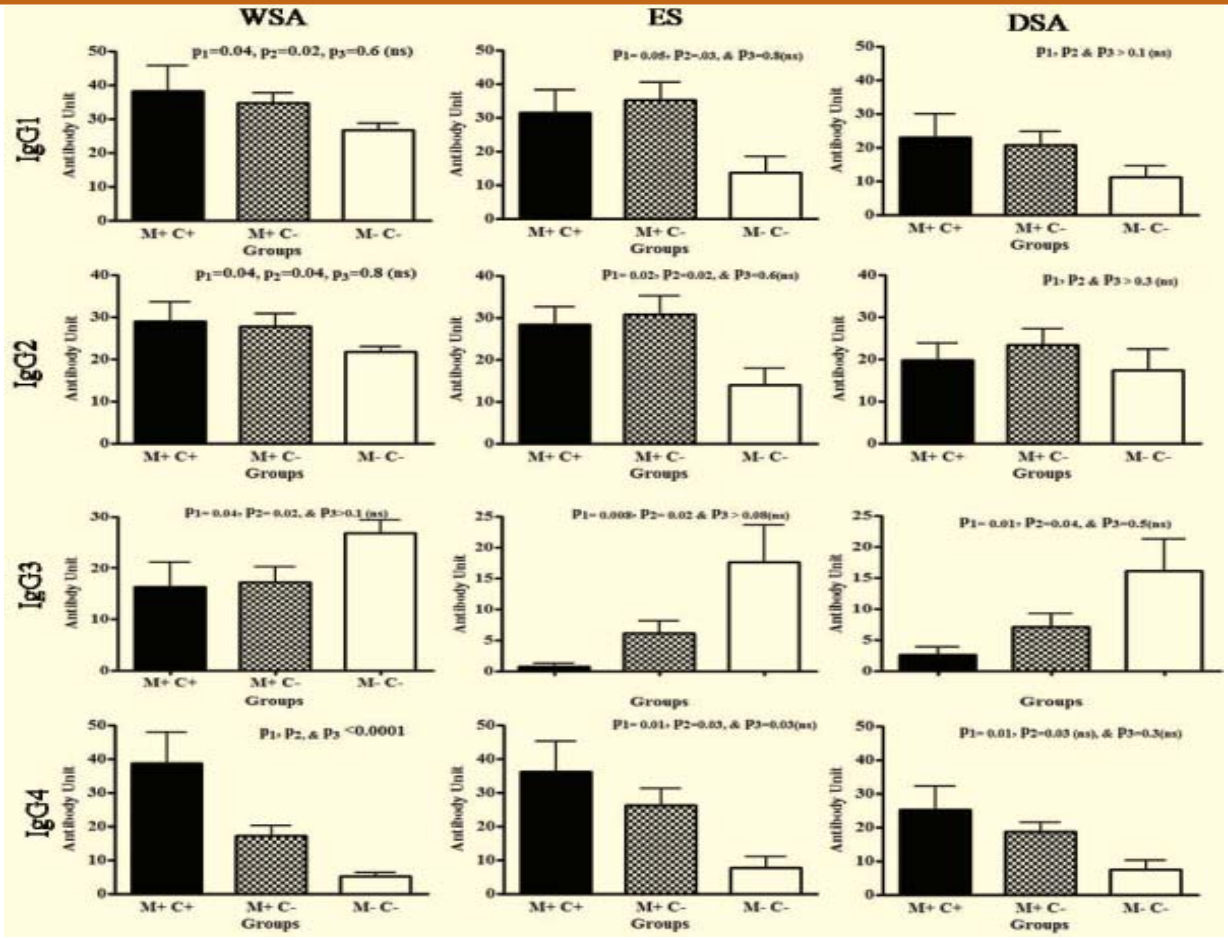
**Parasite collection and antigen preparation**: Live worms of *Setaria digitata* were collected from the bovine intestine from slaughter house of Nandankanan. Water soluble (WSA) and detergent soluble (DSA) antigens were prepared by grinding the worms in PBS and Tris-NP40 respectively. Excretory-Secretory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

**Filarial specific IgG subclass antibodies response**: In order to study the humoral response

**Table 2:** Age, CFA unit and filarial specific IgG subclass response in mother samples.

| | Groups | | | Statistics | | |
|---|---|---|---|---|---|---|
| | M+ C+ | M+ C- | M-C- | M+C+/M-C- | M+C-/M-C- | M+C+/M+C- |
| Sample numbers (%) | 23(15.8) | 43(29.6) | 79(54.4) | | | |
| Mean age, years | 26.05 | 23.92 | 23.2 | *P=0.027* | *P=0.032* | *P= 0.018* |
| CFA GMI* Units | 1735 | 875 | | | | *P= 0.021* |
| Mean Antibody Units | | | | | | |
| Against WSA | | | | | | |
| IgG1 | 36.3 | 42.3 | 31.6 | *P=0.1* | *P=0.05* | *P=0.22* |
| IgG2 | 47.3 | 43.2 | 29.2 | *P=0.01* | *P=0.08* | *P=0.22* |
| IgG3 | 47.85 | 55.1 | 31.8 | *P=0.41* | *P=0.21* | *P=0.22* |
| IgG4 | 60.1 | 42.9 | 7.9 | *P<0.0001* | *P<0.0001* | *P1=0.029* |
| Against DSA | | | | | | |
| IgG1 | 15.2 | 22.0 | 20.6 | *P=0.09* | *P=0.21* | *P=0.13* |
| IgG2 | 34.7 | 33.3 | 31.1 | *P=0.41* | *P=0.21* | *P=0.22* |
| IgG3 | 19.8 | 25.9 | 42.1 | *P=0.02* | *p=0.05* | *P=0.44* |
| IgG4 | 40.0 | 22.4 | 8.7 | *P=0.003* | *P=0.005* | *P=.044* |
| Against ES | | | | | | |
| IgG1 | 37.2 | 41.8 | 23.8 | *P=0.41* | *P=0.21* | *P=0.22* |
| IgG2 | 46.4 | 49.2 | 28.5 | *P=0.07* | *P=0.01* | *P=0.13* |
| IgG3 | 15.3 | 19.8 | 34.3 | *P=0.032* | *P=0.033* | *P=0.41* |
| IgG4 | 48.4 | 31.3 | 12.3 | *P<0.0001* | *P=0.006* | *P=0.07* |

**Fig. 1.** Filarial specific IgG subclass antibody responses to different antigens in cord blood samples.




filarial specific IgG subclass responses were assessed in all the samples against water-soluble antigen (WSA), detergent soluble antigen (DSA) and excretory secretory antigen (ES).

Antibody responses were quantified in both maternal and cord sera by ELISA as shown in table-2 and figure-1. Results of our study shows that filarial specific IgG1, IgG2 and IgG4 antibody responses were

**Table 3**: Filarial specific IgG isotype antibody seropositivity in maternal and cord blood according to infection status against the water soluble antigen.

| Maternal Infection Status | N | IgG1 seropositive | | IgG2 seropositive | | IgG3 seropositive | | IgG4 seropositive | |
|---|---|---|---|---|---|---|---|---|---|
| | | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) |
| Both Positive | 20 | 65 | 40 | 90 | 55 | 80 | 30 | 80 | 70 |
| Mother Positive | 43 | 69 | 51 | 88 | 60 | 60 | 25 | 74 | 42 |
| Both Negative | 63 | 50 | 30 | 85 | 52 | 28 | 8 | 46 | 13 |

**Table 4**: Filarial specific IgG isotype antibody seropositivity in maternal and cord blood according to infection status against the detergent soluble antigen

| Maternal Infection Status | N | IgG1 seropositive | | IgG2 seropositive | | IgG3 seropositive | | IgG4 seropositive | |
|---|---|---|---|---|---|---|---|---|---|
| | | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) |
| **Both Positive** | **20** | **30** | **30** | **55** | **35** | **15** | **5** | **45** | **35** |
| **Mother Positive** | **43** | **44** | **18** | **49** | **32** | **28** | **7** | **48** | **32** |
| **Both Negative** | **63** | **82** | **27** | **78** | **27** | **55** | **5** | **40** | **13** |

**Table 5**: Filarial specific IgG isotype antibody seropositivity in maternal and cord blood according to infection status against the excretory secretory antigen.

| Maternal Infection seropositive | N | IgG1 seropositive | | IgG2 seropositive | | IgG3 seropositive | | IgG4 seropositive | |
|---|---|---|---|---|---|---|---|---|---|
| | | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) | Maternal sera (%) | Cord sera (%) |
| Both Positive | 20 | 50 | 50 | 60 | 35 | 25 | 15 | 60 | 65 |
| Mother Positive | 43 | 58 | 44 | 75 | 45 | 37 | 12 | 78 | 56 |
| Both Negative | 63 | 95 | 14 | 86 | 40 | 68 | 14 | 68 | 22 |

significantly higher in cords of infected mother compared to uninfected mother ($p<0.05$) Filarial specific IgG1, IgG2 and IgG4 antibody response pattern in mothers were similar like cord bloods. Filarial specific IgG3 antibody response was significantly high in cords of uninfected mothers than the cords of infected mothers. However when a comparison was made between M+C+ and M+C- group only IgG4 response was significantly higher in M+C+ groups for both mother and cord samples.

**Filarial specific IgG, IgM and IgE response:** There were reports available that suggest the occurrence of transfer of IgG subclasses from mother to cord but IgM and IgE cannot cross the placental barrier. In order to find out the fact of in-utero sensitization we assessed filarial specific IgM and IgE antibody responses in maternal and cord blood (Fig.-2). Our result shows that filarial specific IgM and IgE antibody responses are significantly higher in mothers than the cords in all groups. IgM levels were very low in cord bloods; interestingly IgE antibody response in cords of M+C+ group was significantly higher than the cords of M+C- and M-C- groups ($P<0.05$) indicates that in utero sensitisation of immune response by filarial antigens. This result strongly supports that antigens can cross the placental barrier and induces the cord to produces antibodies. According to CFA assay results there was no transfer of CFA occurs from mother to cord in M+C- group but still our results showed filarial specific IgE antibody response in cord bloods of the same group, so to find out the fact behind the antibody response we need to quantify the antibody responses against carbohydrate and lipid. We found IgM response in

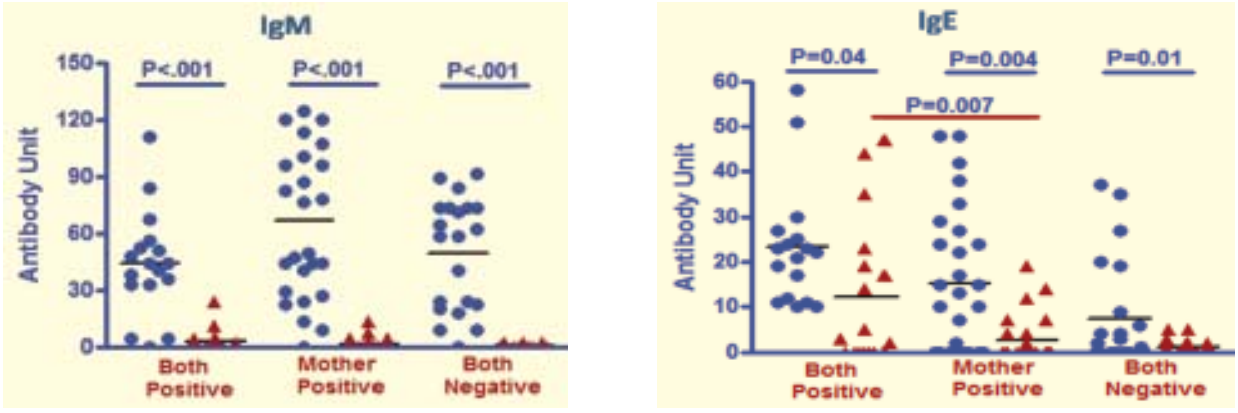



**Fig. 2:** IgM and IgE responses against water soluble antigens in maternal and cord blood samples according to infection status.

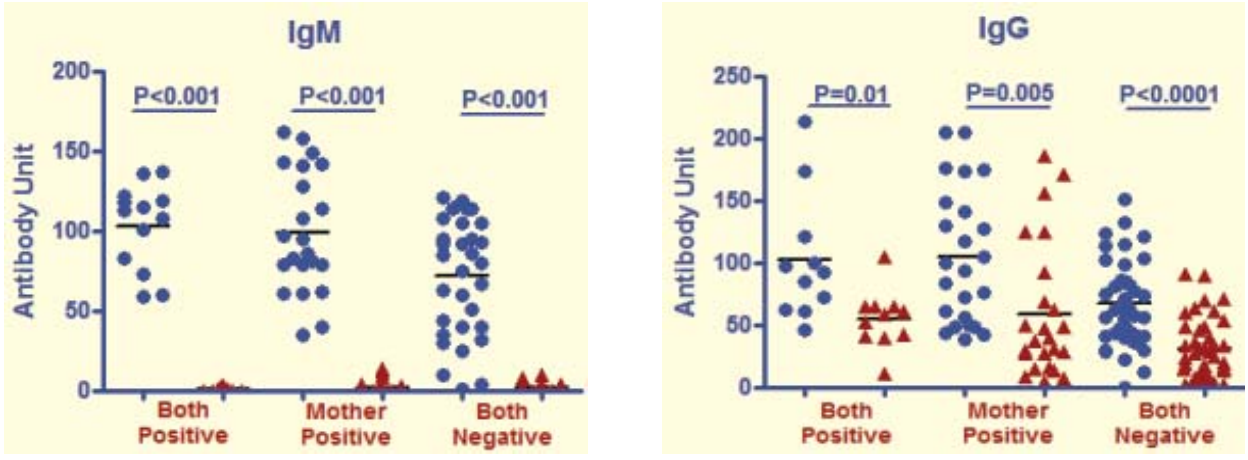



**Fig. 3:** IgG and IgM response against Carbohydrate antigens in maternal and cord blood samples according to infection status.

mothers was significantly higher against purified carbohydrate than other forms of antigens (Fig.-3).

### Quantification of T-regulatory cells and B1 cells

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

T regulatory cells known as the immune suppressors are higher in cord bloods than its respective mothers in positive groups but in CFA negative group it shows just its opposite trend (Figure-4). B1 cells are higher in CFA negative cord than CFA +ve cords both born from CFA +ve mothers which means higher B1 cell population protects the cord from getting infection where as higher T regulatory cells makes the cord more susceptible to infection.

### Quantification of Cytokines

In order to study the effect of maternal infection on neonatal cellular responsiveness several Th1 type (IL-12 and IFN-ã) and Th2 type (IL-5 and IL-10) cytokines were quantified in maternal as well as cord sera by using sandwich ELISA kits of e-biosciences.

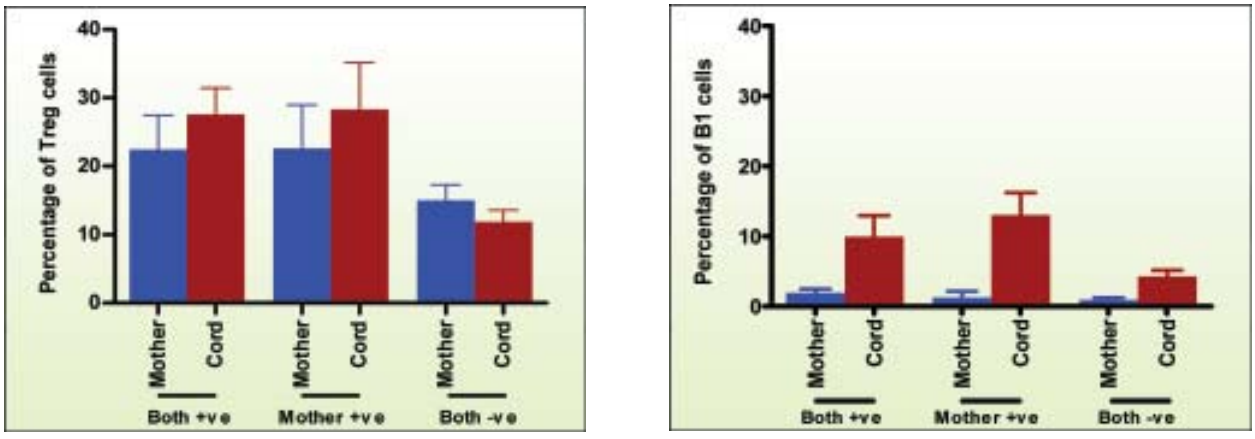



**Fig. 4:** (A) T regulatory cells and (B) B1 cell profile of maternal and cord sera according to infection status.

It was previously reported by different researchers that low levels of IFN-ã and IL-12 were produced by UCBC in response to parasite antigen where as high levels of IL-5 and IL-10 were produced by UCBC that is equivalent to the levels produced by PBMC. In our study plasma levels of Th-1 type of cytokines IL-12 and IFN-ã both were found highest in endemic normal group and lowest in both positive

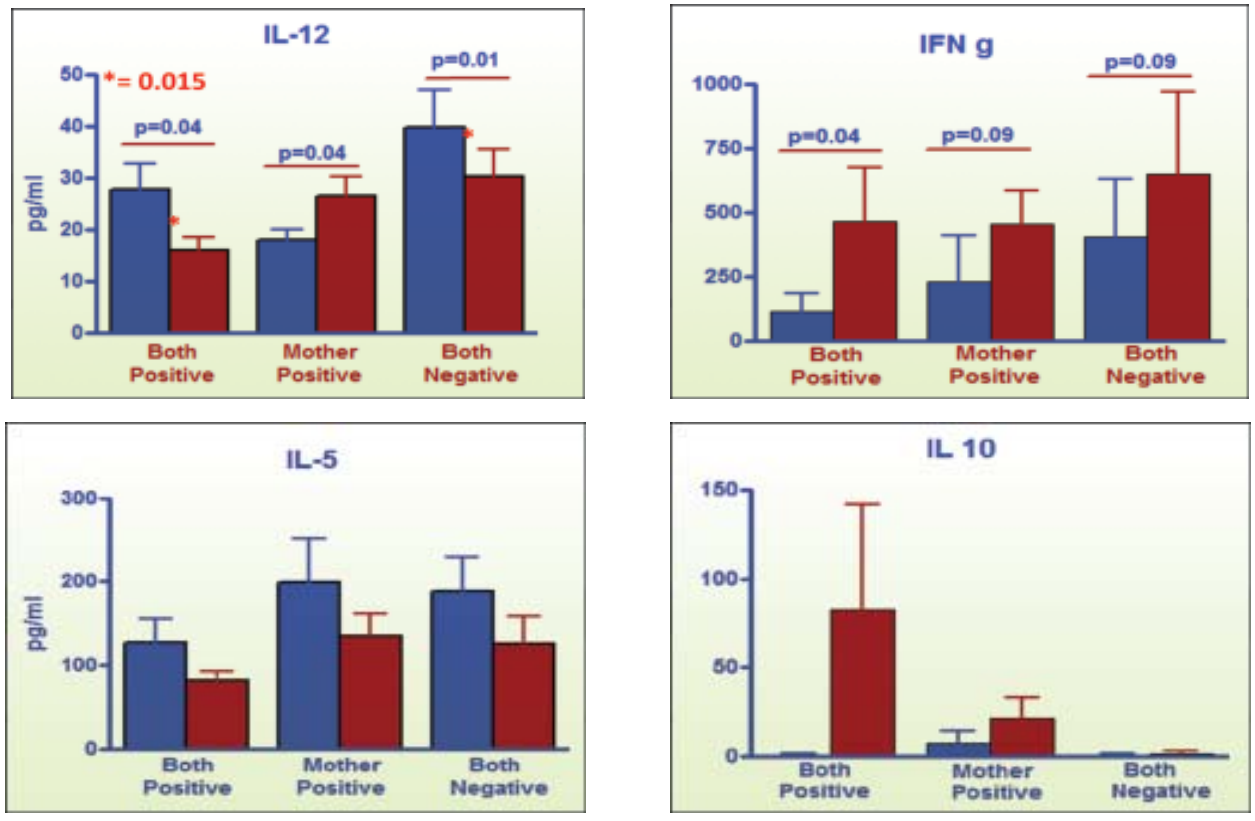



**Fig. 5:** Serum levels of cytokines (Th1 and Th2) in maternal and cord sera according to infection status.

groups where transfer of antigen occurs (Fig. 5). Amongst the groups IL-12 was significantly high in maternal sera than cord sera except in the mother positive group where transfer of antigen could not occur, however IFN ã was always high in cord sera than maternal sera. Plasma levels of Th-2 type of cytokines IL-10 and IL-5 were found to be contradictory, level of IL-10 was highest in both positive group and lowest in endemic normal group where as IL-5 level was vice versa. IL-5 was always high in maternal sera than cord sera and IL-10 was found to be high in cord sera. It was observed that the proinflammatory cytokine IFN-ã production was higher in cords of both negative group than other infected groups but anti-inflammatory cytokine IL-10 production was high in cords of both positive groups. It means production of Inflammatory cytokines gives protection and production of anti-inflammatory cytokines makes the cord more susceptible for infection due suppression of immune system.

### Summary

- Transplacental transfer of circulating filarial antigens from (34%) infected mothers to newborn children had been observed.
- Filarial specific IgM and IgE antibodies response to water soluble antigens and purified carbohydrate are significantly high in cord blood of infected mothers compared to uninfected mothers. All the above data indicates in utero sensitisation of cord blood samples by filarial antigens.
- Filarial specific IgG subclass responses were assessed in cord blood of infected & uninfected mothers against water-soluble antigen (WSA), detergent soluble antigen (DSA) and excretory secretory antigen (ES).
- Increased frequencies of filarial specific IgG4 (a marker of active filarial infection) against all three antigens was observed in children born from infected mother.
- Increased levels of T- regulatory cell population detected in cord blood of filarial infected mother compared to uninfected mothers indicating down regulation of Th1 response in cord blood of infected mothers.
- Plasma levels of IFN-ã and IL-12 is high in CFA– ve neonates than CFA+ve neonates those born from CFA+ve mothers. IL-5 is high in CFA-ve neonates born from CFA+ve mothers and IL-10 is high in CFA+ve neonates born from CFA +ve mothers.
- An Increased levels of IL-10 and decreased levels of IFN-ã have been detected in cord blood of children born to filarial infected mothers indicating that increased T-regulatory cells could down regulate inflammatory responses and may facilitate parasite survival thus leading to carrier state of infection.
- B1 cells are up regulated in neonates born from CFA positive mothers however it is low when there is a transfer of antigen from mother to cord and high in neonates when there is no transfer of antigen.

From the above results it can be presumed that the neonates are sensitised in utero and the antigen presentation in CFA+ve neonates born from CFA+ve mothers is suppressed due to high levels of IL-10 and T-Regulatory cells.

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

Name : Mrs. Buli Kumari Panigrahi
Status : SRF(ICMR)
Guide : Dr. N. Mahapatra
Starting Date : 10th September 2008

### Objectives

- To find out the malariogenic conditions, malaria situation in the command area of the dam and prevalence of the vector and other mosquito species.

- Comparison of malaria incidence & vector fauna in the canal area before and after the construction of the canal.
- Role of environmental factors associated with the vector-bionomics, such as vector density, sporozoite rate and insecticide susceptibility status etc.

### Rationale

The public-health risks associated with large-scale hydro-electric and irrigation projects are well known in India and abroad (Russel, 1938; Oomen et al., 1988; Service, 1989). To accelerate the pace of socio-economic development, the country continues to plan and execute water resource development schemes in large numbers. Though there are positive consequences of these projects like irrigation and water storage, it has also adverse effects on health and environment especially malaria. In Orissa, several dams and canals were constructed within last few decades. But yet, no study was conducted on malaria prevalence, transmission and risk factors pertaining to the transmission before and after the construction of the canal. Moreover, extending irrigation facilities in constructing canals changes the ecology of the area pertaining to breeding and survival of vector mosquito species. Therefore, the transmission and epidemic risk factors of malaria does not remain the same as before. So, an in-depth study is required on malaria transmission and risk factor responsible for the transmission in and around the canal areas hence, this study is being initiated to find out the risk factors associated with spread of malaria in the Rengali Left Bank canal system, which is being constructed newly.

### Progress

### A. Base line data collection on Rengali Left Bank Canal system

Rengali Dam was constructed across River Brahmani, in Angul district. Samal barrage was constructed about 34 km. downstream of Rengali dam. Two canals i.e. Left Bank Canal (LBC) and Right Bank Canal (RBC) are constructed from Samal Barrage. The LBC is divided into 2 parts, LBC-I and LBC-II which will irrigate three malaria endemic blocks of Dhenkanal district such as Parjang (PHC-Parjang), Kamakhyanagar (PHC- Analaberni) and Bhuban (PHC-Mathakargola). (Source: Department of Water Resources, Government of Orissa, October 2008).

Till now only 71 km of the canal has been constructed covering Parjang (0-30 km) and Analabereni (31-71 km) PHC areas. The water has been released up to 30 km of the canal in Parjang PHC area and in Analabereni PHC (31-71 km), the canal is under construction and water has not been released (Dept. of Water Resource Department, 2008).

### Selection of Study villages

The villages selected were from the Parjang PHC (where water has been released) and Analaberni PHC (where water is not released yet). So, villages from Parjang PHC area have irrigation throughout the year due to the water availability in the canal system and the villages of the Analabereni PHC area are basically dry foothill villages (cultivation depends upon rain i.e. from July to September). Study villages are within 1km buffer zone of the irrigation canal command area.

### B. Information on malaria situation:

The data before release of the water in the Parjang area (1999-04) showed the average SPR was 10.8% (5.1-14.6%), API- 9.1 (4.1-13.1) and Pf%-90.8% (77.2-99.8%). The water was released to the above area during 2004. During 2005-2010, post release period the epidemiological parameter showed average SPR-5.9% (1.8-9.3%), API-5.7 (1.5-9.4) and Pf%-96.5% (93-100%).

In Analabereni area, during 1999-2004, the data shows average SPR-18.4% (16.1-21%), API-21.8 (19.3-25.1), Pf%-94.5% (91.8-96.6%). All these parameters showed declining trend during the period coinciding with the post release period (2005-2010), SPR was 11.3% (9.3-15.6%), API-12.8 (8.5-15.5) and Pf%-91.7% (78.8-97.2%).

The malaria incidence between pre (1999-2004) and post release of water period (2005-2010) indicates no significant changes in the Parjang area ($t=1.58$, $df=5$, $P>0.05$). However, there was significant decline in the malaria incidence in the Analabereni area during 2005-10 in comparison to 1999-04 ($t=4.69$, $df=5$, $P<0.01$) (fig.1).

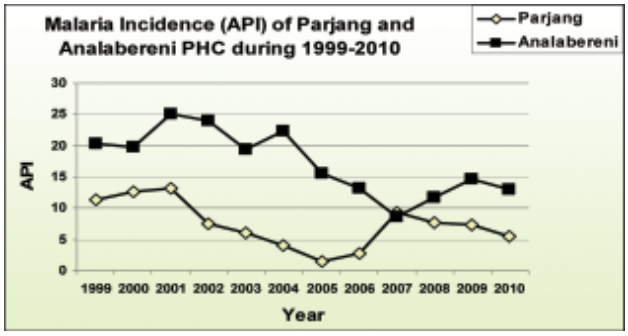



**Fig.1.** Shows API of Parjang and Analabereni PHC respectively from 1999-2010. 2004 is the water release year to the parjang area.

**The observed decrease in mean API in both the areas during 2005-2010 was may be due to the combined effect of NVBDCP control programme and Malaria Mitigation Measures.**

The data of the present study reveals that, in Parjang PHC area the API showed a declining trend from 2004 to 2006. The API was 4.05, 1.51 and 2.69 during 2004, 2005 and 2006 respectively. An extensive control programme taken by the Govt. before the release of water in 2004, might have resulted in reduction of API in the area. Thus, the API came down to 1.51 in 2005 and 2.69 in 2006. As per the NVBDCP guidelines, indoor residual spray (IRS) for the vector control must be done where API was more than 2 for last three consecutive years.

Since the API during the year 2005 decreased to <2 the IRS had not been undertaken in the area which affected the increase of API to 2.69 during 2006 and sudden increase of a very high API of 9.42 in 2007 that lead to undertake IRS in the area and the malaria situation was maintained with the API lower than the baseline level. As compared to Parjang area, in Analabereni area the API was very high i.e 22.24 (in 2004) but the combined effect of routine control programme run by the Govt. and implementation of mitigation measures undertaken in 2006 resulted in 47.1% reduction of API in 2008.

### C. Entomological Survey:

Entomological survey was done in three seasons in the study villages.

### Vector Density

The entomological survey revealed the presence of three efficient malaria vectors, i.e. *Anopheles culicifacies, An. annularis, An. fluviatilis along with An. subpictus, An. vagus, An. hyrcanus, An. karwaris, An. splendidus and An. tessellatus.* Four species of *Culex* mosquitoes *viz., Culex quinquefasciatus, Cx. vishnui and Cx. whitmorie, Cx.gelidus* were also found in both the studied PHCs. The details are given in Table 1.

**The season wise comparison of mean PMHD between the study areas shows:**

- Higher mosquito density (PMHD) in the Analabereni area.

Significant difference of *An. culicifacies* (**t=3.4, P<0.05**) during Rainy season and *An.annularis* (**t=6.78, P<0.05**) during winter season was found.

**Identification of sibling species *An.culicifacies, An.fluviatilis and An.annularis***

Standardization for the identification of sibling species for *An. culicifacies* was done by Allele-specific

**Table. 1:** Per Man Hour Density of Anophelines collected during November 2008-October 2010 in the study villages.

| | Parjang (Mean±SD) | | Analabereni (Mean±SD) | | | |
|---|---|---|---|---|---|---|
| **Species** | **Winter** | **Summer** | **Rainy** | **Winter** | **Summer** | **Rainy** |
| *An.culicifacies* | 2.5±0.35 | 1.8±0.27 | 4±0.11 | 3.8±0.31 | 1.4±0.08 | 4.8±0.13 |
| *An.annularis* | 1.3±0.09 | 1.4±0.43 | 2±0.04 | 2.1±0.13 | 1±0.10 | 2.1±0.02 |
| *An.subpictus* | 5±0.31 | 4.4±0.88 | 6.9±0.27 | 7.2±0.23 | 5±0.12 | 9.7±0.02 |
| *An.vagus* | 4.6±0.97 | 2.6±0.34 | 3.1±0.22 | 5.3±0.93 | 2.3±0.3 | 4.3±0.29 |

SD: Standard Deviation.

**Table. 2.** Antthropophilic Index and sporozoite rate of Anophelines of both the study area.

| 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)

polymerase chain assay (ASPCR) method as per Goswami *et al*, 2006. 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 October 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% & 2.14%) in Parjang and Analabereni PHC respectively (fig.2).

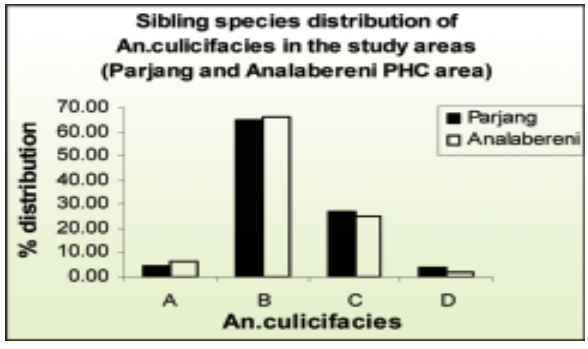



**Fig. 2.** Mosquito samples processed for sibling species of An. culicifacies by Allele-specific polymerase chain assay (ASPCR) (collected during November 2008-October 2010).

Standardization for the identification of sibling species of *An. fluviatilis* was done as per the method of Mohanty *et al*, 2007 (fig.8). 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.

Standardization for the identification of sibling species of *An. annularis* is being done as per the procedure of Alam *et al*, 2007. Only *An.annularis* A species was found in the area.

**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.2).

**Larval Survey:**

Mosquito larval sample were collected from experimental villages from different breeding sites

**Fig.3.** 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.

(fig.3) 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.

### Susceptibility Status

The susceptibility status of important vector species (*An. culicifacies, An. annularis)* to various insecticides like DDT and synthetic pyrethroid were determined using standard WHO method (WHO, 1975).

- The dominant vector *An.culicifacies* and *An.annularis* shows 30-36% resistance to 4% DDT.
- 37-45% resistance to 5% malathion.
- Both the species shows 100% susceptibility to deltamethrin

### 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 (5$^{th}$ 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.

### Risk factors for malaria transmission due to irrigation canal System in the study areas

- Seepage in the canal area (Parjang area).
- Increase of irrigation potential thus increase of breeding habitats of vectors (Parjang area).
- Formation of temporary ponds and breeding spots inside the canal system) in the constructed areas (Analabereni area).
- The poor housing conditions in the vicinity of the irrigation canal may have enhanced human–vector contact (Analabereni area).
- Malaria incidence and entomological study during the study period shows higher in the Analabereni villages, as compared to Parjang villages may be due to
  (a) Increase in breeding spots in the constructed area (breeding spots aroused inside the digging canal) (fig.13).
  (b) More exposure to mosquitoes during out door activities because of poor socio-economic status.
  (c) No electricity to the area, so prolonged host-vector contact.

Poor road communication leads to less coverage by Government control programmes and also difficult to reach the hospital for treatment.

## 4. Bacterial meningitis and pneumonia among pediatric age group in Orissa.

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Status : SRF (ICMR)
Guide : Dr. S. K. Kar

### Introduction

Bacterial meningitis is an important disease especially of early childhood with high case fatality and risk of neurological disorders. The fatality rate associated with this disease is as high as 20-30% in neonates and children world-wide (Xavier et al., 2003) whereas in India and other developing countries fatality rate has been quoted as 16-30%. A wide range of bacteria are associated with meningitis and 80% of all cases of bacterial meningitis are caused by *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococci pneumoniae* (Hart, 2003). Over two third of all the cases of bacterial meningitis occur in children less than 5 years age group. The relative frequency of etiological agents of bacterial meningitis varies with age and geographical region. The burden of disease from bacterial meningitis is higher in low resource setting with poor health infrastructure of developing countries because of high rate of malnutrition generally poor living condition and inadequate access to preventive and curative services which may predispose individuals to infection and opportunities for optimal treatment.

Determination of the etiology of bacterial meningitis and estimating cost of disease are important in guiding vaccination policies. The surveillance of confirmed meningitidis cases, including surveillance of the diversity of causative strains, is essential to managing disease and developing vaccines. Orissa has no surveillance system for bacterial meningitis, and exact rates of meningococcal disease and serogroups. The present study was undertaken to determine the current etiology of bacterial meningitis in Orissa, with particular emphasis on serogroup distribution of Meningococci, Pneumococci and *Haemophillus influenzae* type B.

### Objective

- To isolate *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis* from hospitalized meningitis children under age group of 5 years.
- Identification of the isolates on the basis of their biochemical properties and serological characteristics Surveillance of prevalence of serotypes of *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis* in orissa.
- To assess antibiogram trends in *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitides*.
- Detection of species specific virulence genes of *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis* using specific primers by PCR assay
- Genetic lineage and clonality study of *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis* isolates by PFGE and RAPD PCR assay.

### Plan of work

### Collection of samples

Blood and CSF were collected from the patient for the isolation of *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis*

### Isolation of bacteria

For the isolation of bacteria blood culture was done and then the sample from the blood culture was plated onto blood agar plate and chocolate agar plate. The CSF collected was directly plated onto both the medium for isolation of *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis*. The blood agar plate was used for the isolation of *Streptococci pneumoniae* and *Neissseria meningitides*; the chocolate agar plate was used for isolation of *Haemophillus influenzae*.

### Identification of bacteria

Identification of the bacterial isolates wad done as per the procedure for identification (Manual of Medical Microbiology, ASM press).

Identification of *Streptococci pneumoniae* was done on the basis of bile solubility test, optochin sensitivity test and inulin fermentation followed by serogrouping, *Haemophillus influenzae* was identified on the colony morphology in chocolate agar plate and X and V factor requirement followed by serotyping and *Neissseria meningitides confirmed* on the basis of cytochrome B utilization and carbohydrate utilization and seotyping was done by using specific antisera.

### Antibiogram sensivity pattern

Antibiogram of the isolates of *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis* was carried out by well and disc diffusion method (Kirby, 1966)

### Molecular characterization:

### Detection of virulence gene:

Detection of virulence gene such as capsular transport (*ctr A*) in case of *Neissseria meningitidis* pneumolysin gene (*ply*) in *Streptococci pneumoniae* and capsulation (*bexA*) in *Haemophillus influenzae* by PCR assay.

### Genetic correlation and clonality study

Genetic correlation and clonality study was done by PFGE, RAPD PCR assay, dendogram and sequencing.

### Work done

- 60 samples of suspected meningitis cases from capital hospital were collected from different pediatric age group less than 5 yrs and analysed.
- Out of 60 samples, 36 samples are non-culturable whereas 24 samples are culture positive. Among the culture positive samples in 10 cases the causative organism found to be *Staphylococcus aureus* followed by *Streptococcus pneumoniae (*Suspected) (9 cases) *Staphylococcus sp* (6 cases) and *H.influenzae type b* (one case).
- The association of both *Staphylococcus* aureus and *Streptococcus pneumoniae* was confirmed in three cases.
- It was also recorded that male child are more prone than the female child and the cases are more frequent in children below 1 year.

### Work to be done

- Rapid diagnosis of the causative organism by latex agglutination test and real time PCR method and study their comparative reliability and efficiency with respect to culture method.
- Best method and temperature for storage of the organism.
- Multiplex PCR method for simultaneous identification of *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis* directly from the sample.
- Antibiotic trends of these organism and their resistance marker gene

### Expected outcome

No baseline information is available on the etiology and incidence of bacterial meningitis in Orissa. The study will not only focus on the etiology of bacterial meningitis but also provide the idea on the prevalence of the specific serogroups of the *Streptococci pneumoniae*, *Haemophillus influenzae* and *Neissseria meningitidis* in this region. Further, this will be helpful in designing of Hib, pneumococcal and meningococcal vaccination program. Molecular assay are more accurate and rapid than conventional methods. Therefore, it will help in better and rapid identification of the etiological agents. The antibiotic profile will throw insight on the drug resistivity pattern and will emphasize the better management practices in hospitalized bacterial meningitis.

## 5. Study on micronutrients malnutrition with special reference to vitamin A deficiency and its associations with other micronutrients among children in Orissa.

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Date of joining : 25th June 2009
Guide : Dr. G. Bulliyya

### Background

Vitamin A deficiency (VAD) is one the major public health nutritional problem. Globally, night

blindness affects 5.2 million preschool children (0.9%) and 9.8 million pregnant women (7.8%) population, respectively (WHO 2009). VAD is a public health problem in India including Orissa. An estimated 0.8% Indian children and Orissa 0.3% are suffering from clinical signs (Bitot's spot) of VAD (NNMB 2006). Sub-clinical VAD (low retinol <20ug/dl) affects at a high rates, 190 million (33.3%) preschool children and 19.1 million (15.3%) pregnant women globally. More than half of Indian preschool children (51%) are suffering from sub clinical VAD including Orissa (57%). Although VAD in form of clinical signs declined, sub-clinically remains to be of public health importance. Vitamin A status is associated and influenced by several factors such as morbidity conditions and other nutrients. Children who suffered from infectious diseases (diarrhea, pneumonia, malaria etc) had more of VAD. Iron deficiency deteriorate VA metabolism leading to a reduction in serum retinol. VA improves the mobilization of iron, driving to an increase in the intestinal absorption and utilization of iron (Sommer & West 1996). Vitamin E effects vitamin A metabolism in tissues, and physiological effect of retinol homeostasis. Zinc status influences VA metabolism, absorption, transport and utilization. Oxidative conversion of retinol to retinal requires retinol dehydrogenase enzyme (Smith et al 1973). Other trace elements such as K, Cu etc, play important roles in Vitamin A homeostasis. The present study is being conducted in assessing the vitamin A status and it association with other micronutrients in children in rural areas in Bhubaneswar block, Orissa

### Objective

The general objective is to study the vitamin A status and its association with micronutrients and major trace elements in children under-12 years of age.

### Specific

To assess the vitamin A status and prevalence vitamin A deficiency;

To assess the prevalence of deficiencies of iron, zinc, copper, vitamins E; and

To establish the association of vitamin A with other micronutrients and certain trace elements.

### Material and Methods

A cross sectional study conducted in a population of children below 12 years from Bhubaneswr block. Demographic and anthropometric data (height and weight) collected on 652 children using standard equipments and procedures. A total 260 blood samples collected from children so far and serum samples preserved under -20°C for micronutrient estimations. Blood hemoglobin estimated by cyanomethamoglobin method and heamatological parameters (RBC,WBC, Hct, MCV, MCHC etc) also estimated using MS4 cell counter. Vitamin A levels (serum retinol) were estimated by HPLC (Biery *et al* 1979). Serum vitamin E and vitamin C have been measured by HPLC and spectrophotometric methods respectively. Estimations of ferritin and C-reactive protein, transferrin receptor were done by ELISA methods. Trace elements (Br, K, Ca, Fe, Zn, Cu) were done in a sample of 37 lyophilized serum using proton induced X-ray emission (PIXE) method at Institute of Physics, Bhubaneswar.

### Work progress

From the population of children 36% have under nutrition of which 10% are severe, 41% have stunting of which 16% are severe and 17% have wasting.

Serum retinol levels were estimated in a sample of 168 children (Table 2). Mean concentration of serum retinol is 33.3ìg/dl, male children have significantly higher level of vitamin A than their female counterparts. Similarly female children have more prevalence of vitamin A deficiency (<20ìg/dl) at subclinical level (45%) as compared to male children. Pre-school (0-5yrs) children have significantly higher level of vitamin A than that of school-age children (6-12yrs).

The mean haemoglobin of 249 study sample is 10.21g/dl and boys have greater concentrations than girls. The prevalence of anaemia is 66.7% of which 23%, 24% and 20% are mild, moderate and severe

**Table 1.** Nutritional status of children under12 years in rural Bhubaneswar block.

| Sex & age group | Male 47.7%(n=311) | Female 52.3% (n=341) | Total (n=652) |
|---|---|---|---|
| **Weight-for age** | | | |
| Normal (>2SD) | 56.4 (175) | 71 (242) | 63.7 (417) |
| Underweight (<-2SD) | 33.3 (104) | 18.8 (64) | 26.05 (168) |
| Severe underweight (<-3SD | 10.3 (32) | 10.2 (35) | 10.25 (67) |
| **Height-for-age** | | | |
| Normal (>2SD) | 52.4 (163) | 65.2 (222) | 58.8 (385) |
| Stunting (<-2SD) | 27.8 (86) | 21.7 (74) | 24.8 (160) |
| Severe stunting (<-3SD | 19.8 (62) | 13.1 (45) | 16.4 (107) |
| **Weight-for-height** | | | |
| Normal (>2SD) | 80 (249) | 85.2 (290) | 82.6 (539) |
| Wasting (<-2SD) | 15 (47) | 11.1 (38) | 13.05 (85) |
| Severe wasting (<-3SD | 5 (15) | 3.7 (13) | 4.35 (28) |

categories (Table 3). Serum ferritin, the storage form of iron indictors, reflects iron deficiency in children. The percentage of ferritin deficient children was found to be 32.7% (<50ng/ml). The male children have significantly higher level of ferritin than that of the female children.

The prevalence of vitamin A deficiency is more in children having undernutrition in terms of underweight as well as stunting. Further severe form of undernutrition (<-3SD) is more associated with VAD. (Table 4 & 5)

Vitamin E which is another important antioxidant for body was also estimated. The

**Table 2.** Serum retinol (µg/dl) and vitamin A deficiency in children under12 years in rural Bhubaneswar block

| Age & Sex | N | Mean+SEM | Range | Vitamin A deficiency (retinol) | | |
|---|---|---|---|---|---|---|
| | | | | Normal (10-20) | VAD (<10) | Severe (>20) |
| **Sex** | | | | | | |
| Male | 65 | 41.3+4.78 | 5.08-227.3 | 61.53 (40) | 29.2 (19) | 9.23 (6) |
| Female | 103 | 28.2+2.78* | 5.16-132.6 | 54.36(56) | 40.8 (42) | 4.85(5) |
| **Age group** | | | | | | |
| 0-5 y | 37 | 41.8+6.91 | 5.7– 129.7 | 59.45 (22) | 35.14 (13) | 5.41 (2) |
| 6-12y | 131 | 30.8+2.17* | 5.08-227.3 | 56.49 (74) | 36.64 (48) | 6.87 (9) |
| Total | 168 | 33.3+2.29 | 5.08-227.3 | 57.14 (96) | 36.31 (61) | 6.55 (11) |

Figures in parenthesis are sample number. *Significant P<0.05

**Table 3.** Distribution of hemoglobin (g/dl) and severity of anaemia in study population.

| Age & Sex | N | Mean ± SD | Range | Grades of anemia | | | |
|---|---|---|---|---|---|---|---|
| | | | | Normal | Mild | Moderate | Severe |
| **Sex** | | | | | | | |
| Male | 121 | 9.90 ± 3.81 | 3.7-19.4 | 33.9 (41) | 14.0 (17) | 25.6 (31) | 26.5 (32) |
| Female | 128 | 10.52 ±3.29 | 3.3-20.0 | 32.8 (42) | 30.5 (39) | 21.9 (28) | 14.8 (19) |
| **Age group** | | | | | | | |
| 0-5 y | 64 | 10.08 ± 3.52 | 3.4 -17.8 | 43.7 (28) | 19.5 (9) | 20.3 (13) | 21.8 (14) |
| 6-12y | 185 | 10.26 ±3.59 | 3.3-20.0 | 29.7 (55) | 25.4 (47) | 24.8 (46) | 20.0 (37) |
| Total | 249 | 10.21 ± 3.56 | 3.3-20.0 | 33.3 (83) | 22.5 (56) | 23.7 (59) | 20.5 (51) |

Figures in parenthesis are sample number.

**Table 4.** Vitamin A Deficiency in relation to Weight-for-age (Underweight)

| Weight-for-age | Total % (N) | Non-VAD (>20 µg/dl) | VAD (<20 µg/dl) | Severe VAD (<10µg/dl) |
|---|---|---|---|---|
| Severe (<-3SD) | 7.74(13) | 23.1 (3) | 61.5 (8) | 15.4 (2) |
| Moderate (<-2SD) | 23.21(39) | 35.9 (14) | 46.2 (18) | 18.0 (7) |
| Normal (>-2SD) | 69.05(116) | 68.1(79) | 30.2(35) | 1.7 (2) |
| Total | 168 (100) | 57.1 (96) | 36.3 (61) | 6.6 (11) |

**Table 5.** Vitamin A Deficiency in relation to Height-for-age (Stunting).

| Height-for-age | Total % (N) | Non-VAD (>20 µg/dl) | VAD (<20 µg/dl) | Severe VAD (<10µg/dl) |
|---|---|---|---|---|
| Severe (<-3SD) | 15.48 (26) | 26.92 (7) | 57.7(15) | 15.38 (4) |
| Moderate(<-2SD) | 22.62 (38) | 34.21 (13) | 52.63(20) | 13.16 (5) |
| Normal (>-2SD) | 61.9 (104) | 73.08(76) | 25(26) | 1.92(2) |
| Total | 168(100) | 57.1(96) | 36.3 (61) | 6.6(11) |

prevalence of vitamin E deficiency in children was found to be 27% (<500ìg/dl) and the mean vitamin E level was found to be 972.5ìg/dl. Till now 36 children are found positive to infection whose C - reactive protein level was found to be >10000ng/ml. The mean level of different trace elements were found to be potassium-2213ppm, calcium-284.4ppm, iron-543.4ppm, Zn-9.35ppm, Cu-5.06ppm, Br-7ppm.

1. There is a significant positive correlation found between vitamin A and hemoglobin ($p<0.01$) while there is a significant negative correlation found between hemoglobin and ferritin ($p<0.001$) which possibly suggests free mobilization of iron from serum ferritin store. Since vitamin A is partly responsible for the above mobilization process an increase in vitamin A level indirectly influences depletion of ferritin store.
2. Vitamin A has a significant negative correlation with that of WBC count which probably suggests a inhibitory role of vitamin A on infections.

### Future work

Sample collection for remaining 400 sample out of target 1000 and analysis of blood or serum for vitamin A, E and C, transferrin receptor, C - reactive protein and ferritin from rest of the samples including trace elements from a sub-set of samples. Further data has to be analyzed for thesis writing and submission.

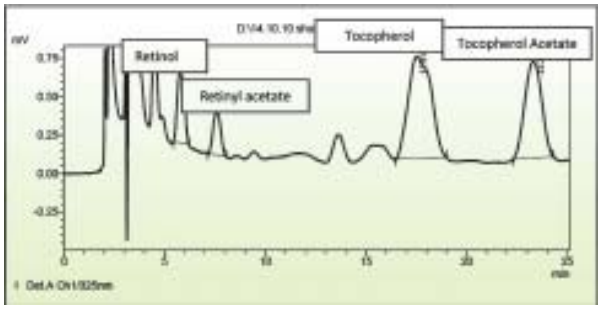


**Fig. 1.** Simultaneous Determination of Vitamin A and E by HPLC.

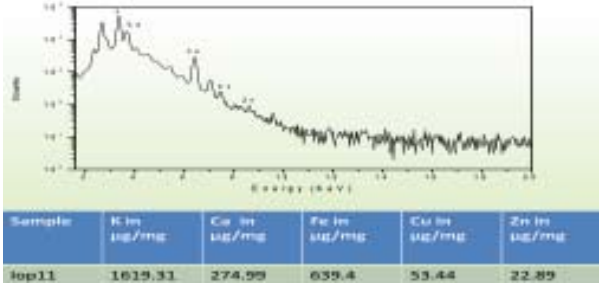


**Fig. 2.** Spectral analysis of trace elements by PIXE.

## 6. Study of Aedes mosquitoes in various parts of Orissa with reference to transmission of arboviral disease.

Name : Biswadeep Das (ICMR- JRF)
Status : JRF(ICMR)
Date of joining : January 2010
Guide : Dr.R.K.Hazra.

### Objectives

- To study the distribution and bionomics of *Aedes* mosquitoes involved in disease transmission in different parts of Orissa.
- To develop a PCR based method for identifying the immature stages of different *Aedes* mosquitoes that will be collected from various parts of Orissa.
- To identify Dengue virus and CHIKV in *Aedes* mosquitoes collected from different regions of Orissa.
- 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. 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. 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.

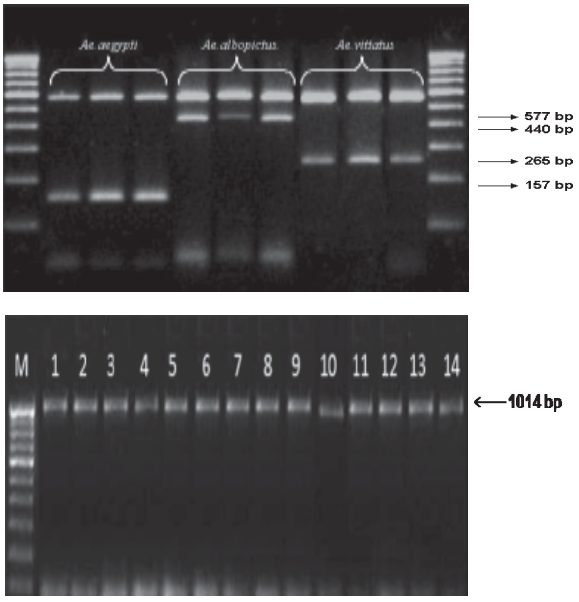


**Fig.1.** 1 % agarose gel photo showing the amplified E1 region of CHIKV.

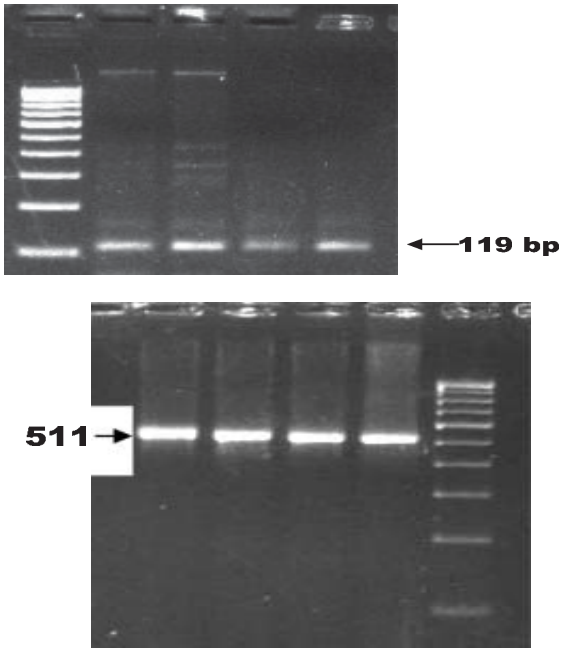


**Fig. 2.** 1.5 % agarose gel photo showing the amplified c-PRM region of DENV. Nested PCR of 511 bp region yielded 119 bp product that corresponds to DENV type II virus.

Arboviral outbreaks have been continuing to occur in the coastal districts of Orissa since 2008. Eight districts were surveyed based upon their high endemicity to chikungunya and dengue that had occurred. Detailed entomological survey was done and detected that *Aedes albopictus* was the most abundant vector with very high container and Breteau

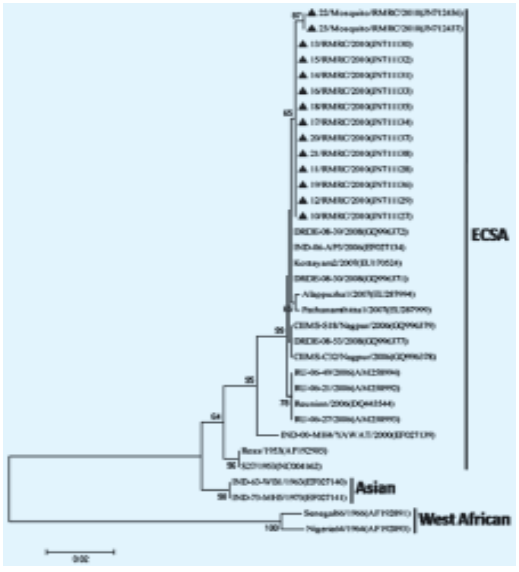


**Fig 3**: Phylogenetic tree of 294 bp sequence of E 1 gene of different genotypes of chikungunya virus showing all CHIKV sequences from Orissa belong to East Central South African genotype.

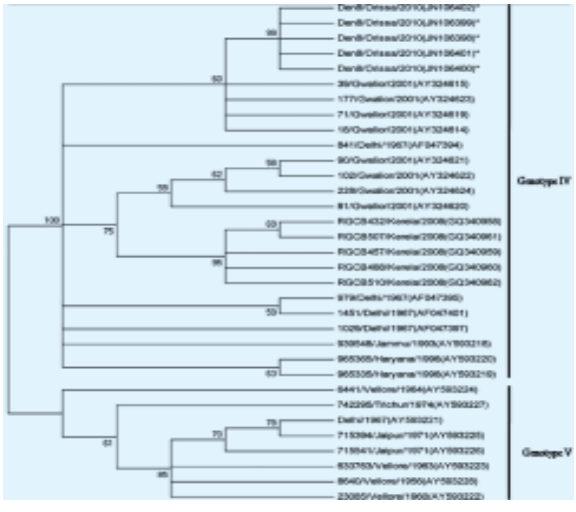


**Fig 4**: Phylogenetic tree of cPRM sequence of DENV showing all Orissa isolates from Malkangiri belong to genotype IV (Cosmopolitan genotype).

indices, thus proving to be the main vector in this region. To assess the clinical and virologic parameters, we collected serum samples from patients in areas where outbreaks were continuing. Chikungunya virus was identified in 70% of cases by both IgM and RT-PCR, thereby indicating that cases were collected during the acute stage of illness. Dengue virus was identified in *Ae. albopictus* larvae and adult species by RT-PCR, thereby indicating transovarial transmission of DENV. Hence molecular methods of detecting CHIKV/DENV can detect the virus at an early stage of the epidemic. Phylogentic analyses of partial E1 gene of CHIKV revealed the circulation of ECSA genotype in the affected areas and c-PRM gene of DENV indicated the circulation of genotype IV (cosmopolitan genotype) of DENV in Malkangiri, Orissa. Thus the more devastating outbreaks of chikungunya virus can be attributed to the circulation of ECSA genotype and genotype IV of DENV-II in these areas.

### Achievements

1. Sequences submitted to Genbank : **HQ010436** (*Aedes vittatus*), **HM486433** (*Aedes aegypti*), **HQ010437** (*Aedes albopictus*), dengue virus (**JN106398-402**), chikungunya virus (**JN711127-JN711138)**.
2. **Das B, Swain S, Patra A, Das M, Tripathy HK, Mohapatra N, Kar SK, Hazra RK (2012).**The development and evaluation of a single step multiplex PCR to differentiate the aquatic stages of morphological similar *Aedes* (Subgenus: Stegomyia) mosquitoes. *Tropical Medicine and International Health*, Vol 17, Issue 2, pg- 235-243.

## 7. Studies on Genetic Aspects of essential hypertension in different population groups of Orissa.

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Status : JRF(CSIR)
Date of joining : 24th sept 2009
Guide : Dr. M.R. Ranjit.

Hypertension is a pathophysiological condition in which the blood pressure remains abnormally higher than normal. There are two classes of clinical hypertension: 1. Primary or Essential Hypertension: with unknown etiology which accounts for 90-95% cases and 2. Secondary Hypertension which results secondarily from renal disease, endocrine disorders, or other identifiable cause. Worldwide there were 972 million hypertensive adults in the year 2000 and is expected to reach 1.56 billion by 2025 (Kearney PM *et al*, 2005). In India there were 65.5 million hypertensives in 2004 (Gupta R, 2004) and with the current rate of hypertension, it will have the largest number of people with hypertension in the world, with the potential of becoming the '**Hypertension capital of world**' (Joshi SR, Parikh RM, 2007). In case of essential hypertension, there are both genetic and environmental factors involved. The candidate genes include those of Renin-Angiotensin-Aldosterone system (Renin, Angiotensinogen), Sympathetic Nervous System (Alpha and Beta adrenorecptors), Renal transporters (Na-K-2Cl cotransporter, Epithelial Sodium Channel), Vascular endothelium (endothelial Nitric Oxide Synthase, endothelin, Endothelin receptors A etc. and the environmental factors include obesity, high alcohol intake, high salt intake (in salt-sensitive patients), aging, sedentary lifestyle, stress, low potassium intake, low calcium intake, low dietary fiber etc. Genetic studies on essential hypertension have been carried out in different parts of India but no report is available for Orissa. So this study has been initiated. Besides, associations with various blood groups (Maxwell RDH, Maxwell KN, 1955; Bhattacharya et al, 2010) and hemoglobin levels have also been observed (Hilde M, Hilden T, 2009). Therefore this study also aims to investigate the association of these parameters with hypertension in Orissa population.

### Objectives

1. To investigate the distribution of candidate gene polymorphisms associated with essential hypertension.
2. To find association of the studied polymorphisms with clinical expression of essential hypertension.

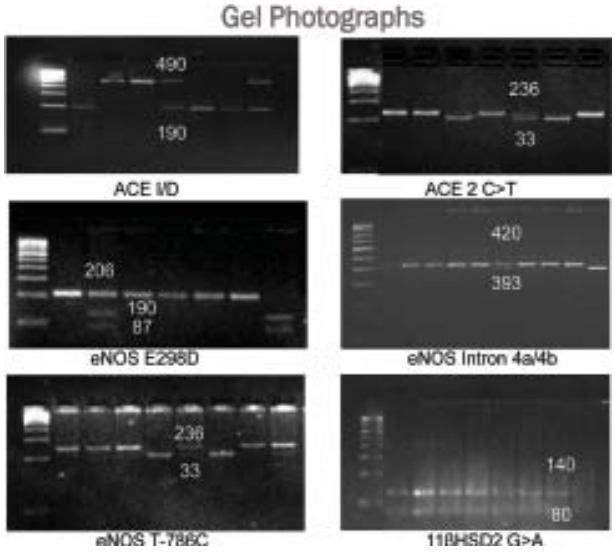
Blood was collected from the subjects by veinipuncture and stored in EDTA vials. From fresh whole blood hemoglobin and blood group were estimated. Plasma was then separated and stored at -20°C for biochemical analysis. Age, height, weight and waist measurements were recorded and BMI was calculated (weight 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 and the data was statistically analysed. Lipid profiling and kidney function tests (urea and Creatinine) were done.

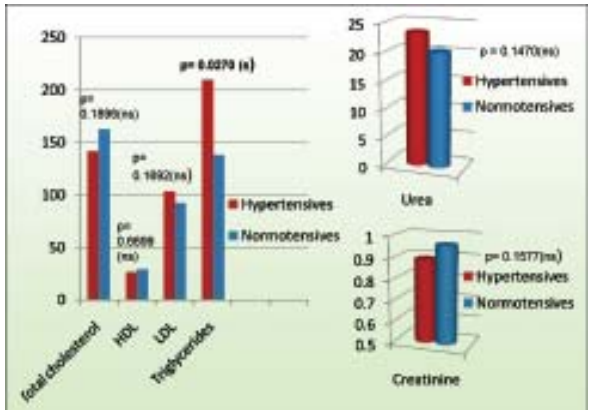
49 hypertensives and 47 age and sex matched controls were studied. No significant association of any of the studied polymorphisms was observed with hypertension. However, smaller p values were observed in case of ACE I/D and eNOS T-786C polymorphisms and C allele of the latter. The p values obtained in case of ACE ID and ID+DD genotypes

**Clinical Characteristics**

| Characteristics | Hypertensives | Normotensives | p value |
|---|---|---|---|
| Mean Age +/- SEM | 50.96+/-1.407 | 47.36+/-1.304 | 0.0644 (ns) |
| Male/Female | 34/15 | 33/14 | 1.0 (ns) |
| Mean Height+/-SEM | 162.4+/-1.499 | 162.3+/-1.323 | 0.9553 (ns) |
| Mean weight+/-SEM | 64.24+/-1.593 | 61.56+/-1.476 | 0.2210(ns) |
| Mean BMI+/-SEM | 24.34+/-0.4741 | 23.33+/-0.4424 | 0.1243(ns) |
| Mean Hb+/-SEM | 12.88+/-0.4478 | 13.55+/-0.5594 | 0.3590(ns) |

**Graphs and photographs showing the results are presented below**




were 0.29 and 0.36 respectively with II as reference and the TC and TC+CC genotypes of T-786C polymorphism showed p values of 0.21 and 0.10 respectively with TT as reference group. The p value obtained for C allele of T-786C genotype was 0.0594. Therefore an increase in sample number may show an association. Triglyceride levels were significantly higher in hypertensives than in controls ($p<0.05$); there was however, no difference in other biochemical parameters. No association was found with any of the blood groups or hemoglobin although hemoglobin levels were lower in hypertensives.

### Further plan

More number of subjects will be enrolled, polymorphisms will be added and the work will be carried further.

## 8. 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

### Introduction

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, 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, so also adults that have compromised immune system and elderly people. 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, Myxo/paramyxovirus, Togavirus and many Flaviviruses. In India the observed viruses are Flaviviruses Nipahvirus, Enteroviruses Chandipuravirus, Myxo/paramyxoviruses, Chikungunya and Herpesviruses.

**Objectives**

1. To identify the causative viral agents of encephalitis.
2. To study the clinical presentation of the viruses causing encephalitis.

**Materials and Methods**

334 Patients were enrolled for the study after being physically and clinically diagnosed by concerned physician. Clinical and demographic information were recorded on predesigned format together with physical examination. Samples(Serum/CSF) were collected as per standard guidelines of venipuncture and lumberpuncture. Samples were aliquoted and stored at -20$^0$C and -80$^0$C for serology and PCR respectively . Samples were subjected to serology and PCR. IgM ELISA were done for HSV I & II, Measles, Dengue,Varicella,enterovirus and JEV. A viral pathogen was regarded as etilogy if one of the following criteria was met (i) CSF and/or serum have virus specific antibodies.(ii) PCR amplification in blood or CSF.

**Results**

The study group comprised of 334 patients with mean age of 7 ± 3.1 and male to female ratio of 1.6:1 in pediatric group while mean age 35 ± 2.9 and male to female ratio 1.4:1 in adult group. Etiological diagnosis was reached or considered probable in 64(19%) by serology (IgM ELISA and PCR) cases out of the suspected cases as described in table 2.

**Table 1.** Age and Sex distribution of the study group.

| Age Group | Male | Female | Total |
|---|---|---|---|
| <1 | 23 | 13 | 36 |
| 1-15 | 118 | 76 | 194 |
| 16-30 | 28 | 12 | 40 |
| 31-45 | 21 | 14 | 35 |
| 45-60 | 9 | 5 | 14 |
| >60 | 9 | 6 | 15 |

**Table2.** Diagnosis of the suspected cases.

| Etiology | No. Of cases | %age |
|---|---|---|
| HSV | 52 | 14 |
| Measles | 5 | 1.4 |
| Dengue | 4 | 0.8 |
| Varicella | 2 | 0.6 |
| Enterovirus | 1 | 0.2 |
| JEV | 1 | 0.2 |

PCR was conducted for 110 acute phase samples obtained from suspected HSV, while 40 out of this acute CSF samples have also been retrospectively tried to amplify for enterovirus, JEV and WNV cases. 5 were positive for HSV out of the total samples processed while the rest were negative for others. During the Dengue outbreak that occurred in Odisha during Sept-Oct (2011) cases of dengue encephalitis were also

**Table 3.** Clinical features of patient with viral encephalitis.

| Clinical signs | %Age |
|---|---|
| Fever | 100 |
| Headache | 95 |
| Vomiting | 83 |
| Convulsion | 100 |
| Altered Sensorium | 100 |
| Meningeal Sign | 90 |
| Cranial nerve Palsy | 70 |

The amplification of the Herpes simplex virus and Dengue have been shown in fig1. and fig.2 respectively.

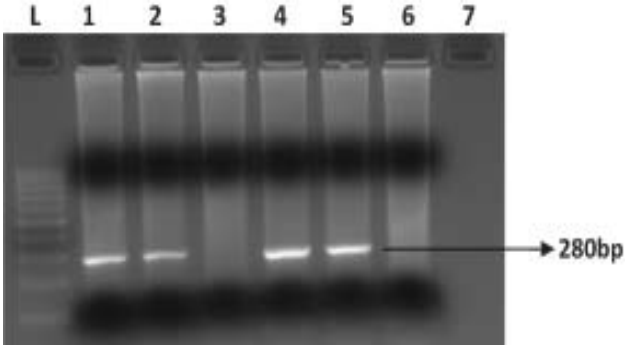



**Fig.1** Amplified DNA from CSF samples of the suspected HSV encephalitis. L(ladder),1-6 are sample and 7 is Negative control.

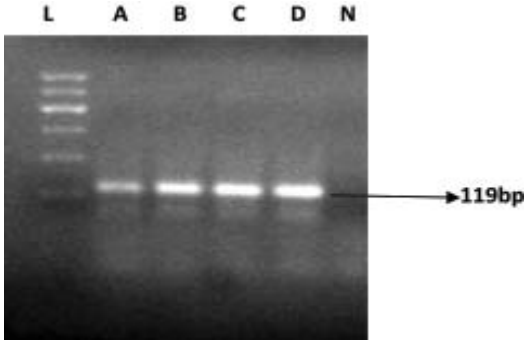



**Fig. 2.** Amplified RNA from serum samples of suspected Dengue. L( Ladder), A-D are samples,N-Negative control. Sample A is from the suspected acute viral encephalitis case.

suspected. Two were found NS1antigen positive in serum sample while one in each from IgM ELISA and PCR amplification.

## Discussion

This hospital based study has provided a preliminary idea about the causative agents for viral encephalitis in eastern Odisha. The most prevalent virus was Herpes virus followed by others in few cases including measles, varicella (Post infectious). Single case from each of Enterovirus and JEV has been found so far as negative samples for HSV have not been retrospectively studied.

Cases of Dengue encephalitis during the outbreak have once again shown the ability of the virus to become neurotropic and alarmed the health personnel to consider Dengue as a potential candidate of being causative organism for viral encephalitis during such outbreak.

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

Name : Priyadarsi Girija Sankar Sethy
Status : SRF(ICMR) Complected
Guide : Dr. G. Bulliyya, Scientist-D

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). 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.

## Objectives

To evaluate the prevalence of protein energy malnutrition among preschool children; To assess the prevalence of micronutrient deficiencies (Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Bromine (Br), Selenium (Se) and Lead (Pb)) and to establish association between PEM and various micronutrient deficiencies. The current study will explore the correlation between micronutrients and magnitude of PEM.

## Materials and Methods

*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), a 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. A 30 cluster PPS sampling method is adopted.

## Laboratory tests

Haemoglobin by Cyanmethaemoglobin (INACG, 1985), serum ferritin by ELISA, micro-nutrients (Potassium, Calcium, Manganese, Iron, Cobalt, Nickel, Copper, Zinc, Bromine, Selenium and Lead by using PIXE by Proton-induced X-ray emission at Institute of Physics.

## Results

The nutritional status of children (n=1100) 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 46.6 % 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 % (<-2SD) with 1.0% severely stunted (<-3SD) indicating the long duration malnutrition. Approximately 19% 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 74.4% were found anaemic (66.4 girls &72.1 boys), 21% found to have iron-deficiency anaemic. The mean haemoglobin levels (10.2 g/dl) among the children were below the cut of values. The proportion of mildly anaemia (57.8%) dominated over moderate (11.8%) grade of anaemia. Severe anaemia (<7 g/dl) among the children was 3.8%. The mean value of trace elements like K, Ca, Mn, Fe, Co, Ni, Cu, Zn, Se, Br and Pb are given in Table1. It is found that the value of trace elements such as Ca, Co, Cu, Fe, K, Mn & Zn increased with age, whereas Se, Ni, Br, and Pb do not are increased with age. Iron deficiency is correlated positively with Zn, and negatively with Cu. Pb levels inversely related with Fe, Ca, Zn and Mn. Some correlations were found among the trace elements in the serum of children. The total analysis is yet to be complete.

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

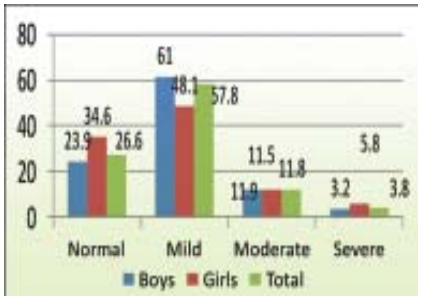



**Fig.2** Prevalence of Anaemia (Normal e"11, Mild 9-11, moderate 7-9, Severe<7g/dl)

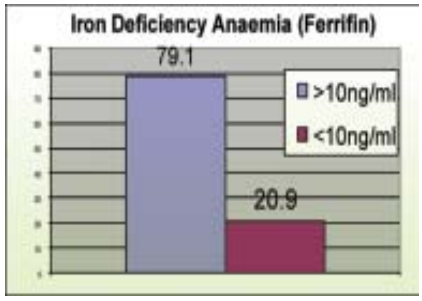



**Fig. 3** Iron deficiencyAnaemia

**Homogiious mix. ————Palate ———— Sample ———— PIXE ————--— Peaks of Trace Elements**

**Of Serum & Graphite Holder**

**Fig.4.** Process of Analysing Trace element by using PIXE.

**Table 1**. Serum concentrations of trace elements (PPM ) in preschool children, Orissa, India.

| Trace elements | Mean ± SD | 95% CI | Earlier studies |
|---|---|---|---|
| Potassium | 529.4±20.4 | 455.6 - 589.1 | 3.5-5.0 mmol=L (Iverson etal,2007) |
| Calcium | 276.8±17.3 | 243.0 - 378.2 | 2.05-2.55 mmol=L (Iverson etal,2007) |
| Iron | 428.6±18.3 | 315.1 – 524.0 | 10.7-26.9 mmol=L (Iverson etal,2007) |
| Manganese | 19.5±3.62 | 5.82 - 32.98 | 0.08±0.147PPM (Esfahani etal.,2007) |
| Cobalt | 3.4± 2.09 | 0.03 - 7.48 | 0.05±0.077 PPM (Esfahani etal.,2007) |
| Nickel | 3.2± 0.81 | 1.80 - 9.75 | 0.056 ±0.057 PPM (Esfahani etal.,2007) |
| Cupper | 4.07±0.91 | 2.28 - 6.53 | 3.61±0.10 umol/L (Ugwuja etal., 2007) |
| Zinc | 15.1±2.6 | 1.3 - 20.7 | 15.15±0.19µmol/L (Ugwuja etal., 2007) |
| Selenium | 1.0±0.9 | 0.0 - 4.7 | 0.90µmol/L (Ania etal., 2002) |
| Bromine | 60.1±10.8 | 2.9 - 79.2 | 69.2±8.39 µmol/L (Miura etal,1999) |
| Lead | 0.37±0.81 | 0.0 - 3.5 | 0.207 ± 0.105 ìmol/L (Yonghua *etal*, 2011) |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## 10. Molecular analysis of different anophelines and their vectorial attributes in different geographical regions of Orissa.


Name : Sunita Swain

Status : SRF (ICMR)

Guide : Dr. R. K. Hazra, Scientist-D


(PhD thesis submitted Sept. 2011 in Utkal University.)


## Summary

The baseline entomological data of anophelines such as vector density, vectorial capacity and host preference is crucially needed to assess the epidemiological impact of malaria control activities directed against vectors.

In Orissa, recent information on malaria transmission intensity is insufficient. Therefore a study was initiated to know the anopheline species composition and their relation to malaria transmission in nine ecologically different sites of Orissa. The present survey, which is the study on malaria transmission dynamics in Orissa, complements previous data on anopheline species distribution in the state. A total 7642 female anopheline mosquitoes belonging to 23 species were collected by different methods from four geographical zones of Orissa. *An. culicifacies* was the dominant species (23.7%) followed by *An. annularis* (23.3%) and *An. subpictus* (18.6%). Boudh represented with the highest number of

species (20). *An. culicifacies* was found to be the main malaria vector, *An. annularis* playing a secondary role, overcoming *An. fluviatilis* generally regarded as a main vector throughout its range. Among the three species, *An. fluviatilis* is highly anthropophilic wheras *An. culicifacies*, *An. annularis* are mainly zoophilic but due to high density they play significant role in malaria transmission. These biological variations indicate that they are more efficient malaria vectors with a capacity to sustain malaria transmission at lower vector population densities. The feeding behaviour of all the malaria vectors varied along their geographic distribution, partly because of the high degree of human population heterogeneity and land use. The significant degree of exophagic biting behaviour observed for the species may be partly as a result of the location of the villages along the different altitudes. Dwellings have incomplete walls and the habits of the human population facilitate human-vector contact and enhanced exophagy of mosquitoes. Out of 23 species of anophelines distributed throughout the nine districts of the state, five species (An.culicifacies, An.fluviatilis, An.annularis, An.varuna, An.subpictus) were found to be infected with the malaria parasite. The detection of spread of drug-resistant *P. falciparum* in a population, before any pathological symptoms detected in humans is possible by analyzing the anopheline vectors, transmitting malaria. In the present study we developed a new strategy to detect the spread of chloroquine-resistant (CQR) strains of *P. falciparum* by the major malaria vectors prevalent in selected endemic regions of Orissa, India. This approach of studying the anophelines to search for drug resistant parasites may also help in tackling the sporadic cases of drug resistance due to spreading of malaria.

For accurate knowledge of the species distribution and biology of anophelines has been limited due to the absence of reliable diagnostic characteristics. In this study, a multiplex polymerase chain reaction (PCR) assay was developed based on the sequence of the D3 region of 28S rDNA to distinguish between four members (*An. fluviatilis*, *An. culicifacies*, *An. varuna* and *An. aconitus*) of three subgroups (Minimus, Aconitus, Culicifacies) of the Funestus group of Myzomyia and three members (*An. annularis*, *An. pallidus* and *An. philippinensis*) of the Annularis group of the Neocellia series of the *Anopheles* subgenus Cellia, prevalent in Orissa, India. This assay can be applied as an unbiased confirmatory method for the identification of morphological variants, imperfectly preserved specimens and life stages for which taxonomic keys do not allow a definitive species determination.

Studies on the relationship of various vectors and non-vectors of malaria from the evolutionary point of view are important. In the present study, the phylogenetic relationships among the species of the anophelines of subgenus Cellia are inferred from the mitochondrial genes COI and COII, the ribosomal RNA gene, in particular the D3 region, and Internal Transcribed Spacer 2 (ITS2) region. The molecular phylogeny obtained in this work matches with that of the classical morphological taxonomy reasonably well, and was useful in properly defining species positions and resolving the ambiguity that normally arises due to morphological taxonomy. The correct arrangement of the various anopheline taxa as per the traditional morphological character-based classification of anophelines was there when we considered the D3 region of 28S rRNA gene and ITS2 region of rDNA. However, the arrangement of the taxa did not match with that of the morphological classification in some aspects, when we considered the COI and COII region of mitochondrial DNA. It may have been due to the variable degree of the rate of evolution of the different genes within the organism. Thus, a proper selection of those particular genes that evolve at the rate that is reflected at the species differentiation level could help to construct the correct phylogenetic relationship among the

anophelines and could be used to correlate with the grouping pattern done from the morphological perspective.

Insecticide resistance in malaria vectors is a growing concern in many countries, which requires immediate attention because of the limited chemical arsenal available for vector control. The current extent and distribution of this resistance in many parts of the Orissa state is unknown and yet such information is essential for the planning of effective malaria control interventions. Malaria has recently been resurged in some parts of Orissa. An attempt was made to asses the current status of insecticide resistance in the main malaria vector, *An. culicifacies*. Following the exposure of females of *An. culicifacies* to DDT impregnated papers, 6 out of 9 populations showed resistant. In this study, *An. culicifacies s.l* was found resistant to DDT, which might suggest the presence of *kdr*. In Keonjhar district, the L1014F *kdr* frequency was higher and both heterozygous and homozygous resistant mosquitoes were observed. For *An. culicifacies* molecular forms, the *kdr* genotypes were unequally distributed among bioassay survivors and non-survivors. This study however, found only the molecular A and B form of *An. culicifacies* with the *kdr* mutation. Therefore, based on the present study, it is suggested that areas under the influence of *An. culicifacies* should be covered under spraying of synthetic pyrethroids. Also the time of spray should coincide with the peak prevalence time of the vector species. These strategies may produce good epidemiological results. The results of this study highlight the importance of standardized longitudinal insecticide resistance monitoring and the urgent need for studies to monitor the impact of this resistance on malaria vector control activities.

*Anopheles* innate immunity affects *Plasmodium* development and is a potential target of innovative malaria control strategies. The completion of the *An. gambiae* genome has greatly facilitated research in this direction, and various anti-malarial immunity genes have now been identified. So far little attention has been devoted to examining polymorphism of immunity genes in natural malaria vector populations and its integrity in non-vectors. The extent and distribution of nucleotide diversity in immunity genes might provide insights into the evolutionary forces that condition pathogen-vector interactions. The discovery of polymorphisms is an essential step towards association studies of susceptibility to infection. In the present work we investigated patterns of genetic diversity of three anti- *Plasmodium* genes in various malaria vectors and non-vectors. We selected Defensin, Cecropin and Relish genes of *An. culicifacies*, *An. fluviatilis*, *An. vagus* and *An. aconitus*. During the result analysis of the immune genes, it was found that there are very rare non-synonymous mutations occurred in the coding sequence and most of the mutations are of synonymous nature resulting less deleterious effects on the polypeptide. The non-synonymous mutations are marked in *An. vagus*, which is a non-vector for malaria as compared to vector species. The nucleotide diversity (Pi) of all studied loci was quite low in all the studied species both in the synonymous and non-synonymous substitutions. The levels of divergence of these loci between different species were low except Relish gene was most divergent, with Dxy values range from 0.009 to 0.265. Cecropin and Relish did not show any signs of positive selection, and in particular, showed little or no differentiation between malaria vectors and non-vector species, indicating that these genes are largely subject to purifying selection. In the three of the studied loci comparisons Defensin showed a significant excess of non-synonymous polymorphisms. Some PCR error is expected to be present in the Defensin data set. Since a majority of possible mutations are non-synonymous, random errors will bias the observed number of non-synonymous polymorphisms upward. We can further investigate towards the direction of in vitro and in vivo studies on the effects of the identified mutations

on the overall effects of *Plasmodium* development inside the vector. Thus this will lead to the deciding factors of vector competency in anophelines.

### Achievements

- **Swain S**, Mohanty A, Tripathy HK, Mahapatra N, Kar SK, Hazra RK. Molecular identification and phylogeny of Myzomyia and Neocellia series of *Anopheles* subgenus Cellia (Diptera: Culicidae). ***Infection Genetics Evolution*** **2010** Oct;10(7):931-9.
- Das B, **Swain S,** Patra A, Das M, Tripathy HK, Mohapatra N, Kar SK, Hazra RK (2011).The development and evaluation of a single step multiplex PCR to differentiate the aquatic stages of morphological similar *Aedes* (Subgenus: Stegomyia) mosquitoes. ***Tropical Medicine and International Health*** **Feb: 17 (i): 235-243.**
- Total **42** DNA sequences submitted to the NCBI, GenBank with accession no. **EU366352-62**, **FJ159579-07** and **FJ176742-43**.




Interaction with students by SAC member during poster session of the SAC meeting at RMRC, BBSR.

# Publications and Information

**"Health & Nutrition" Book release on the occasion of ICMR Centenary Year Celebration at RMRC, Bhubaneswar**

# Publications

## Publications in 2010


1. Babu BV, Kar SK. Domestic violence in Eastern India: factors associated with victimization and perpetration. ***Public Health***. 2010.124(3):136-148.
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## Facilities

### 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

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.

### 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 Journal consortia through ICMR/ NML.

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 2011 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 leased lines. Presently two leased lines are connected to LAN server. One 4 MBPS ICMR-NIC Lerased line and one 100 MBPS NKN Leased line. Now Internet browsing and accessing all online journals through consortia is available in 24X7 formula.

**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.

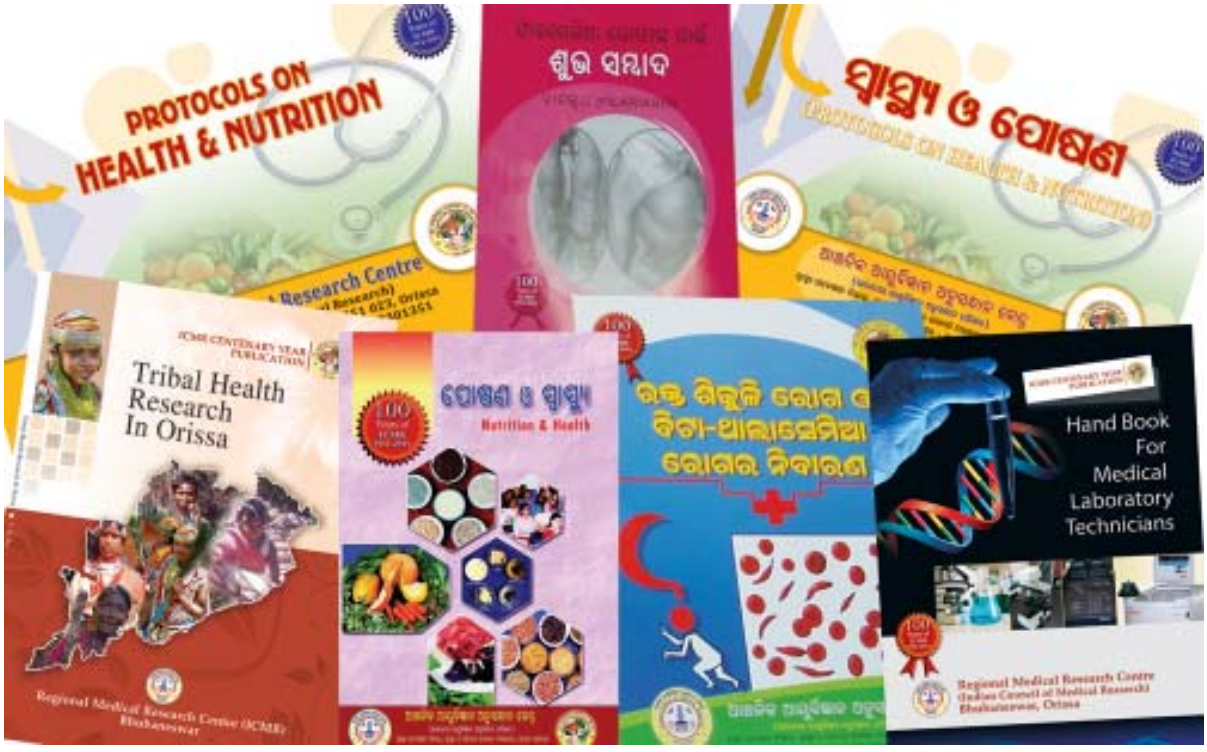
| Journal | Web site |
|---|---|
| Science | http://www.sciencemag.org |
| NEJM | http://content.nejm.org |
| BMJ | http://www.bmj.com |
| Lancet | http://www.sciencedirect.com |
| Nature | http://www.nature.com |

### ERMED Consortia

In this consortium total 1515 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




Centenary Year Publications

(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 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 up to 1995 .

### Publication Cell

### Centenary Year Publications

On the eve of centenary year celebration of ICMR (1911-2011) the Centre published the following publications.

1. Tribal health Research inOrissa (Book)
2. Hand Book for Medical Laboratory Technicians ( Book)
3. Protocols on Health and Nutrition ( Flip Chart-English)
4. Protocols on Health and Nutrition ( Flip Chart-Odia)
5. Filariasis Book Let ( Odia)
6. Sickle cell & Beta Thallasemia ( Odia)
7. Nutrition and Health ( Odia)

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.

### 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 (Mrs. Puspita Mishra and Miss. Nibedita Senapati) 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.

### Scientific Lecture

1. Dr. D.A Gadkari, Ex-Director, National Institute of Virology, Pune delivered a talk at RMRC Auditorium on 20th August 2010 on "Evolution of Influenza Virus and Pandemic H1N1". Scientists and Research scholars of RMRC, Doctors of Kalinga Hospital and Docts from State Health Dept. Were participated in the delibaration.
2. A series of lecture was organised for 10th Professional Development Course participants by RMRC scientists on 29th Sept. 2010 . The participants were district level Medical officers from Jharkhand, Chhatisgarh, Orissa and Andaman Nikobar under the banner of Public Health management and health sector reforms at SIHFW, Orissa. Dr. B.B. Pal, Sci-D, Dr. A.S.Kerketta, Sci-D and Dr. B. Dwibedi, Sci-B delivered the lecture on Diahoeal Diseases, Malaria, Hepatitis and Filariasis.
3. Dr. Kabi Prasad Mishra, Sr. Consoltant Cardiologist delivered a talk on "Science and Spirituality" on 5th October 2010 at RMRC Auditorium where all famili members of RMRC employees were invited.

### H1N1 Vaccination

H1N1 Vactination was undertaken for all employees of RMRC, Bhubaneswar by Capital Hospital, Bhubaneswar on 20th August in the RMRC Laboratory under the direct supervision of CMO, Capital Hospital, bhubaneswar.

### Hindi Day Observation

The Centre organised Hindi Day on 15th Sept. 2011 at RMRC Auditorium . Dr. Rajveer Singh, Principal Scientist, Directorate of Water Management , Bhubaneswar was the chief speaker on this occasion. Dr. S.K Kar, Director , Dr. Dasarathi Das, Scientist-D and Hindi Cell incharge were also delivered speech on the occasion. Other speakers were Dr. N. Mahapatra, Sc-E, Dr. M.R. Ranjit, Sci-D, Dr. A. Mahapatra, Sci-D, Dr. B. Dwibedi, Sci-B and Mr. P.K.Jangid, T.O . The vote of thanks were given by Dr. B. Sahooo, Lib & Inf. Officer.

### 24th SAC Meeting

The 24th SAC meeting was held on 19-20 oct 2010 at this Centre. In two days meeting all the ongoing and completed projects of the scientists were discussed. The poster presentation session was held on 2nd day morning where all the Ph.D scholars were presented their work progress on posters. Dr. D.S. Agarwal was the chairman of 24th SAC meeting and Dr. Lalit Kant, Chief ECD, ICMR, New Delhi was ICMR nominee and Dr. S.K.Kar, Director was the member Secretary.

### M Sc Dissertation

During this year following six M.Sc. students from various universities have undertaken their M.Sc. dissertation work under various scientists for partial fulfilment of the M.Sc. degree.

### Ph.D Awarded

Sudhansu Sekhar Nishank, SRF (CSIR) has been awarded PhD degree from Utkal University for his thesis on "Molecular characterization of â thalassaemia and its clinical significance in Orissa" in 2011. Dr. M.R.Ranjit, Scientist-E was the co-guide of the Ph.D work.

### Ph.D Submitted

1. Miss Upasana Sahu, SRF (RMRC) has completed her PhD thesis on "Role of microparticles and ABCA1 transporter in the pathogenesis of cerebral malaria" and submitted to Utkal University for the examination in 2011.
2. Miss Ronally Rout, SRF (UGC) has submitted her thesis on the topic "Molecular mechanism of rosetting in severe falciaprum malaria" for PhD examination to Utkal University.
3. Mr G Dhangda Majhi, SRF(CSIR) has submitted his thesis on the subject "Role of NO synthase in the pathogenesis of severe malaria" for PhD examination to Utkal University.

### Observation of National Science day

RMRC, Bhubaneswar organised National Science Day on 26th Feb. 2011 by conducting a series of lecture by our scientists at Vanivihar High School, Bhubaneswar. Dr. Dasarathi Das cientist-D and Dr. B. Dwibedi, Scietist-C talked on Infectious Diseases and Role of Hand Washing in prevention of diseases.

### Hindi Day Celebration

The centre organized Hindi Week Celebration from 11-14 September 2011.During the Hindi week observation debate competaation was held and prizes were distributed to the winners.

### ICMR Centenary Year Celebration

The Indian Council of Medical Research observed Centenary Year celebration from 15th Nov. 2010 to 15th Nov. 2011. On the occasion of Centenary year celebration the centre organized the 8th Joint Annual conference of the Indian Society for Malaria

and other communicable diseases (ISMOCD) and the Indian association of Epidemiologists (IAE) from 15th to 17th April 2011 at RMRC, Bhubaneswar. Dr. Shiv Lal, President ISMOCD & IAE and Dr. AC Dhariwal, Secretary, ISMOCD & IAE and Dr. V.M Katoch, Secretary, DHR & DG, ICMR and chief patron of the conference delivered the scientific communinity. Dr. S.K.Kar, Director, RMRC was the Chairman local organising committee and Dr. A. Mahapatrea, Scientist-D and Dr. M.R. ranbjit, Scientist-E were local organising secretary. In the scientific session, total 19 Guest lectures, 33 Oral Presentation and 105 Poster presentations werepresented by various eminent scientists & students throughout the country.

### Centenary Year Coin

On the eve of Centenary year celebration of ICMR, the Centre have distributed 15gram silver coin carring both ICMR logo and Centenary year logo . Shri Sanjiv Datta, F.A ICMR and Mr. Arun Baroka, Sr. DDG, Admn, ICMR were present in the coin distribution meeting held on 5th September 2011.

### Opening of BSL-2 Lab & TB Cultire Facility

The Centre organised a meeting for opening of BSL-2 Laboratory and TB Culture Facility on 5th September 2011 in the RMRC, Auditorium. Mrs. Anu Garg, Commisonner-cum Secretary, Health & Family Welfare, Govt. Of Odisha was the chief Guest, Dr. P.K.Das, DMET, Govt. Of Odisha was the chief speaker, Sri Sanjiv Datta, Financial Advisor, ICMR and Sri Arun Baroka, Sr. DDG, Admn, ICMR were guest of honour of the meeting.Dr. S.K.Kar, Director RMRC have given the welcome address to the gatherings.

### Inauguration of RMRC, Field Unit, Raygada

RMRC field unit at Raygada was inaugurated by honarable Minister of Health & family Welfare on 6th September 2011 at Raygada CDMO premices. In a colourful inauguration ceremony the opening Remarks was addressed by Dr. S. K. Kar, Director, RMRC, Bhubaneswar followes by welcome Address by Dr. Nitin Bhanudas Jalwa, DM & Collector, Raygada. Shri Prasanna Achrya Honble Minister of Health & FW. Govt. of Odisha, Sri Lal Bihari Himirika, Honble Minister of SC, ST, Development & Welfare, Govt. of Orissa, Shri Jagannath Saroka, President, Zilla Parishad and MLA, Gunupur were address the doctors, paramedical staff and media peronnel. Dr. Benudhar nayak, CDMO, Raygada has given the vote of thanks.


Dr. Nitin Bhanudas Jalwa, DM & Collector, Raygada addressing in the RMRC field unit inauguration meeting

### Budget and Resource Generation

The total sanction budget in respect of the Centre (Non-Plan & Plan) for the year 2010-11 is Rs. 696.20 lakhs and sanctioned budget for 2011-12 is Rs. 576.00 lakha up to Nov. 2011. The Head wise expenditure for 2010-11 of the budget is shown below. The resource generation during 2010-11 is 3 Crore and for 2011-12 is 1.5 crore from the extramural grant and Ph.D program through UGC, CSIR and others.

**BUDGET OF RMRC (10-11) (In Lakhs), SOURCE : ICMR**

| Establishment | Administrative Expenses | Contractual Service | Others | Equipment | Capital |
|---|---|---|---|---|---|
| 391.00 | 101.41 | 67.50 | 8.90 | 11.81 | 115.58 |



Pre-visit of Director & Scientific team to the CDMO office Kalahandi for establishment of RMRC field unit at Bhawanipatna.

## Meeting /Seminar Attended

**Dr. S. K. kar, Director**

1. Participated as a core member of the board in the meeting of the Assessment Board for promotion of ICMR Scientists under the Flexible Complementing Scheme for scientists holding post of Scientist- B to Scientist-F held during 7-9th January 2011 at ICMR Headquarters, New Delhi.
2. Participated in the Expert Group Meeting held at Delhi on Development of Virology Grade 1 lab on 25th Jan 2011 and presented annual progress and data.
3. Participated as Chairman in the protocol development meeting on Hib Survellance on 28th January 2011 at ICMR Headquaters, Delhi.
4. Participated in Expert Group Meeting for the " Monograph on Filariasis" at ICMR Headquarters 1st February 2011.
5. Attended Expert group meeting on monograph on filariasis at ICMR on 1st &3rd February 2011.
6. Participated in the Expert Group meeting to review award of Research Fellowship at ICMR Headquarters, New Delhi on 21st February 2011.
7. Participated at a workshop organized by DHS, Orissa on "Public Health Functions and Functionries" at TMST conference Hall Bhubaneswar on 26th February 2011.
8. Attended Human ethical committee meeting on 9th February 2011.
9. Attended Animal Ethical Committee Meeting on 10th February 2011.
10. Attended Expert group meeting on Fellowships at ICMR Hqrs. On 21st February 2011.
11. Attended Press sensitization workshop on MDA and delivered talk on "Addition of Albendazole-advantage thereof" at Hotel Kalinga Ashoka on 4th February 2011.
12. Attended SAG meeting at ICMR on 17th to 19th March 2011.
13. Attended and delivered a talk on "Filariasis: Epidemiology, Diagnosis, Clinical features and treatment". at Rajendra Memorial Institute for Medical Sciences, Patna on 22nd March 2011.
14. Participated in Press sensitization workshop on MDA-2011 for elimination of Lymphatic Filariasis held at Hotel, Kalinga Asoka, Bhubaneswar on 4th March 2011 and delivered a talk on " Advantage of Addition of Albendazole".
15. Participated as an invited member in the Scientific Advisory Group Meeting held at Delhi on 17th March 2011 and presented talk on annual scientific progress.
16. Participated as a member of AO Selecting Committee Meeting at ICMR, New Delhi on 19th July 2011.
17. Participated in investigato's meeting on Hib Meningitis at National Institute of Epidemiology, Chennai on 28th July 2011.
18. Participated in Tribal Health Meeting at RMRC, Dibrugarh on 9th-10th August 2011.
19. Attended Filariasis Meeting at New Delhi on 8th September 2011.
20. Attended Selecting Committee Meeting of LDCE at ICMR, New Delhi on 25th&26th September 2011.
21. Attended Brain Storming Meeting at ICMR, New Delhi on 5th October 2011.
22. Participated Hypertension protocol Meeting at ICMR, New Delhi on 11th November 2011.
23. Attended as Chief Speaker in the inaugural session of School of pharmaceutical Sciences, Bhubaneswar on 12th July 2011.
24. Attended 2nd Governing Body meeting of API at IMA House, Cuttack on 24th July 2011.
25. Attended NAMS Golden Jubilee celebration and delivered talk on "Lymphatic filariasis Pathogenesis and Management" on 14th October 2011.
26. Attended Workshop on Research Methodology at SUM Hospital and delivered talk on"Current

status and future avenues of biomedical research in Orissa" with special emphasis to a Territory Care Health Centre on 16th September 2011.

27. Delivered welcome address at STS Workshop at RMRC, BBSR on 27th to 29th September 2011.
28. Attended Brain Storming meeting on Vector Forum at NIMR, Delhi on 4th & 5th October 2011.
29. Delivered a talk on " Lymphatic Filariasis, pathogenesis and Challenges" at the meeting organized at RMRC for WHO team visiting the Centre on 13th October 2011.
30. Attended Centinery Celabration Meeting at ICMR, New Delhi on 15th November 2011.
31. Attended RMRC, Dibrugarh SAC Meeting on 22nd & 23rd November 2011.
32. Attended Foundation Day Celebration Meeting at NJIL, Agra and delivered a talk on " Vector Borne Disease and public health importance in India" on 17th December 2011.
33. Attended VCRC SAC Meeting at Pandicherry on 20th & 21st December 2011.

**Dr. N. Mahapatra**

1. Attended review meeting on malaria control with commissioner cum secretary (State Health Department) every month.
2. Attended SRC meeting on Biotechnology of Utkal University as a member on the month of November 2010 and February 2011.
3. Attended joint meeting of the technical and monitoring held on 16th November 2010.
4. Attended meeting to control mosquito menaces and appropriate control measures in urban areas (BMC & CMC) on 16th March 2011.
5. Attended Director's meeting on 8th and 9th May 2011at NewDelhi.

**Dr. M.R.Ranjit**

1. Attended the National Conference on "Environment Degradation and its impact on Mankind" organized by Punjabi University, Patiala from 19th -20th November 2010.Presented the paper entitled "Epidemiology of malaria in Orissa and possible impact of climate change on it"
2. Attended and Delivered a lecture entitled "Severe malaria: more questions than answers to the process" on 23rd November 2010, at Global Exchange Lecture Course, jointly organized by NIMR, Delhi & EMBO, Germany held from 21 November to 04 December 2010 at New Delhi.

**Dr. A.K.Satapathy**

1. Dr A. K. Satapathy attended 8th joint Annual conference of The Indian Society for malaria and other Communicable Diseases & The Indian associaton of Epidemiologist held at Bhubaneswar from 15-17April 2011

**Dr. G. Bulliyya**

1. Participated training Programme on Scaling Up of Water Productivity in Agriculture for Productivity' of Directorate of Water Management, Bhubaneswar at Nathpur, Khurda district and delivered a talk on role of water in communicable diseases in achieving food and nutrition security on January 15, 2011.
2. Attended National Seminar on Medical Anthropology in India: Biocultural Perspectives at Utkal University, Bhubaneswar and presented a paper on' intervention strategies to control anaemia in adolescent in a tribal block of Gajapati district on March 25, 2011.
3. Participated in the Joint Annual Conference of the Indian Society for Malaria and Other Communiable Diseases & The Indian Association of Epidemiologists', at Swosti Plaza, organized by RMRC, Bhubaneswar during April 15-17, 2011.
4. Attended consultation Meeting on 'Food and Nutrition Security' at M.S.Swaminathan Research Foundation, Jeypore, Koraput and presented a paper on 'Nutritional status of Vulnerable Sections of Population and Food Security' on April 18, 2011.

5. Attended as resource person of Annual Health Survey Orientation Meeting of Partener Institutions for the CAB Component of DLHS-4 at National Institute of Health & Family Welfare, New Delhi on April 20, 2011.
6. Participated State-level Workshop on Health Promotion of Adolescent Health Project organized by Multi Applied System at Hotel Suryansh, Bhubaneswar on July 30, 2011.
7. Participated scientific Workshop on Breastfeeding.The Global Standard" organized by Nestle Nutrition Institute, South Asia Region at Puri and presented a paper on Role of breastfeeding in the context of achieving child survival MDG: Time trends and regional variations on July 31, 2011.
8. Attended 'Workshop on Tribal Health Research Forum' on the eve of International Indigenous People's Day at Regional Medical Research Centre, Dibrugarh. Presented 'RMRC-Bhubaneswar ongoing Research Contribution on Tribal studies for the year 2010-11 on August 9-10, 2011.
9. Acted as Panel member for the "State-level workshop on Issues of Tribal Development" organized by SCSTRTI & UNDP, Bhubaneswar at Crown Hotel, Bhuabneswar, presented a paper on 'Food and Nutrition security of tribal people in Orissa'.
10. Attended Research Project Planning Workshop on "Root and Tuber Crops for Food Security in Asia Pacific" organized by International Potato Centre of Central Tuber Crops Research Institute, at Hotel Trident, Bhubaneswar on October 13, 2011.
11. Attended 51st Annual Conference of The National Academy of Medical Sciences (NAMS) at Institute of Medical Sciences and SUM Hospital, Bhubneshwar on October 15, 2011.
12. Participated District-level Training on Adolescent Reproductive and Sexual Health (ARSH) as a resource person at Hotel Center Park, Bhawanipatna, Kalahandi ditrict, on October 21, 2011.
13. Attended meeting of the ICMR Tribal Health Research Forum" at the Institute of Immunohaematology, Mumbai on 6th December, 2011 and presented 'RMRC-Bhubaneswar Research Contribution on Tribal studies for the period Sep-Nov 2011.

**Dr. R. K. Hazra**

1. Attended the National Symposium on climate changed Vector Borne diseases held at the Asian Society Kolkata on 6th Feb 2010 and deliver guest lecture.
2. Participated EMBO Global Exchange Lecture Course on Molecular and Evolutionary Genetics of Malaria, National Institute of Malaria Research, New Delhi, India & delivered a lecture on 23rd November 2010 on Phylogenetic analysis.
3. Attended 8th Joint Annual conference of the Indian Society for Malaria and other communicable diseases (ISMOCD) and the Indian association of Epidemiologists (IAE) at Bhubaneswar from 15th to 17th April 2011.
4. Attended and presented a paper entitled "Emergence of abroural diseases in Orissa at the XV symposium ds Jabalpur, India from 15-18 Oct, 2011.

**Dr B. B. Pal**

**1.** Attended the National Seminar on "Medical Anthropology in India- Bio-cultural prospectives " and presented the paper on " Sosio-Cultural behavior associated with cholera outbreak in the tribal areas of Orissa 24-25th March,2911,Utkal University ,Vani Vihar ,Orissa.

**2.** Attended Annual conference of the Indian society for malaria and other communicable diseases & the Indian Association of Epidemologist, 15-17th April, RMRC, Bhubaneswar, Orissa.

**3.** Dr B.B.Pal was invited and attended National seminar on "Trends in microbial Bio-remediation of Environmental soil, 24-25th Sept 2011, OUAT, Bhubaneswar, Orissa.

### Dr. A. S. Kerketta

1. Attended 8th Joint Annual conference of the Indian Society for Malaria and other communicable diseases (ISMOCD) and the Indian association of Epidemiologists (IAE) at Bhubaneswar from 15th to 17th April 2011.

### Dr. A. Mahapatra

1. Acted as organising secretary 8th Joint Annual conference of the Indian Society for Malaria and other communicable diseases (ISMOCD) and the Indian association of Epidemiologists (IAE) at RMRC Bhubaneswar from 15th to 17th April 2011.
2. Attended 36th all India Sociological Conference held at Ravenshaw University, Cuttack from 27-29, Nov. 2010.
3. Participated Symposium on Vectors and vector Borne Diseases held at RMRC(T) Jabalpur during 15th to 17th Oct 2011.
4. Attended National Seminar on "Medical Anthropology in India: Bio-cultural Perspectives" held during 24th to 25th March at Utkal University, Bhubaneswar.

### Dr B. Dwibedi

1. Delivered a talk at Post Graduate Dept. of Zoology, Utkal University, Vani Vihar, Bhubaneswar on 3rd February 2011.
2. Attended a Workshop on Public Health Function and Functionaries at Directorate of Health Services, Odisha- Conference Hall of TMST (Behind Capital Hospital), Bhubaneswar on 26th February 2011.
3. Attended HIV/ Malaria meeting at NARI, Pune on 25th May 2011.
4. Attended Tribal Health Forum Meeting at RMRC, Dibrugarh on9th August 2011.
5. Delivereda lecture on Seromolecular markers of Hepatitis B virus infection and their clinical relevance at NISER, Bhubaneswar on 3rd September 2011.

6 Attended and delivered a talk in a Workshop on Research Methodology at IMS, SUM Hospital, Bhubaneswar on 16th September 2011.

7. Attended Meeting of the Sub-committee for R & D Projects pertaining to Biotechnology at Conference Hall of Institute of Life Sciences, Bhubaneswar16th September 2011.
8. Awarded WHO fellowship and attended fellowship programme at the Royal Tropical Institute (KIT), Netherlands from 10th to 21st October 2011.

### Dr. B. Sahoo

1. Participated ICMR Librarian meeting on Library Modernization held on 22nd June 2011 at NIOH , Ahemdabad and presented a paper on "Present scenario of RMRC, Library and future prospective".
2. Attended a seminar on "E- Contents" organized by Informatics India Limited, Bangalore on 23rd November 2011 at Hotel May Fare, Bhubaneswar.

### Students

1. Mrs. Buli Kumari Panigrahi, SRF has attended International Symposium on Recent Advances in Ecology and Management of Vectors and Vector Borne Diseases, from 1-3 December, 2010, Gwalior and presented a Paper entitled "Effect of a modified ecosystem on malaria situation in two endemic areas of Orissa, India.
2. Biswadeep Das has attended and awarded best poster prize at the XII symposium of National Academy of vector borne diseases at RMRCT, Jabalpur, India from 15-18- Oct, 2011.

25th SAC meeting

## 25th Scientific Advisory Committee

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   Regional Medical Research Centre
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   PO: BJB Nagar, BBSR 751014
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## Staff position

### Scientists:

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| Dr. Bhagirathi Dwibedi, M.B.B.S, M.D | Scientist-C |

### Research & Technical Staff

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**STUDENTS:**

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| Sunita Swain M.Sc. | SRF (ICMR) |
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| | |
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| | |
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| Dr. A.R Mohanta | Asst. Research Scientist |
| Mrs. Haraprava Sahu | Social Worker |
| Mr. G.C. Mantri | Lab. Technician |
| Mr. R.K. Sahoo | Driver |
| Mr. Santosh Kumar Juharsing | Field Attendant |

Inauguration of Biometric Attendance System in RMRC, Bhubaneswar


**Dr. V.M. Katoch, Secretary DHR & DG, ICMR laying the foundation stone for establishment of OPD in the RMRC premises.**

**Hon'ble Minister of Health & Family Welfare, Govt. of Odisha, Sri Prasana Acharya lighting the lamp for RMRC field unit inauguration at Rayagada**


# Regional Medical Research Centre

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